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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Title: 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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Declaration of interests

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.

Highlights

- COco is a prospective cohort study conducted in the Netherlands, 2020-2021.

- Population of non-medical contact professions with regular re-exposure to SARS-CoV-2.

- COco evaluates the dynamics of antibody levels following exposure to SARS-CoV-2.

- Very low seroreversion rates were found, suggesting long-term natural immunity.

- IgG antibody levels initially decreased, but remained detectable up to 12 months.

Abstract

Objective To evaluate dynamics of antibody levels following exposure to SARS-CoV-2 during 12 months in Dutch non-vaccinated hairdressers and hospitality staff.

Methods In this prospective cohort study, blood samples were collected every three months for one year, and analyzed using a qualitative total antibody ELISA and a quantitative IgG antibody ELISA. Participants filled out 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 (B) and 95% confidence intervals (95%CI) 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 the 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 up to 12 months in all participants. Higher age (B, 10-years increment: 24.6, 95%CI: 5.7-43.5) and higher BMI (B, 5kg/m² increment: 40.0, 95%CI: 2.9-77.2) were significantly associated with a higher peak of antibody levels.

Conclusions In this cohort, SARS-CoV-2 antibodies persisted for up to one year after initial seropositivity, suggesting long-term natural immunity.

Keywords: SARS-CoV-2 antibodies, cohort study, natural infection, contact profession, COVID-19 Serological Testing, Immunoglobulin G

INTRODUCTION

Since the start of the Coronavirus Disease 2019 (COVID-19) pandemic in early 2020, over 564 million confirmed cases and over 6 million deaths have been reported globally (July 22nd, 2022)(WHO, 2022a). In the Netherlands (total population: 17.6 million), 8.3 million inhabitants have officially been diagnosed with COVID-19 (WHO, 2022b). This number is an underestimation of the actual number of cases, since not all people were tested for severe acute respiratory syndrome coronavirus 2 (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 (Chvatal-Medina et al., 2021).

Research on the natural immune response following exposure to SARS-CoV-2 could aid to better understanding the duration of protective immunity. Such 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 versus 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 (Chvatal-Medina et al., 2021, Post et al., 2020). However, such populations may not adequately represent the general population, while it is especially important for policymakers to have knowledge on 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 (Anand et al., 2021, Dan et al., 2021, Deisenhammer et al., 2021, Demonbreun et al., 2021, He et al., 2021, Kučinskaitė-Kodzė et al., 2021, Luo et al., 2021, Petersen et al., 2021). 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 (Luo et al., 2021). 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 (Mioch et al., 2021). Antibodies were measured 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 which 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 has been described previously (Mioch et al., 2021). Hairdressers and hospitality staff (n=497) were recruited in June/July 2020; individuals were followed up to one 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 during approximately one year. During venipuncture, 3.5ml blood was drawn and serum was analyzed by the Microvida Laboratory for Medical Microbiology using the qualitative Wantai SARS-CoV-2 total antibody ELISA (Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China) per the manufacturer's instructions (Mioch et al., 2021). The manufacturer defined an absorbance to cut-off ratio (A/C.O.) ≥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. ≥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 (Nilsson et al., 2021).

For the additional quantitative analyses, we included all samples from all participants who had a seropositive (A/C.O. ≥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 (RIVM) using the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) 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 (EUROIMMUN, 2020). Antibody levels ≥11 relative units per milliliter (RU/ml) were considered seropositive, ≥8-<11RU/ml borderline, and <8RU/ml seronegative. For in-depth analyses of IgG antibody dynamics, we selected all participants with ≥1 seropositive result before their final visit. Additionally, we considered inclusion of participants with ≥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 ≥50% decrease were considered fluctuating antibody levels. Other changes were labeled as stable antibody levels. To calculate rates (percentage in antibody level changes per three 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 (Mioch et al., 2021). After enrolment, participants answered weekly a follow-up questionnaire (supplementary questionnaire 1), which collected information on health and work in the past week. Once every three 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 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 having had contact with someone who tested positive for SARS-CoV-2 (yes/no). Severity of COVID-19 related symptoms was divided into two categories, based on whether participants did or did not report fever.

