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MAJOR ARTICLE

# SARS-CoV-2 Seroprevalence and Antibody Kinetics Among Health Care Workers in a Spanish Hospital After 3 Months of Follow-up

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***Background.*** At the COVID-19 spring 2020 pandemic peak in Spain, prevalence of SARS-CoV-2 infection in a cohort of 578 randomly selected health care workers (HCWs) from Hospital Clínic de Barcelona was 11.2%.

***Methods.*** A follow-up survey 1 month later (April-May 2020) measured infection by rRT-PCR and IgM, IgA, and IgG to the receptor-binding domain of the spike protein by Luminex. Antibody kinetics, including IgG subclasses, was assessed until month 3.

***Results.*** At month 1, the prevalence of infection measured by rRT-PCR and serology was 14.9% (84/565) and seroprevalence 14.5% (82/565). We found 25 (5%) new infections in 501 participants without previous evidence of infection. IgM, IgG, and IgA levels declined in 3 months (antibody decay rates 0.15 [95% CI, .11–.19], 0.66 [95% CI, .54–.82], and 0.12 [95% CI, .09–.16], respectively), and 68.33% of HCWs had seroreverted for IgM, 3.08% for IgG, and 24.29% for IgA. The most frequent subclass responses were IgG1 (highest levels) and IgG2, followed by IgG3, and only IgA1 but no IgA2 was detected.

***Conclusions.*** Continuous and improved surveillance of SARS-CoV-2 infections in HCWs remains critical, particularly in high-risk groups. The observed fast decay of IgA and IgM levels has implications for seroprevalence studies using these isotypes.

**Keywords.** COVID-19; SARS-CoV-2; seroprevalence; antibodies; health care workers; longitudinal cohort; kinetics.

Since the start of the coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), there have been 2 priority questions: to establish the prevalence and incidence of the infection and to unravel whether cases are protected from future reinfections and/or disease. Among the 34.2 million confirmed SARS-CoV-2 infections and over 1 million deaths, as of October 2020 [1], health care workers (HCWs) continue to be one of the populations at higher risk due to close contact with COVID-19 patients [2]. Most infections in HCWs are asymptomatic or mild [2–6] but undetected infections can put their fellow HCWs and patients at risk. Prompt identification of cases by real time reverse-transcriptase polymerase chain reaction (rRT-PCR) screenings at hospitals is crucial to avoid new infections, isolations, and quarantines in HCWs.

We previously reported the prevalence of SARS-CoV-2 in a cohort of 578 HCWs from a large hospital in Barcelona, Spain, at the peak of the spring 2020 pandemic (baseline, 28 March to 9 April 2020) [3]. We found that 9.3% (95% confidence interval [CI], 7.1–12.0) of the participants were seropositive and the cumulative prevalence of SARS-CoV-2 infection (considering a past or current positive result to either antibody testing or rRT-PCR) was 11.2% (95% CI, 8.8–14.1). The seroprevalence was relatively low but higher than the 7% estimated in the general population in Barcelona 1 month later according to a large national seroprevalence study [7]. Our findings were consistent with other studies in HCWs [4, 5, 8], although prevalence of up to 44% had also been reported in other countries [9]. Importantly, 40% of the infections in our HCW cohort had not been previously detected [3].

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This cohort is being followed up for 1 year to assess seroconversion and to understand naturally acquired immunity to COVID-19 by evaluating the kinetics of antibody responses, including IgG subclasses that have barely been explored [10, 11]. Each IgG subclass is involved in different antibody functions beyond viral neutralization through the differential binding of Fc receptors or to complement, therefore this characterization is relevant to understand the mechanisms of immune protection [12].

Here, we determined the prevalence of SARS-CoV-2 by antibody serology and rRT-PCR 1 month after the baseline. We measured IgM, IgG, and IgA isotypes and subclasses, and assessed the factors associated with new infections as well as levels and kinetics of antibodies after 3 months of follow-up.

## METHODS

### Study Design and Population

We performed the second cross-sectional survey (27 April to 6 May 2020) of a 4-stage seroprevalence study in a cohort of 578 HCWs who had been randomly selected and recruited from a total of 5598 HCWs registered at Hospital Clínic of Barcelona (HCB) [3]. Participants were invited to a follow-up visit 1 month later (month 1 visit) and those with any evidence of previous infection were invited again 2 months later (month 3 visit). The study population included HCWs who deliver care and services directly or indirectly to patients. Further information on the study population can be found in the Supplementary Materials. We collected a nasopharyngeal swab for SARS-CoV-2 rRT-PCR at month 1 and a blood sample for antibody and immunological assessments at month 1 and 3. For participants isolated at home due to a COVID-19 diagnosis or in quarantine, data and sample collection took place at their households. Written informed consent was obtained from all study participants prior to study initiation. The study was approved by the Ethics Committee at HCB (reference number, HCB/2020/0336). Data for each participant were collected in a standardized electronic questionnaire as previously described [3].

