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Factors influencing the immune response over 15 months after SARS-CoV-2 infection: A longitudinal population-wide study in the Faroe Islands

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Background. The durability of SARS-CoV-2 antibody response and the resulting immunity to COVID-19 is unclear.

Objectives. To investigate long-term humoral immunity to SARS-CoV-2.

Methods. In this nationwide, longitudinal study, we determined antibody response in 411 patients aged 0–93 years from two waves of infections (March to December 2020) contributing 1063 blood samples. Each individual had blood drawn on 4–5 occasions 1–15 months after disease onset. We measured total anti-SARS-CoV-2 receptor-binding domain (RBD) antibody using a qualitative RBD sandwich ELISA, IgM, IgG and IgA levels using an quantitative in-house ELISA-based assay and neutralizing antibodies (NAbs) using an in-house ELISA-based pseudoneutralizing assay. IgG subclasses were analyzed in a subset of samples by ELISA-based assay. We used nonlinear models to study the durability of SARS-CoV-2 antibody responses and its influence over time.

Results. After 15 months, 94% still had detectable circulating antibodies, mainly the IgG isotype, and 92% had detectable NAbs. The distribution of IgG antibodies varied significantly over time, characterized by a biphasic pattern with an initial decline followed by a plateau after approximately 7 months. However, the NAbs remained relatively stable throughout the period. The strength of the antibody response was influenced by smoking and hospitalization, with lower IgG levels in smokers and higher levels in hospitalized individuals. Antibody stability over time was mainly associated with male sex and older age with higher initial levels but more marked decrease.

Conclusions. The humoral immune response to SARS-CoV-2 infection varies depending on behavioral factors and disease severity, and antibody stability over 15 months was associated with sex and age.

Keywords: Faroe Islands, infection-acquired immunity, longitudinal study, SARS-CoV-2 antibodies, vaccination-acquired immunity

Introduction

Between January 2020 and 1 July 2022, more than 560 million people worldwide were infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has been documented that clinical manifestations of coronavirus disease 2019 (COVID-19) range from asymptomatic to severe disease course [1]. However, less is known about the long-term durability of SARS-CoV-2-specific antibody responses following symptomatic infection and the protective capacity towards SARS-CoV-2 reinfection. Understanding the kinetics of waning immunity and the magnitude of antibody responses following SARS-CoV-2 infection at the individual and population levels is crucial for future decisions on managing the pandemic and ongoing strategies for global vaccine strategies [2].

SARS-CoV-2 infection is associated with the development of variable levels of antibodies with neutralizing activity. However, we are limited by the length of reported follow-up data to know the expected duration of protection against COVID-19 following infection. The durations are defined by the end of the conducted studies rather than the disappearance of antibodies. Few studies have prospectively assessed the long-term immunity beyond 12 months after infection. We have previously reported that SARS-CoV-2 antibodies persist for at least 12 months [3], which is in line with other findings [4-8]. A few long-term prospective studies have been conducted. An Italian study found that anti-Spike (S) receptor-binding domain (RBD) IgG persisted in 96.8% of subjects 14 months after SARS-CoV-2 infection [9]. A study from France found persistence of anti-RBD antibodies up to 13 months after infection and that they may reduce the risk of reinfection [10]. In a Spanish study, seropositivity was 96.9% up to 322–379 days post symptom onset [11]. Overall, studies show considerable heterogeneity in immune responses between individuals. In line with these results, reinfections relative to the overall incidence were relatively rare in the Faroe Islands until the emergence of the Omicron variant in December 2021. Before the Omicron variant became the most prevalent, there had been 4477 individuals with COVID-19 among the 53,600 inhabitants, and only one reinfection was recorded.

Predicting the durability of immunity against SARS-CoV-2 is important and longitudinal studies are needed. In two prospective COVID-19 patient cohorts in the Faroe Islands from the first (March to April 2020) and second wave (August to December 2020), we have investigated long-term humoral immunity to SARS-CoV-2. We report binding (IgG, IgM, and IgA) and neutralizing antibodies (NAbs) to the SARS-CoV-2 RBD domain up to 15 months and further explore potential correlates of immune activity to demographic and clinical data. Additionally, we further investigate the antibody response after vaccination in a subgroup of the participants.

Methods

Study design and participants

All consecutive patients with COVID-19 confirmed by reverse transcription polymerase chain reaction (RT-PCR) testing of an oropharyngeal swab from the first wave (3 March to 22 April 2020) and second wave (3 August to 25 December 2020) in the Faroe Islands were invited to participate in this prospective longitudinal observational study. The date of infection was registered as the day of symptom onset, or if asymptomatic, the day of positive RT-PCR testing. In December 2020, the recruitment was less systematic as not all patients were invited ($n = 35$ participants of 78 eligible from 1–26 December 2020). Members of the COVID-19 task force who followed all COVID-19 patients during their disease course asked the patients for permission to be contacted by the research team [12]. Participation included a blood sample shortly after recovery and consecutive blood samples approximately 1, 3–4, 7, 12, and 15 months after recovery, allowing for cross-sectional and longitudinal analyses of SARS-CoV-2-specific antibodies. Additionally, they answered a short questionnaire regarding sociodemographic and behavioral factors and comorbidities.

The symptoms during the acute phase were recorded, and persistent symptoms were documented through follow-up phone calls [1, 13]. The vaccination roll out began in the Faroe Islands on 30 December 2020, with the Pfizer-BioNTech vaccine (BNT162b2) as the only vaccine.

The study was approved by the Faroese Research Ethical Committee and the Data Protection Agency. All participants provided written informed consent.

Determination of antibody levels

Total antibody levels were measured using a qualitative RBD sandwich ELISA according to

the manufacturer's instructions (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., China). Quantitative determination of IgG, IgM, and IgA antibodies was performed using an in-house ELISA-based assay, as described previously [14] (see Supplementary Information for a brief description of the method). The thresholds for assay positivity were defined as 6 AU/ml, 22.4 AU/ml, and 2.41 AU/ml for IgG, IgM, and IgA, respectively. Low and high IgG levels were defined as ≤22.05 and >22.05 AU/ml, respectively. Low and high IgM levels were defined as ≤40 and >40 AU/ml, respectively. Low and high IgA levels were defined as ≤8.28 and >8.28 AU/ml, respectively. The sensitivity and specificity of the assays were defined as 94.3% sensitivity and 99.5% specificity, 63.4 % sensitivity and 99.3% specificity, and 61.4% sensitivity and 99.1% specificity for IgG, IgM, and IgA, respectively [14].

Virus NAbs measurement

As a proxy for measuring virus NAbs, we used an in-house produced ELISA-based pseudoneutralizing assay that measures the interaction between the ACE-2 host receptor and RBD to estimate the degree of inhibition of virus NAbs against RBD, as described previously [15] (see Supplementary Information for a brief description of the method). The threshold for virus NAbs assay positivity was defined as 25%, with a sensitivity of 92.2% and a specificity of 95.4%.

Analyses of IgG subclasses

A total of 20 nonvaccinated individuals from the first wave who had contributed with four samples, with equal sex and age distribution were selected for analyses of IgG subclasses levels as described previously using an ELISA-based assay [16] (Table S1, see Supplementary Information for a brief description of the method).

Statistical analyses and prediction models

GraphPad Prism version 9.2.0 (GraphPad Software, La Jolla, CA) was employed to estimate the antibody levels. Estimation of IgM, IgG, IgA levels and IgG subclasses 1 (IgG1) and 3 (IgG3) levels was interpolated by regression analyses using a four-parameter logistic curve fitting. Results were given in AU/ml (in a 1:200 dilution, the calibrator was defined to contain 2 AU/ml for IgM, IgG, and IgA; and 200 AU/ml for IgG1 and IgG3). A seronegative sample is defined by an AU/ml value below the assay positivity threshold. Seropositive samples are then classified into low and high antibody levels by an AU/ml below the median or above the median, respectively. To evaluate linearity between total IgG levels and IgG1 and IgG3 levels, a simple linear regression was performed. R (version 4.1.0 for Windows, R Foundation for Statistical Computing) was employed for the statistical analyses.

A generalized mixed model with zero-inflated Gamma distribution was used to represent and analyze IgG levels. The days from infection were represented using two natural cubic splines to nonlinear model trends. Modeling of antibody levels was represented from the date of infection up to 472 days. IgG levels were also represented and analyzed using a linear-mixed model from the date of infection and up to 472 days. A generalized mixed model with binomial distribution was used to represent and analyze IgA responses and NAbs index. IgA levels and NAbs index were transformed into a binary variable defined as positive (>2.41 AU/ml) or negative response, and neutralizing (>25%) or non-neutralizing, respectively. Due to the different distribution of antibody levels in vaccinated individuals after the date of infection, IgG levels were represented with four natural cubic splines. Interactions between days and age group (<30, >30–50, >50 years), days and sex, and days and waves were analyzed. For IgG level analysis, interactions between days and smoking (ever smoking), days and hospitalization (hospitalized), days and symptoms at onset, days and body mass index (BMI) (normal [18.5–24.9], overweight [25–29.9], obese [>30]), days and chronic disease, and days and medication were analyzed separately. For IgA response analysis, interactions between days and hospitalization were analyzed separately. *P*-values from generalized mixed model analyses were calculated using Type II Wald chi-square tests (details of each model analyses can be found in Table S2). All results are stratified by wave due to different sampling periods. Antibody levels were log10 transformed to obtain normality (and back-transformed when reported). A more detailed description of the generalized mixed models can be found in the Supplementary Information.

