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SARS-CoV-2 antibodies persist up to 12 months after natural infection in healthy employees working in non-medical contact-intensive professions

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ABSTRACT

Objectives: This study aimed to evaluate dynamics of antibody levels after exposure to SARS-CoV-2 for 12 months in Dutch non-vaccinated hairdressers and hospitality staff.
Methods: In this prospective cohort study, blood samples were collected every 3 months for 1 year and analyzed using a qualitative total antibody enzyme-linked immunosorbent assay (ELISA) and a quantitative immunoglobulin (Ig)G antibody ELISA. Participants completed questionnaires, providing information on demographics, health, and work. Differences in antibody levels were evaluated using Mann–Whitney U and Wilcoxon signed-rank tests. Beta coefficients (β) and 95% confidence intervals (CIs) were calculated using linear regression.
Results: Ninety-five of 497 participants (19.1%) had ≥1 seropositive measurement before their last visit using the qualitative ELISA. Only 2.1% (2/95) seroreverted during follow-up. Of 95 participants, 82 (86.3%) tested IgG seropositive in the quantitative ELISA too. IgG antibody levels significantly decreased in the first months (P <0.01) but remained detectable for up to 12 months in all participants. Older age (β, 10-years increment: 24.6, 95% CI: 5.7-43.5) and higher body mass index (β, 5kg/m² increment: 40.0, 95% CI: 2.9-77.2) were significantly associated with a higher peak of antibody levels.
Conclusion: In this cohort, SARS-CoV-2 antibodies persisted for up to 1 year after initial seropositivity, suggesting long-term natural immunity.

Introduction

Since the start of the COVID-19 pandemic in early 2020, over 564 million confirmed cases and over 6 million deaths have been reported globally (July 22, 2022) [33]. In the Netherlands (total population: 17.6 million), 8.3 million inhabitants have officially been diagnosed with COVID-19 [34]. This number is an underestimation of the actual number of cases because not all people were tested for severe SARS-CoV-2 infections. Hence, a significant percentage of the population has developed natural immunity against SARS-CoV-2 at some point during the pandemic [2].

Research on the natural immune response after exposure to SARS-CoV-2 could aid a better understanding of the duration of

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protective immunity. This information is crucial in assisting public health decisions, for example, for estimating the effects of restricting social activities, but also in weighing the benefit of vaccinating more people worldwide vs providing extra doses to those having developed natural immunity.

In most studies examining natural immunity after COVID-19, the study population consisted of patients who had a severe infection, usually requiring hospitalization or healthcare workers [2,28]. However, such populations may not adequately represent the general population, and it is especially important for policymakers to have knowledge of the duration of protective immunity in the overall population.

A small number of studies investigated the immune response after natural SARS-CoV-2 infection in a generic population [1,3–5,14,19,21,26]. These studies report contrasting data regarding the duration of detectable antibody levels. Various factors may have influenced these seemingly contrasting findings, such as differences in assay used or study population. Many studies have a cross-sectional design, while the dynamics of antibody development after infection can vary widely among individuals [21]. Hence, longitudinal studies are needed to improve our understanding of the duration of natural immunity against SARS-CoV-2 in the general population.

Previously, we introduced COco, a Dutch cohort study evaluating antibodies against SARS-CoV-2 in 497 hairdressers and hospitality staff [23]. Antibodies were measured for up to 12 months in non-vaccinated individuals. Here, we evaluated the dynamics of antibody levels in this healthy population of individuals who frequently have contact with other people at work, thereby being potentially exposed to the coronavirus while not being trained to take measures to prevent infection, similar to the general population. We studied both the presence and quantity of antibodies over time and tested whether baseline variables were associated with antibody peak levels and dynamics.

Methods

Study design and population

COco is a prospective cohort study that evaluated SARS-CoV-2 antibodies in non-medical contact-based professions in the province of North-Brabant in the Netherlands. Its design, recruitment, and population have been described previously [23]. Hairdressers and hospitality staff (n = 497) were recruited in June/July 2020; individuals were followed for up to 1 year during four visits or until vaccination. No participants were hospitalized for COVID-19. In the current study, we selected participants who tested seropositive before their last visit to analyze antibody titers over time. Participation was voluntary after providing written informed consent.

