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# The Fall in Antibody Response to SARS-CoV-2: a Longitudinal Study of Asymptomatic to Critically Ill Patients Up to 10 Months after Recovery

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**ABSTRACT** The aim of this study was to assess the long-term dynamics and factors associated with the serological response against the severe acute respiratory syndrome coronavirus 2 after primary infection. A prospective longitudinal study was conducted with monthly serological follow-up during the first 4 months, and then at 6, 8, and 10 months after the disease onset of all recovered adult in- and outpatients with coronavirus disease 2019 (COVID-19) attending Udine Hospital (Italy) during the first wave (from March to May 2020). A total of 546 individuals were included (289 female, mean age 53.1 years), mostly with mild COVID-19 (370, 68.3%). Patients were followed for a median of 302 days (interquartile range, 186 to 311). The overall seroconversion rate within 2 months was 32% for IgM and 90% for IgG. Seroreversion was observed in 90% of patients for IgM at 4 months and in 47% for IgG at 10 months. Older age, number of symptoms at acute onset, and severity of acute COVID-19 were all independent predictors of long-term immunity both for IgM ($\beta$, linear regression coefficient, 1.10, $P = 0.001$; $\beta$ 5.15 $P = 0.014$; $\beta$ 43.84 $P = 0.021$, respectively) and for IgG ($\beta$ 1.43 $P < 0.001$; $\beta$ 10.46 $P < 0.001$; $\beta$ 46.79 $P < 0.001$, respectively), whereas the initial IgG peak was associated only with IgG duration ($\beta$ 1.12, $P < 0.001$). IgM antibodies disappeared at 4 months, and IgG antibodies declined in about half of patients 10 months after acute COVID-19. These effects varied depending on the intensity of the initial antibody response, age, and burden of acute COVID-19.

**KEYWORDS** SARS-CoV-2 IgG, SARS-CoV-2 IgM, SARS-CoV-2 antibodies, SARS-CoV-2 serology, longitudinal study

Emerging data on the serological response following infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) suggest that most individuals have detectable antibody levels at late stages of infection (3 to 4 weeks after acute onset) (1–3). However, the seroconversion rate and antibody duration for SARS-CoV-2 vary significantly across studies, depending on the serological tests used, the disease stage, and the study design. The available literature provides evidence mainly on hospitalized patients

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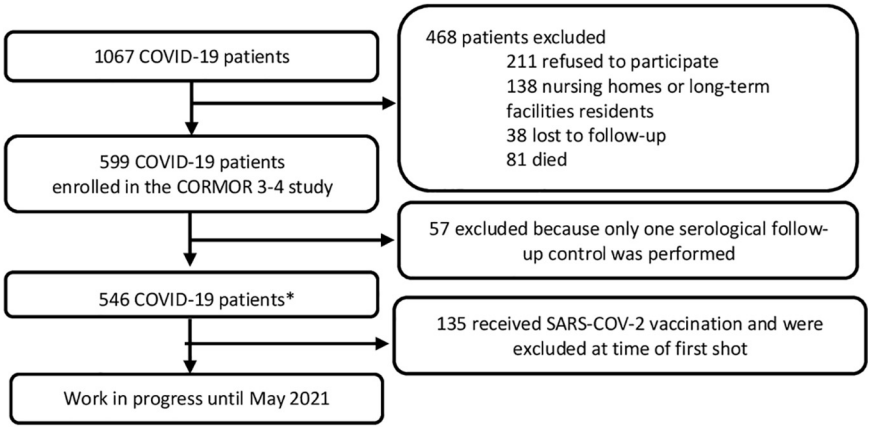


**FIG 1** Serological follow-up (up to February 2021). Flow diagram of in- and outpatients with COVID-19 included.

or subgroups of interest, such as health care workers (HCWs), with little attention given to asymptomatic patients (4–8). In addition, most studies have a limited follow-up at post-symptom onset. As such, knowledge of the factors associated with long-term longevity and magnitude of humoral immunity remain largely unknown (2, 9–13).

The aims of this prospective study were (i) to perform a longitudinal assessment over a 10-month follow-up period of anti-SARS-CoV-2 antibodies in a wide spectrum of individuals ranging from asymptomatic to severely infected who recovered from COVID-19 after the first wave and (ii) to comprehensively characterize predictors of the serological response against SARS-CoV-2.

## MATERIALS AND METHODS

**Study setting and population.** We performed this study at Udine Hospital (Italy), a 1,000-bed tertiary-care teaching hospital identified as a regional referral center for COVID-19 patients and serving approximately 350,000 citizens. The methods and findings are reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (14).

The target population was a cohort of all consecutive adult in- and outpatients (≥18 years) attending the Infectious Disease Department with a diagnosis of COVID-19 from 1 March (the day of the first COVID-19 diagnosis at our hospital) to 30 May 2020. Patients willing to participate in the study and who completed at least two serological follow-up visits up to 28 February 2021 were enrolled. The participant flow diagram over time is reported in Fig. 1. Given that in Italy the SARS-CoV-2 vaccination campaign started on 27 December 2020, those patients who received the vaccination and/or had a SARS-CoV-2 reinfection diagnosis were followed in a separate cohort.