Parametric or nonparametric tests were applied as indicated, for example, chi-squared or Fisher's exact test, Friedman test, two-sided Wilcoxon signed rank test, Mann Whitney U test, Kruskal Wallis test, and Spearman rank correlation tests. *P*-value less than 0.05 was considered significant.

Results

Characteristics of the cohort

Out of 484 eligible COVID-19 cases in the study period, 411 (85%) participated in this study during the two waves, of which only 14 were hospitalized during acute illness. Out of 187 cases in the first wave, 174 (93%) participated (Table 1). There were up to five samplings during the study period (median and 5%–95% percentile): 89 days (43–131), 210 days (180–244), 363 days (319–373), 455 days (401–463), and 453 (430–463) after infection with 168, 139, 163 (n = 6 vaccinated), 155 (n = 39 vaccinated), and 15 (n = 13 vaccinated) participants providing samples, respectively. One hundred and twenty eight delivered four consecutive samples, of which 36 were vaccinated. Of note, 28% (n = 49) of the participants were vaccinated at some time during the study period.

During the second wave, 297 COVID-19 cases were registered, of which 237 individuals participated (79%) (Table 1). The five samplings occurred (median [5%–95% percentile]) 27 days (15–44), 125 days (82–155), 203 days (152–233), 306 days (245–318), and 313 (308–316) with 220, 221 (n = 2 vaccinated), 221 (n = 10 vaccinated), 170 (n = 66 vaccinated), and three (n = 3 vaccinated) participants providing samples, respectively. A total of 158 participants delivered four samples, of which 64 were vaccinated. Of note, 32% (n = 76) were vaccinated at some time during the study period. Cohort characteristics are depicted in Table 1, Fig. 1, and Fig. S1.

Seropositivity assessed by different methods

At the first sampling, 99.4% and 96.4% of the participants had either total antibodies and/or IgG, IgA, and IgM in wave 1 and wave 2, respectively. More than 98% of the participants had detectable antibody levels at the third sampling median 363 (wave 1) and 203 (wave 2) days after infection, while this decreased to 94% and 76% during the last sampling, respectively (Table 2). At first sampling in wave 2—taken within the first month—the proportion with detectable NAbs was only 83% but increased to above 90% in the second sampling, while NAbs were relatively stable (>90%) at all time points in wave 1. Only a few participants had five samples, which in most cases was taken after vaccination, and thus the fifth sampling is not shown in the table. Upset plots presenting overlap among measurements at each sampling show that for all sampling periods in both waves, the majority of individuals were seropositive with Wantai, total antibody (IgG, IgM, and/or IgA), NAbs, and IgG, while IgM and IgA were not detectable (Figs S2, S3, and S4) [17]. Median antibody levels are found in Table S3.

When comparing total antibodies and IgG, there was significant concordance in seropositivity assessed by Wantai and the direct antibody ELISA. However, this was not observed in the last sampling or when comparing total antibodies with IgM and IgA. NAbs levels were significantly correlated with IgG levels (rho > 0.5 and rho > 0.7) and also to a lesser degree with IgM levels (rho > 0.2) in all samplings for waves 1 and 2, respectively.

IgG was the most abundant isotype at all sampling periods; it was the most prevalent detected isotype at all samplings except in the first sampling in the second wave where all isotypes were present in combination (Fig. S4). Similar results can be observed when the different isotypes are divided in high and low levels (Fig. S5). Further, the majority had detectable IgG levels at all samplings (Fig. S4). Levels of IgG and IgA were significantly correlated at each sampling and over time for both waves whereas correlation with IgM was only observed at the first sampling time and was stronger in wave 2 (Fig. S6).

Distribution of IgG levels in circulation over time after SARS-CoV-2 infection

Figure 2 depicts the IgG levels over time in individuals after SARS-CoV-2 infection. The distribution of IgG antibodies varied significantly over time ($p < 0.001$), characterized by a waning of IgG levels from infection onset. Age was a significant influencing factor on the circulating IgG levels ($p = 0.0009$), where the older population (>50 years) produced higher IgG levels compared to the younger groups (e.g., IgG levels at day 60 after infection for a female individual >50 years: 34.4 AU/ml, 95% confidence interval [CI]: 26.4–43.9 AU/ml; between 30–50 years: 20.0 AU/ml, 95% CI: 15.4–25.5 AU/ml; and <30 years: 23.7 AU/ml, 95% CI: 18.4–29.9 AU/ml). An interaction between days from infection and sex was found ($p = 0.0002$), showing a faster decrease in males compared with females. For example, the decrease for a man aged 30–50 years would be 51.7%, 95%

Table 1. *Study cohort characteristics and clinical information from COVID-19 patients in the Faroe Islands from March to December 2020, stratified in two waves (*n = *174 and* n = *237)*

	First wave (n = 174)		Second wave (n = 237)	
Sex, *n* (%)				
Female	93	53.4	126	53.2
Male	81	46.6	111	46.8
Age				
Age (years), median (5%–95% percentile)	41.1	11.0–70.4	33.8	11.7–73.0
Age distribution, *n* (%)				
0–17	19	10.9	29	12.2
18–34	54	31.0	92	38.8
35–49	40	23.0	56	23.6
50–66	46	26.4	41	17.3
67+	15	8.6	19	8.0
Smoking status[b], *n* (%)				
Ever smoker	77	45.8	86	41.7
Never smoker	91	54.2	120	58.3
Daily medication use[c], *n* (%)				
Yes	64	37.0	50	25.5
Self-reported comorbidities[d], *n* (%)				
Yes	57	32.9	69	34.5
Most prevalent comorbidities, *n* (%)				
Hypertension	27	15.6	21	10.5
Hypercholesterolemia	8	4.6	19	9.5
Type 2 diabetes	5	2.9	5	2.5
Asthma	16	9.2	8	4.0
Hypothyroidism	7	4.1	3	1.5
Body mass index (BMI)[e]				
BMI, median (95% percentile)	25.8	17.2–34.6	25.0	17.9–37.0
BMI group distribution, *n* (%)				
Normal (<24.9)	55	41.0	99	49.7
Overweight (25–29.9)	58	43.3	64	32.2
Obese (>30)	21	15.7	36	18.1
Symptoms[a] during the acute phase[f], *n* (%)	167	96	191	91.4
Disease severity during the acute phase, *n* (%)				
Hospitalized	8	4.7	6	2.9
Number of participants at each sampling, *n* (number of vaccinated individuals)				
First sampling	168		220	
Second sampling	139		221(2)	
Third sampling	163 (6)		221(10)	
Fourth sampling	155 (39)		170 (66)	
Fifth sampling	15 (13)		3 (3)	
Days from infection to blood sample taken, median (5%–95% percentile)				
First sampling	89	43–131	27	15–44
Second sampling	210	180–244	125	82–155

(*Continued*)

Table 1. *(Continued)*

	First wave ($n = 174$)		Second wave ($n = 237$)	
Third sampling	363	319–373	203	152–233
Fourth sampling	455	401–463	306	245–318
Fifth sampling	453	430–463	313	308–316

[a]At least one symptom reported.
[b]Missing data ($n = 6$; $n = 31$).
[c]Missing data ($n = 1$; $n = 41$).
[d]Missing data ($n = 1$; $n = 37$).
[e]Missing data ($n = 40$; $n = 38$).
[f]Missing data ($n = 0$; $n = 28$).

CI: 48.6–54.4%, of IgG levels from day 30 to day 120 after the infection, compared to a decrease of 39.8%, 95% CI: 35.2–43.2%, for a woman of the same age and for the same period of time. An interaction between days from infection and age was observed ($p = 0.008$), characterized by the more marked decrease of IgG for the older group (>50 years: 30% decrease, 95% CI: 26.9%–32.6%, from day 60 to day 120 after infection) compared to the younger group (<30 years: 25.4% decrease, 95% CI: 22.3%–28.1%, from day 60 to day 120 after infection). Moreover, an interaction between days from infection and the wave was found to be significant ($p < 0.001$), indicating that IgG dynamics over time are different between waves. These differences arise likely due to the differences in the sampling and the time after infection (wave 1 has a longer span than wave 2).

