Persistence and baseline determinants of seropositivity and reinfection rates in health care workers up to 12.5 months after COVID-19

Carlota Dobaño (✉ carlota.dobano@isglobal.org)

ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona <https://orcid.org/0000-0002-6751-4060>

Anna Ramirez

IDIAP Jordi Gol <https://orcid.org/0000-0001-8263-1292>

Selena Alonso

ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona <https://orcid.org/0000-0003-4029-9716>

Josep Vidal-Alaball

Institut Català de la Salut <https://orcid.org/0000-0002-3527-4242>

Gemma Ruiz-Olalla

ISGlobal

Marta Vidal

ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona

Rocio Rubio

ISGlobal

Emma Cascant

ISGlobal

Daniel Parras

IDIBAPS <https://orcid.org/0000-0002-2677-4245>

Natalia Rodrigo Melero

CRG

Pau Serra

IDIBAPS

Carlo Carolis

Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona

<https://orcid.org/0000-0003-4240-1139>

Pere Santamaria

Faculty of Medicine, University of Calgary

Anna Forcada

Gerència territorial de Catalunya Central

Jacobo Mendioroz

ICS

Ruth Aguilar

ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona <https://orcid.org/0000-0003-3277-3107>

Gemma Moncunill

ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona <https://orcid.org/0000-0001-5105-9836>

Anna Ruiz-Comellas

IDIAP Jordi Gol

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Abstract

We assessed the duration and baseline determinants of antibody responses to SARS-CoV-2 spike antigens and the occurrence of reinfections in a prospective cohort of 173 Spanish primary health care worker patients followed up initially for nine months and subsequently up to 12.5 months after COVID-19 symptoms onset. Seropositivity to SARS-CoV-2 spike and receptor binding domain antigens up to 149–270 days was 92.49% (90.17% IgG, 76.3% IgA, 60.69% IgM). In a subset of 64 health care workers who had not yet been vaccinated by April 2021, seropositivity was 96.88% (95.31% IgG, 82.81% IgA) up to 322–379 days post symptoms onset. There were four suspected reinfections detected by passive case detection, two among seronegative individuals (five and seven months after the first episode), and one low antibody responder. Antibody levels significantly correlated with fever, hospitalization, anosmia/hypogeusia, allergies, smoking and occupation. Stable sustainment of IgG responses raises hope for long-lasting COVID-19 vaccine immunity.

Introduction

A key question to understand the evolution of the COVID-19 pandemic is the duration of immune response generated to SARS-CoV-2. Most patients induce a robust humoral and cellular response [1] but with high heterogeneity and a percentage of non-responders. Diversity in epitope specificity, quality and functional capacity of antibodies will likely affect the efficacy of the immunity mediated. Antibodies elicited after exposure to SARS-CoV-2 have been associated with protective immunity up to 6 months [2–5], although we do not yet have a correlate of protection, and reinfections occur seemingly at a low frequency. The spike (S) protein on the virus surface is considered the main target of protective antibodies, and the component of the leading vaccines [6] already under implementation. Functional neutralizing antibodies highly correlate with IgG levels to the receptor binding domain (RBD) of S [1], but IgA and IgM also have neutralizing properties [7].

Despite an increasing understanding of the nature of antibody responses, their longevity remains to be defined as the pandemic evolves. The duration of protective antibodies is a critical question as reinfection rates may increase if immunity wanes. Although initial reports indicated a decline in antibodies after 3 months [8], subsequent studies have shown relatively stable antibody levels, mostly IgG, over a period of up to 6 months and beyond [1–5, 9–17]. As massive global immunization campaigns advance, this knowledge will give clues as to how long COVID-19 vaccine immunity might last and how preexisting SARS-CoV-2 antibodies and other baseline variables could affect vaccine effectiveness.

Methods

Study subjects

Demographic and clinical data were collected to characterize the factors associated with disease presentation, presence of sequelae, long COVID-19 and reinfection in a cohort of 173 primary health care workers (HCW) in Barcelona, Spain, recruited at the first peak of the pandemic (March-April 2020). Baseline symptoms recorded included fever, shivers, headache, asthenia, myalgia, arthralgia, dyspnea, chest pain, cough, sputum production, hemoptysis, anosmia, hypoageusia, odynophagia, tachycardia, dizziness and thrombosis. For the multivariable regression analysis, symptoms were grouped into categories: digestive, otolaryngology, neurological, ophthalmology, and skin disorders. Baseline information collected included also history of previous allergies and smoking habits.