**Acute COVID-19 and baseline definitions.** Diagnosis of COVID-19 infection was established as confirmed or suspected. Patients with a positive nucleic acid amplification test (NAAT) such as real-time PCR (RT-PCR) for SARS-CoV-2 in respiratory tract specimens were considered confirmed cases, whereas those with a negative SARS-CoV-2 NAAT but suggestive laboratory or imaging findings and/or positive serology were considered suspected cases (15).

Patients were classified using the COVID-19 disease severity scale as follows: asymptomatic, mild disease (without pneumonia), moderate disease (with pneumonia), severe disease (with severe pneumonia), critical disease, including acute respiratory distress syndrome (ARDS), sepsis and/or septic shock (16). Specifically, for the analysis, patients were classified into three groups: (i) asymptomatic, (ii) mild, and (iii) moderate to critical disease.

Both symptomatic and asymptomatic patients were also categorized according to their place of treatment—the intensive care unit (ICU) group, the hospital ward group (admitted to the infectious disease, emergency, or pulmonology units), and the outpatient group (e.g., asymptomatic identified with contact tracing for close contacts of patients with diagnosis of COVID-19).

Viral RNA shedding was defined as the interval from the first to last positive NAAT for SARS-COV-2.

**Data collection and measures.** For each included patient, a database was populated that included demographic data, comorbidities, chronic therapy, clinical and laboratory data, and treatments recorded in the acute phase. Data were then populated prospectively by accessing hospital and microbiology databases by using a standardized protocol for the data collection.

**Serological response definitions.** Seroconversion was defined as the development of at least one positive serological sample, and seronegativity, as the absence of any positive serological samples within 2 months of infection onset. Seroreversion was defined as a decline in antibody levels below the

positivity threshold after initial seroconversion. IgM max and IgG max were defined as the highest IgM and IgG values, respectively, reached by each patient within 2 months of the infection onset. The changes in seropositivity and the magnitude of antibody titers were described in the overall study cohort, stratified by the presence or absence of symptoms, preexisting conditions, sex, age, existing phenotypes, ethnicity, work, acute disease onset, severity of acute disease, and RNA viral shedding.

**Laboratory methods.** RT-PCR was performed following the recommendations of the World Health Organization for COVID-19 clinical management and outbreak control purposes (16).

The identification of cases with the COVID-19 virus was based on the detection of unique sequences of virus RNA by NAAT such as RT-PCR with confirmation by nucleic acid sequencing on respiratory samples. The following genes were investigated: E gene for screening and then RdRp and N genes of SARS-CoV-2 for confirmation. The viral RNA was extracted using automated RNA extraction with the ELITe InGenius SP200 system (ELITechGroup), and real-time quantitative PCR (RT-qPCR) was performed using a LightMix Modular SARS and Wuhan CoV E-gene kit on a LightCycler 480 II instrument (Roche). The specimens were considered positive if the cycle threshold ($C_T$) value for at least one of the three genes was $\leq$36.

Anti-SARS-CoV-2 serologies were obtained from venous blood samples. SARS-CoV-2 antibodies (immunoglobulin G [IgG] and M [IgM] antibodies) were assessed using iFlash-SARS-CoV-2 (Shenzhen Yhlo Biotech Co. Ltd., China, distributed in Italy by Pantec SRL), a paramagnetic particle chemiluminescence immunoassay (CLIA) against SARS-CoV-2 N and S protein. The Flash-SARS-CoV-2 test was chosen after an internal validation test and according to the available literature (17). According to the manufacturer's instructions (V1.0 English Fd. 2020-02-20), the IgM and IgG cutoffs were considered to be 10.0 kAU/liter. The test performance has been documented to have a sensitivity and specificity of 86.1% and 99.2%, respectively, for IgM and 93.7% and 96.3%, respectively, for IgG (17).

**Ethical issues.** This study was approved by the Ethics Committee of the Friuli Venezia Giulia Region (CORMOR 3-4 protocol; CEUR-2020-OS-219 and CEUR-2020-OS-205). All procedures were carried out in accordance with the ethical standards of the University of Udine and the Azienda Sanitaria Universitaria Friuli Centrale and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all subjects before collection of the data and performance of the serological tests.

**Statistical analysis.** Patient demographic and clinical characteristics were presented with absolute values and percentages for categorical variables and means or medians (standard deviation [SD] or interquartile ranges [IQRs]) for continuous variables. The Shapiro-Wilk test was used to assess whether data were normally or nonnormally distributed. Patients were divided into two groups (symptomatic and asymptomatic). Categorical variables were compared using the $\chi^2$ test or Fisher's exact test, while quantitative variables were compared using the *t* test or Mann-Whitney U test, as appropriate. Univariate and multivariate linear regression were performed to estimate the association between the antibody persistence of IgG (and IgM) and clinical/demographic variables, by calculating the $\beta$ (linear regression coefficient) and 95% confidence intervals (CIs). The clinical variables considered were the severity of acute COVID-19 (16), the presence of symptoms, the number of acute symptoms of COVID-19, the ICU admission, the days of viral shedding, and the maximum IgG and IgM values within 2 months. The demographic variables were gender, age, body max index (BMI), comorbidities, smoking and alcohol habits, HCW status, and chronic medication. The multivariate analyses included all variables significant at $P \leq 0.10$ in the univariate analysis, taking into account potential collinearities. Quadratic linear regression of IgG and IgM was performed to fit a curve estimating the distribution of antibodies over time. Additionally, 134 subjects in the study provided longitudinal blood samples over the entire follow-up period, allowing for longitudinal assessment of IgG and IgM values. Linear mixed models were used to analyze the distribution of IgG and IgM over time, adjusting for clinical/demographic variables. A sensitivity analysis was performed on this subgroup of patients (supplemental material). Statistical analyses were performed using STATA 16.1.