To study the different combination of isotype responses over time, we transformed the IgA and IgM responses into a binary variable and used them to model IgG over time. In Fig. S7, we observed a significant association of positive IgA responses in those individuals with higher IgG levels over time ($p < 0.0001$). A similar but weaker association was observed for positive IgM responses (Fig. S8, $p = 0.02$), suggesting that individuals who develop higher levels of IgG are related to positive IgA and/or IgM responses.

We also modeled the IgG dynamics using a linear model, allowing for calculation of the decreasing rate of IgG antibodies over time (Fig. S9). Following the same tendency as seen in the nonlinear model, interactions between days from infection and age, sex, and waves were observed ($p = 0.002$, $p = 0.05$, and $p < 0.001$, respectively). This is characterized by a 30-day faster decline of IgG levels in older individuals compared to the youngest group (female >50 years: 8.1%, 95% CI: 7.1%–9.1%; female 30–50 years: 6.3%, 95% CI: 5.4%–7.7%; and female <30 years: 4.1%, 95% CI: 3.0%–5.2%). The 30-day waning was more pronounced in the male population by approximately 2.2% compared to the female population. Moreover, individuals infected in the second wave had an approximately 5.1% greater decrease of IgG every 30 days since infection.

Effect of covariates in the IgG dynamics after SARS-CoV-2 infection

Ever smoking significantly affected the development of IgG antibodies in individuals with SARS-CoV-2 infection ($p = 0.0002$, Fig. 3), being lower for smokers, independently of the age (e.g., IgG levels day 60 from infection in a nonsmoker and smoker female between 30–50 years: 24.2 AU/ml, 95% CI: 18.2–31.4 AU/ml; and 16.2 AU/ml, 95% CI: 12.4–21.1 AU/ml, respectively).

Hospitalization also significantly influenced the development of IgG antibodies in infected individuals ($p = 0.0003$, Fig. 4), being higher for individuals who required hospitalization during SARS-CoV-2 infection (e.g., IgG levels day 60 from infection in a nonhospitalized and hospitalized female between 30–50 years: 18.7 AU/ml, 95% CI: 14.4–23.7 AU/ml; and 58.3 AU/ml, 95% CI: 31.9–99.0 AU/ml, respectively). Hospitalized older individuals appeared to develop higher IgG levels compared to the younger groups (female 30–50 years: 58.3 AU/ml, 95% CI: 31.9–99.4 AU/ml; female >50 years: 99.2 AU/ml, 95% CI: 53.55–168.62 AU/ml, day 60 after infection). Albeit not significant, there was a tendency to develop higher levels of IgG antibodies (4% approximately) in individuals who were symptomatic at infection onset ($p = 0.05$, Fig. S10).

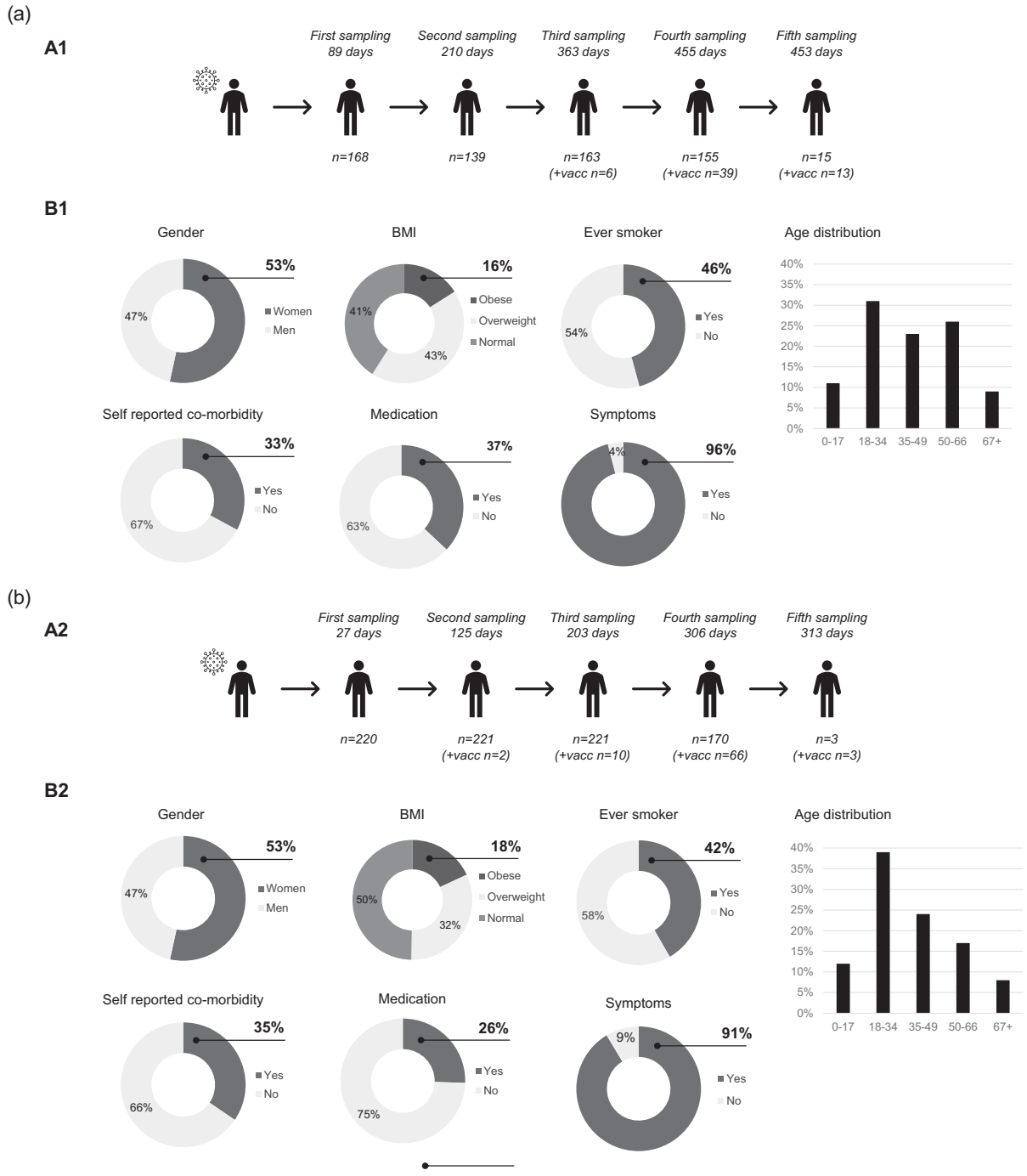

Fig. 1 *(a) Illustration depicting median days since disease and participation at each sampling in wave 1 (A1) and wave 2 (A2); (b) Characteristics of the study population in wave 1 (B1) and wave 2 (B2).*

Table 2. *Seropositivity in polymerase chain reaction (PCR)-confirmed COVID-19 cases from the Faroe Islands in the period March to December 2020 was assessed by different methods per sampling and stratified according to waves*

	Seropositivity wave 1 *n* (%)	Seropositivity wave 2 *n* (%)
First sampling	***n =168***	***n = 220***
Total antibodies (Wantai)	167 (99.4)	212 (96.4)
Any antibody[a]	163 (97.0)[b]	212 (96.4)[b]
IgG	159 (94.6)[b]	207 (94.1)[b]
IgM	28 (16.7)	116 (52.7)[b]
IgA	74 (44.0)	173 (78.6)[b]
Neutralizing antibodies	155 (92.3)	183 (83.2)[b]
Second sampling	***n = 139***	***n = 219***
Total antibodies (Wantai)	138 (99.3)	215 (98.2)
Any antibody[a]	131 (94.2)[b]	208 (95.0)[b]
IgG	128 (92.1)[b]	208 (95.0)[b]
IgM	10 (7.2)	22 (10.0)
IgA	58 (41.7)	94 (42.9)
Neutralizing antibodies	133 (96.4)[b]	205 (93.6)[b]
Third sampling	***n = 157***	***n = 211***
Total antibodies (Wantai)	155 (98.7)[b]	208 (98.6)[b]
Any antibody[a]	150 (95.5)[b]	194 (91.9)[b]
IgG	148 (94.3)[b]	194 (91.9)[b]
IgM	7 (4.5)	16 (7.6)
IgA	52 (33.1)	59 (28.0)
Neutralizing antibodies	146 (93.0)[b]	184 (87.2)[b]
Fourth sampling	***n = 116***	***n = 104***
Total antibodies (Wantai)	115 (99.1)	103 (99.0)
Any antibody[a]	109 (94.0)	79 (76.0)
IgG	105 (90.5)	76 (73.1)
IgM	4 (3.4)	3 (2.9)
IgA	22 (19.0)	44 (23.1)
Neutralizing antibodies	107 (92.2)	95 (91.3)

[a]IgG, IgM, and/or IgA detected.
[b]Compared with seropositivity measured with the qualitative method (total antibodies—Wantai), Fisher's exact test; $p < 0.05$.

