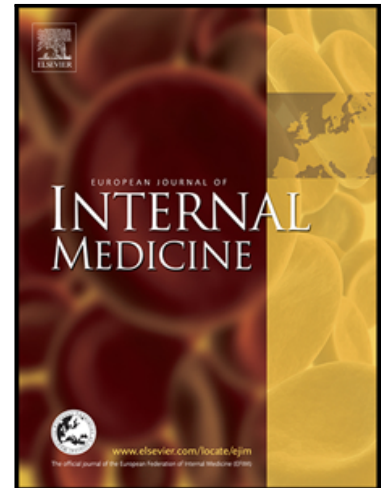


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kinetics of anti-SARS-CoV-2 antibodies over time. Results of 10 month follow up in over 300 seropositive Health Care Workers

Jose Felipe Varona MD , Rodrigo Madurga PhD ,
Francisco Peñalver MD , Elena Abarca MD , Cristina Almirall MD ,
Marta Cruz MD , Enrique Ramos , Jose María Castellano MD

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1 Title page

2 **kinetics of anti-SARS-CoV-2 antibodies over time.**

3 **Results of 10 month follow up in over 300 seropositive Health Care Workers.**

4 Jose Felipe Varona, MD,^{1,2} Rodrigo Madurga, PhD^{3,4} Francisco Peñalver, MD,⁵ Elena Abarca, MD,⁶
5 Cristina Almirall MD,⁶ Marta Cruz MD,⁶ Enrique Ramos MD,⁶ Jose María Castellano, MD^{2,3,7,8}

6

7 ¹ Departamento de Medicina Interna, Hospital Universitario HM Montepíncipe, HM Hospitales,
8 Madrid, Spain.

9 ² Facultad de Medicina, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain.

10 ³ Fundación de Investigación, HM Hospitales, Madrid, Spain.

11 ⁴ Facultad de Ciencias Experimentales, Universidad Francisco de Vitoria, Madrid, Spain.

12 ⁵ Departamento de Seguridad, Salud y Bienestar de HM Hospitales, Madrid, Spain.

13 ⁶ Servicio de Laboratorio, HM Hospitales, Madrid, Spain.

14 ⁷ Departamento de Cardiología, Centro Integral de Enfermedades Cardiovasculares (CIEC), Hospital
15 Universitario HM Montepíncipe, Madrid, Spain.

16 ⁸ Centro Nacional de Investigaciones Cardiovasculares (CNIC), Instituto de Salud Carlos III, Madrid,
17 Spain.

18

19 **Corresponding Author:**

20 Jose Felipe Varona Arche, MD, PhD

21 Hospital Universitario HM Montepíncipe, Boadilla del Monte, Madrid, Spain.

22 Email: jfvarona@hmhospitales.com

23 Address: Avd Montepíncipe 25, Boadilla del Monte, Madrid 28660

24 Telephone number: +0034 699511091

25

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1 **Highlights**

- 2 - More than 75% HCW maintained seropositivity against SARS-CoV-2 after 9 months
- 3 - Two distinct antibody dynamic profiles were observed: declining vs. stable
- 4 - Factors associated with stable profile were age, higher baseline-titers and high-exposure
- 5 to COVID-19.
- 6 - Estimated mean time to loss antibodies was 375 days from baseline.

7 **Abstract**

8 **Background:** The kinetics of the antibodies against severe acute respiratory syndrome coronavirus
9 2 (SARS-CoV-2) needs to be evaluated since long-term duration of antibody remains largely
10 unknown, particularly in infected healthcare workers (HCW).

11 **Methods:** Prospective study, evaluating the longitudinal profile of anti-SARS-CoV-2 antibody titers
12 in a random sample of 331 seropositive healthcare workers (HCW) of Spanish Hospitals Group.
13 Serial measurements of serum IgG-anti-SARS-CoV-2 were obtained at baseline (April-May,2020),
14 and in 2 follow-up visits. Linear mixed models were used to investigate antibody kinetics and
15 associated factors.

16 **Results:** A total of 306 seropositive subjects (median age: 44.7years;69.9 % female) were included
17 in the final analysis. After a median follow-up of 274 days between baseline and final
18 measurement, 235(76.8%) maintained seropositivity. Antibody titers decreased in 82.0%, while
19 remained stable in 13.1%. Factors associated with stability of antibodies over time included
20 age \geq 45 years, higher baseline titers, severe/moderate infection and high-grade exposure to
21 COVID-19 patients. In declining profile, estimated mean antibody half-life was 146.3
22 days(95%CI:138.6-154.9) from baseline. Multivariate models show independent longer durability
23 of antibodies in HCW with high-risk exposure to COVID-19 patients (+14.1 days;95%CI:0.6-40.2)
24 and with symptomatic COVID-19 (+14.1 days;95%CI:0.9-43.0). The estimated mean time to loss
25 antibodies was 375(95% CI:342-408) days from baseline.