## RESULTS

**Study population at onset of acute COVID 19.** Overall, during the study period, 1,067 COVID-19 patients were diagnosed at our hospital (Fig. 1), and a total of 546 were eligible for the study and completed at least two serological follow-up control visits. Patients' baseline characteristics, clinical presentation, and viral shedding according to symptomatic/asymptomatic status at acute COVID-19 onset are summarized in Table 1.

**Serological features and dynamics of SARS-CoV-2 IgM and IgG according to disease severity.** In all, 3,041 blood samples were collected from the 546 patients and tested for antibodies against SARS-CoV-2. Patients were followed for a median of 302 days (IQR, 186 to 311). A median of 6 samples were tested (IQR, 4 to 7; range, 2 to 10).

The frequency and timing of IgM/IgG seroreversion, robustness of IgM/IgG max, and median IgM/IgG titers from symptom onset to follow-up, classified according to asymptomatic and symptomatic status with different grades of disease severity, were significantly different and are presented in Fig. 2 and Tables 2 and 3.

Overall, the seroconversion rates for IgM within 2 months was 32%, and only 5 of

**TABLE 1** Patients' baseline characteristics, clinical presentation, and viral shedding according to symptomatic/asymptomatic status at acute COVID-19 onset[a]

| Characteristic | Total (*N* = 546) | Symptomatic (*n* = 502) | | Asymptomatic (*n* = 44) | *P* value |
|---|---|---|---|---|---|
| | | Moderate, critical and severe (*N* = 128)[b] | Mild (*N* = 374)[b] | | |
| Gender, [*n* (%)] | | | | | 0.002 |
| Female | 292 (53.5) | 51 (39.8) | 158 (42.2) | 25 (56.8) | |
| Male | 254 (46.5) | 77 (60.2) | 216 (57.7) | 19 (43.2) | |
| Age, median [yrs (IQR)] | 54 (42–64) | 60.5 (51.5–71.5) | 52 (39–62) | 52 (40–64) | <0.001 |
| BMI, median (IQR) | 25.2 (22.7–28.3) | 26.8 (24.3–29.0) | 24.6 (22.2–27.7) | 25.9 (22.3–28.7) | <0.001 |
| Ethnicity [*n*/*N* (%)] | | | | | 0.503 |
| Native Italian | 480/521 (92.1) | 109/123 (88.6) | 334/359 (93.0) | 37/39 (94.9) | |
| European | 38/521 (7.3) | 13/123 (10.6) | 23/359 (6.4) | 2/39 (5.1) | |
| Non-European | 3/521 (0.6) | 1/123 (0.8) | 2/359 (0.6) | 0/39 (0) | |
| Smoking habit [*n*/*N* (%)] | | | | | 0.012 |
| Smoker | 78/544 (14.3) | 7 (5.5) | 65/373 (17.4) | 6/43 (13.9) | |
| Nonsmoker | 356/544 (65.4) | 88 (68.7) | 241/373 (64.6) | 27/43 (62.8) | |
| Ex-smoker | 110/544 (20.2) | 33 (25.8) | 67/373 (18.0) | 10/43 (23.3) | |
| Alcohol habit [*n*/*N* (%)] | | | | | 0.849 |
| Nondrinker | 269/538 (50) | 62/124 (50) | 183/371 (49.3) | 24/43 (55.8) | |
| Drinker | 266/538 (49.4) | 61/124 (49.2) | 186/371 (50.1) | 19/43 (44.2) | |
| Abuser | 3/538 (0.6) | 1/124 (0.8) | 2/371 (0.5) | 0/43 (0) | |
| Occupation [*n*/*N* (%)] | | | | | <0.001 |
| Exposed to public | 141/504 (28.0) | 40/120 (33.4) | 93/347 (26.8) | 8/37 (21.6) | |
| Not exposed to public | 92/504 (18.2) | 19/120 (15.8) | 67/347 (19.3) | 6/37 (16.2) | |
| HCW | 119/504 (23.6) | 12/120 (10.0) | 91/347 (26.2) | 16/37 (43.2) | |