An interaction between days from infection and BMI was found ($p = 0.01$, Fig. 5), indicating different IgG dynamics in individuals with different BMI categories. Obese individuals appeared to have a more rapid decrease of IgG levels after infection compared to normal-weight individuals (e.g., decrease of IgG levels from day 60 to day 160 after infection in normal weight and obese females between 30–50 years: 35.2%, 95% CI: 32.2–37.6%; and 51.2%, 95% CI: 48.6%–53.5% AU/ml, respectively).

On the other hand, suffering from a chronic disease or the effect of medication intake did not influence the development of IgG antibodies nor in their dynamics after SARS-CoV-2 infection (Figs S11 and S12, respectively).

To evaluate the influence of age, smoking and hospitalization on the development of higher levels of IgG, a generalized mixed model with binomial distribution was used to represent and analyze IgG levels. In Fig. S13, we observed a significant effect of age ($p = 0.04$) on the development of high or low/negative IgG levels after infection. Here, older individuals developed higher IgG levels after infection onset and had a more dramatic decrease of this isotype over time compared to younger individuals. Figure S14 depicts the effect of smoking on the levels of IgG after infection, where we can

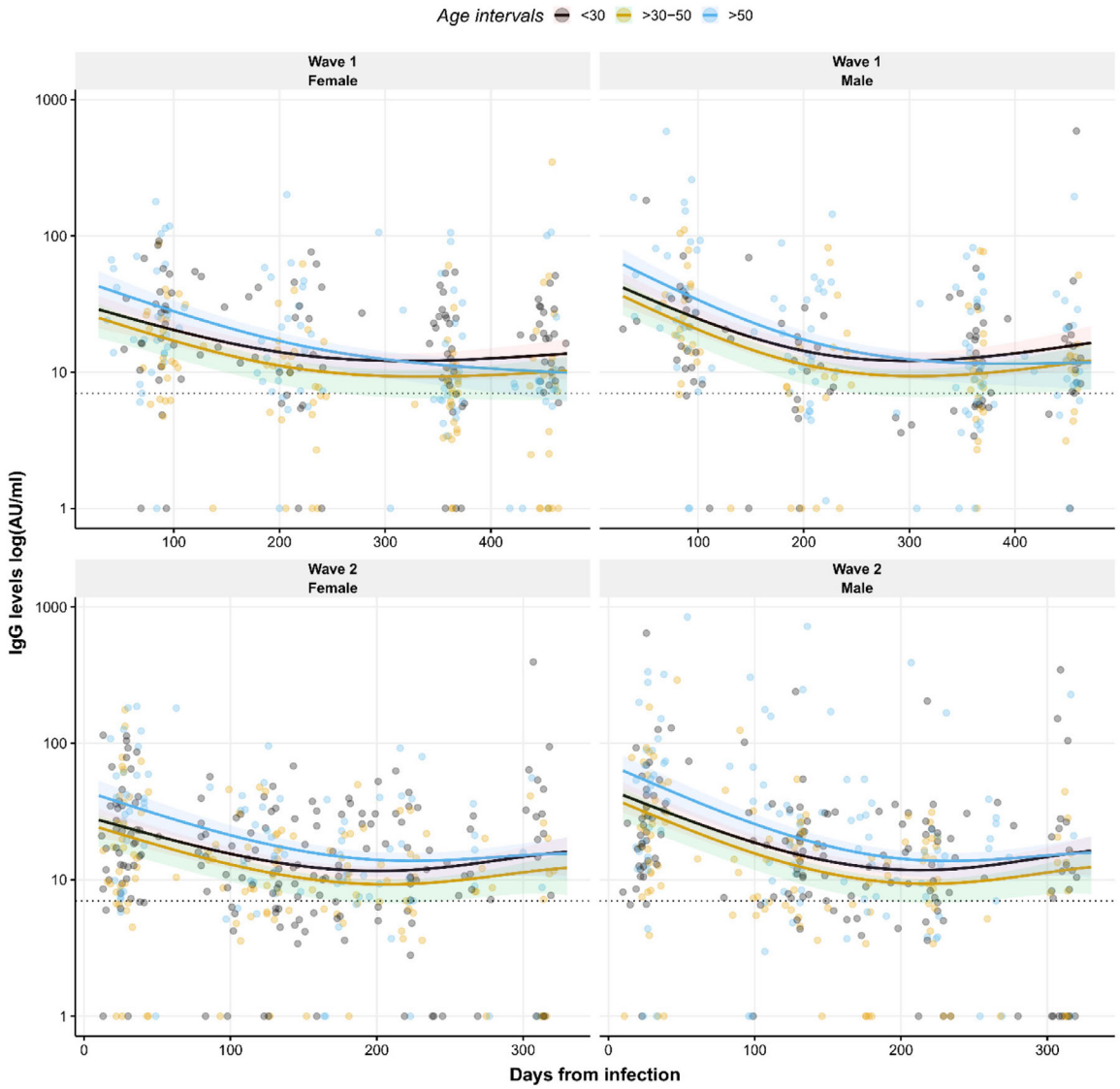

Fig. 2 *Dynamics of IgG levels against receptor-binding domain in circulation over time in individuals after SARS-CoV-2 infection using a nonlinear model. Distribution of IgG levels, log(AU/ml), over time (days from infection onset) in individuals from wave 1 (upper Fig. 1) and wave 2 (lower Fig. 1). The left and right panels represent the distribution of IgG levels in females and males, respectively. Black, yellow, and blue colors represent individuals with age <30, 30–50, and >50 years, respectively. The circles represent the observed levels of IgG antibodies in circulation. The solid lines represent the predicted levels of IgG antibodies in circulation. The horizontal dotted line indicates the assay positivity threshold. The 95% confidence interval is represented as the shadowed areas, with the center in the predicted (mean) value.*

observe a significant effect ($p = 0.008$) of smoking related to the development of lower levels of IgG compared to nonsmokers. Figure S15 shows the significant influence of hospitalization on the development of higher IgG levels compared to non-hospitalized individuals ($p = 0.008$).

IgG subclasses

The four IgG subclasses targeting SARS-CoV-2 RBD were analyzed in all samples, with overall IgG1 and IgG3 responses higher than IgG2 and IgG4 responses. The latter two had very weak response and thus we could not make a proper