1 **Conclusions:** We present the first study measuring the kinetics of antibody response against SARS-
2 CoV-2 in HCW beyond 6 months. Most participants remained seropositive after 9 months but
3 presented a significant decline in antibody-titers. Two distinct antibody dynamic profiles were
4 observed (declining vs. stable). Independent factors associated with longer durability of antibodies
5 were symptomatic infection and higher exposure to COVID-19 patients.

6

7 **Key Words**

8 SARS-CoV-2; COVID-19, Antibodies; antibody kinetics; immune response; Durability; Persistence;
9 Healthcare Workers; Healthcare Personnel

10 **Text**

11 **INTRODUCTION**

12 The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome
13 coronavirus 2 (SARS-CoV-2), has led to an unprecedented health emergency, causing over 125
14 million confirmed cases and over 2.5 million deaths worldwide as of March 2021.¹ Understanding
15 the long-term immunological response in infected individuals will be central in defeating the
16 COVID-19 pandemic, and understanding humoral kinetics, timing, and persistence of SARS-CoV-2
17 antibodies after natural infection, will be essential to ensure an appropriate vaccination strategy.

18 Reports studying the immunological response in previous coronaviruses outbursts have shown
19 that after infection, a specific IgG response is elicited and sustained for 1-2 years declining
20 thereafter,^{2,3} but available data for SARS-CoV-2 is very limited beyond 4 months⁴, especially in
21 health care workers (HCW), a population particularly exposed to SARS-CoV-2 with potential high
22 risk for reinfection. Indeed, the scarce published longitudinal data on seroprevalence of HCW

1 shows a wide variability on the percentage of subjects who remain seropositive after 4 months of
2 infection, ranging from 42 to 96% in predominantly small follow up cohorts.⁵⁻⁸. Furthermore, there
3 is almost no data about asymptomatic infected HCW.

4 To the best of our knowledge, this is the first study to evaluate the durability and to characterize
5 the longitudinal profile of anti-SARS-CoV-2 antibody levels beyond 6 months in a large cohort of
6 seropositive health personnel. In this context, we conducted a 10-month follow-up serological
7 quantitative study to determine the kinetics of the humoral response against SARS-CoV-2 infection
8 in a representative sample of over 300 seropositive HCW of Grupo HM Hospitales (GHM), a chain
9 of 17 Hospitals across 4 regions of Spain. Furthermore, we analyzed different variables to identify
10 clinical and demographic factors associated with stability/declining of antibody levels and
11 seroreversion.

12 The current work expands on a transversal study of seroprevalence of the entire population of
13 workers of Grupo HM-Hospitales, over 6000 HCW, to identify both symptomatic and
14 asymptomatic individuals who presented anti-SARS-CoV-2 antibodies, the results of which have
15 been recently published.⁹

16 **METHODS**

17 **Study design, population, setting and procedures**

18 Prospective study, measuring the evolution of serum IgG anti-SARS-CoV-2 titers among employees
19 of the GHM who presented a positive result for SARS-CoV-2 antibodies after the first COVID-19
20 pandemic wave in Spain. The baseline study was carried out between April-June,2020, including
21 6038 employees (mean age:43.8;71%female) and 662(11.0%) presented IgG anti-SARS-CoV-2 at
22 baseline (39% asymptomatic), the results of which have been published.⁹

1 A sample corresponding to 50% of all seropositive HCW was randomly selected by simple random
2 sampling from the baseline study, and its longitudinal profile of anti-SARS-CoV-2 antibodies was
3 evaluated (n=331). Serial blood measurements were collected at baseline (April13th–May28th
4 ,2020) and in 2 follow-up visits (last measurement: January12th-February9th,2021). Those HCW
5 with a significant increase in the antibody titer during the follow-up were specifically studied to
6 rule out reinfection by evaluating clinical data and performing PCR test.

7 In January 2021, the COVID-19 vaccination of all healthcare personnel was implemented in all
8 Grupo HM-Hospitales workers and, as a result, 185 (55.4%) workers of the study received the first
9 dose before the last serological extraction, with a median (IQR) time between the vaccination (first
10 dose) and the third serological sample of 11 (8-13) days, without no impact in antibody titers
11 waning (slopes between the last two measurements were -0.49[-0.71,-0.21]A.U./month for not
12 vaccinated and -0.34[-0.71,-0.19]A.U./month for vaccinated individuals [Mann-Whitney U test,
13 $p=0.17$]; Figure S1 in the Supplement).

14 **Quantification of antibodies against SARS-CoV-2.**

15 We used the Enzyme Immunoassay (ELISA) developed by DIA.PRO to measure serum anti-
16 nucleocapsid (anti-N) and anti-spike (anti-S) protein IgG antibody titers against SARS-CoV-2 in a
17 venous blood sample for all the measurements of the study. This serum test has a clinical
18 sensitivity estimated at 98%-100% and a specificity >98% (Enzyme immunoassay for the
19 determination of IgG antibodies to COVID-19 in human serum and plasma DIA.PRO IFU). Serum
20 IgG titers were considered negative (non-reactive) with a result less than 0.90 arbitrary units
21 (AU)/mL (<0.90 AU/mL), positive (reactive) with a result greater than 1.10 AU/mL (>1.10AU/mL)
22 and indeterminate with a result in the interval between 0.900 and 1.10(0.90≤x≤1.10AU/mL).