| Retired | 93/504 (18.4) | 37/120 (30.8) | 51/347 (14.7) | 5/37 (13.5) | |
| Other | 59/504 (11.7) | 12/120 (10.0) | 45/347 (13.0) | 2/37 (5.4) | |
| Comorbidities (*n* [%]) | | | | | 0.001 |
| 0 | 259 (47.4) | 46 (35.9) | 197 (52.7) | 16 (36.4) | |
| 1 | 163 (29.8) | 40 (31.3) | 104 (27.8) | 19 (43.2) | |
| 2 | 69 (12.6) | 18 (14.1) | 43 (11.5) | 8 (18.2) | |
| 3 | 35 (6.4) | 15 (11.7) | 19 (5.1) | 1 (2.3) | |
| ≥4 | 20 (3.7) | 9 (7.0) | 11 (2.9) | 0 (0) | |
| Comorbidities [*n*/*N* (%)] | | | | | <0.001 |
| Hypertension | 122/534 (22.8) | 45/126 (35.7) | 65/3604 (17.9) | 12 (27.3) | |
| Obesity[c] | 89/546 (16.3) | 25/128 (19.5) | 56/374 (15.0) | 8/44 (18.2) | 0.424 |
| Diabetes | 31/541 (5.7) | 15 (11.7) | 14/369 (3.8) | 2 (4.5) | 0.006 |
| Chronic respiratory disease[d] | 20/541 (3.7) | 5 (3.9) | 15/369 (4.1) | 0 (0) | 0.563 |
| Cardiovascular disease[e] | 7/541 (1.3) | 4 (3.1) | 3/369 (0.8) | 0 (0) | 0.125 |
| Liver disease | 10/541 (1.8) | 3 (2.3) | 6/369 (1.6) | 1 (2.3) | 0.658 |
| Psychiatric disorders[f] | 6/541 (1.1) | 0 (0) | 5/369 (1.4) | 1 (2.3) | 0.211 |
| Immunosuppression[g] | 8/539 (1.5) | 3/127 (2.4) | 5/368 (1.4) | 0 (0) | 0.487 |
| Under chronic medication [*n*/*N* (%)] | 260/539 (48.2) | 80/127 (63.0) | 155/368 (42.1) | 25 (56.8) | <0.001 |
| Symptoms at onset [*n*/*N* (%)] | | | | | <0.001 |
| 0 | 44/541 (8.1) | 0/125 (0) | 0/372 (0) | 44 (100) | |
| 1 | 110/541 (20.3) | 19/125 (15.2) | 91/372 (24.5) | 0 (0) | |
| 2 | 102/541 (18.8) | 34/125 (27.2) | 68/372 (18.3) | 0 (0) | |
| 3 | 94/541 (17.4) | 17/125 (13.6) | 77/372 (20.7) | 0 (0) | |
| 4 | 84/541 (15.5) | 29/125 (23.2) | 55/372 (14.8) | 0 (0) | |
| ≥5 | 107/541 (19.8) | 26/125 (20.8) | 81/372 (21.8) | 0 (0) | |
| Management [*n*/*N* (%)] | | | | | <0.001 |
| Outpatients | 394/541 (72.8) | 1/125 (0.8) | 350/372 (94.1) | 43 (97.7) | |
| Inpatients | | | | | |
| Ward | 125/541 (23.1) | 102/125 (81.6) | 22/372 (5.9) | 1 (2.3) | |
| ICU | 22/541 (4.1) | 22/125 (17.6) | 0/372 (0) | 0 (0) | |
| Viral shedding (days) | 19 (14–25) | 21 (14–29) | 19 (14–25) | 15 (11–19) | <0.001 |

[a]Data are *n* (%), *n*/*N* (%), median (IQR). Abbreviations: BMI, body mass index; HCWs, health care workers; ICU, intensive care unit; IQR, interquartile range.
[b]Disease severity scale (16).
[c]Obesity was defined as a body mass index (BMI) >30 kg/m$^2$.
[d]Pulmonary disease: asthma, chronic obstructive pulmonary disease.
[e]Cardiovascular disease: heart failure, ischemic heart disease, tachyarrhythmia, valvular heart disease, venous thromboembolism.
[f]Depression, anxiety.
[g]Immunosuppressed patients were defined as those receiving corticosteroid treatment at a dose of ≥20 mg prednisone or the equivalent for ≥4 weeks, with neutropenia (absolute neutrophil count, <500/mm$^3$), or with anticancer chemotherapy and/or biologics in the previous 6 months before the COVID-19 onset. Overweight was defined as a body mass index (BMI) of >25 kg/m$^2$, and obesity as a BMI of >30 kg/m$^2$.