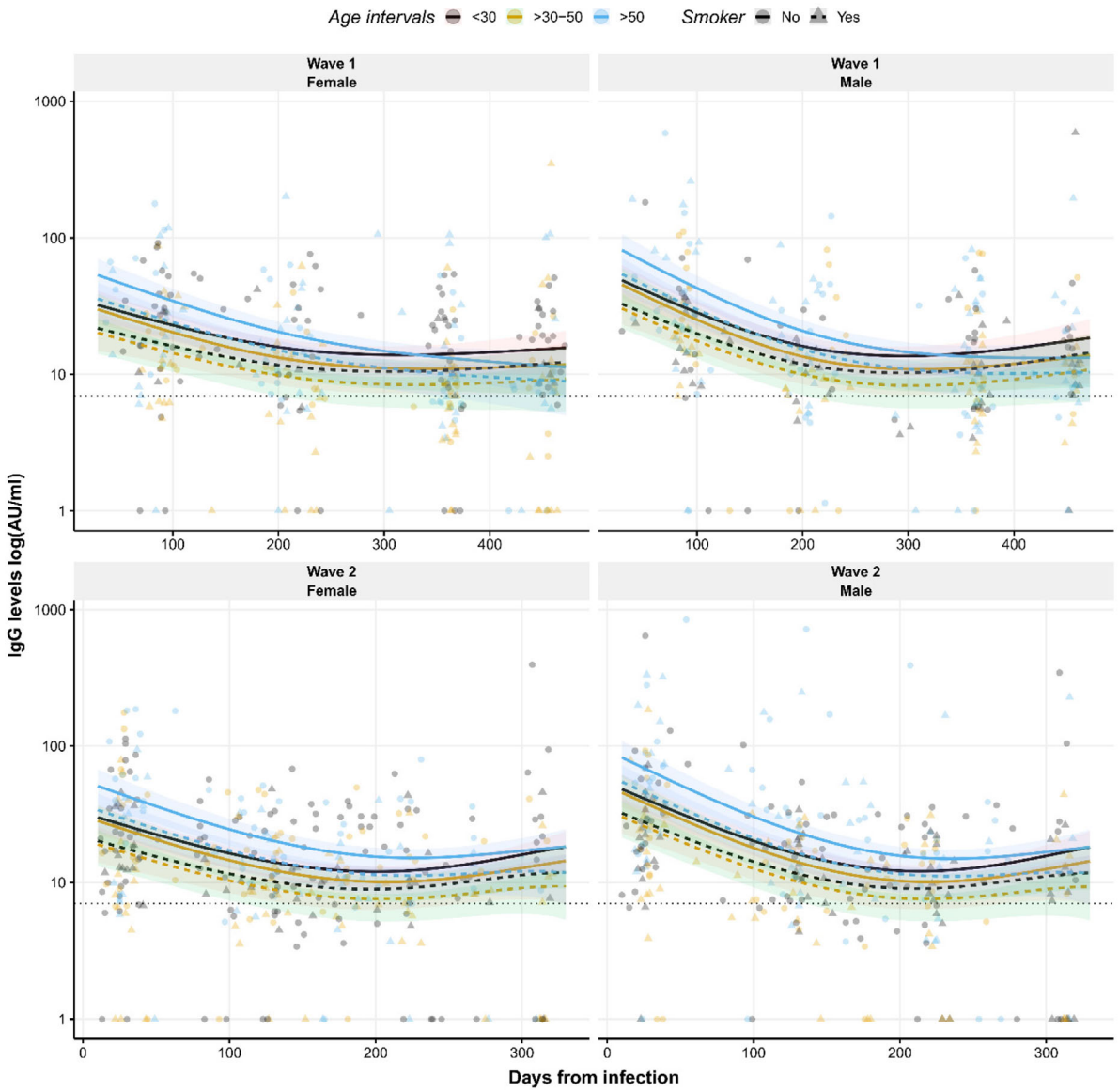

Fig. 3 *Smoking affects IgG levels against receptor-binding domain in circulation over time in individuals after SARS-CoV-2 infection. Distribution of IgG levels, log(AU/ml), over time (days from infection onset) in individuals from wave 1 (upper Fig. 2) and wave 2 (lower Fig. 2). The left and right panels represent the distribution of IgG levels in females and males, respectively. Black, yellow, and blue colors represent individuals with age <30, 30–50, and >50 years, respectively. The solid and dashed lines represent the predicted levels of IgG antibodies in never and ever smokers, respectively. The circles and triangles represent the observed levels of IgG antibodies in circulation in nonhospitalized and hospitalized individuals, respectively. The horizontal dotted line indicates the assay positivity threshold. The 95% confidence interval is represented as the shadowed areas, with the center in the predicted (mean) value.*

interpolation. The IgG subclass analyses showed that IgG1 and IgG3 were the majority IgG subclasses induced by SARS-CoV-2 infection. There was high correlation between IgG1 and total IgG (rho = 0.95) and no correlation between IgG3 and total IgG (rho = 0.16); that is, the IgG response is mainly driven by IgG1 (Fig. S16). Both IgG1 and IgG3 levels diminish significantly over time ($p < 0.001$ and $p = 0.05$, respectively), with the highest level in the first sample (Table S1).

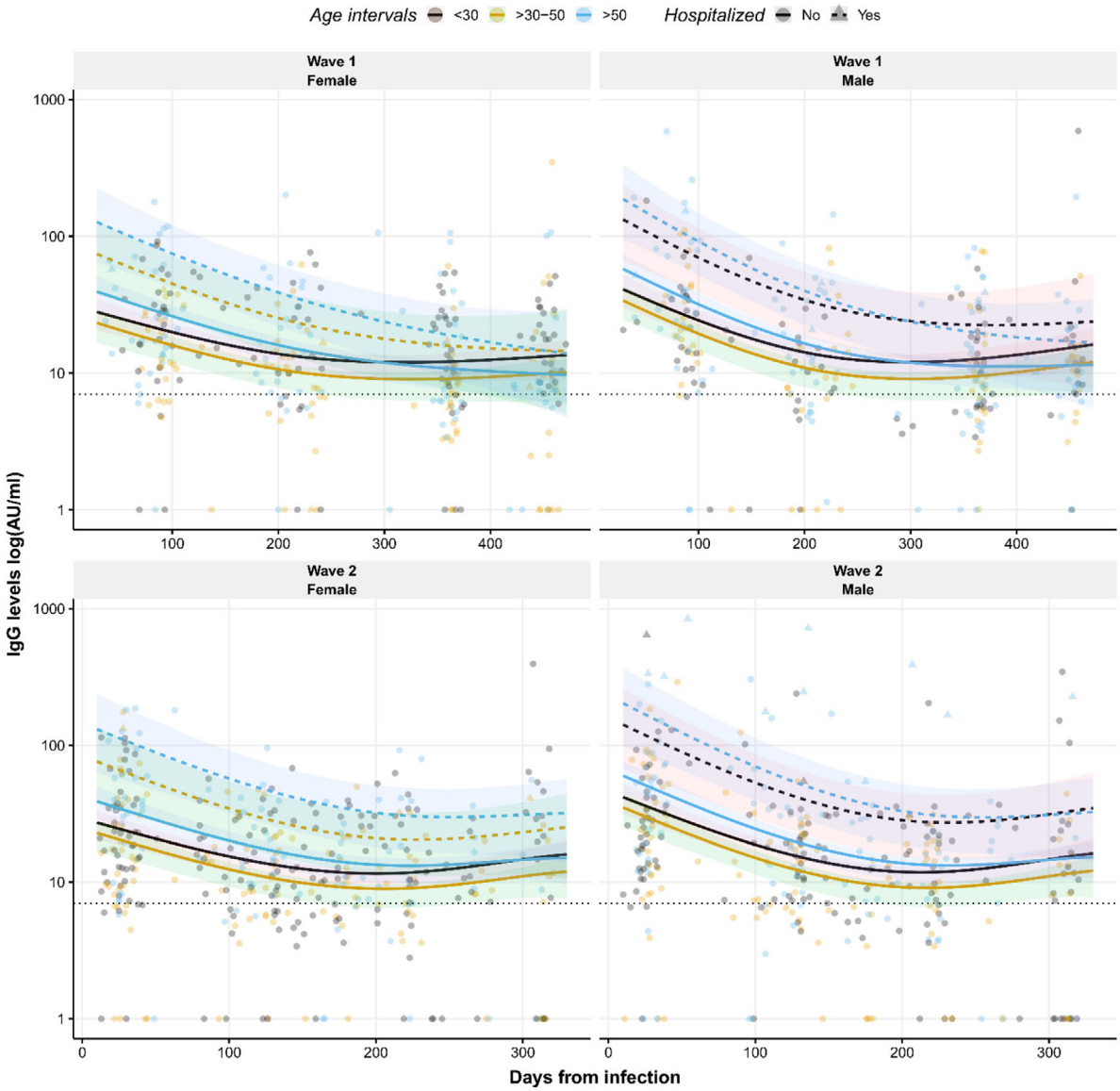

Fig. 4 *Effect of hospitalization on IgG levels against receptor-binding domain in circulation over time in individuals after SARS-CoV-2 infection. Distribution of IgG levels, log(AU/ml), over time (days from infection onset) in individuals from wave 1 (upper Fig. 3) and wave 2 (lower Fig. 3). Left and right panels represent the distribution of IgG levels in females and males, respectively. Black, yellow, and blue colors represent individuals with age <30, 30–50, and >50 years, respectively. The solid and dashed lines represent the predicted levels of IgG antibodies in nonhospitalized and hospitalized individuals, respectively. The circles and triangles represent the observed levels of IgG antibodies in circulation in nonhospitalized and hospitalized individuals, respectively. The horizontal dotted line indicates the assay positivity threshold. The 95% confidence interval is represented as the shadowed areas, with the center in the predicted (mean) value.*

IgG1 levels were significantly higher than IgG3 levels for all samplings ($p < 0.001$, $p = 0.01$, and $p = 0.02$, respectively) except in the fourth sampling, where the levels were comparable ($p = 0.63$). Men had higher levels for all time points and isotypes, albeit only significantly in the first sample. There were no correlations between, respectively, IgG1 and IgG3 and age, comorbidity, medication, symptoms at baseline, or BMI.