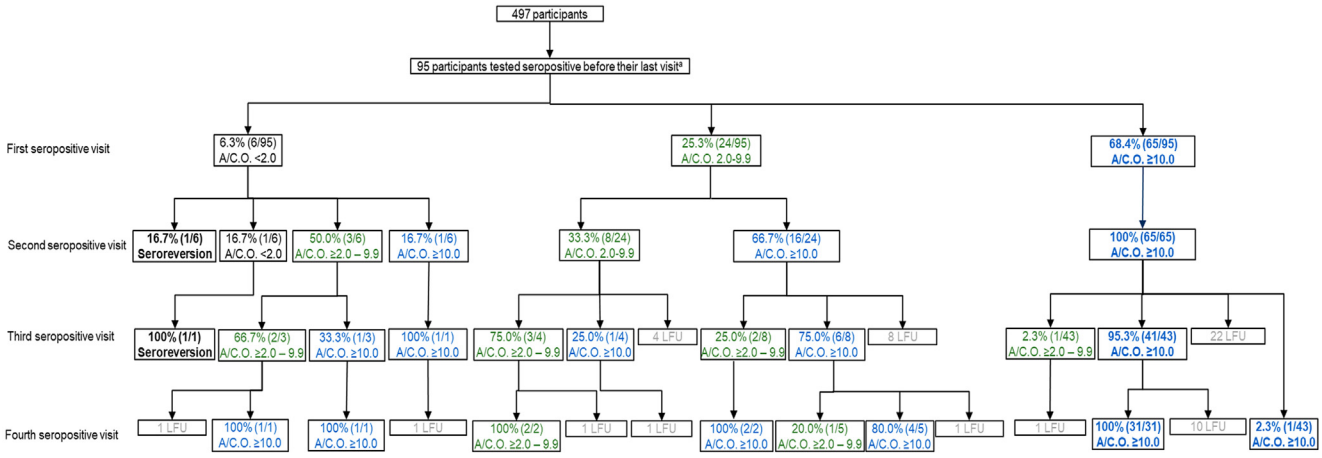
Data collection and analyses

Blood samples were collected from each participant at four timepoints for approximately 1 year. During venipuncture, 3.5 ml blood was drawn, and serum was analyzed by the Microvida Laboratory for Medical Microbiology using the qualitative Wantai SARS-CoV-2 total antibody enzyme-linked immunosorbent assay (ELISA) (Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China) per the manufacturer's instructions [23]. The manufacturer defined an absorbance to a cut-off ratio (A/C.O.) $\geq$1.1 as seropositive, A/C.O. 0.9-1.0 borderline seronegative, and A/C.O. $<$0.9 seronegative. Additionally, we divided seropositivity with an A/C.O. $\geq$10.0, 2.0-9.9, and 1.1-1.0 into strongly seropositive, seropositive, and weakly seropositive, respectively. As such, these test results were evaluated qualitatively and semi-quantitively, similar to earlier studies [24].

For the additional quantitative analyses, we included all samples from all participants who had a seropositive (A/C.O. $\geq$1.1) or borderline seronegative (A/C.O. 0.9-1.0) sample in the qualitative ELISA at a measurement before their final visit. These samples allowed evaluation of antibody dynamics after an initial seropositive measurement. In addition, we randomly selected several participants who were seropositive at their final visit only to evaluate agreement between the two assays. The samples were analyzed by the National Institute for Public Health and the Environment using the Anti-SARS-CoV-2 QuantiVac ELISA (immunoglobulin [Ig]G) test (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). This is a quantitative ELISA determining the concentration of IgG antibodies against the S1 antigen (including receptor-binding domain [RBD]) of SARS-CoV-2. It was performed on participants' serum using the EUROIMMUN Analyzer I platform per the manufacturer's instructions. This ELISA has an estimated sensitivity and specificity of 90.3% and 99.8%, respectively [7]. Antibody levels $\geq$11 relative units per milliliter (RU/ml) were considered seropositive, $\geq$8-$<$11 RU/ml borderline, and $<$8RU/ml seronegative. For in-depth analyses of IgG antibody dynamics, we selected all participants with $\geq$1 seropositive result before their final visit. Additionally, we considered inclusion of participants with $\geq$1 borderline test result before their final visit. Three participants had a borderline test result but no seropositive test result before the last visit. One participant was included for in-depth analyses, as the participant had a borderline test result followed by a seropositive test result during the last two visits. The other two participants with borderline test results were excluded from the analyses: these participants only had borderline and seronegative test results using the quantitative ELISA, while all four test results using the more sensitive qualitative ELISA were seropositive. Therefore, we assumed that the quantitative measurements were incorrect, and therefore, we excluded these from the analyses.

To signify the development of IgG antibody levels, we calculated differences between participants' highest and lowest antibody levels. When antibody levels had decreased by 50% or increased by 100%, this was labeled as decreasing or increasing antibody levels, respectively. Doubled antibody concentrations after an initial $\geq$50% decrease were considered fluctuating antibody levels. Other changes were labeled as stable antibody levels. To calculate rates (percentage in antibody level changes per 3 months), we subtracted the highest from the baseline antibody concentration and divided it by the time between those measurements if participants had increasing antibody levels; for those with decreasing antibody levels, the antibody level peak and antibody concentration at the last visit were used. Participants with fluctuating antibody levels were censored for rate calculations when antibodies increased.

In addition, three web-based questionnaires were collected. The baseline questionnaire, collecting information such as demographics, has been published previously [23]. After enrollment, participants answered weekly a follow-up questionnaire (Supplementary questionnaire 1), which collected information on health and work in the past week. Once every 3 months, the weekly questionnaire was expanded with questions regarding SARS-CoV-2 transmission risk outside work (Supplementary questionnaire 2). We analyzed data from the baseline questionnaire; the variables we used are described in more detail in the Supplementary data. Furthermore, we analyzed several variables from the follow-up questionnaires: symptoms possibly related to COVID-19, positive polymerase chain reaction (PCR) test result (yes/no), not shaking hands as a measure to prevent SARS-CoV-2 transmission (yes/no), being part of contact tracing (yes/no) and have had contact with someone who tested positive for SARS-CoV-2 (yes/no). The severity of COVID-19-related symptoms was divided into two categories based on whether participants did or did not report fever.