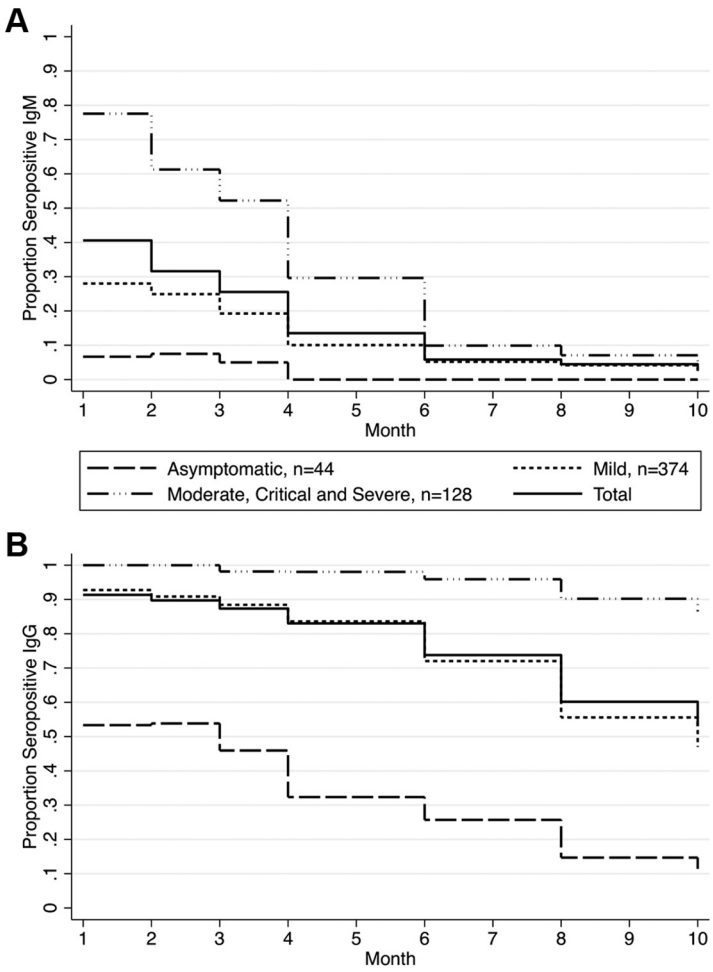


**FIG 2** (A and B)Longitudinal assessment of anti-SARS-CoV-2 IgM (A) and IgG (B) in patients who recovered from COVID-19, according to the grade of severity of acute disease.

44 (11.4%) asymptomatic patients developed a virus-specific IgM antibody response. The IgM max value was recorded at a median of 56 days (IQR, 33 to 63), and IgM was generally not detected beyond 4 months after acute onset (90th percentile equal to 135 days) (Fig. 2 and Tables 2 and 3).

Overall, the seroconversion rates for IgG within 2 months were 90%. The IgG max value was recorded a median of 55 days (IQR, 35 to 62) after the onset of symptoms, and overall rates of IgG seroreversion at 10 months were around 47% (Fig. 2 and Tables 2 and 3).

**TABLE 2** Serological evolution according to symptomatic/asymptomatic status at acute COVID-19 onset[a]

| Parameter | Total (*n* = 546) | Data for symptomatic patients (*n* = 502) | | Data for asymptomatic patients (*n* = 44) | *P* |
|---|---|---|---|---|---|
| | | Moderate, critical and severe[d] (*n* = 128) | Mild[d] (*n* = 374) | | |
| IgM seroconversion[b,c] | 171/516 (33.1) | 77/120 (64.2) | 90/355 (25.3) | 4/41 (9.8) | 0.001 |
| IgG seroconversion[b,c] | 465/516 (90.1) | 119/120 (99.2) | 323/355 (91.0) | 23/41 (56.1) | <0.001 |
| IgM max[b,c] | 6 (2–24) | 30.5 (12–87) | 4 (1−14) | 1 (1–3) | <0.001 |
| IgG max[b,c] | 77 (48–98) | 90.5 (69–115) | 75 (44-95) | 18 (1–74) | <0.001 |
| Persistence of IgG[b], days | 245 (123–308) | 305 (224–313) | 208 (122-306) | 75 (0–180) | <0.001 |
| Persistence of IgM[b], days | 34 (0–93) | 83 (32–120) | 0 (0-92) | 0 (0–0) | <0.001 |

[a]Data are *n* (%), *n*/*N* (%), median interquartile range (IQR).
[b]Measured in kAU/liter.
[c]Within 2 months from symptoms onset.
[d]Disease severity scale (16).

**TABLE 3** Median IgM and IgG titers according to symptomatic and asymptomatic status at acute COVID-19 onset[a]

| Months from onset | No. of observations | Data for symptomatic patients | | Data for asymptomatic patients | *P* value[b] |
|---|---|---|---|---|---|
| | | Moderate, critical and severe | Mild | | |
| IgG[c] | | | | | |
| 1 | 338 | 74.5 (61–103) | 70 (52–95) | 14.5 (0–74) | <0.001 |
| 2 | 488 | 80 (63–95) | 66 (34–85) | 16 (2–65) | <0.001 |
| 3 | 465 | 83 (69–93) | 57 (22–84) | 10.5 (2–47) | <0.001 |
| 4 | 422 | 78 (58–95) | 46 (18–79.2) | 8.5 (3–29.8) | <0.001 |
| 6 | 365 | 61 (35–86) | 26 (12–57) | 13.5 (4.5–37) | <0.001 |
| 8 | 333 | 42 (21–67) | 17.5 (7–40.3) | 8.7 (3–11) | <0.001 |
| 10 | 288 | 32.9 (19.3–54.4) | 14.8 (6.5–33.7) | 7.2 (3.1–17.6) | <0.001 |
| IgM[c] | | | | | |
| 1 | 338 | 37.9 (11–97) | 3.5 (1–12) | 1 (1–3) | <0.001 |
| 2 | 487 | 18 (4–43.1) | 3 (1–11) | 1 (0.8–3) | <0.001 |
| 3 | 464 | 12 (5–22) | 3 (1–8) | 1 (0.4–2) | <0.001 |
| 4 | 419 | 6 (2–12) | 2 (1–5) | 1 (0–1) | <0.001 |
| 6 | 365 | 3.6 (1–6.5) | 1 (1–3) | 1 (0.6–2) | <0.001 |
| 8 | 334 | 2 (1–4) | 1 (1–2.2) | 1 (0.4–1.5) | 0.028 |
| 10 | 288 | 1 (0.5–2.2) | 0.7 (0.4–1.4) | 0.5 (0.3–1.6) | 0.371 |