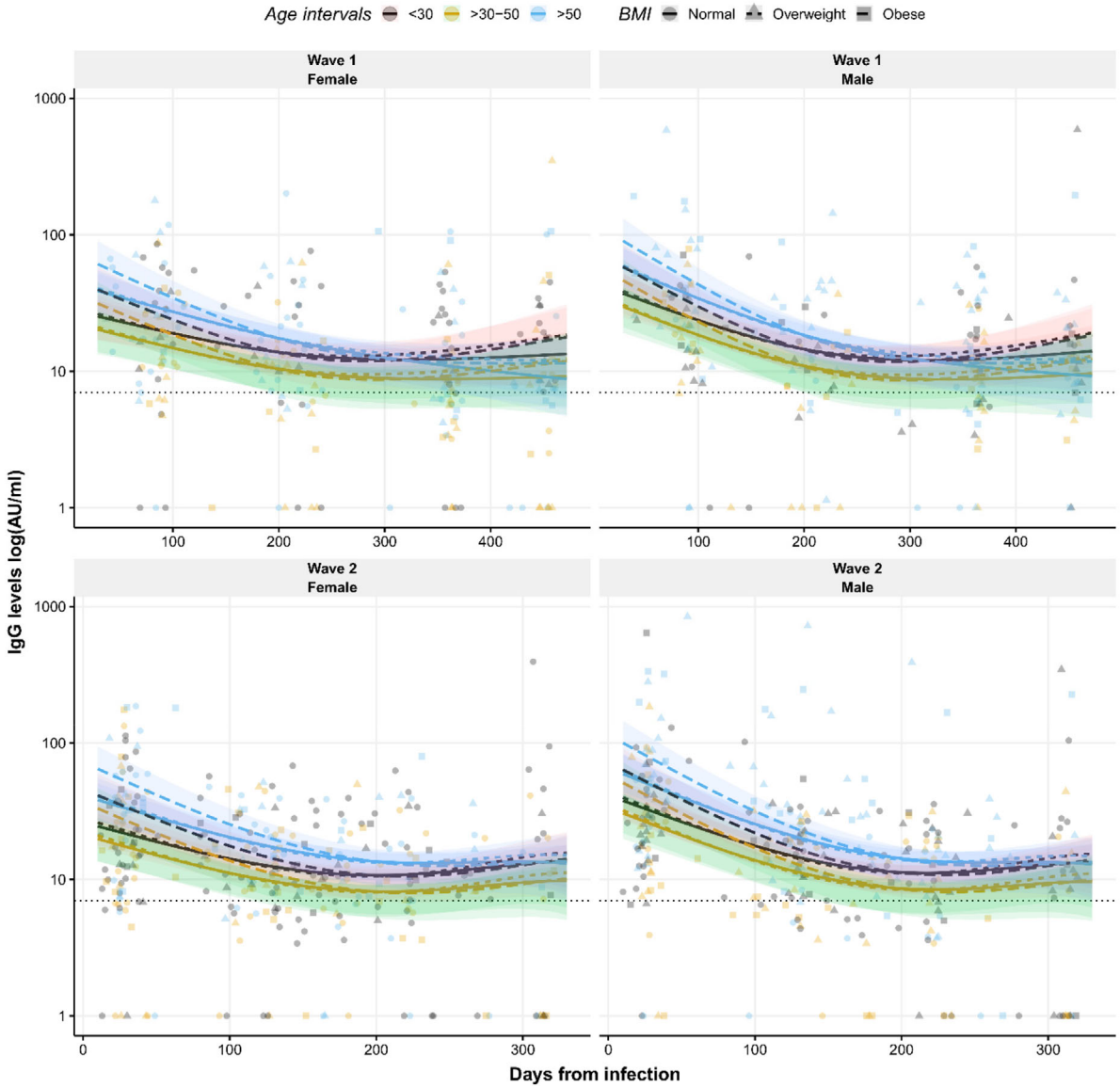

Fig. 5 *Effect of body mass index (BMI) IgG levels against receptor-binding domain in circulation over time in individuals after SARS-CoV-2 infection using a nonlinear model. Distribution of IgG levels, log(AU/ml), over time (days from infection onset) in individuals from wave 1 (upper Fig. 4) and wave 2 (lower Fig. 4). Left and right panels represent the distribution of IgG levels in females and males, respectively. Black, yellow, and blue colors represent individuals with age <30, 30–50, and >50 years, respectively. The solid, short-dashed, and long-dashed lines represent the predicted levels of IgG antibodies in normal weight, overweight, and obese individuals, respectively. The circles, triangles, and squares represent the observed levels of IgG antibodies in circulation in normal weight, overweight, and obese individuals, respectively. The horizontal dotted line indicates the assay positivity threshold. The 95% confidence interval is represented as the shadowed areas, with the center in the predicted (mean) value.*

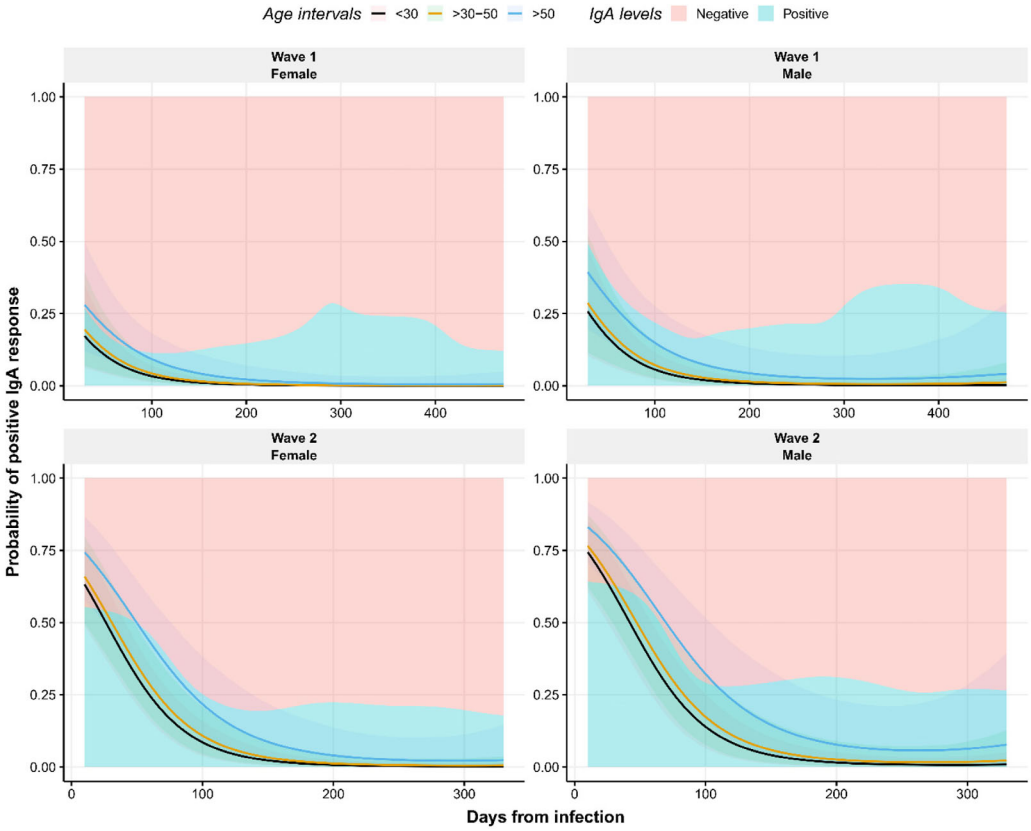

Fig. 6 *Observed and predicted probability of positive IgA responses against receptor-binding domain over time in individuals after SARS-CoV-2 infection. Distribution of positive IgA response (probability) over time (days from the infection onset) in individuals from wave 1 (upper Fig. 5) and wave 2 (lower Fig. 5). Left and right panels represent the distribution of IgA levels in females and males, respectively. Black, yellow, and blue colors represent individuals with age <30, 30–50, and >50 years, respectively. Blue and pink backgrounds represent the conditional density estimation of positive and negative IgA responses, respectively. The 95% confidence interval is represented as the shadowed areas, with the center in the predicted (mean) value.*

IgA responses after SARS-CoV-2 infection and influencing factors

As observed in the levels of circulating IgG levels after SARS-CoV-2 infection, the probability of measuring positive IgA responses varies significantly over time ($p < 0.0001$), characterized by a waning of detectable IgA responses since infection onset (Fig. 6).

A tendency for higher IgA responses can be observed in older individuals (e.g., probability of positive IgA response female >50 years: 17.6%, 95% CI: 8.0%–31.3%) compared to younger groups (e.g., probability of positive IgA response female <30 years: 8.5%, 95% CI: 3.5%–16.8%) ($p = 0.05$), and was also observed for males, for example (probability of positive IgA response male <30 years: 13.6%, 95% CI: 5.9%–25.6%) ($p = 0.07$). In addition, a significant difference of IgA responses was observed between waves ($p = 0.003$). Albeit not significant, hospitalization affected the probability of positive IgA responses (e.g., probability of positive IgA response in hospitalized and nonhospitalized female 30–50 years: 54.6%, 95% CI: 19.3%–86.4%; and 24.4%, 95% CI: 15.6%–35.3%, respectively) ($p = 0.1$, Fig. S17).