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 $n \geq 5$ 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 (Kaplan and Meier, 1958). 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 (B) and their respective 95% confidence intervals (95%CI) for variables associated with the IgG antibody level peak. Univariable logistic regression model was used to calculate odds ratios and their respective 95% confidence intervals 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% confidence intervals 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 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 BMI was 25kg/m² (range 16-38kg/m²).

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 prior to 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 Body Mass Index (BMI) below 25kg/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.

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.

Since follow-up time differed between participants, we calculated the rate of change of antibody levels per three months (Figure 2E). Only a minority (11.0%) of participants had a decrease in antibody levels of more than 50% per three months; the majority (69.5%) had a decrease in antibody levels between 0 and 50% per three 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 42RU/ml, interquartile range (IQR) 25-98RU/ml versus median 29RU/ml, IQR 16-75RU/ml, p=0.002) and second and third measurements (n=48, median 27RU/ml, IQR 14-80RU/ml versus median 20RU/ml, IQR 10-52RU/ml, p<0.001), but not between the third and last measurements (n=37, median 19RU/ml, IQR 10-51RU/ml versus median 21RU/ml, IQR 10-60RU/ml, p=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 6-7RU/ml). When conducting subgroup analyses evaluating antibody level changes in those with confirmed PCR test results (n=29), similar results were observed: antibody levels decreased significantly between the first and second measurement (median 42RU/ml, IQR 26-187RU/ml versus median 25RU/ml, IQR 17-76RU/ml), p=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

76RU/ml, min-max 14-191RU/ml), followed by participants with decreasing (n=38, median 56RU/ml, min-max 13-312RU/ml), stable (n=36, median 41RU/ml, min-max 12-660RU/ml) and increasing antibody levels (n=5, median 15RU/ml, min-max 9-52RU/ml). Participants with increasing antibody levels had significantly lower baseline antibody levels than those with decreasing (p=0.006) or stable (p=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 (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=0.099 and p=0.103, respectively).

Since previous studies suggested that those with severe disease developed higher antibody levels (Chvatal-Medina et al., 2021, Lynch et al., 2020), we evaluated whether disease severity predicted antibody levels and antibody level dynamics in our cohort (Figure 4). Since 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 83RU/ml, 95%CI 20-146RU/ml versus n=24, mean 26RU/ml, 95%CI 16-36RU/ml, p=0.050). However, this difference was particularly driven by one participant with fever. She reported a SARS-CoV-2 reinfection between the third and fourth measurement and therefore, antibody levels increased from 22RU/ml to 412RU/ml. After excluding her from the analyses, those with fever still had higher mean antibody levels at the last visit (n=13, mean 58RU/ml, 95%CI 25-91RU/ml versus n=24, mean 26RU/ml, 95%CI 16-36RU/ml), but this difference was not

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.

BMI, body-mass index; min, minimum; max, maximum.

Table 2: Associations between participant characteristics and longitudinal changes in SARS-CoV-2 IgG antibody levels

Dependent variable: Decreasing antibody level (yes/no)	
Independent variable	OR (95%CI)
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)
BMI (5 kg/m² increment)	0.7 (0.4 – 1.3)
Current smoker (ref: not currently smoking) **- Smoking (5 cigarettes per day increment)**	1.2 (0.4 – 3.8) 2.7 (0.9 – 8.3)
Current alcohol user (ref: currently no alcohol use) **- Alcohol quantity (7 alcohol units per week increment)**	0.9 (0.2 – 3.7) 1.2 (0.8 – 1.7)

BMI, Body-mass index; CI, confidence interval; OR, odds ratio; Ref, reference.

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

Table 3 Associations between participant characteristics and peak of SARS-CoV-2 IgG antibody levels

Dependent variable: Peak of antibody level	**Univariable**	**Bivariable**
Variables	B (95%CI)	B (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)
BMI (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)

CI, confidence interval; B, beta coefficient; Ref, reference; *, p<0.05.

In the second column, beta coefficients and their 95% confidence intervals 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.