[a]Data are median interquartile range (IQR).
[b]Measured in kAU/liter.
[c]Bonferroni correction was applied.

**Risk factors associated with longevity of SARS-CoV-2 IgM and IgG serological response.** Results of risk factors associated with the duration of SARS-CoV-2 IgM and IgG serological response at univariate analysis are listed in Tables 4 and 5. In the multivariate linear regression analysis, older age, number of symptoms at acute onset, and severity of acute COVID-19, were all independent predictors of long-term immunity both for IgM ($\beta$ 1.10 $P = 0.001$; $\beta$ 5.15 $P = 0.014$; $\beta$ 43.84 $P = 0.021$, respectively) and for IgG ($\beta$ 1.43 $P < 0.001$; $\beta$ 10.46 $P < 0.001$; $\beta$ 46.79 $P < 0.001$, respectively). The robustness of the initial IgM max titer was also independently associated with both IgM ($\beta$ 0.16 $P < 0.001$) and IgG response longevity ($\beta$ 0.15 $P < 0.001$), and the initial IgG max was significantly conditioned with IgG duration ($\beta$ 1.12, $P < 0.001$) (Tables 4 and 5). The adjusted-$R$ squared results were 0.39 and 0.17 for the multivariate linear regression of IgG and IgM, respectively.

**Sensitivity subanalysis.** A sensitivity subanalysis was performed in 134 patients that provided all longitudinal serial measurements of IgM/IgG levels, and it confirmed the analyzed data on the general population (see Fig. S1 and Tables S1 to S4 in the supplemental material).

## DISCUSSION

In this prospective longitudinal study on a wide spectrum of patients who recovered from COVID-19 after the first wave and whose clinical status ranged from asymptomatic to severely infected, we examined the long-term evolution of seroconversion for both IgG and IgM following SARS-CoV-2 infection. We found that SARS-CoV-2 IgM disappeared, while IgG antibodies declined in half of COVID-19 survivors within 10 months after acute COVID-19. These effects were faster in younger adults and in patients with low acute disease severity and with a weak initial serological response. The strengths of the present study lie in the (i) size of the population analyzed ($n = 546$) regardless of the manifestation of the diseases at the onset (asymptomatic versus symptomatic), thus providing more generalizability data beyond specific populations, (ii) duration of the follow-up of 10 months, substantially longer than in previous reports, and (iii) longitudinal prospective evaluation of humoral immunity after the first wave (2, 5–9, 11, 18).

Once infected with SARS-CoV-2, most patients develop virus-specific antibodies, followed by a decline in antibody responses in the late convalescent period (3 to 9 months postinfection) (4, 19, 20). Moreover, our data show higher seroconversion

**TABLE 4** Univariable and multivariable analysis of risk factors associated with persistence of SARS-CoV-2 IgM antibody[a]

| Risk factors | $\beta$ | 95% CI | *P* value | $\beta$ | 95% CI | *P* value |
|---|---|---|---|---|---|---|
| Gender | −6.59 | (−21.71, 8.53) | 0.392 | | | |
| Age | 1.41 | (0.91, 1.90) | <0.001 | 1.09 | (0.48, 1.70) | 0.001 |
| BMI | 0.38 | (−1.22, 1.99) | 0.640 | | | |
| Smoking habit | | | | | | |
| Smoker versus nonsmoker | −7.91 | (−31.85, 16.03) | 0.516 | | | |
| Ex-smoker versus nonsmoker | 9.29 | (−9.54, 28.13) | 0.333 | | | |
| Ex-smoker versus smoker | 17.20 | (−10.33, 44.74) | 0.220 | | | |
| Alcohol habit | | | | | | |
| Drinker versus nondrinker | −5.84 | (−21.16, 9.48) | 0.454 | | | |
| Abuser versus nondrinker | −56.89 | (−165.04, 51.26) | 0.302 | | | |
| Abuser versus drinker | −51.05 | (−159.22, 51.13) | 0.354 | | | |
| HCW and in contact with public | −16.41 | (−32.34, −0.49) | 0.043 | −2.24 | (−18.33, 13.86) | 0.785 |
| No. of comorbidities | 8.12 | (1.34, 14.89) | 0.019 | −2.04 | (−10.68, 6.59) | 0.642 |
| Under chronic medication | 21.18 | (6.11, 36.26) | 0.006 | 5.66 | (−13.65, 24.97) | 0.564 |
| Severity of acute COVID-19[b] | | | | | | |
| Mild versus asymptomatic | 43.45 | (16.24, 70.66) | 0.002 | 27.97 | (−5.56, 61.50) | 0.102 |
| Moderate, critical and severe versus asymptomatic | 81.17 | (52.23, 110.21) | <0.001 | 44.33 | (7.06, 81.60) | 0.020 |
| Moderate, critical and severe versus mild | 37.72 | (21.51, 53.93) | <0.001 | 16.36 | (−2.31, 35.03) | 0.086 |
| Symptomatic | 55.35 | (27.97, 82.73) | <0.001 | | | |
| No. of symptoms at onset | 6.50 | (2.67, 10.33) | 0.001 | 4.90 | (0.64, 9.17) | 0.024 |
| Management | | | | | | |
| Ward versus outpatients | 39.42 | (22.66, 56.17) | <0.001 | | | |
| ICU versus outpatients | 35.31 | (2.50, 68.11) | 0.035 | | | |
| ICU versus ward | −4.11 | (−38.65, 30.43) | 0.815 | | | |
| Viral shedding, days | 0.65 | (−0.19, 1.49) | 0.127 | | | |
| Viral shedding >14 days | 12.70 | (−5.27, 30.66) | 0.166 | | | |
| IgG max within 2 months[c] | 0.29 | (0.09, 0.49) | 0.005 | 0.05 | (−0.17, 0.27) | 0.647 |
| IgM max within 2 months[c] | 0.22 | (0.15, 0.29) | <0.001 | 0.16 | (0.08, 0.24) | <0.001 |