1 The ELISA test used detects IgG antibodies against specific subunits of spike and nucleocapsid
2 proteins with a established proportion in the solid phase. This specificity would explain in part why
3 the antibody titers are not affected in vaccinated subjects as compared with other available kits.

4 **Statistical analysis**

5 Individuals were classified according to their antibody profile over time as: stable (n=40) when the
6 variation in antibody titers were lower than 25%, declining (n=251) when the loss of antibody
7 titers was higher than 25% overtime and others (n=15) for those profiles that show an increase of
8 more than 25% in antibody titer at some time point.

9 Summary statistics were performed as absolute and relative frequencies (%). Chi-squared tests (or
10 Fisher tests when needed) were used to study the dependence between stable and declining
11 profiles and age (categorized by median), sex, symptoms, infection category, grade of exposure to
12 COVID-19, receiving SARS-CoV-2 vaccine or not and baseline antibody titer (categorized by
13 terciles). Those variables with a p value <0.2 were considered for univariable and multivariable
14 logistic regression with the antibody profile (stable or declining) as dependent variable.

15 Linear mixed models were used to investigate antibody waning for those individuals with declining
16 profile. Assuming antibody levels fell exponentially, the natural log transformed titers were
17 modelled over time. Correlated random intercept and slope were allowed. The univariable effect
18 of covariates, including age, sex, grade of exposure to COVID-19, infection category and
19 symptoms, on the fixed effect intercept and slope was analysed. A multivariable model including
20 all the analysed covariates was performed to study independent effects on antibody waning.

21 Analyses were performed using R 4.0.4 and the lme4 library.¹⁰

1 Seroreversion was defined as the loss of antibody titers below the diagnostic threshold of 1.1A.U.
2 The association of the different variables with seroreversion was analysed using Chi-squared test
3 or Fisher test if required.

4 **RESULTS**

5 Three hundred and six HCW were included in the final analysis (25 subjects were excluded due to
6 incomplete data). Serological testing was performance between 13-April 2020 and 9-February
7 2021, with a median (IQR) [range] time from the baseline to last measurement of 274 (265-280)
8 [156-294] days. Median age was 44.7 years (IQR 34.9-53.2; range 21.3 to 80.2 years) and 69.9%
9 were females. Demographic and clinical characteristics are summarized in **table 1**.

10 ***DYNAMIC ANTIBODY PROFILE***

11 We identified two distinct antibodies dynamic profiles according to the quantitative antibody
12 concentration kinetics, which we have categorized as: declining, which included those subjects
13 who presented a significant decrease in antibody titers (defined as a decline > 25% between the
14 first and the last serological testing) (n=251; 82.0%); and stable, for those who presented a
15 variation in antibody titers less than 25% throughout the study period (n= 40; 13.1%). **Figure 1**
16 shows the tendency of IgG antibody levels over time, from baseline visit (April-June 2020) to last
17 follow-up visit (January-February 2021), while **table 1** shows the demographic and clinical
18 characteristics according to antibody dynamic profile.

19 Fifteen subjects (4.9%) showed a significant increase (>25%) in antibody titer (Figure S2 in the
20 Supplement shows their dynamic profile). We believe this increase probably represents either
21 baseline measurement being carried out during the increasing phase for IgG or possible re-

1 infection (these HCW were specifically evaluated to detect reinfection), and these subjects were
2 therefore excluded from the analysis.

3 Factors associated with persistence/declining anti-SARS-CoV-2 IgG antibody levels are shown in
4 **table 2**, highlighting that stable profile was observed more frequently in the third tercile of
5 baseline levels of antibody titers ($p < 0.0001$), age > 45 ($p = 0.0004$), high-grade risk exposure (p
6 $= 0.031$), and moderate/severe infection ($p = 0.003$). Univariate logistic regression models identified
7 age ≥ 45 years (OR:4.1; 95% CI: 1.9 - 9.4; $p = 0.0005$), third tercile of baseline antibody level (OR=
8 19.8; 95% CI: 5.7 - 125; $p < 0.0001$), high-risk exposure (OR:2.9; 95% CI:1.2-7.8; $p = 0.024$) and
9 moderate/severe infection (OR:4.3;95% CI: 1.7-10.3; $p = 0.001$) as variables significantly associated
10 with stable/non-declining antibody titers. The multivariate logistic regression model showed that
11 age, baseline antibody titer and exposure risk are independently and positively associated with the
12 stable antibody profile.

13 ***KINETICS ON DECLINING OF ANTIBODIES.***

14 We considered the antibody titer of the first measurement as the baseline antibody levels. It is
15 worth mentioning that 75 individuals reported a positive PCR with median (IQR) of 28 (22 - 37)
16 days prior to the first measurement.