Effect of vaccination on IgG levels and IgA responses in infected individuals

A proportion of the participants in the study received a COVID-19 vaccine after infection when this was available to the population (30 December 2021 and onward). As expected, a variation in IgG

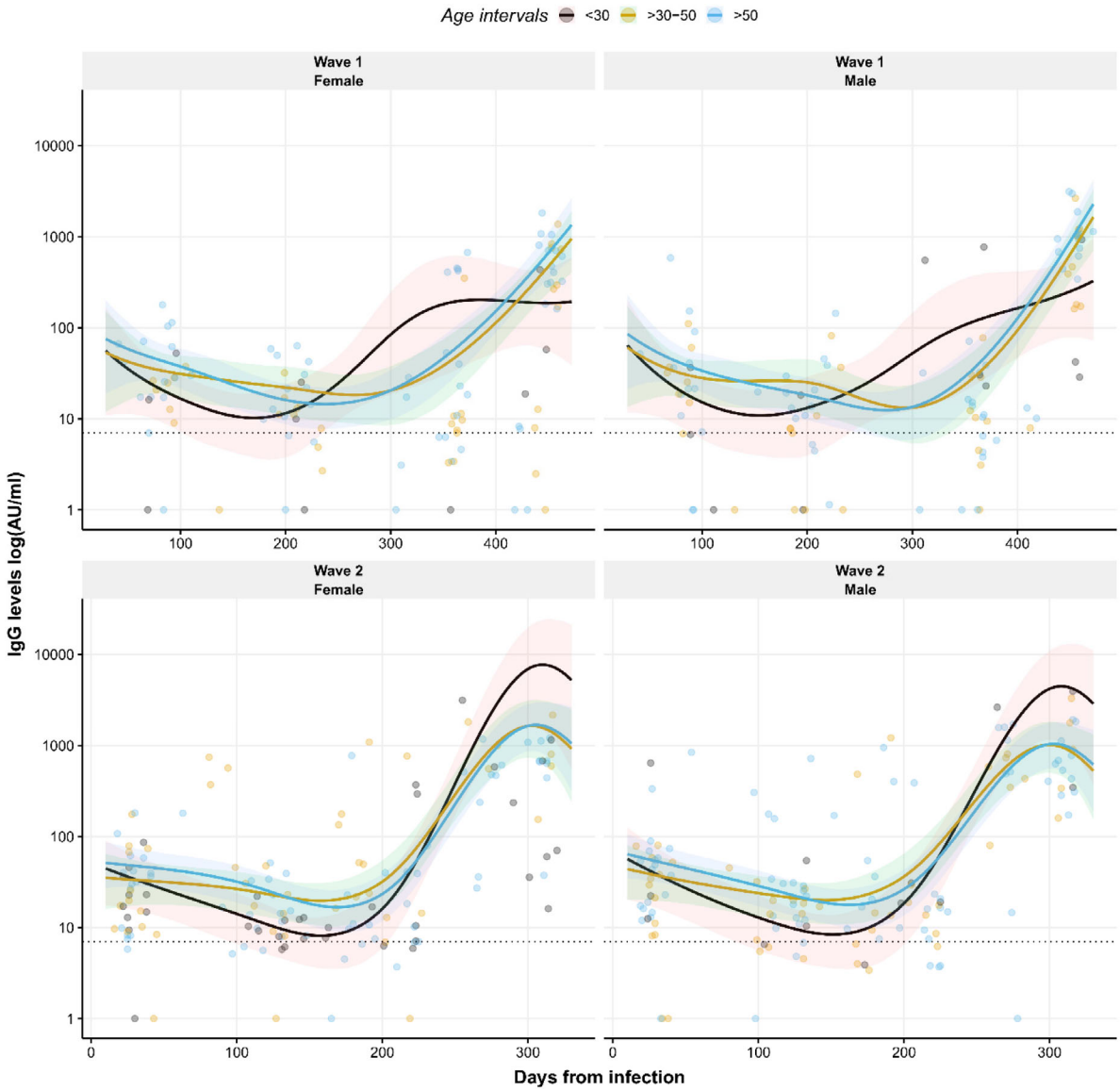

Fig. 7 *Effect of vaccination on IgG levels against receptor-binding domain in circulation over time in individuals vaccinated after SARS-CoV-2 infection. Distribution of IgG levels, log(AU/ml), over time (days from the infection) in individuals previously infected and vaccinated with BNT162b2 in wave 1 (upper Fig. 6) and wave 2 (lower Fig. 6). Left and right panels represent the distribution of IgG levels in females and males, respectively. Black, yellow, and blue colors represent individuals with age <30, 30–50, and >50 years, respectively. The circles represent the observed levels of IgG antibodies in circulation. The solid lines represent the predicted levels of IgG antibodies in circulation. The horizontal dotted line indicates the assay positivity threshold. The 95% confidence interval is represented as the shadowed areas, with the center in the predicted (mean) value.*

levels was observed ($p < 0.001$, Fig. 7), which is also evident in the different dynamics of IgG levels in both waves ($p < 0.001$), mostly due to the longer span of time between infection and vaccination for individuals infected during wave 1 compared to wave 2. It was observed that the peak levels of IgG antibodies are reached after vaccination, most pronounced in wave 2. As observed in other reports [18], younger individuals mounted a higher level of IgG antibodies compared to older

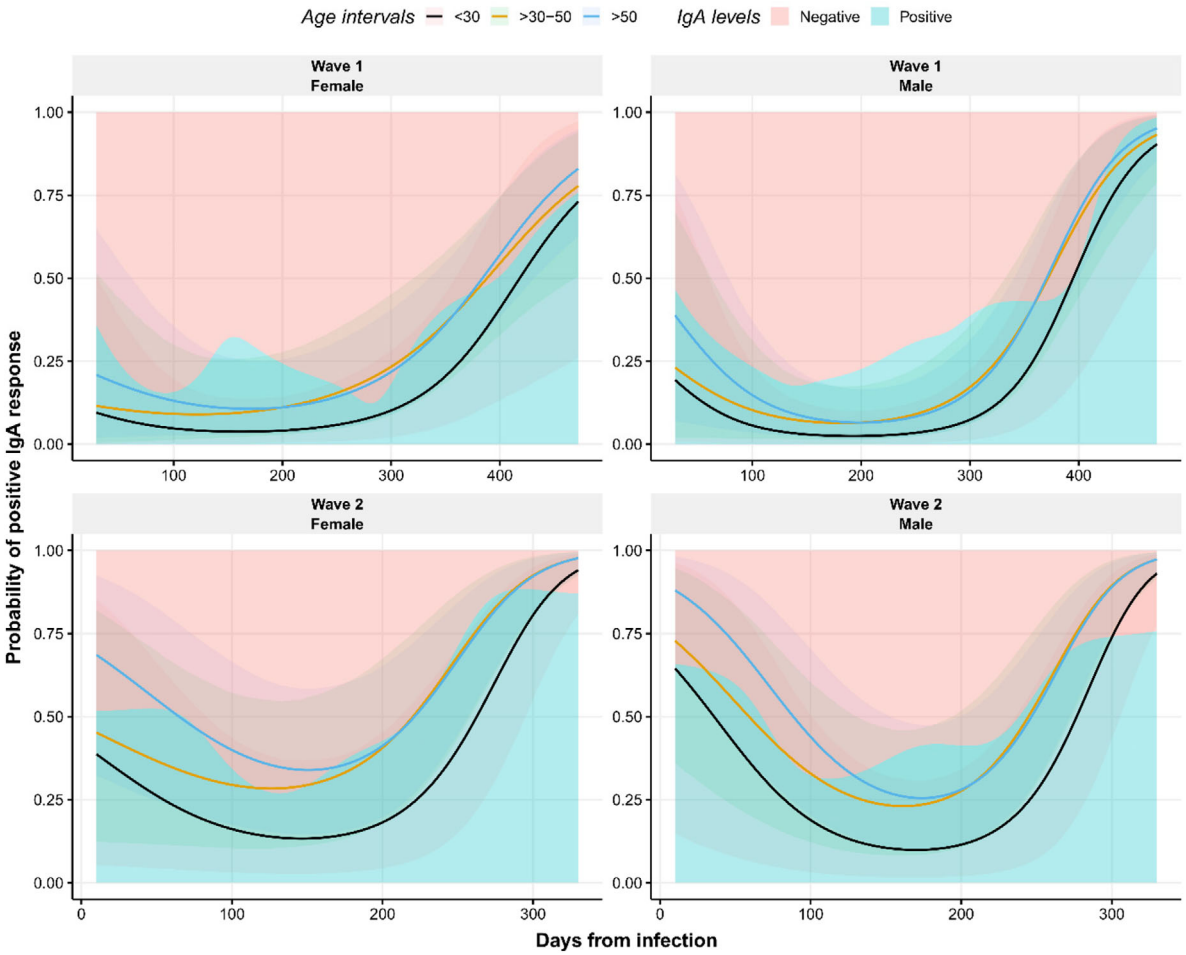

Fig. 8 *Effect of the vaccination on the probability of positive IgA responses against receptor-binding domain over time in individuals after SARS-CoV-2 infection. Distribution of positive IgA response (probability) over time (days from the infection onset) in individuals from wave 1 (upper Fig. 7) and wave 2 (lower Fig. 7). Left and right panels represent the distribution of IgA levels in females and males, respectively. Black, yellow, and blue colors represent individuals with age <30, 30–50, and >50 years, respectively. Blue and pink backgrounds represent the conditional density estimation of positive and negative IgA responses, respectively. The 95% confidence interval is represented as the shadowed areas, with the center in the predicted (mean) value.*

individuals (female <30 years: 6923.8 AU/ml, 95% CI: 1614.5–19,750.7 AU/ml; female 30–50 years: 1636.7 AU/ml, 95% CI: 739.4–3118.2 AU/ml; and female >50 years: 1643.9 AU/ml, 95% CI: 863.2–2846.8 AU/ml) (Fig. 7, bottom panels), which was not observed in individuals infected in wave 1 (Fig. 7, top panels), probably due to the low number of young participants in this group.