[a] Participants were excluded if their first seropositive test was at the last visit

A/C.O.: absorbance to cut-off ratio; A/C.O. <2.0: weak seropositive; A/C.O. ≥10: strong seropositive; LFU: lost to follow-up (either due to last visit or no-show)

Figure 1. Flow chart depicting the percentage of participants with a seropositive test result[a], per visit, as evaluated using the qualitative enzyme-linked immunosorbent assay.
[a]Participants were excluded if their first seropositive test was at the last visit.
A/C.O.: absorbance to cut-off ratio; A/C.O. <2.0: weak seropositive; A/C.O. ≥10: strong seropositive; LFU: lost to follow-up (either due to last visit or no-show).

Statistical analyses

Descriptive statistics and frequencies were used to analyze baseline characteristics. Baseline characteristics were compared using chi-square tests or Fisher's exact test (for cell frequency n ≥5 or n <5, respectively) for dichotomous categorical variables and Mann–Whitney U tests for numerical variables. The ordinal categorical variable education (low, middle, and high) was analyzed using a Mann–Whitney U test. Seroreversion curves were constructed using the Kaplan–Meier method [17]. Differences in antibody levels were evaluated using Mann–Whitney U tests for independent samples (e.g., comparing participants with and without fever) and Wilcoxon signed-rank tests for dependent samples (differences in antibody levels within individuals at different visits). Non-parametric tests were used due to the non-normal distribution of antibody levels, as determined using Shapiro–Wilk tests. Uni- and bivariable linear regression models were used to calculate beta coefficients (β) and their respective 95% confidence intervals (CIs) for variables associated with the IgG antibody level peak. Univariable logistic regression model was used to calculate odds ratios and their respective 95% CIs for variables predicting a decrease in antibody levels over time. P <0.05 was considered statistically significant. Trend lines were drawn based on the mean antibody levels of all participants at 0, 3.25, 7.25, and 10.75 months. Analyses were conducted using SPSS Statistics 24.0. The survival curve and boxplot were plotted using R4.1.2; scatterplots, including trend lines and 95% CIs, were constructed using Microsoft Excel.

Results

In total, 497 individuals were included in the COco-study. In June/July 2020, 11.3% (56/497) of participants tested positive for SARS-CoV-2 antibodies using the qualitative total antibody ELISA. This percentage increased to 13.7% (62/454), 25.1% (103/410), and 32.0% (110/344) in subsequent measurements in September/October 2020, January 2021, and February-June 2021, respectively. In total, 95 participants (19.1%) had at least one seropositive measurement before their last follow-up visit. Six of 95 participants (6.3%) had a weak seropositive test result at their first seropositive measurement, while 69.4% (65/95) had a strong seropositive test result (Figure 1). Of the 65 strongly seropositive participants, all but one (98.5%) remained strongly seropositive at all follow-up visits. In the study population, only two participants seroreverted (Supplementary Figure 1); both initially had weak seropositivity (Figure 1). Antibody levels of the other four participants with initial weak seropositive test results increased at subsequent visits. All participants who had an A/C.O ≥2.0 at their first seropositive measurement kept having seropositive measurements of A/C.O ≥2.0 at follow-up visits (Figure 1). Hence, the qualitative total antibody ELISA suggested limited seroreversion.

Of the 95 participants with a seropositive measurement before their last follow-up visit in the qualitative ELISA, 82 participants were SARS-CoV-2 IgG seropositive before their last follow-up visit in the QuantiVac IgG ELISA. Baseline characteristics of the 82 participants are summarized in Table 1. The majority (72.0%) were women, had a middle-level education (56.1%), and worked in the hospitality industry (61.0%). The median age was 39 years (range 18-68 years). Most participants were born in the Netherlands (97.6%), had no chronic disease (77.8%), did not smoke (82.9%), and used alcohol (90.2%, of whom 56.8% on average ≤1 alcohol unit per day). Median self-reported body mass index (BMI) was 25 kg/m^2 (range 16-38 kg/m^2).

The two participants who seroreverted in the qualitative ELISA were not IgG seropositive (or borderline) in the quantitative assay. We further examined these two participants. Both reported COVID-19-related symptoms before the initial qualitative seropositive test result, including fever in one participant. However, both participants did not have PCR-confirmed COVID-19, as they had symptoms before recruitment when PCR testing was limited available in the Netherlands. Both participants were women aged 17-30 years and 30-50 years. They did not have a chronic disease, did not smoke, reported minimal or no alcohol use, and had a BMI below 25 kg/m^2. As such, we were unable to identify potential determinants for their weak immune response but also cannot confirm that they had truly been SARS-CoV-2 infected.

Table 1
Baseline characteristics of participants with at least one seropositive SARS-CoV-2 immunoglobulin G test result before the last follow-up visit.