17 The mean trajectory of IgG antibody levels in each individual with declining profile was modelled
18 using a linear mixed model (**Figure 2A, Table 3, Table S1**). Estimated mean antibody half-life was
19 146.3 (95%CI: 138.6-154.9) days from baseline. The estimated mean baseline antibody titer was
20 6.55 (95% CI: 6.20 - 6.93) arbitrary units (A.U.). Initial antibodies titer were correlated with longer
21 estimated antibody half-lives (**Figure 2B**, Spearman's rank $R^2 = 0.55$, $p < 0.0001$).

1 The effect of demographic and other covariates on the antibody's trajectories was marginal for
2 longer half-lives for age, high-risk exposure and previous COVID-19 symptoms (**Table 3; Table S1**
3 **and Figure S3-4** in the Supplement). Multivariate model showed that high-risk exposure to COVID-
4 19 patients (+14.1 days; 95%CI:0.6-40.2) and previous COVID-19 symptoms (+14.1 days; 95%CI: 0.9
5 -39.8) were independently associated with higher antibodies durability. Age \geq 45 years (+10.5
6 days;95%CI:-1.4-33.2) showed marginal evidence of association with longer half-life of the
7 antibodies.

8 **Table S2** in the Supplement shows the median (IQR) for baseline, last follow-up and change in IgG
9 antibody titer, considering exposure risk to COVID-19 patients, infection category and baseline
10 levels of antibodies.

11 **SEROREVERSION.**

12 The percentage of participants who experienced seroreversion (defined as an antibody titer >1.1
13 AU/mL at baseline and ≤ 1.1 AU/mL at the follow-up visits) was 23.2 % (71/306).

14 Median (IQR) time to seroreversion was 275 (224-280) days, as most seroreversion events were
15 observed at the third visit. Seroreversion percentages, depending on demographics and other
16 covariates are summarized in **Table 4.**

17 In order to have a projection of the time to loss of antibodies, we used a linear mixed baseline
18 model in order to extrapolate the mean time to cross the threshold of 1.1A.U. (mean time to loss
19 antibodies), finding that, on average, subjects will have detectable concentrations of IgG anti
20 SARS-COV-2 up to 375 (95%CI:342-408) days from baseline (**Figure 2A**).

21 **Reinfection**

1 Fifteen subjects did not present a declining or stable longitudinal antibody profile. Evaluating their
2 profile and available clinical data, we found one probable case of PCR-documented reinfection
3 (**Table S3** in the Supplement).

4 **DISCUSSION**

5 The aim of the current study was to investigate the immunological response in antibody levels
6 over time (mean follow up over nine months) in a large and representative cohort of more than
7 300 HCW who tested positive for IgG anti-SARS-CoV-2 antibodies after the first wave of COVID-19
8 outbreak in Spain. To the best of our knowledge, this is the first report evaluating the kinetics of
9 SARS-CoV-2 antibodies in HCW beyond 6 months. These results provide further insight into the
10 nature of post-infection immunity.

11 The results showcase a high percentage of healthcare personnel maintaining detectable antibody
12 levels beyond 9 months after infection (median follow-up 274 days) with more than 75% of
13 subjects remaining seropositive, but with a significant decline in antibody levels in most of them.
14 Only 13.1% of the population presented stable concentrations of antibodies in the study follow up
15 period. A recent work carried out in HCW in Britain estimated a mean antibody half-life of 85 days
16 from the maximum positive IgG titer, after a median follow-up time of 4 months.⁸ The results of
17 our study expand on this observation and demonstrate a significant longer durability of antibodies
18 with a mean half-life of 146 days from the first detectable measurement, after a mean follow-up
19 higher than 9 months.

20 Our results are inconsistent with previous works in HCW which included significantly shorter
21 follow up periods. Some studies reported a rapid loss of SARS-CoV-2 specific antibodies within the
22 first three months after infection, alerting about a short-lasting humoral protection.^{6,7} These
23 differences may reflect, among other issues, differences in baseline characteristics of the study

1 population (symptomatic category of SARS-CoV2 infection, age, baseline antibody titers...) and,
2 more importantly, different sensitivity of the immunoassays used to detect specific antibodies
3 against SARS-CoV-2. One of the strengths of the current work is that the same immunoassay (from
4 the same company, with same badge of production) was used in the complete population
5 throughout the study testing for both serum anti-nucleocapsid (anti-N) and anti-spike (anti-S)
6 protein IgG antibodies.

7 Here, we report a novel finding about the dynamic profile of the humoral response against SARS-
8 CoV-2. While most subjects present a declining profile, a subgroup shows a stable (non-declining)
9 antibody titer profile during at least the first 10 months after infection. Further characterization of
10 this stable profile shows a significant correlation with high baseline antibody levels, but other
11 factors as well. Interestingly, a higher degree of exposure to COVID-19 patients is associated to
12 stability in antibody levels, with stable profile being observed more predominantly in first line
13 HCW. This thought-provoking observation may be signaling a potential mechanism of viral re-
14 exposure in maintaining antibody levels in HCW directly involved with the care of COVID-19
15 patients.