### SARS-CoV-2 Laboratory Analyses

Methods for SARS-CoV-2 detection by rRT-PCR followed the CDC-006-00019 Centers for Disease Control and Prevention (CDC)/Deputy Director for Infectious Diseases (DDID)/National Center for Immunization and Respiratory Diseases (NCIRD)/Division of Viral Diseases protocol, as previously described [3] (Supplementary Material). Immunoglobulin M (IgM), immunoglobulin G (IgG), and immunoglobulin A (IgA) antibodies to the receptor-binding domain (RBD) of the spike glycoprotein of SARS-CoV-2, kindly donated by the Krammer laboratory (Mount Sinai, New York) [13], were measured as in the baseline survey of this cohort [3]. IgG and IgA subclass assays were performed following a similar Luminex protocol (Supplementary Material) [14]. For the assay cutoff calculation, the median fluorescence intensities (MFIs) of 47 prepandemic controls were first $\log_{10}$-transformed to approximate a normal distribution. Then, the value obtained from 10 to the power of (mean + 3 standard deviations of the $\log_{10}$-transformed MFIs) was applied to nontransformed MFIs of test samples as the cutoff.

### Statistical Analysis

We tested the association between variables with the $\chi^2$ or Fisher exact test (for categorical variables), *t* Student, or Wilcoxon Sum Rank tests (for continuous quantitative variables). Univariable logistic models were run to evaluate factors associated with seropositivity. The effect of infection on antibody levels was analyzed using multilevel mixed-effects linear regression models incorporating Gaussian random intercepts. This resulted in an estimate of the rates of antibody dynamics (decay), assuming a single exponential model. For the analysis of antibody levels by different factors, antibody levels corresponded to participants who were seropositive at month 0 and to those who seroconverted from month 0 and month 1 (cumulative seropositive data). The LOESS (locally estimated scatterplot smoothing) method was used to fit a curve to depict kinetics of antibody levels over time. Statistical comparisons were performed at 2-sided significance level of .05 and 95% CIs were calculated for all estimations. Analyses were undertaken using Stata/SE software version 16.1 and R studio version R-4.0.2 [15] (packages *tidyverse* and *pheatmap*).

## RESULTS

### Demographic Characteristics of Individuals Without Previous Evidence of Infection (Month 1 Survey)

One month after baseline, 566 of 578 HCWs were visited again (2.1% lost to follow-up) and blood sample was obtained in 565 of them. From the total of 566 individuals, 65 (11.5%) had previous evidence of infection at baseline by serology or rRT-PCR [3], thus the remaining 501 (88.5%) individuals had no evidence of infection. Of those, 359 (71.7%) were female, 268 (53.5%) were younger than 45 years, and the mean age was 42 years. Half of the individuals (239/501) were nurses, auxiliary nurses, or stretcher-bearers, 25.5% (128/501) were physicians, 7.6% (38/501) were laboratory or other technicians; 50.3% (252/501) worked in COVID-19 units, and 76.4% (383/501) had direct contact with COVID-19 patients since the last visit. Eleven per cent (54/501) of participants reported having had COVID-19–compatible symptoms in the previous month and 21.2% (106/501) had comorbidities (Supplementary Table 1).

### SARS-CoV-2 Infections at Month 1 of Follow-up

At the month 1 visit, the cumulative prevalence of infection measured by either rRT-PCR or serology was 14.9% (84/565). Nine participants had a positive rRT-PCR in the 28 days following the initial study visit, and only 3 of these positive rRT-PCRs were detected at the second survey. The seroprevalence at month 1 was 14.5% (82/565) for either IgM and/or IgG and/

or IgA and 10.1% for IgM, 11.3% for IgG, and 11.5% for IgA (Supplementary Figure 1). There was an absolute increment of 25 SARS-CoV-2 infections detected by rRT-PCR or serology, 5% among the 501 previously uninfected individuals (4% of all individuals at month 1). Among these 25 individuals, infection was detected only by antibody serology (IgM/IgG/IgA) in 16, by antibody serology and rRT-PCR in 7, and only by rRT-PCR in 2. The latter 2 seronegative individuals at month 1 had a positive rRT-PCR result more than 20 days before the survey.

Having had COVID-19–compatible symptoms during the follow-up month was associated with experiencing a SARS-CoV-2 infection between month 0 and 1 with an OR of 6.6 (95% CI, 2.8–14.4) and $P < .0001$ in univariable analysis (Table 1). The professional category was also associated with infections; laboratory and other technicians had the highest odds of being infected (OR, 13.3; 95% CI, 1.5–115.8; $P = .048$). Sex, age, comorbidities, working in COVID-19 units, or having had direct contact with patients since the last visit were not associated with SARS-CoV-2 infection.

#### SARS-CoV-2 Antibody Seroreversion Rate

From the 54 seropositive HCWs at baseline, 1 did not have a sample available at month 1, 3/36 (8.3%) had seroreverted for IgM, 1/44 (2.3%) for IgG, and 5/47 (10.6%) for IgA. From the 82 HCWs seropositive at month 1, 66 were followed up at month 3. Of those 66, at month 3, 38/49 (77.6%) had seroreverted for IgM, 2/54 (3.7%) for IgG, and 13/53 (24.5%) for IgA. Two individuals who had seroreverted at month 1 (1 for IgG and 1 for IgA) had detectable antibody levels again at month 3. In total, there were 41/60 (68.3%) seroreversions for IgM, 2/65 (3.1%) for IgG, and 17/70 (24.3%) for IgA. From the remaining 512 HCWs who were seronegative at recruitment, a total of 31 HCWs (6.1%) seroconverted during the follow-up month for at least 1 immunoglobulin. Of those, 8 had a positive rRT-PCR detected at baseline or before. Separately by isotypes, 21 seroconverted for IgM, 20 for IgG, and 23 for IgA. There were 5 individuals who seroconverted for IgM only, 3 for IgG only, and 6 for IgA only.