		Total group (N = 82)	Decreasing antibody levels (N = 38)	Non-decreasing antibody levels (N = 44)	*P*-value
Sex	Male	23 (28.0%)	14 (36.8%)	9 (20.5%)	0.100
	Female	59 (72.0%)	24 (63.2%)	35 (79.5%)	
Age, in years	Median (minimum-maximum)	39 (18-68)	42 (18-62)	37 (18-68)	0.477
	18-29 years	25 (30.5%)	11 (28.9%)	14 (31.8%)	
	30-50 years	33 (40.2%)	13 (34.2%)	20 (45.5%)	
	51-68 years	24 (29.3%)	14 (36.8%)	10 (22.7%)	
Born in the Netherlands	No	2 (2.4%)	1 (2.6%)	1 (2.3%)	1.000[b]
Household size	Median (minimum-maximum)	3 (1-15)	3 (1-5)	3 (1-15)	0.784
	One person (participant lives alone)	6 (7.3%)	1 (2.6%)	5 (11.4%)	
	Two persons	27 (32.9%)	16 (42.1%)	11 (25.0%)	
	Three persons	22 (26.8%)	8 (21.1%)	14 (31.8%)	
	Four persons	19 (23.2%)	11 (28.9%)	8 (18.2%)	
	Five persons	5 (6.1%)	2 (5.3%)	3 (6.8%)	
	>5 persons	3 (3.6%)	0 (0.0%)	3 (6.8%)	
Education level	Low	16 (19.5%)	6 (15.8%)	10 (22.7%)	0.716
	Middle	46 (56.1%)	23 (60.5%)	23 (52.3%)	
	High	20 (24.4%)	9 (23.7%)	11 (25.0%)	
Work setting	Hairdresser	32 (39.0%)	13 (34.2%)	19 (43.2%)	0.406
	Hospitality industry	50 (61.0%)	25 (65.8%)	25 (56.8%)	
Workplace location	Breda	40 (48.8%)	22 (57.9%)	18 (40.9%)	0.585[c]
	Roosendaal	18 (22.0%)	6 (15.8%)	12 (27.3%)	
	Etten-Leur	5 (6.1%)	1 (2.6%)	4 (9.1%)	
	Oosterhout	4 (4.9%)	2 (5.3%)	2 (4.5%)	
	Other municipalities	15 (18.3%)	7 (18.4%)	8 (18.3%)	
Working hours	Median (minimum-maximum)	30 (8-70)	35 (8-60)	26 (8-70)	0.166
	8-20 hours	24 (29.3%)	11 (28.9%)	13 (29.5%)	
	21-40 hours	33 (40.2%)	12 (31.6%)	21 (47.7%)	
	≥ 40 hours	25 (30.5%)	15 (39.5%)	10 (22.7%)	
Chronic disease	Yes[a]	19 (23.2%)	12 (31.6%)	7 (15.9%)	0.094
Body mass index, in kg/m^2	Median (minimum-maximum)	25 (16-38)	24 (17-33)	25 (16-38)	0.320
	Normal weight	46 (56.1%)	22 (57.9%)	24 (54.5%)	
	Overweight	29 (35.4%)	14 (36.8%)	15 (34.1%)	
	Obesity	7 (8.5%)	2 (5.3%)	5 (11.4%)	
Current smoker	Yes	14 (17.1%)	7 (18.4)	7 (15.9%)	0.763
Current smokers: number of cigarettes per day	Median (minimum-maximum)	7 (1-20)	10 (1-20)	2 (1-10)	0.128
	< 10	8 (57.1%)	2 (28.6%)	6 (85.7%)	
	10-20	6 (42.9%)	5 (71.4%)	1 (14.3%)	
	> 20	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Current alcohol use	Yes	74 (90.2%)	34 (89.5%)	40 (90.9%)	0.827
Current alcohol users: alcohol units per week	Median (minimum-maximum)	6 (0.5-41)	8 (0.5-41)	4 (0.5-34)	0.068
	0.5-7	42 (56.8%)	16 (47.1%)	26 (65.0%)	
	8-14	12 (16.2%)	9 (26.5%)	3 (7.5%)	
	15-21	8 (10.8%)	4 (11.8%)	4 (10.0%)	
	> 21	12 (16.2%)	5 (14.7%)	7 (17.5%)	

Baseline characteristics of participants with decreasing and non-decreasing antibody IgG antibody levels were compared using Wilcoxon rank sum tests (numerical variables) or chi-squared tests (categorical variables).
All values are n (%) unless specified otherwise.
[a]Patients were considered to have a chronic disease if they reported a chronic illness and/or used medication chronically.
[b]Due to small participant groups (n <5), a Fisher's exact test was used.
[c]We compared those working in Breda/Roosendaal to those working in other cities or villages.

We evaluated longitudinal changes in individuals' IgG antibody levels (Figure 2). Participants were divided into two groups based on changes in antibody levels over time: participants with (n = 38, 46.3%) and without decreasing (n = 44, 53.7%) antibody levels. Baseline characteristics of these two groups did not differ significantly (Table 1). Participants without decreasing antibody levels were further divided into those with stable (n = 36, 43.9%), increasing (n = 5, 6.1%), and fluctuating (n = 3, 3.7%) antibody levels (Figure 2A), and did not have significantly different baseline characteristics (Supplementary Table 1). We hypothesized that those with fluctuating antibodies had a rise in antibody levels after an initial decline due to re-exposure to SARS-CoV-2. Indeed, one participant with fluctuating antibody levels reported that she tested SARS-CoV-2 positive before antibody levels increased again, but the other two participants did not report any re-exposure to SARS-CoV-2.

Because follow-up time differed between participants, we calculated the rate of change of antibody levels per 3 months (Figure 2E). Only a minority (11.0%) of participants had a decrease in antibody levels of more than 50% per 3 months; the majority (69.5%) had a decrease in antibody levels between 0 and 50% per 3 months.