16 Information of antibody titers beyond 4 months after first positivity is also scarce in the general
17 population. Most of the cohorts with up to 5-6 months follow-up have showed high percentages in
18 persistence of seropositivity (80% or higher).¹¹⁻¹⁶ However, the majority of them have also
19 reported a relevant decrease in antibody titers over time, with one of them estimating a 2-fold
20 decrease of IgG antibodies time of 158 days.¹⁵ In our cohort, the model estimated a mean time to
21 loss antibodies of 375 days from baseline. This finding is in the line of studies in previous SARS-CoV
22 and MERS-CoV outbreaks in which specific IgG antibodies were shown to persist for 1-2 years
23 post-infection and decline thereafter.^{2,3}

24 **Factors associated with stability in antibody titers**

1 Higher baseline antibody titers have been associated with an increased probability to sustain
2 detectable antibody levels over time compared to lower baseline antibody levels.⁶⁻⁸ In the current
3 study, baseline antibody levels were positively correlated with half-lives of antibody and HCW who
4 presented baseline antibody levels in the third tercile showed a significant longer half-life of
5 antibodies (Figure 2B). This finding has been associated with the severity of SARS-CoV-2 infection.
6 Previous reports have described that levels of antibodies against SARS-CoV-2 in the acute phase
7 correlate to clinical disease severity and then decline after 3 months since onset of illness.¹⁷⁻¹⁹
8 While a recent study reported that the antibodies are persistent for at least 6 months in severe
9 COVID-19 patients,²⁰ reports in asymptomatic infection and pauci-symptomatic disease have
10 showed a weak and non-sustained humoral immunity response against SARS-CoV-2.^{21,22}
11 Our findings, expanding previous reports, suggest that moderate/severe COVID-19 patients
12 present significantly higher levels of baseline antibodies, but no significant difference in the
13 decreasing rate over time, compared to subjects with mild COVID-19 (half-life of antibodies was
14 significantly longer in symptomatic compared with asymptomatic COVID-19, but not in
15 moderate/severe compared to asymptomatic/ mild infection). In contrast, HCW with high-grade
16 risk exposure to COVID-19 patients showed not only significantly more frequency of stable
17 dynamic antibody response over time, but also longer antibody half-life and lower estimated
18 monthly decrease in antibody titers compared to HCW with low-grade exposure (Table S2). This
19 finding adds validity to the previously mentioned mechanism that viral re-exposure could be
20 playing a role in this more stable dynamic antibody profile in first line HCW.
21 Finally, in our study, age above 45 was also independently associated with stable dynamic
22 antibody profile and longer antibody half-life. This finding is consistent with a previous study
23 reporting that increasing age was associated with longer durability of anti-SARS-CoV-2 antibodies.⁸

1 **Reinfection**

2 Whether the rate of decline in anti-SARS-CoV-2 antibodies is associated with an increased risk of
3 reinfection and disease remains to be determined. This issue has special implications in first line
4 HCW, since their inherent particular risk due to close contact with COVID-19 patients. In the
5 current work we detected a single case of possible reinfection. This is in the line with the findings
6 of Lumley *et al* who reported that the presence of anti-SARS-CoV-2 antibodies is associated with a
7 significant reduction in risk of SARS-CoV-2 reinfection in the ensuing 6 months, with only 2
8 asymptomatic cases of re-infection and no symptomatic infection detected in seropositive
9 subjects.²³

10 **Implications**

11 The results of the current study suggest an important role for serological screening and follow-up
12 and may help in our understanding of the humoral response in HCW exposed to SARS-COV-2.

13 These in turn may have potential implications in the current vaccination strategy. Results from
14 ongoing long-term studies will help better understand the kinetics of the humoral response and
15 their association to clinical manifestations of the disease.

16 **Limitations**

17 The current study has several limitations. One of the main is that we have non-acute information
18 on timing of infection onset to evaluate antibody kinetics. However, our study is based on a real-
19 world HCW cohort with a wide range of symptoms, including 40% of asymptomatic infected HCW,
20 and in this setting does not allow to accurately obtain this information. Another limitation that
21 needs to be pointed out is that the methodology used quantitatively measures antibodies against
22 the spike and nucleocapsid proteins of SARS-CoV-2 (anti-S and anti-N antibodies) but does not

1 identify the presence of neutralizing antibodies. The measurement of anti-S and anti-N antibodies
2 is much more accessible in a general clinical setting, and supporting this strategy, several reports
3 have shown that anti-SARS-CoV-2 antibodies to the spike protein correlates with neutralizing
4 antibodies and are responsible for neutralizing activity against SARS-CoV-2.^{24,25}

5 **Conclusions**

6 In conclusion, our results demonstrate that most of infected HCW maintain seropositivity levels of
7 IgG anti-SARS-CoV-2 antibodies after 9 months, but with a significant decline in levels. A subgroup
8 of subjects shows a stable, sustained (non-declining) dynamic profile over time, associated to high
9 baseline antibody titers, higher age and high-grade exposure to COVID-19 patients. A longer
10 durability in half-life of antibodies was associated with symptomatic infection and high-risk
11 exposure to COVID-19 patients. Finally, based on the current results, we were able to extrapolate
12 a time to loss of detectable antibody titers of 375 days, in line with the immunological response
13 demonstrated in previous coronarviridae outbreaks.