There were 5 seronegative HCWs at month 1 but their previous rRT-PCR was positive. Time since the first positive rRT-PCR ranged from 20 to 47 days. Two of these HCWs were asymptomatic.

#### IgA, IgG, and IgM Levels in Seropositive HCWs

Overall, IgM, IgG, and IgA levels decreased from baseline to month 3, with antibody decay rates at month 3 of 0.14 (95% CI, .11–.18), 0.66 (95% CI, .53–.82), and 0.12 (95% CI, .09–.16), respectively (Table 2). The estimated time to seroreversion was 1.95 months (95% CI, 1.74–2.22; $P < .0001$), 19.41 months (95% CI, 12.84–39.75; $P < .0001$), and 1.95 months (95% CI, 1.71–2.25; $P < .0001$) for IgM, IgG, and IgA, respectively. Despite the overall decay in antibodies, IgG levels increased in 28/43 individuals from baseline to month 1 and in 6/52 individuals from month 1 to month 3 (Figure 1). In adjusted models by days since onset of symptoms, antibody decay rates were similar (Supplementary Table 2). No differences in antibody kinetics were observed between asymptomatic and symptomatic individuals (Figure 1).

IgA levels were higher in seropositive HCWs reporting having had COVID-19–compatible symptoms ($P < .001$) and a similar trend was observed for IgM ($P = .057$; Figure 2). In addition, IgM levels were higher in those seropositive HCWs with symptoms for >10 days compared to seropositive HCWs with shorter duration of symptoms, and a similar trend was observed

**Table 1. Factors Associated With SARS-CoV-2 Infections From Recruitment to Month 1 (n = 501)**

| Factor | Odds Ratio | 95% CI | *P* |
|---|---|---|---|
| Sex | | | |
| Male | 1 | | .6218 |
| Female | 1.27 | .50–3.24 | |
| Age[a] | 0.98 | .95–1.02 | .3346 |
| Job category | | | |
| Other | 1 | | .0480 |
| Technician | 13.03 | 1.47–115.76 | |
| Physician | 2.77 | .30–25.25 | |
| Nurse/auxiliary services | 5.54 | .72–42.55 | |
| Directly involved in clinical care | | | |
| No | 1 | | .9103 |
| Yes | 1.06 | .41–2.70 | |
| COVID-19 unit | | | |
| No | 1 | | .8615 |
| Yes | 1.07 | .48–2.40 | |
| Comorbidities[b] | | | |
| No | 1 | | .5192 |
| Yes | 0.70 | .23–2.08 | |
| Smoker | | | |
| No | 1 | | .2982 |
| Yes | 1.58 | .67–3.77 | |
| Frequency of smoking[c] | | | |
| Occasional smoker | 1 | | .7742 |
| Social smoker | 1.38 | .15–12.52 | |
| Regular smoker | 1.00 | - | |
| Direct contact with patients since the last visit | | | |
| No | 1 | | .9569 |
| Yes | 0.97 | .38–2.50 | |
| Telematic work in the last 2 months | | | |
| No | 1 | | .2546 |
| Yes | 0.56 | .21–1.52 | |
| Any symptom of COVID-19 (within the last month) | | | |
| No | 1 | | < .0001 |
| Yes | 6.55 | 2.77–15.44 | |

Abbreviation: CI, confidence interval.

[a]Odds ratio per unit increase.

[b]Comorbidities include: heart and liver disease, diabetes, chronic respiratory and renal disease, cancer and autoimmune disease, and other immunological disorders.

[c]n = 104.

**Table 2. Rate of Antibody Decay Calculated by Mixed-Effects Linear Models**

| | IgM (n = 104) | | IgG (n = 125) | | IgA (n = 132) | |
|---|---|---|---|---|---|---|
| **Predictors** | **Estimate (95% CI)** | *P* | **Estimate (95% CI)** | ***P*** | **Estimate (95% CI)** | ***P*** |
| Time point | | | | | | |
| M 0 | 1 | <.0001 | 1 | < .0001 | 1 | <.0001 |
| M 1 | 0.49 (.39–.62) | | 1.16 (.95–1.42) | | 0.34 (.26–.44) | |
| M 3 | 0.14 (.11–.18) | | 0.66 (.53–.82) | | 0.12 (.09–.16) | |
| Intercept, MFI[a] | 12 082.59 (9410.89–15 512.76) | <.0001 | 11 099.44 (8520.29–14 459.32) | <.0001 | 6018.07 (4529.06–7996.62) | <.0001 |
| Random effects | | | | | | |
| Variable (intercept) | 0.34 (.19–.61) | | 0.57 (.35–.92) | | 0.59 (.35–.99) | |
| Variable (residual) | 0.25 (.18–.35) | | 0.23 (.17–.32) | | 0.40 (.30–.55) | |
| Intraclass correlation | 0.57 (.40–.74) | | 0.71 (.57–.82) | | 0.59 (.43–.73) | |

Intraclass correlation describes how strongly measures from the same subject resemble each other.

Abbreviations: CI, confidence interval; M, month; MFI, median fluorescence intensity.

[a]Estimated antibody baseline level.