In addition, we analyzed group changes in antibody levels across the four measurements (Figure 2G). Antibody levels decreased significantly both between the first and second (n = 82, median 42 RU/ml, interquartile range [IQR] 25-98 RU/ml vs median 29RU/ml, IQR 16-75RU/ml, *P*-value = 0.002) and second and third measurements (n = 48, median 27 RU/ml, IQR 14-80 RU/ml vs median 20 RU/ml, IQR 10-52 RU/ml, *P* <0.001), but not between the third and last measurements (n = 37, median 19 RU/ml, IQR 10-51 RU/ml vs median 21 RU/ml, IQR 10-60 RU/ml, *P*-value = 0.541). Of the 37 participants who had three follow-up visits after initial seropositivity, all had detectable antibodies at the fourth visit, but IgG levels in six participants (16.2%) were below the borderline of seropositivity (range 6-7 RU/ml). When conducting subgroup analyses evaluating antibody level changes in those with

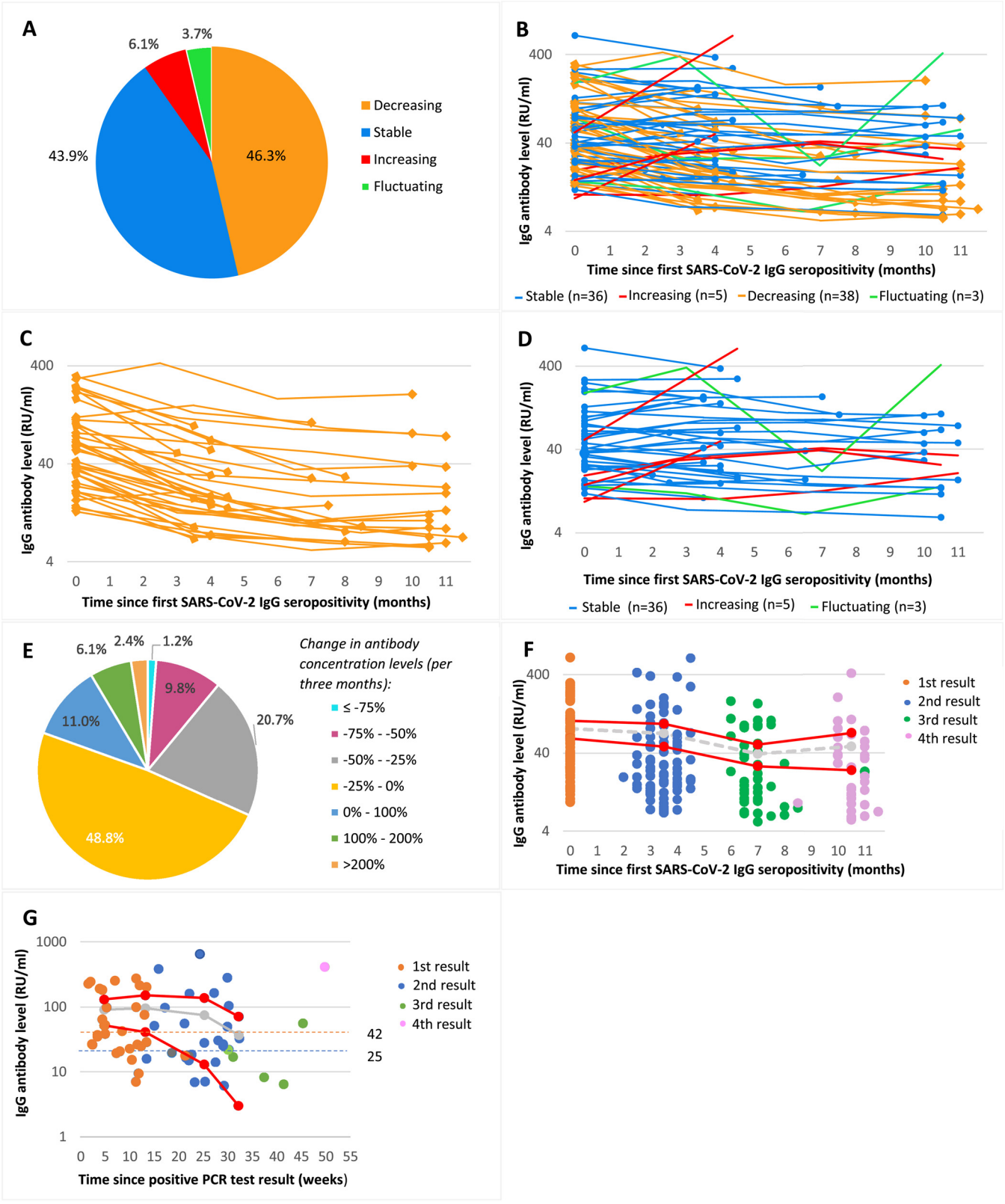


Figure 2. Longitudinal changes in IgG antibody levels of COco-study participants. Participants were included when having at least one follow-up measurement after their first seropositive test result. Participants (n = 82) were grouped based on whether they had decreasing or non-decreasing (stable, increasing, or fluctuating) antibody levels (A). Longitudinal changes were plotted for the whole group (B) and we separated participants by decreasing antibody levels (n = 38) (C) and non-decreasing antibody levels (n = 44) (D). Participants (n = 82) were grouped based on the percentage decrease or increase in antibody concentration per 3 months (E). Antibody levels of the whole group per measurement were plotted. The gray line indicates the trendline; red lines indicate 95% confidence intervals (F). Finally, antibody levels were plotted for the group of participants reporting a positive PCR test (n = 29). The gray line indicates the trendline; red lines indicate the 95% confidence intervals. The striped orange line indicates the median of the first measurement, and the striped, blue line indicates the median of the second measurement after the self-reported positive PCR test (G).
Ig, immunoglobulin; PCR, polymerase chain reaction; RU/ml, relative units per milliliter.