14 **Ethics approval**

15 The protocol was approved by the Ethics Committee of HM Group (GHM) (Comité Ético de
16 Investigación con Medicamentos de HM Hospitales) (ref. no. 20.04.1611/1640-GHM).

17 **Funding**

18 This work has not received any funding

19 **Data availability**

20 The data underlying this article will be shared on reasonable request to the corresponding author

21 **Author Contributions**

1 All authors conceptualized and designed the study, J.F.V., R.M. and J.M.C. drafted the manuscript
2 and made final revisions, and all authors critically revised, read and approved the final manuscript.

3 Conflict of Interest Statement

4 The authors of the manuscript:
5 “kinetics of anti-SARS-CoV-2 antibodies over time.
6 Results of 10 month follow up in over 300 seropositive Health Care Workers”
7 Declared No Conflict of Interest

8 TABLES

9 Table 1. **Demographic and clinical characteristics, by antibody dynamic profile.**

10 COVID-19: Coronavirus Disease 2019; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; IgG: Immunoglobulin G.

11 ¹ Fifteen subjects (4.9%) showed a significant increase (>25%) in antibody titer and were excluded from the analysis.

		All ¹ (n = 306)	Declining (n = 251)	Stable (n = 40)	P value
Age (years)	< 45 years	156 (51.0%)	135 (88.2%)	9 (5.8%)	0.0004
	≥45 years	150 (49.0%)	111 (75.5%)	31 (20.7%)	
Sex	Female	214 (69.9%)	183 (85.5%)	23 (10.7%)	0.071
	Male	92 (30.1%)	68 (73.9%)	17 (18.5%)	
Baseline IgG anti-SARS-CoV-2 Antibody Titer	T1	102 (33.3%)	88 (86.3%)	2 (2.0%)	< 0.0001
	T2	101 (33.0%)	92 (91.1%)	6 (5.9%)	
	T3	103 (33.7%)	71 (68.9%)	32 (31.1%)	
Exposure Risk	Non-high grade	94 (30.7%)	84 (89.4%)	6 (6.4%)	0.031
	High grade	212 (69.3%)	167 (78.8%)	34 (16.0%)	
COVID-19 Symptoms	No	128 (41.8%)	110 (85,9%)	12 (9.4%)	0.14
	Yes	178 (58.2%)	141 (79,2%)	28 (15.7%)	
Infection Category	Asymptomatic/ Mild	281 (91.8%)	235 (83,6%)	31 (11,0%)	0.003
	Moderate/Severe	25 (8.2%)	16 (64,0%)	9 (36,0%)	
Vaccinated	No	121 (39.5%)	95 (78,5%)	21 (17,4%)	0.11
	Yes	185 (60.5%)	156 (84,3%)	19 (10,3%)	

12 Table 2. **Univariate and multivariate logistic regression models** for identification factors
13 associated with a stable profile.

14 COVID-19: Coronavirus Disease 2019; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; IgG: Immunoglobulin G.

		Univariate Model	P value	Multivariate Model	P value
		OR (95% CI)		OR (95% CI)	
Age (years)	< 45 years	Ref.	-	Ref.	-
	≥45 years	4.1 (1.9 - 9.4)	0.0005	3.1 (1.3 - 8.0)	0.012
Sex	Female	Ref.	-	Ref.	-
	Male	2.0 (1.0 - 3.9)	0.05	1.7 (0.7 - 3.8)	0.23
Baseline IgG anti-SARS-CoV-2 Antibody Titer	T1	Ref.	-	Ref.	-
	T2	2.9 (0.6 - 19.9)	0.20	2.5 (0.5 - 17.8)	0.28
	T3	19.8 (5.7 - 125.0)	< 0,0001	13.0 (3.6 - 84.1)	0.0008
Exposure Risk	Non-high grade	Ref.	-	Ref.	-
	High grade	2.9 (1.2 - 7.8)	0.024	2.8 (1.1 - 8.3)	0.037
Infection Category	Asymptomatic/ Mild	Ref.	-	Ref.	-
	Moderate/Severe	4.3 (1.7 - 10.3)	0.001	2.2 (0.7 - 6.9)	0.16
COVID-19 Symptoms	No	Ref.	-	Ref.	-
	Yes	1.8 (0.9 - 3.9)	0.10	1.4 (0.6 - 3.5)	0.51
Vaccinated	No	Ref.	-	Ref.	-
	Yes	0.55 (0.28 - 1.1)	0.082	0.66 (0.30 - 1.4)	0.29

1 Table 3. Model to estimate mean half-life of antibodies and its variation considering
2 demographic and clinical characteristics.