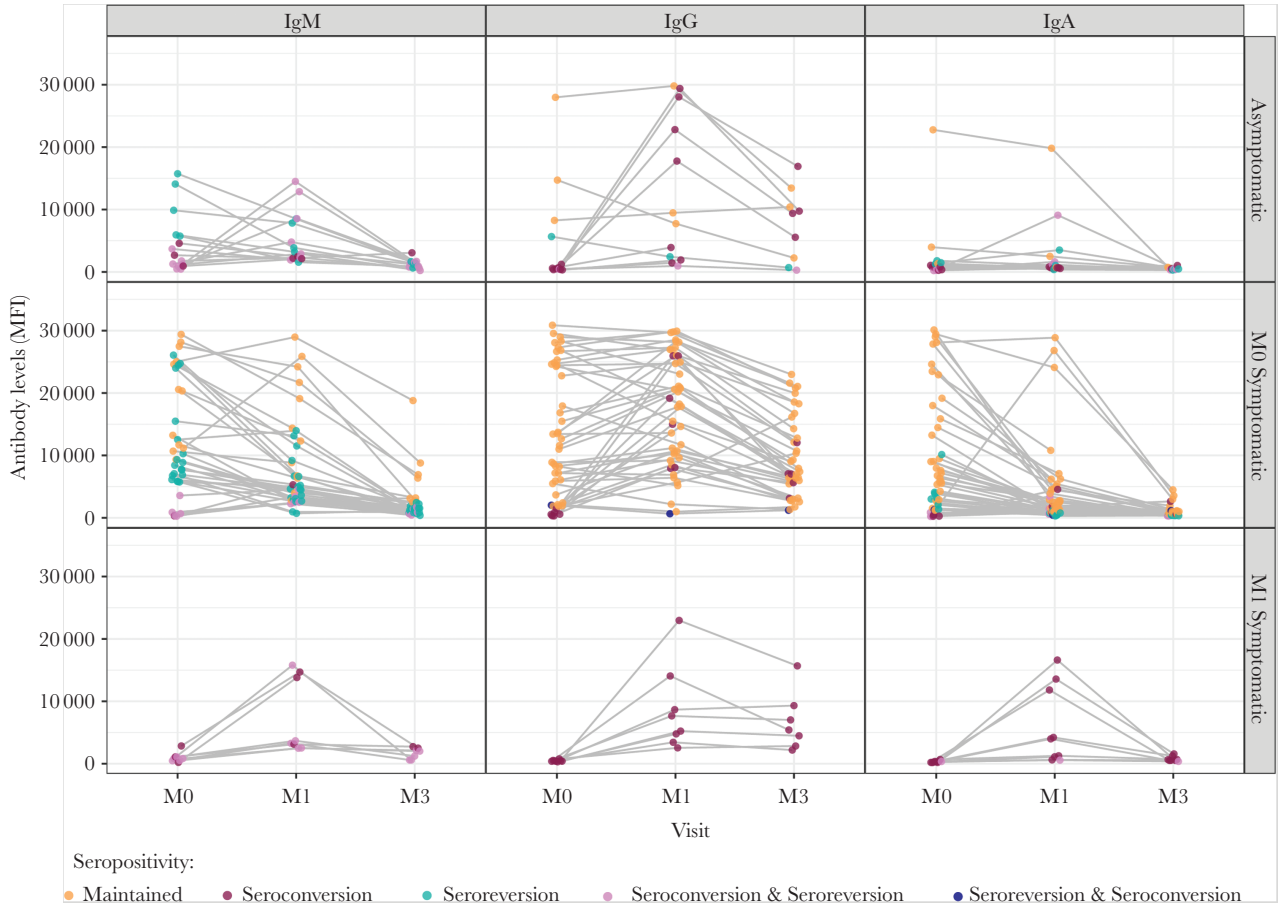


**Figure 1.** Kinetics of SARS-CoV-2 antibodies in seropositive individuals during the 3 months of follow-up. Levels (MFI) of IgM, IgG, and IgA against receptor-binding domain of the SARS-CoV-2 spike glycoprotein stratified by asymptomatic participants and participants who reported COVID-19–compatible symptoms for the first time at recruitment (M 0) or at M 1. No participants reported symptoms for the first time at M 3. Lines indicate paired samples. Yellow dots depict individuals (IgM, n = 12; IgG, n = 41; IgA, n = 35) who had detectable antibody levels at all study visits when antibody levels where measured; burgundy dots, individuals who seroconverted for a particular isotype at M 1 (IgM, n = 7; IgG, n = 21; IgA, n = 17); green dots, individuals who seroreverted between M 0 and M 1 (IgM, n = 3; IgG, n = 0; IgA, n = 4) or M 1 and M 3 (IgM, n = 21; IgG, n = 1; IgA, n = 6); pink dots, individuals who seroconverted from M 0 to M 1 and then seroreverted (IgM, n = 17; IgG, n = 1; IgA, n = 7); and blue dots, individuals who seroreverted and seroconverted again (IgM, n = 0; IgG, n = 1; IgA, n = 1). Abbreviations: COVID-19, coronavirus disease 2019; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; M, month; MFI, median fluorescence intensity; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. See online version for color figures.