A similar distribution in IgA responses after vaccination was observed (Fig. 8), where the probability of positive IgA responses significantly varied due to vaccination ($p < 0.001$), as expected. As reported previously, infected older individuals appeared to have a higher probability of positive IgA responses than younger individuals (female >50 years: 92.2%, 95% CI: 81.4%–97.6%; and female <30 years: 80.6%, 95% CI: 55.3%–94.8%). A different dynamic was observed between waves ($p = 0.0003$), probably due to the longer span of time between infection and vaccination for individuals infected during wave 1 compared to wave 2.

Discussion

In this nationwide longitudinal study, we evaluated the presence of SARS-CoV-2-specific antibodies in individuals aged 0–93 years with asymptomatic, mild, moderate, and severe disease up to 15 months after infection. We found that 94% of all participants had detectable levels of antibodies, mainly IgG, 92% NAbs, while even a higher proportion (99%) displayed total antibodies. The results show different dynamics of antibody levels over time in infected individuals characterized by a waning of IgG levels from infection onset, with a biphasic pattern with an initial decline followed by a plateau after approximately 7 months in both waves, although the IgG dynamics otherwise were different in the two waves. Regardless of the decrease in IgG levels, the neutralizing capacity of circulating antibodies remained high, indicating a high efficiency of antibodies induced by infection.

In line with the IgG dynamics observed in our study, Wang et al. [19] showed that after an initial decline, between 6 and 12 months after infection the concentration of NAbs remains unchanged, and Turner et al. found [20] that antibodies declined rapidly in the first 4 months after infection and then more gradually over the following 7 months, remaining detectable at least 11 months after infection [20], which is consistent with our results with longer follow-up. The authors argue that consistent with the longevity of bone marrow plasma cells, infection with SARS-CoV-2 leads to persistent anti-RBD antibodies in serum and corresponding neutralizing responses [19, 20]. The biphasic pattern with an initial decline followed by a plateau is consistent with the expectation that a proportion of the plasma cells in an acute immune reaction become memory plasma cells [21, 22]. This is an indication of a shift from antibody production by short-lived plasma cells to antibody production by memory plasma cells. Even if antibody levels last very long, it is essential that they be able to neutralize the virus. Our results show a high correlation between NAbs and IgG levels, indicating that infection-acquired immunity is effective for up to 15 months. Only one reinfection was reported of the 4477 individuals diagnosed with COVID-19 by 17 December 2021 in the Faroes. This supports our finding with prolonged protection after infection with COVID-19 up to 15 months and the protective effect of recovery from the previous infection, as found in other studies [23].

Consistent with what was previously reported [24–26], we find that IgG1 and IgG3 are the most prevalent subclasses, with IgG1 mainly driving the IgG response. However, we find that men have significantly higher levels, which is contrary to Luo et al., who found that sex had no effect on IgG subclasses while comorbidity and older age did [24]. In contrast, Tandhavanant et al. found that that IgG3 levels had higher correlation with total IgG and that age and sex were not associated with IgG subclass detection [26].

The vast majority of participants did mature detectable antibodies initially. Only ~3% of the participants did not mature a detectable antibody response in the first sampling. This is lower than in other studies, for example, a study from Wuhan, China, where 5.4% of convalescent plasma donors had undetectable levels [7] and a study from Germany where 5.6% had undetectable antibodies [27]. However, the proportion with detectable NAbs was only 83% in the sample taken within the first month after active disease and increased to 94% in the second sampling, probably due to a lag time from onset to mounting NAbs. IgA was only persistent in 19% of the participants and IgM only in 3%, 15 months after infection, while the fraction with no detectable IgG titers remained constant over time. However, the probability of measuring positive IgA responses varies significantly over time, characterized by a waning of detectable IgA responses since infection onset. As with IgG, higher IgA response was observed in older individuals and in males.

There was substantial heterogeneity in the antibody response among the participants. Older individuals produced higher IgG levels than the younger ones but had a more marked decrease of IgG. This was also found for men. We found that smoking, BMI, and hospitalization affected the development of IgG antibodies with lower levels in smokers, faster decrease in people with high BMI, and higher levels in those hospitalized. These factors may influence the level of protective immunity and risk of reinfection. Still, the majority of patients presented with antibodies in the last sampling, albeit at declining levels. Only a few studies have, to our knowledge, longitudinally assessed antibody response beyond 10 months. A study from United States—including 764 serum samples from 250 patients aged 18+ years collected 6 ($n = 72$) and 12 months ($n = 19$) after infection [28]—concluded that humoral responses to SARS-CoV-2 infection

are robust on longer time scales, including those arising from milder infections. However, the magnitude and durability of the antibody response after infection was lower and more variable in younger participants who did not require hospitalization for COVID-19. They found that older age correlated positively with both higher IgG antibodies and NAb levels when controlling for COVID-19 hospitalization status [28], consistent with our findings.

Similarly, a US study with 77 patients, median 49 years, who experienced mild infections [20] reported that SARS-CoV-2 spike antibodies decline rapidly in the first 4 months, then more gradually over the following 7 months, remaining detectable at least 11 months after infection. A study from Wuhan, China, including 869 COVID-19 convalescent plasma donors aged 18–55 years with sampling 1–2 months, 6–7 months, and 11–12 months after infection [7] reported a positive rate of an IgG antibody response against RBD-IgG exceeding 90% initially and 70% at 12 months post diagnosis and a downward trend with stabilization after 9 months. Further, they reported higher IgG levels among males and a positive correlation with age, consistent with our findings. A German study including 963 individuals aged 18–79 years with predominantly mild COVID-19 conducted longitudinal analyses in a subgroup ($n = 137$) and found that 94.4% had detectable SARS-CoV-2 antibodies initially (~7 weeks) and 73% at last follow-up, concluding that humoral IgG response persists for as long as 10 months. They found age, symptomatic infection, disease severity, and sex to be predicting factors of SARS-CoV-2-neutralizing activity [27]. Although supporting our findings, unfortunately, none of the studies mentioned included smoking as an explanatory variable. Our results showing lower antibody levels among smokers agree with observations from a Swiss study [29] and are supported by findings that smokers have been shown to respond with lower antibody levels to a variety of respiratory pathogens [30].

A subgroup of participants provided samples after they had been vaccinated. Individuals who have been infected prior to vaccination have been shown to generate higher levels of IgG antibodies [18, 31] and a longer half-life, which is also indicated in our data as we observed a steep rise in the IgG titers to levels above those observed after infection. However, our data do not yet allow for comparison of half-time because we only have one time point after vaccination.

One strength of our study is that it is population based, with a high participation rate (92% and 79% from wave 1 and 2, respectively) limiting bias towards an overrepresentation of more severe cases. Antibody response was assessed by both serum antibodies and NAbs, which offer informative assessment of antiviral activity of patient sera against viral infection. Another strength is that we include four consecutive samples from 92 and 94 nonvaccinated individuals, respectively from wave 1 and 2, permitting assessment of antibody dynamics over time. Furthermore, we include all age groups, that is, also children. Still, the number of participants may be a limitation when analyzing data from both waves separately leading to low numbers in some categories, for example, age groups.

Conclusion

In conclusion, we find that the vast majority of people mount a robust and long-lasting immune response after being infected with SARS-CoV-2. The results show that IgG antibody responses against SARS-CoV-2 RBD domain are preserved at least 15 months after infection, after an initial decline during the first 7 months. Moreover, sex, age, smoking status, and need for hospitalization influenced the initial level of SARS-CoV-2 antibodies, while sex, age, and BMI influenced the decay of antibodies over time. Furthermore, our results document that people who develop infection-acquired immunity to SARS-CoV-2 and subsequently are vaccinated produce a higher antibody response. Although antibodies only represent a part of the immune response, our results strongly suggest that previously infected individuals have a robust humoral immune response that reduces the risk of SARS-CoV-2 reinfection for a period of at least 15 months.

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Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported

Author contributions

Maria S. Petersen: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing – original draft; Writing – review and editing. Cecilie B. Hansen: Formal analysis; Writing – review and editing. Jóhanna Ljósá Hansen: Formal analysis; Writing – review and editing. Ida Jarlhelt: Formal analysis; Writing – review and editing. Bjarni á Steig: Writing – review and editing. Lars F. Møller: Writing – review and editing. Marin Strøm: Writing – review and editing. Guðrið Andorsdóttir: Funding acquisition; Writing – review and editing. Shahin Gaini: Funding acquisition; Writing – review and editing. Pál Weihe: Conceptualization; Funding acquisition; Writing – review and editing. Peter Garred: Conceptualization; Formal analysis; Funding acquisition; Methodology; Supervision; Writing – review and editing.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary Information: Brief description of the methods. ■