3 COVID-19: Coronavirus Disease 2019; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; IgG:
4 Immunoglobulin G.

		Univariate model		Multivariate model	
		Estimate	95% CI	Estimate	95% CI
Baseline model	Initial titer	6.55	(6.20 - 6.93)	6.05	(5.31 - 6.89)
	half-life	146.3	(138.6 - 154.9)	121.4	(109.1 - 136.6)
Age model	Initial titer : < 45 years	6.49	(6.03 - 6.98)	-	-
	half-life : < 45 years	141.1	(131.4 - 152.2)	-	-
	Δ Initial titer: > 45 years	0.36	(-0.32 - 1.23)	0.33	(-0.30 - 1.23)
	Δ half-life : > 45 years	10.1	(-5.0 - 35.5)	10.5	(-1.4 - 33.2)

Sex model	Initial titer : Male	6.81	(6.12 - 7.57)	-	-
	half-life : Male	146.2	(132.3 - 163.5)	-	-
	Δ Initial titer: Female	-0.34	(-0.99 - 0.57)	0.35	(-0.35 - 1.36)
	Δ half-life : Female	0.1	(-13.3 - 26.6)	-0.5	(-9.7 - 17.5)
Exposure Risk model	Initial titer : Low risk	6.48	(5.89 - 7.12)	-	-
	half-life : Low risk	135.8	(124.7 - 149.1)	-	-
	Δ Initial titer: High risk	0.12	(-0.56 - 1.03)	0.22	(-0.41 - 1.12)
	Δ half-life : High risk	16.3	(-0.1 - 46.4)	14.1	(0.6 - 40.2)
Infection Category model	Initial titer : Asymptomatic/Mild	6.53	(6.17 - 6.92)	-	-
	half-life : Asymptomatic/Mild	145.6	(137.7 - 154.4)	-	-
	Δ Initial titer: Moderate/ Severe	0.36	(-0.97 - 2.24)	0.07	(-1.01 - 1.82)
	Δ half-life : Moderate/ Severe	11.3	(-17.2 - 70.5)	3.1	(-13.9 - 43.0)
COVID-19 Symptoms model	Initial titer : No	6.26	(5.76 - 6.80)	-	-
	half-life : No	138.0	(127.9 - 149.8)	-	-
	Δ Initial titer: Yes	0.54	(-0.16 - 1.45)	0.34	(-0.30 - 1.25)
	Δ half-life : Yes	15.4	(-0.5 - 43.1)	14.1	(0.9 - 39.8)

1 Table 4. Demographic and clinical characteristics, by seroreversion status.

2 Statistical inference was performed using Chi-squared test or Fisher test when needed.

3 COVID-19: Coronavirus Disease 2019; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; IgG:

4 Immunoglobulin G.

		Non-seroreversion (n = 235)	Seroreversion (n = 71)	P value
Age (years)	< 45 years	112 (71.8%)	44 (28.2%)	0.045
	≥45 years	123 (82.0%)	27 (18.0%)	
Sex	Female	161 (75.2%)	53 (24.8%)	0.4
	Male	74 (80.4%)	18 (19.6%)	
Baseline IgG anti-SARS-CoV-2 Antibody Titer	T1	49 (48.0%)	53 (52.0%)	< 0.0001
	T2	83 (82.2%)	18 (17.8%)	
	T3	103 (100.0%)	0 (0.0%)	
Exposure Risk	Non-high grade	70 (74.5%)	24 (25.5%)	0.62
	High grade	165 (77.8%)	47 (22.2%)	
COVID-19 Symptoms	No	96 (75.0%)	32 (25.0%)	0.62

	Yes	139 (78.1%)	39 (21.9%)	
Infection Category	Asymptomatic/ Mild	211 (75.1%)	70 (24.9%)	0.034
	Moderate/Severe	24 (96.0%)	1 (4.0%)	
Vaccinated	No	99 (81.8%)	22 (18.2%)	0.12
	Yes	156 (84,3%)	19 (10,3%)	

1 FIGURES

2 **Figure 1. Declining and Stable dynamic profiles**, according to the rate of change IgG anti SARS-
3 CoV-2 antibody levels over time, from baseline visit (April-June 2020) to last follow-up visit
4 (January-February 2021).

5

6 Figure 2. **Linear mixed model** for the SARS-CoV-2 IgG antibodies decline. A) Overall mean
7 trajectory from the baseline observed level. Mean trajectory and 95% confidence interval are
8 shown as solid line and dashed area. The dashed grey line represents the limit of the observed
9 period of time and the dashed red line represents the titer threshold of 1.1 A.U. B) Comparison of
10 baseline observed titers and the estimated antibody half-life per subject.