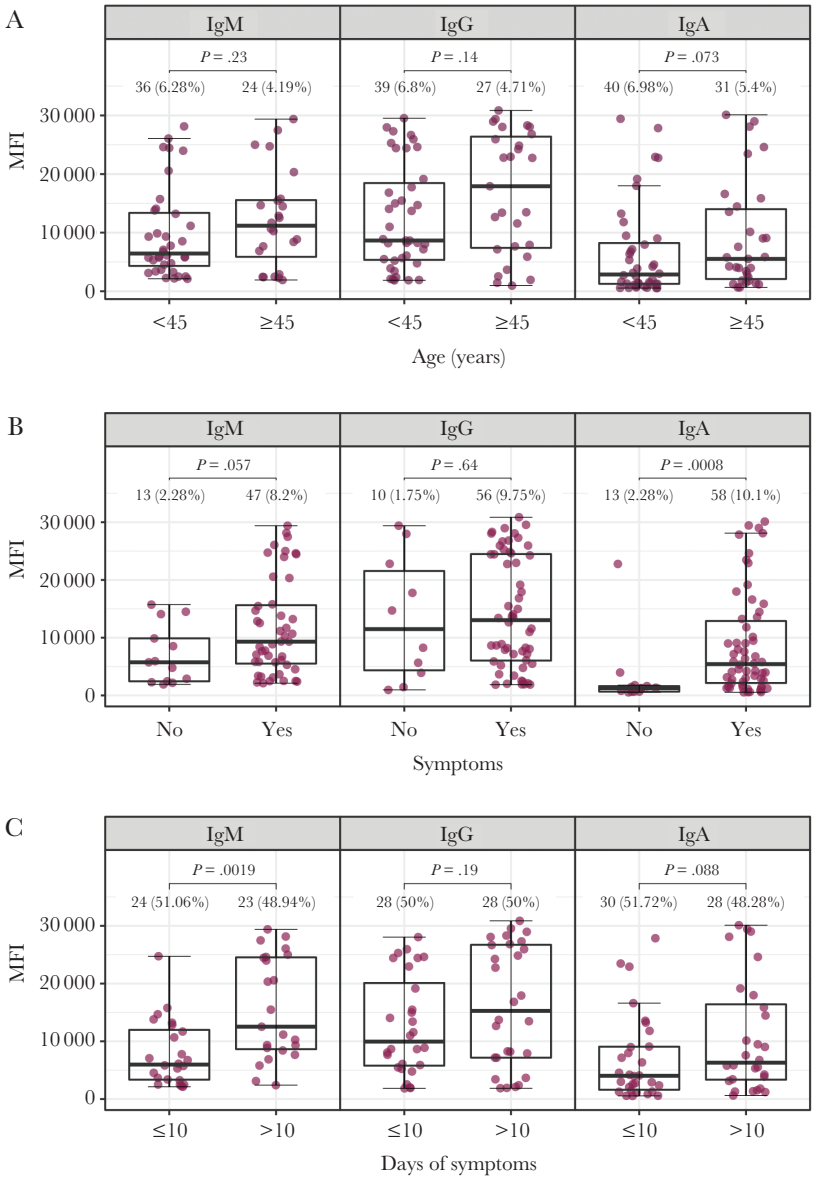


**Figure 2.** SARS-CoV-2 antibody levels by demographic and clinical variables. Levels (MFI) of IgM (n = 60), IgG (n = 66), and IgA (n = 71) against receptor-binding domain of the SARS-CoV-2 spike glycoprotein stratified by (*A*) age, (*B*) presence of symptoms, and (*C*) duration of symptoms. Graphs show data from accumulative month 0 and month 1 seropositive individuals: month 0 antibody levels from seropositive individuals at month 0 plus month 1 antibody levels from individuals who seroconverted from month 0 to month 1. Percentages indicate the sum of proportions of seropositive subjects from recruitment and month 1 within each category of the x-axis with respect to the total number of samples from each visit (*A* and *B*) or the proportion of individuals within each category of the x-axis with respect to the total number seropositive symptomatic (*C*). The center line of boxes depicts the median MFI; the lower and upper hinges correspond to the first and third quartiles; the distance between the first and third quartiles corresponds to the IQR; whiskers extend from the hinge to the highest or lowest value within 1.5 × IQR of the respective hinge. Wilcoxon rank test was used to assess statistically significant differences in antibody levels between groups. Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; MFI, median fluorescence intensity; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

for IgA (Figure 2). Age and sex were not associated with antibody levels (Figure 2 and Supplementary Figure 2).

Among HCWs with positive rRT-PCR, IgM levels peaked around 20 days since the first positive rRT-PCR, declined during 30 days after the positive rRT-PCR, and then seemed to stabilize (Figure 3A). IgA levels followed a similar pattern with a slightly earlier peak. In contrast, IgG levels increased until 50 days since the first positive rRT-PCR and a decrease was observed thereafter but milder than for IgM and IgA. Similar kinetics were observed for antibody levels since onset of symptoms among seropositive HCWs reporting having had symptoms (Figure 3B). However, antibodies peaked some days later compared to the kinetics by days since rRT-PCR.

### Antibody Subclasses

IgG subclasses were measured only in IgG-seropositive samples. IgG1 had the highest levels (and correlated with IgG), followed by IgG2, IgG3, and IgG4 (Figure 4A and 4B).