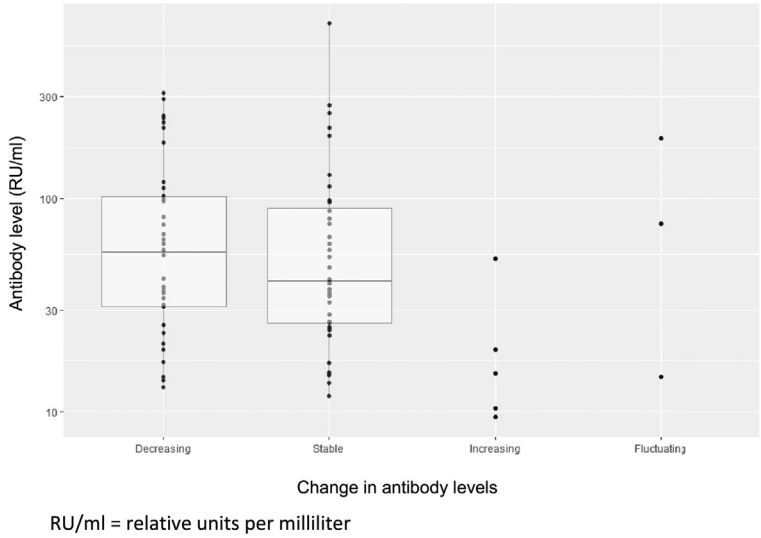


Figure 3. Antibody concentration of the whole group at the first seropositive measurement, divided into decreasing (n = 38), stable (n = 36), increasing (n = 5), and fluctuating (n = 3) antibody levels plotted in a boxplot combined with a scatterplot.
RU/ml, relative units per milliliter.

Table 2
Associations between participant characteristics and longitudinal changes in SARS-CoV-2 immunoglobulin G antibody levels.

Dependent variable: Decreasing antibody level (yes/no) Independent variable	Odds ratio (95% confidence interval)
Fever (ref: no fever)	1.3 (0.5-3.1)
Female sex (ref: male sex)	0.4 (0.2-1.2)
Age (10-years increment)	1.1 (0.8-1.6)
Chronic disease (ref: no chronic disease)	2.4 (0.9-7.0)
Body mass index (5 kg/m² increment)	0.7 (0.4-1.3)
Current smoker (ref: not currently smoking)	1.2 (0.4-3.8)
- Smoking (five cigarettes per day increment)	2.7 (0.9-8.3)
Current alcohol user (ref: currently no alcohol use)	0.9 (0.2-3.7)
- Alcohol quantity (seven alcohol units per week increment)	1.2 (0.8-1.7)

Odd ratios and their 95% confidence intervals were calculated using a univariable logistic regression model.
Ref, reference.

confirmed PCR test results (n = 29), similar results were observed: antibody levels decreased significantly between the first and second measurement (median 42 RU/ml, IQR 26-187 RU/ml vs median 25 RU/ml, IQR 17-76 RU/ml), *P*-value = 0.007), but IgG antibodies remained detectable in all participants for the study duration (Figure 2G).

Next, we aimed to identify variables associated with higher antibody levels and/or antibody level dynamics.

First, we evaluated whether antibody levels at the first seropositive measurement were associated with longitudinal antibody responses (Figure 3). Participants with fluctuating antibody levels had the highest concentration of IgG antibodies at baseline (n = 3, median 76 RU/ml, min-max 14-191 RU/ml), followed by participants with decreasing (n = 38, median 56 RU/ml, min-max 13-312 RU/ml), stable (n = 36, median 41 RU/ml, min-max 12-660 RU/ml), and increasing antibody levels (n = 5, median 15 RU/ml, min-max 9-52 RU/ml). Participants with increasing antibody levels had significantly lower baseline antibody levels than those with decreasing (*P*-value = 0.006) or stable (*P*-value = 0.011) antibody levels; no other significant differences were observed.

In further analyses (Table 2), we did not identify any measured baseline characteristic that significantly predicted decreasing antibody levels. Those with a chronic disease seemed to have more frequently decreasing antibody levels (odds ratio [OR] 2.4, 95% CI 0.9-7.0), while women seemed to have decreasing antibody levels less frequently (OR 0.4, 95% CI 0.2-1.2), but these differences were not statistically significant (*P*-value = 0.099 and *P*-value = 0.103, respectively).

Because previous studies suggested that those with severe disease developed higher antibody levels [2, 22], we evaluated whether disease severity predicted antibody levels and antibody level dynamics in our cohort (Figure 4). Because participants were not hospitalized for COVID-19, we grouped participants based on whether they reported fever. While participants with fever had higher antibody levels at every visit, these differences were only significant at the last measurement (n = 14, mean 83 RU/ml, 95% CI 20-146 RU/ml vs n = 24, mean 26 RU/ml, 95% CI 16-36 RU/ml, *P*-value = 0.050). However, this difference was particularly driven by one participant with fever. She reported SARS-CoV-2 reinfection between the third and fourth measurement, and therefore, antibody levels increased from 22 RU/ml to 412 RU/ml. After excluding her from the analyses, those with fever still had higher mean antibody levels at the last visit (n = 13, mean 58 RU/ml, 95% CI 25-91 RU/ml vs n = 24, mean 26 RU/ml, 95% CI 16-36 RU/ml), but this difference was not significant (*P*-value = 0.095). Both participants with and without fever had a tendency toward decreasing antibody levels over time (Figure 4).