[a]BMI, body mass index; CI, confidence interval; HCW, health care worker; ICU, intensive care unit; $\beta$, linear regression coefficient.
[b]Disease severity scale (16).
[c]Measured in kAU/liter.

rates for IgG (90%) than IgM (32%) and the expected early IgM seroreversion after the onset of illness, in contrast to the persistence of IgG antibodies, but also reveal IgG loss in around 50% of COVID-19 survivors 10 months after their recovery (5, 21). Despite similarities between SARS-CoV-2 and previous severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) outbreaks, which showed a long duration of immunity detectable up to 2 to 3 years, our findings agree more with observations of antibody persistence after infection with common seasonal human coronaviruses (HCoVs), which decline between 6 and 12 months after infection (1, 5, 8, 9, 22). These findings might suggest that (i) the presence of IgM antibodies to SARS-CoV-2 should be interpreted carefully on the basis of the specific clinical and epidemiological scenario, (ii) the seroprevalence surveys may underestimate the true nature and the extent of the population's exposure to SARS-CoV-2, and (iii) stringent thresholds applied in SARS-CoV-2 IgG assays may lead to underdetection of asymptomatic or mild infections, but if assay thresholds are lowered to improve sensitivity, this might also result in reduced specificity (5, 10, 21).

Furthermore, our longitudinal study showed that the magnitude and duration of the antibody response against SARS-CoV-2 is heterogeneous and varies widely between individuals (5, 7, 9, 13, 23). The correlation of humoral immunity with older age is likely to have significant implications for protective immunity in a vulnerable population (1, 5, 13). On the one hand, aging leads to immunosenescence, with a reduced capacity to fight novel SARS-CoV-2 infections and mount adequate responses

**TABLE 5** Univariable and multivariable analysis of risk factors associated with persistence of SARS-CoV-2 IgG antibody[a]

| Risk factors | $\beta$ | 95% CI | *P* value | $\beta$ | 95% CI | *P* value |
|---|---|---|---|---|---|---|
| Gender | −9.40 | (−27.59, 8.79) | 0.310 | | | |
| Age | 1.96 | (1.39, 2.52) | <0.001 | 1.46 | (0.84, 2.08) | <0.001 |
| BMI | 1.42 | (−0.41, 3.25) | 0.128 | | | |
| Smoking habit | | | | | | |
| Smoker versus nonsmoker | −31.00 | (−58.41, −3.59) | 0.027 | −1.83 | (−25.21, 21.55) | 0.921 |
| Ex-smoker versus nonsmoker | 9.90 | (−13.06, 32.86) | 0.397 | −0.27 | (−19.35, 18.81) | 0.978 |
| Ex-smoker versus smoker | 40.91 | (8.74, 73.07) | 0.013 | 1.56 | (−26.06, 29.18) | 0.912 |
| Alcohol habit | | | | | | |
| Drinker versus nondrinker | −8.30 | (−26.61, 10.01) | 0.374 | | | |
| Abuser versus nondrinker | 85.48 | (−34.34, 205.30) | 0.162 | | | |
| Abuser versus drinker | 93.77 | (−26.07, 213.62) | 0.125 | | | |
| HCW and in contact with public | −14.50 | (−33.59, 4.58) | 0.136 | | | |
| No. of comorbidities | 9.14 | (0.77, 17.50) | 0.032 | −5.94 | (−15.12, 3.23) | 0.204 |
| Under chronic medication | 19.12 | (0.86, 37.37) | 0.040 | −6.78 | (−26.81, 13.26) | 0.507 |
| Severity of acute COVID-19[b] | | | | | | |
| Mild versus asymptomatic | 100.64 | (68.21, 133.07) | <0.001 | 26.00 | (−8.67, 60.68) | 0.141 |
| Moderate, critical and severe versus asymptomatic | 159.01 | (123.79, 194.24) | <0.001 | 43.93 | (5.18, 82.68) | 0.026 |
| Moderate, critical and severe versus mild | 58.37 | (38.43, 78.32) | <0.001 | 17.92 | (−2.05, 37.90) | 0.079 |
| Symptomatic | 115.96 | (82.98, 148.95) | <0.001 | | | |
| No. of symptoms at onset | 16.78 | (12.19, 21.37) | <0.001 | 10.94 | (6.57, 15.31) | <0.001 |
| Management | | | | | | |
| Ward versus outpatients | 61.08 | (40.21, 81.95) | <0.001 | | | |
| ICU versus outpatients | 74.20 | (30.33, 118.07) | 0.001 | | | |
| ICU versus ward | 13.12 | (−33.18, 59.42) | 0.578 | | | |
| Viral shedding, days | 1.86 | (0.83, 2.89) | <0.001 | 0.37 | (−0.49, 1.24) | 0.397 |
| Viral shedding >14 days | 29.98 | (9.02, 50.93) | 0.005 | | | |
| IgG max within 2 mos[c] | 1.50 | (1.29, 1.71) | <0.001 | 1.14 | (0.92, 1.36) | <0.001 |
| IgM max within 2 mos[c] | 0.24 | (0.15, 0.34) | <0.001 | 0.15 | (0.07, 0.23) | <0.001 |