Five cross-sectional surveys were done between September to November 2020, and January to April 2021, to obtain venous blood for assessing maintenance of anti-SARS-CoV-2 seropositivity and analyze baseline factors associated with antibody levels. Participants were not systematically monitored for potential asymptomatic reinfections. Vaccinated HCW were excluded from this analysis.

The study protocol was approved by the IRB *Comitè Ètic d'Investigació Clínica IDIAP Jordi Gol* (codes 20/094-PCV and 20/162-PCV) and written informed consent was obtained from participants.

Laboratory analyses

Levels of IgM, IgA and IgG to RBD and S recombinant proteins expressed from plasmids donated by F. Krammer (Mount Sinai, NY) were quantified in plasma by Luminex, as described [18]. The cutoff for seropositivity was calculated with 128 prepandemic samples as 10 to the mean plus 3 standard deviations of $\log_{10}$ -transformed mean fluorescence intensity values.

Data analysis

Antibody levels were correlated with days since onset of symptoms, and results expressed in Spearman coefficient (ρ) and p values. Univariable and multivariable stepwise linear regression models were fit to determine the effect of baseline variables on the antibody levels ($\log_{10}$) in the full cohort before the start of vaccination (December 2020). Models were selected based on the Akaike information criterion, Bayesian information criterion and adjusted r-square parameters. A transformed beta value (%) of the log-linear model was calculated with the formula: $((10^{\beta}) - 1) \times 100$, giving the difference (in percentage) in antibody levels when comparing to the reference group for categorical variables or for a one-unit change for continuous variables, for easier interpretation of the beta value results. All p-values were considered statistically significant when <0.05 . All data collected were managed and analyzed using the R software version 3.6.3.

Results And Discussion

Most clinical cases in this cohort of HCW were mild-moderate COVID-19, with 24 hospitalized, and 64 presenting with sequelae. Median age was 49 years (IQR 41-58), 137 were females, and there were 13 smokers and 31 ex-smokers.

We did not detect a significant decline in antibody levels as a function of time since symptoms onset (Figure 1). The percentage of seropositivity 149-270 days after symptoms onset combining RBD and S antigens was 60.69% for IgM, 76.30% for IgA, and 90.17% for IgG, consistent with the expected longer duration of the latter isotype. Unexpectedly, seropositivity was quite sustained also for IgM and IgA, considered to be isotypes of shorter duration. Computing all immunoglobulins, seroprevalence 5-9 months after the initial COVID-19 episode was as high as 92.49%, indicating very stable persistence of responses. Furthermore, 64 of 173 HCW not yet vaccinated were tested in January and April 2021, and the overall percentage of seropositivity up to 322-379 days after onset of symptoms in this subset was still as high as 96.88% (IgG 95.31%, IgA 82.81%, IgM 25.0%). These 64 HCW had a seropositivity of 98.44% at 5-9 months after the initial COVID-19 (IgG 95.31%, IgA 87.50%, IgM 37.50%).

There were four suspected reinfections (Table 1). Before the second positive polymerase chain reaction (PCR) diagnosis, two symptomatic cases were seronegative, one asymptomatic was seropositive with low antibodies, and one had unknown serostatus. We attempted to recover the viral RNA from the first episodes for genome sequencing and demonstration of different strains but unfortunately it was not stored. This data set provides some indication of the frequency of reinfection in 173 primary infections with three likely reinfections (interval >90 days as per CDC guidelines) and one suspected reinfection (<90 days between primary and reinfection). Therefore, there was a minimal overall rate of symptomatic reinfection of 2/173 (1.16%). This rate contrasts with what we found in another HCW cohort that we followed up for 7-month seroprevalence in which no reinfections were detected [19, 5]. It could be that primary HCW are more at risk of reinfection than hospital-based HCW, although it should be pointed out that is based on limited sample size. The study also provides some evidence that a lack of S antibody response is a risk factor for symptomatic reinfection while positive serology leads to asymptomatic reinfection (Table 1). This is relevant due to the strong correlation ($\rho=0.9$) between antibody levels to S and RBD with neutralizing function that are thought to confer protection [5].