Analyses of other baseline characteristics indicated that older age (β, 10-years increment: 24.6, 95% CI 5.7-43.5) and BMI (β, 5 kg/m² increment: 40.0, 95% CI: 2.9-77.2) were significantly asso-

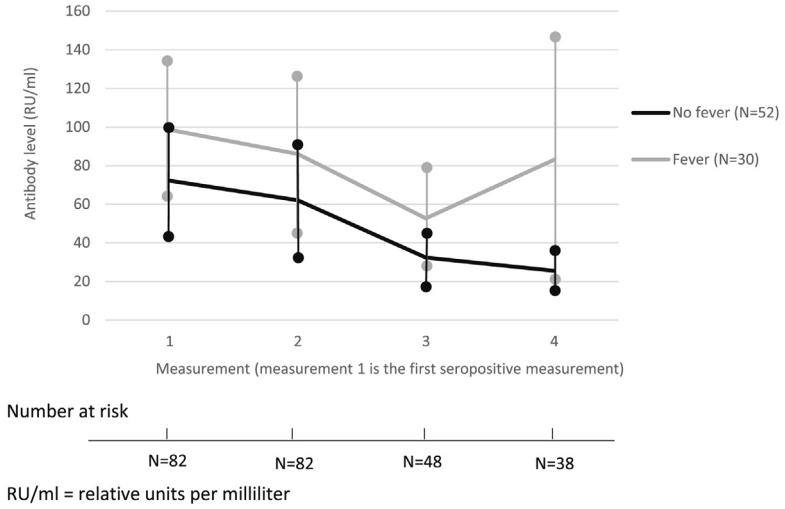


Figure 4. Longitudinal changes in the mean immunoglobulin G antibody levels of the whole group. Participants were grouped based on whether they had fever (fever: n = 52; no fever: n = 30). Vertical lines indicate 95% confidence intervals.
RU/ml, relative units per milliliter.

Table 3
Associations between participant characteristics and peak of SARS-CoV-2 immunoglobin G antibody levels.

Dependent variable: Peak of antibody level Variables	Univariable β (95% CI)	Bivariable β (95% CI) after adjusting for fever
Fever (ref: no fever)	24.2 (–32.6-80.9)	-
Female sex (ref: male sex)	–44.2 (–104.5-16.1)	–43.4 (–103.9-17.1)
Age (10-years increment)	**24.6 (5.7-43.5)***	**25.0 (6.0-43.9)***
Chronic disease (ref: no chronic disease)	47.8 (–16.4-112.0)	46.3 (–18.2-110.8)
Body mass index (5 kg/m² increment)	**40.0 (2.9-77.2)***	**42.2 (4.9-79.5)***
Current smoker (ref: not currently smoking)	–41.7 (–114.0-30.7)	–41.4 (–113.9-31.1)
-Smoking (5 cigarettes per day increment)	–8.2 (–40.8-24.3)	–10.0 (–40.9-20.9)
Current alcohol user (ref: currently no alcohol use)	26.7 (–65.7-119.0)	26.9 (–65.6-119.4)
- Alcohol quantity (7 alcohol units per week increment)	–15.0 (–36.4-6.5)	–15.0 (–36.4-6.5)

In the second column, beta coefficients and their 95% CIs were calculated using a univariable linear regression model. The last column reports beta coefficients for the variable listed in the first column, after adjusting for severity of COVID-19-related symptoms (fever/no fever) using a bivariable linear regression model.
CI, confidence interval; β, beta coefficient; Ref, reference.
* P <0.05.

ciated with antibody peak levels (Table 3). These associations remained significant after adjusting for disease severity (β: 25.0, 95% CI: 6.0-43.9, and β: 42.2, 95% CI: 4.9-79.5, respectively).

Discussion

In this longitudinal cohort study on SARS-CoV-2 antibody levels in Dutch hairdressers and hospitality staff, we found little evidence for waning natural immunity. IgG antibody levels decreased somewhat in about half of participants during the follow-up of up to 12 months but remained detectable. In the qualitative Wantai SARS-CoV-2 total antibody assay, two participants seroreverted, having a seropositive test result followed by a seronegative test result. The seropositivity could not be confirmed by the quantitative IgG assay. Evaluating the A/C.O. ratio, using the Wantai assay semi-quantitatively [24], these two participants only had weak seropositivity with an A/C.O. ratio just above the cut-off for seropositivity, as determined by the manufacturer. This suggested a very weak immune response or false positive test result. All other participants continued to have detectable immune responses.

Abovementioned results match with other published studies on natural immunity after COVID-19. Previous studies in healthcare workers or severely ill patients with COVID-19 generally reported that antibodies remain detectable for a long time after natural infection [8,9,11,15,20,27,30,35]. However, these are subpopulations that may not reflect the general population, as healthcare workers are trained to work with protective equipment, and severely ill patients with COVID-19 require hospitalization while most patients in the general population do not [32]. Our results demonstrate that antibodies also remain detectable in a group comparable to a general population.