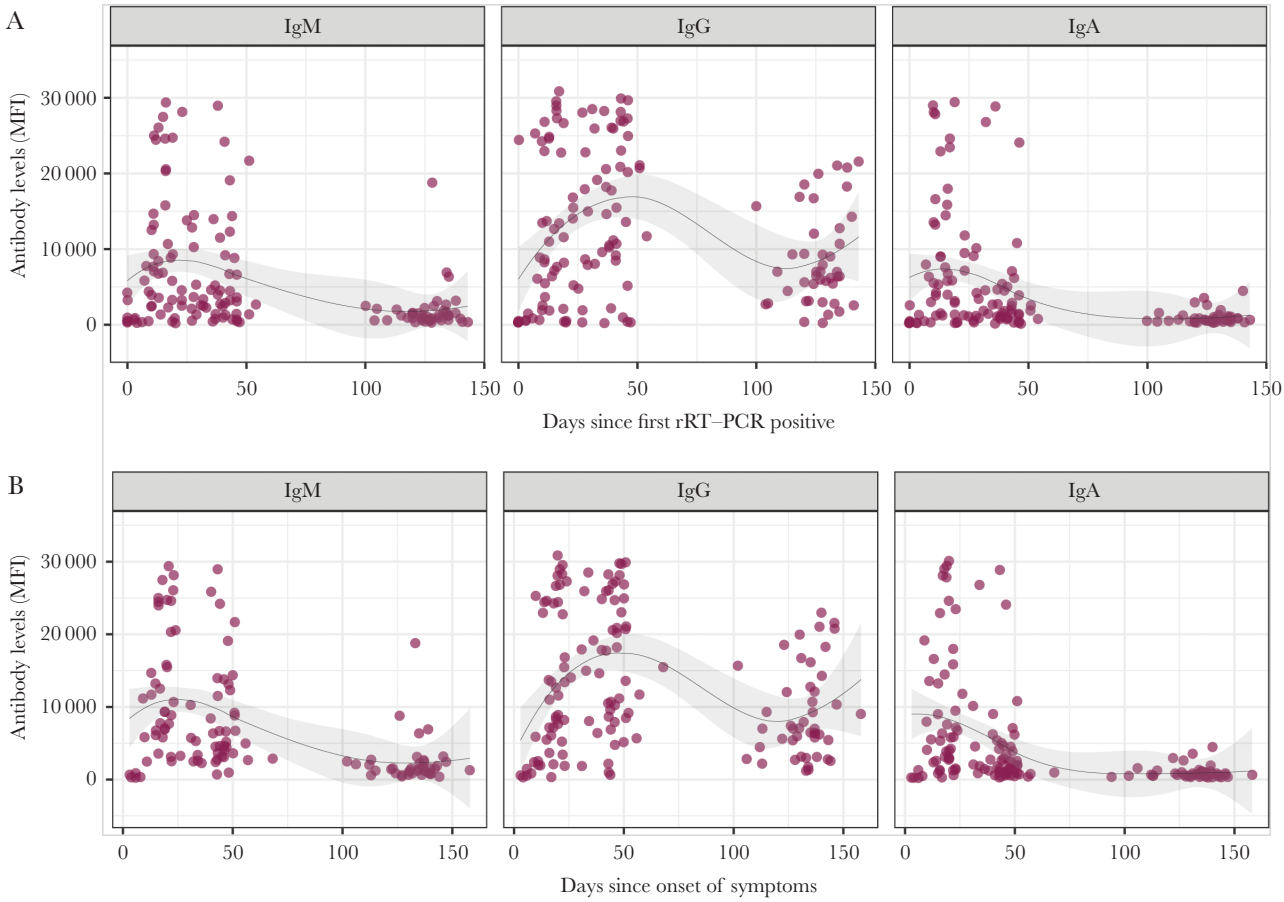


**Figure 3.** SARS-CoV-2 antibody levels by time since first rRT-PCR and onset of symptoms. Levels (MFI) of IgM, IgG, and IgA against receptor-binding domain of the SARS-CoV-2 spike glycoprotein by (*A*) days since the first positive rRT-PCR, and (*B*) days since onset of any symptom. Graphs show pooled month 0, month 1, and month 3 data. Data in (*A*) are only for individuals with any rRT-PCR positive (n = 142). Data in (*B*) are only for seropositive individuals at any visit since onset of any symptom compatible with COVID-19 (n = 121 for IgM, n = 145 for IgG, and n = 149 for IgA). The fitted curves were calculated using the LOESS method. Shaded areas represent 95% confidence intervals. Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; LOESS, locally estimated scatterplot smoothing; MFI, median fluorescence intensity; rRT-PCR, real time reverse-transcriptase polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Approximately 55% of the IgG-positive samples had detectable IgG1 at month 0 and 1, and 60% and 73% had IgG2 at month 0 and 1, respectively (Figure 4C). Around 45% and 58% had detectable IgG3, and only 2% and 3% had IgG4 at month 0 and 1, respectively, but this increased to almost 70% at month 3 despite very low levels (Figure 4C). IgG subclass levels were maintained or increased from month 0 to 1, whereas IgG1 and IgG2 decreased from month 1 to 3 and 20/52 (38.5%) HCWs seroreverted for IgG2. The samples with the highest levels of IgG3 were IgG1 negative (Figure 4B).

IgG2 levels were higher in those IgG-seropositive HCWs who had more than 10 days of symptoms compared to those with lower duration of symptoms but no other associated factors were found (Figure 4D and 4E; Supplementary Figure 3).

Regarding IgA subclass, only IgA1 and no IgA2 was detected (Supplementary Figure 4).

## DISCUSSION

After 1 month of follow-up, we found a prevalence of 5% infections in HCWs without a previous COVID-19 diagnosis or evidence of past infection. This is a substantial amount of new infections considering that (1) the accumulated prevalence of infection at recruitment was 11.2%, (2) the peak of the pandemic had already passed, (3) personal protective equipment was available, (4) regular rRT-PCR screenings had been implemented for several weeks at the hospital, and (5) the population had been confined for almost 1.5 months. Interestingly, 64% of these infections were detected by serology only, probably reflecting infections occurring 1 to 3 weeks before this survey.