[a] $\beta$, linear regression coefficient; BMI, body mass index; CI, confidence interval; HCW, health care worker; ICU, intensive care unit.
[b] Disease severity scale (16).
[c] Measured in kAU/liters.

to vaccines, while on the other, it contributes to the development of a chronic state of inflammation called "inflammaging," which leads to poorer outcomes, with acute disease and a robust and prolonged humoral response (24, 25). Therefore, our data seem to add interesting insights into how aging influences the immune system at a time when vaccination planning and responses in frail elderly patients are key topics (25–27). In contrast to previous studies, we did not find any relationship with gender, preexisting comorbidities, or other phenotypes (5, 7, 9).

Our findings on differences in duration of immune response according to disease severity at acute onset have been found in SARS, MERS, and more recently, in COVID-19 (5, 7, 9, 13, 23). Of interest, we found a novel association of increasing humoral immunity in relation to the number of symptoms at acute COVID-19 onset. Most studies of antibody kinetics have been carried out among symptomatic patients, while data regarding asymptomatic SARS-CoV-2 patients over time are still scarce (3, 21, 28–30). Our study highlights that the serological response and kinetics are significantly different between symptomatic and asymptomatic patients even 10 months after symptom onset, in a context of very prolonged follow-up and extensive assessment of asymptomatic patients ($n$ = 55) compared to available studies in this field (3, 30). In keeping with published data, asymptomatic individuals maintained low levels of IgG with rapid seroreversion. Interestingly, in contrast to a recent study (30), most of our asymptomatic patients (88.6%) did not develop an IgM humoral response within 2 months. The absence of IgM might depend on the time of test performance after the disease onset

and on the type of serological assay. The humoral response of asymptomatic patients underlines the limitations of serosurvey studies and provides interesting insights, since it may reflect cross-protection against other seasonal HCoVs (22), or it could be the result of the complex balance between the individual immune state and the inflammatory response against SARS-CoV-2, manifesting itself in an asymptomatic infection (3, 21, 28, 30).

The robustness of the initial humoral response was associated with IgM and IgG antibody duration. These results are in line with previous studies (13, 23), allowing a prediction of long-term antibody duration up to 10 months after acute COVID-19 on the basis of the intensity of the initial antibody response.

Our study has several limitations. First, it was performed at a single center, and a number of patients were lost to follow-up. As this may have introduced a selection bias, we performed a sensitivity analysis that assessed the robustness of the study. Second, test accuracy, levels of detection, differences in the half-lives of antibodies, and competition between IgM and IgG may be assay-dependent, and emergence of SARS-CoV-2 variants of concern may condition different humoral response (5, 17, 29). The interassay validation and the standardization of serological assays are essential to improving our understanding of antibody kinetics and longevity. Lastly, neutralizing antibody and tests to assess B cell- and T cell-mediated adaptive immunity were not performed; the complexity of this test prevents routine testing on a large scale, and the utility of assessing long-term immunity against SARS-CoV-2 remains undetermined (23, 29).

**Conclusions.** Our longitudinal study showed that SARS-CoV-2 IgM disappeared within 4 months, while IgG antibodies declined in half of patients within 10 months after acute COVID-19. The magnitude and duration of humoral immunity against SARS-CoV-2 is heterogeneous and varies widely between individuals, depending on age, burden of disease at acute onset, and intensity of the initial antibody response. Further large-scale prospective longitudinal studies are needed to determine the longevity of humoral immunity against SARS-CoV-2 as a surrogate of individual protection against reinfection after natural infection and vaccination.

## SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, PDF file, 0.4 MB.

**SUPPLEMENTAL FILE 2**, PDF file, 0.2 MB.

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