11 REFERENCES

- 12 1) World Health Organization (WHO). Coronavirus disease (COVID-19).
13 <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>
- 14 2) Lin Q, Zhu L, Ni Z, Meng H and You L. Duration of serum neutralizing antibodies for SARS-
15 CoV-2: Lessons from SARS-CoV infection. *Journal of microbiology, immunology, and*
16 *infection*. 2020.
- 17 3) O Murchu E, Byrne P, Walsh KA et al. Immune response following infection with SARS-CoV-
18 2 and other coronaviruses: A rapid review. *Reviews in medical virology*. 2020:e2162.
- 19 4) Arkhipova-Jenkins I, Helfand M, Armstrong C et al. Antibody Response After SARS-CoV-2
20 Infection and Implications for Immunity : A Rapid Living Review. *Ann Intern Med*. 2021
21 Mar 16. Epub ahead of print.
- 22 5) Patel MM, Thornburg NJ, Stubblefield WB et al. Change in antibodies to SARS-CoV-2 over
23 60 days among health care personnel in Nashville, Tennessee. *Jama*. 2020;324:1781-1782.
- 24 6) Self WH, Tenforde MW, Stubblefield WB et al. Decline in SARS-CoV-2 antibodies after mild
25 infection among frontline health care personnel in a multistate hospital network—12
26 States, April–August 2020. *Morbidity and Mortality Weekly Report*. 2020;69:1762.
- 27 7) Marot S, Malet I, Leducq V et al. Rapid decline of neutralizing antibodies against SARS-
28 CoV-2 among infected healthcare workers. *Nature communications*. 2021;12:1-7.

- 1 8) Lumley SF, Wei J, O'Donnell D et al. The duration, dynamics and determinants of SARS-
2 CoV-2 antibody responses in individual healthcare workers. *Clinical Infectious Diseases*.
3 2021.
- 4 9) Varona JF, Madurga R, Peñalver F et al. Seroprevalence of SARS-CoV-2 antibodies in over
5 6000 healthcare workers in Spain. *International Journal of Epidemiology*. 2021.
- 6 10) Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4.
7 *Journal of Statistical Software*. 2015; 67 (1): 1-48
- 8 11) Wajnberg A, Amanat F, Firpo A et al. Robust neutralizing antibodies to SARS-CoV-2
9 infection persist for months. *Science*. 2020 Dec 4;370(6521):1227-1230
- 10 12) Sherina N, Piralla A, Du L et al. W. Persistence of SARS-CoV-2-specific B and T cell
11 responses in convalescent COVID-19 patients 6-8 months after the infection. *Med (N Y)*.
12 2021 Mar 12;2(3):281-295.
- 13 13) Pradenas E, Trinité B, Urrea et al. Stable neutralizing antibody levels 6 months after mild
14 and severe COVID-19 episodes. *Med*. 2021.
- 15 14) Dan JM, Mateus J, Kato Y et al. Immunological memory to SARS-CoV-2 assessed for up to 8
16 months after infection. *Science*. 2021.
- 17 15) den Hartog G, Vos ER, van den Hoogen LL et al. Persistence of antibodies to SARS-CoV-2 in
18 relation to symptoms in a nationwide prospective study. *Clinical Infectious Diseases*. 2021.
- 19 16) He Z, Ren L, Yang J et al. Seroprevalence and humoral immune durability of anti-SARS-CoV-
20 2 antibodies in Wuhan, China: a longitudinal, population-level, cross-sectional study.
21 *Lancet*. 2021 Mar 20;397(10279):1075-1084.
- 22 17) Jeewandara C, Jayathilaka D, Gomes L et al. SARS-CoV-2 neutralizing antibodies in patients
23 with varying severity of acute COVID-19 illness. *Scientific Reports*. 2021;11:1-7.
- 24 18) Legros V, Denolly S, Vogrig M et al. A longitudinal study of SARS-CoV-2-infected patients
25 reveals a high correlation between neutralizing antibodies and COVID-19 severity. *Cell Mol*
26 *Immunol*. 2021 Feb;18(2):318-327.
- 27 19) Seow J, Graham C, Merrick B et al. Longitudinal observation and decline of neutralizing
28 antibody responses in the three months following SARS-CoV-2 infection in humans. *Nature*
29 *Microbiology*. 2020;5:1598-1607.
- 30 20) Han Y, Liu P, Qiu Y et al. Effective virus-neutralizing activities in antisera from the first
31 wave of survivors of severe COVID-19. *JCI insight*. 2021;6.
- 32 21) Anichini G, Gandolfo C, Terrosi C et al. Antibody response to SARS-CoV-2 in infected
33 patients with different clinical outcome. *J Med Virol*. 2021 Apr;93(4):2548-2552.

- 1 22) Long Q-X, Tang X-J, Shi Q-L et al. Clinical and immunological assessment of asymptomatic
2 SARS-CoV-2 infections. *Nature medicine*. 2020;26:1200-1204.
- 3 23) Lumley SF, O'Donnell D, Stoesser NE et al. Antibody status and incidence of SARS-CoV-2
4 infection in health care workers. *New England Journal of Medicine*. 2021;384:533-540.
- 5 24) Zost SJ, Gilchuk P, Case JB et al. Potently neutralizing and protective human antibodies
6 against SARS-CoV-2. *Nature*. 2020;584:443-449.
- 7 25) Ju B, Zhang Q, Ge J et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection.
8 *Nature*. 2020;584:115-119.
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