The single factor showing the highest strength of association with newly detected past or present SARS-CoV-2 infection was having had any symptom compatible with COVID-19 in the previous month. Around 60% of the infected individuals were asymptomatic, which is a higher proportion than what we had

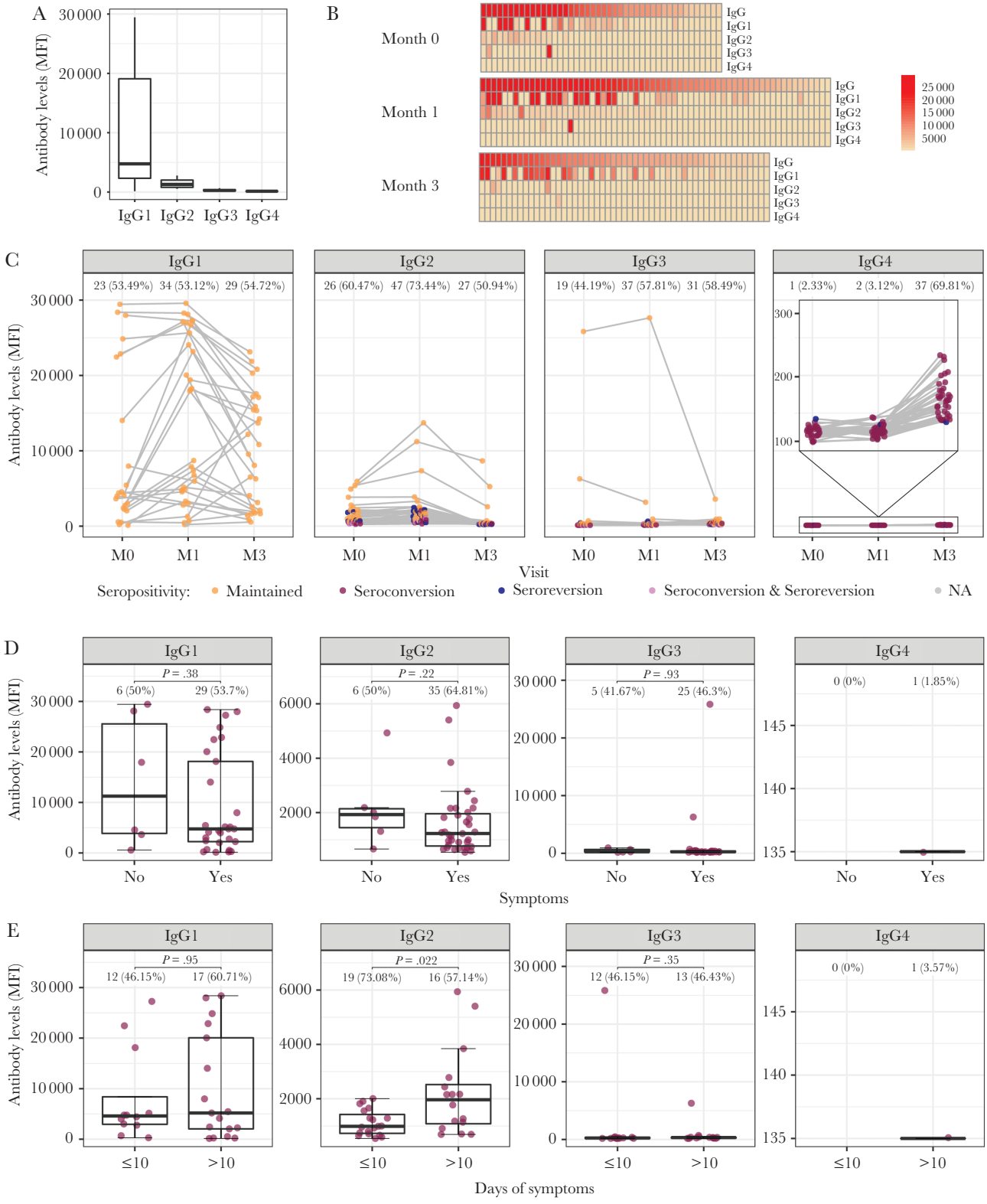


**Figure 4.** SARS-CoV-2 IgG subclass responses in IgG-seropositive individuals. All panels show levels (MFI) of IgG1, IgG2, IgG3, and IgG4 against receptor-binding domain of the SARS-CoV-2 spike glycoprotein. *A*, Boxplots. *B*, Heatmaps showing the MFI of IgG and IgG subclasses for all IgG-seropositive individuals at recruitment, month 1, and month 3 separately. *C*, Kinetics of IgG subclass levels in seropositive individuals from month 0 to month 3. Lines indicate paired samples; yellow dots depict individuals who had detectable antibody levels at all study visits when antibody levels where measured; burgundy and blue dots show individuals who seroconverted and seroreverted, respectively, for the represented subclass between visits. *D*, IgG subclass levels stratified by symptoms. *E*, IgG subclass levels stratified by days of symptoms. *A*, *D*, and *E*, Data from accumulative month 0 and month 1 seropositive individuals: month 0 antibody levels from seropositive individuals at month 0 plus month 1 antibody levels from individuals who seroconverted from month 0 to month 1. Percentages indicate the proportion of seropositive subjects within each category of the x-axis. The center line of

previously reported at baseline [3], although it is in line with other studies reporting from 20% to 80% asymptomatic infections [16, 17]. Consistently, we also found that working in a COVID-19 unit was not associated with SARS-CoV-2 infections [3] but, curiously, we found that technicians had an increased risk. This may be due to a decreased perception of risk in this group in contrast to other job categories that may take more precautions due to their direct contact with COVID-19 patients.