Studies examining natural immunity in the general population are more limited and have contrasting results regarding the duration of detectable antibodies after COVID-19. While most studies are in line with our findings [1,3], a study in non-hospitalized individuals in Chicago reported that only half of the initially seropositive participants (N = 87) had detectable IgG SARS-CoV-2 antibodies after 3-4 months [5]. A potential explanation for these seemingly contrasting data is that sample collection and assay differed. Demonbreun et al. [5] used a self-sampled dried-blood spot assay, which may be less accurate, as the estimated sensitivity ranges between 81.5% and 89.4% [25]. Alternatively, our participants may have continued to have detectable antibodies due to frequent re-exposure at work, resulting in natural boosting. Regardless, our subpopulation is relatively comparable to the current general population: as most countries have reduced or eliminated restrictive measures, people are regularly exposed to the virus, similar to our participants who were exposed due to social interactions at work, but at times, particularly when circulation of the virus increases, people take measures to prevent exposure, similar to our study population too. As such, our study contributes to an expanding body of literature suggesting long-term natural immunity after a

SARS-CoV-2 infection in the general population. This real-world evidence will aid policymakers and others in making decisions regarding social restrictions and vaccination, both for SARS-CoV-2 (for example, in populations or countries with low vaccine-induced immunity) and potential introductions of new coronaviruses.

We aimed to identify variables associated with antibody peak levels and dynamics. Lower baseline antibody levels were associated with increasing antibody levels over time but likely reflected a short time between infection and measurement. Older age and BMI were associated with higher antibody peak levels. This is in contrast with studies that found a negative association between antibody levels and BMI after COVID-19 vaccination [18,31], but is in agreement with a previous study examining the immune response after natural COVID-19 infection in Dutch blood donors, which used data unrelated to COco[29]. This positive association between antibody peak levels after natural infection and age or BMI could be related to the increased risk for severe COVID-19 disease in these populations, eventually resulting in stronger antibody responses [2,10,22,36]. Chronic disease seemed to be associated with decreasing antibody levels in our cohort, potentially due to a (relative) immune deficiency, but this difference was not significant. Although not significant either, chronic disease seemed to be associated with higher antibody peak levels (β: 47.8, 95%CI: -16.4-110.8). Potentially, comorbidities are associated with increased disease severity, which in turn correlates with higher antibody levels [10].

Previous studies in hospitalized patients similarly suggested that severe illness was associated with higher SARS-CoV-2 antibody responses [2]. Another longitudinal study, in which the population primarily consisted of recovered, non-hospitalized patients, also found an association between fever and higher IgG concentrations [29]. Although participants who reported fever generally had higher antibody levels in our cohort, these differences were not significant, potentially due to the small sample size or recall bias.

A strength of our study is that our participants had diverse levels of education and socioeconomic status, in contrast to most studies that overrepresent the highly educated [6]. Another strength is the longitudinal measurements within individuals. While more frequent blood sample collection would have been preferred, we consider our data more reliable than cross-sectional data, as we and others have shown that antibody peak levels vary widely between individuals [29].

This study has several limitations. We evaluated SARS-CoV-2 total antibody and IgG levels but did not include neutralizing antibodies. However, the IgG ELISA measured anti-RBD IgG, and because RBD is the main target for neutralizing antibodies, we assumed similar results [16]. Second, participants had frequently not been tested by PCR due to limited availability, and PCR test results were self-reported. As a result, our study may include false positive test results, and we may have missed individuals without detectable antibody levels after infection. However, the specificity of both used antibody tests is high (≥99%) [7,13], and cross-reactivity with other respiratory viruses, including non-SARS-CoV-2 coronaviruses, was non-existent [12]. Third, our population size limited the statistical power to identify variables associated with decreasing antibody levels. Pooled analyses may provide more details. Finally, we had not objectively confirmed the time of COVID-19 infection in all participants, while the time between infection and first measurement will affect antibody levels. However, dynamics remained similar when analyzing the subgroup of participants whose date of positive PCR test was available.

In conclusion, after an initial immune response due to a natural SARS-CoV-2 infection, we observed a reduction in antibody levels in approximately half of the studied seropositive hairdressers and hospitality staff in the Netherlands. However, most participants continued to have detectable antibody levels for up to 1 year. Therefore, our real-world data results suggest long-term immune protection after natural infection. Future studies should investigate whether this detectable natural immune response also results in less (severe) reinfections after 1 year, especially considering that this virus is continuously evolving into new variants.

Declarations of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approval

The COco-study has been approved by the Medical Research Ethics Committees United (MEC-U) at Nieuwegein, The Netherlands (project number A20.247/R20.041). It follows laws and guidelines on research with human subjects, including international standards such as the Declaration of Helsinki.

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CRediT authorship contribution statement

Dymphie Mioch: conceptualization, methodology, investigation, formal analysis, writing - original draft. **Leonard Vanbrabant:** methodology, formal analysis, writing - review & editing. **Johan Reimerink:** methodology, formal analysis, writing - review & editing. **Sandra Kuiper:** conceptualization, methodology, formal analysis, writing - review & editing, funding acquisition. **Esther Lodder:** conceptualization, methodology, formal analysis, writing - review & editing. **Wouter van den Bijllaardt:** conceptualization, methodology, investigation, formal analysis, writing - review & editing. **Jan Kluytmans:** conceptualization, methodology, investigation, formal analysis, writing - review & editing. **Michel D. Wissing:** conceptualization, methodology, investigation, formal analysis, writing - original draft, writing - review & editing, supervision, funding acquisition, visualization.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.11.025.

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