Overall, IgM and IgA levels decreased substantially within 3 months and we estimated around 2 months to IgM and IgA seroreversion. Of note, from the seropositive HCWs at month 1, 78% had seroreverted for IgM and 25% for IgA by month 3. In contrast, estimated time to seroreversion for IgG was over 19 months and many individuals had an increase in the antibody levels during the first month, probably due to the short time since infection and the delayed peak response of IgG compared to IgA and IgM. The fastest decrease of IgM and IgA and the observed curves of antibody levels by days since the first positive rRT-PCR are consistent with previously reported data [18, 19] and with the expected patterns of an antibody response: IgM and IgA peak and then decline early after an infection and are typically short-term responses, while IgG peaks and decays later to a stable titer that is maintained over time. Also, IgG has a half-life of 21 days (less for IgG3) [20], whereas the half-life of IgA and IgM is 4–9 days [21, 22]. Nevertheless, emerging data indicate that SARS-CoV-2 IgG responses may wane quickly over time [23] to undetectable levels in a considerable proportion of individuals [24, 25]. In our study, over a period of 3 months, only 2 individuals seroreverted for IgG. Antibody decay and seroreversion has enormous implications for the correct interpretation of serosurveys and could indicate waning protection and difficulties to achieve herd immunity.

We confirmed that IgA levels are higher in symptomatic individuals compared to the asymptomatic ones, and that IgM levels positively correlate with the duration of symptoms [3]. Despite not having found statistical differences for IgG levels, increasing evidence suggest that levels and duration of antibodies are also higher in symptomatic and in moderate-severe patients than mild cases [24, 26]. In addition, we found 5 nonresponders with more than 20 days since the first positive rRT-PCR. Lower antibody levels in asymptomatic and mild cases and antibody nonresponders would also affect seroprevalence studies and could imply lower protection to reinfection, although infected individuals also mount T-cell responses [27], which may independently protect from infection.

More than half of the seropositive HCWs for IgG had IgG1 and IgG2 responses, whereas IgG3 responses were detected in fewer individuals. This finding differs from 2 other studies showing dominance of IgG3 and almost no IgG2 responses in COVID-19 patients [10, 11]. IgG subclass responses increased from month 0 to month 1 in most of the individuals and, interestingly, many seroconverted during this month of follow-up for IgG2 and IgG3, but IgG1 and IgG2 levels decreased by month 3 and about 40% of HCWs seroreverted for IgG2. IgG4 responses, albeit very low, were detected at month 3 but not before. Overall, antibody levels were higher for IgG1 than the other isotypes, following the relative abundance of these isotypes in plasma (IgG1>IgG2>IgG3>IgG4). We did not find any factor associated with IgG subclass levels, with the exception of IgG2 levels positively correlating with longer duration of symptoms. Class-switch recombination to IgG2 occurs after IgG1 during the course of the immune response to limit inflammation [28]. While IgG1 are typically proinflammatory and have effector functions resulting in infection clearance through efficient binding to Fc receptors and complement, IgG2 has decreased binding [28]. The association of IgG2 levels with duration of symptoms could reflect an anti-inflammatory response elicited by higher persistence of viruses and inflammation. Conversely, IgG2 could be contributing to the persistence of symptoms through competition with IgG1, causing less efficient clearance of viruses.

The main limitation of this study is the small sample size for the analysis of factors associated with SARS-CoV-2 infection. In addition, there may be a recall bias in some reported data such as symptoms. Antibody responses were only analyzed using 1 antigen and other viral proteins may elicit different responses in different individuals [14], thus we could have slightly underestimated the overall seroprevalence of infection. Finally, kinetics of antibody responses and antibody decay rates have to be interpreted with caution as only 3 timepoints have been analyzed and rates may change depending on the baseline levels, and if levels are measured at the peak response or at the later steady-state period. Data from the next time points will complete the kinetics information.

Our findings reinforce the importance of strengthening SARS-CoV-2 surveillance among HCWs. Despite having implemented regular rRT-PCR screenings, SARS-CoV-2 infections may go undetected. The lower antibody levels in the asymptomatic and mild cases, and the decay of IgA and IgM, have implications for seroprevalence studies as these isotypes may be undetectable 1–2 months post infection. Although

boxes depicts the median of MFIs; the lower and upper hinges correspond to the first and third quartiles; the distance between the first and third quartiles corresponds to the IQR; whiskers extend from the hinge to the highest or lowest value within 1.5 × IQR of the respective hinge. Wilcoxon rank test was used to assess statistically significant differences in antibody levels between groups. Abbreviations: IgG, immunoglobulin G; IQR, interquartile range; MFI, median fluorescence intensity; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. See online version for color figures.

we could not show any evidence on IgG antibody decay after around 2 months from initial infection, we hope that subsequent surveys might provide some insight into this decay. Longer follow-up visits of this cohort will allow assessment of the duration of IgG and IgG subclass responses and their role in protection from disease and reinfection.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

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