Stepwise multivariable regression analyses showed that the baseline factors most consistently and significantly associated with higher levels of antibodies 5-9 months after infection were having been admitted to hospital, presenting fever (n=131), anosmia and/or hypogeusia (n=106), and having had previous allergies (n=24) (Table 2). Specifically, for anti-S IgG, HCW with fever had 2.5 times higher levels, patients with anosmia and/or hypogeusia had 2.6 times higher levels, and those with allergies had 1.9 times higher levels, than patients without those conditions. Baseline factors associated with lower levels of IgA and IgG included being a nurse (n=68) or a physician (n=70) compared to other occupation categories working in primary health care centers including customer and social services staff (n=35), and smoking. For anti-S IgA, physicians had 34.84% and nurses 45.67% lower levels than the other jobs, and smokers had 46.17% less than non-smokers (Table 2). Nurses included eight auxiliary nurses, and physicians included one dentist. Other factors were associated with only certain isotypes. Presenting with sputum and/or hemoptysis (n=13) was associated with higher IgM levels, and shivers (n=86) were associated with higher IgAs. Of note, hospitalized patients had 2.1 times higher IgM levels to RBD than non-hospitalized. Age correlated positively with IgGs, having 1.39% higher antibody levels to RBD with each year of age older (Table 2). Higher IgGs (and IgAs less strongly) positively correlated with duration

of symptoms (median 24 days, IQR 13-36; S $\rho=0.229$ $P=0.002$; RBD $\rho=0.246$, $P=0.001$) and number of symptoms (median 10, IQR 6-12; S $\rho=0.351$ $P<0.001$; RBD $\rho=0.364$, $P<0.001$). All other variables, symptoms, or sequelae, were either not statistically significantly associated with antibody levels or weakly in univariable models.

Previous acute phase studies showed that COVID-19 severity was associated with higher antibody responses. Here, hospitalization was associated with higher immunoglobulin levels many months after convalescence, suggesting that severity does not affect stability of memory B cell and plasma cells producing antibodies [2–4, 20]. Common symptoms like fever and highly specific symptoms like alteration in smell and taste were also associated with higher antibodies. Interestingly, having previous allergies also correlated positively with higher antibody levels, which to our knowledge has not been reported. This could be related to disease exacerbation and increased risk of respiratory infections associated with some allergies [21] although this relationship remains unclear. Lower antibody levels in nurses and physicians than other HCW could indicate a lower exposure due to PPE use and higher awareness of risks [19]. Smoking had previously been associated with lower antibody responses [22, 23] and we show that this effect persists after several months primarily affecting IgA, the main mucosal antibody.

In conclusion, despite the large heterogeneity in antibody levels induced by SARS-CoV-2 infection, most HCW patients remained seropositive for anti-S antibodies up to 12.5 months after COVID-19. The findings that after PCR reversion, 2 out of 13 seronegatives had another symptomatic episode, and that one low responder had a second (asymptomatic) infection, are consistent with a protective role for antibodies [24]. Considering that antibody levels achieved by COVID-19 immunization are usually higher than those elicited following natural infection, based on this study it could be speculated that immune memory induced by first generation vaccines could also be long-lasting, therefore reducing the probability that periodical boosters might be required to sustain protective immunity, at least within the first year. Furthermore, data indicates that naïve people should be prioritized for vaccination over those who had suffered COVID-19 since the latter maintain antibodies for at least a year.

Declarations

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AUTHOR CONTRIBUTIONS

Designed the study: CD, GM, JVA, ARC, ARM. Recruited and followed up patients and collected clinical data: ARM, JVA, AF, JM, ARC. Performed the laboratory analysis: SA, RR, MV. Performed the statistical analysis: GRO, SA. Produced the proteins for immunoassays: DP, NRM, PS, CC, PS. Coordinated or managed the study and/or laboratory work: EC, RA, GM, CD. Wrote the first draft: CD; Revised and approved the manuscript: all.

Conflict of interest statement

Authors declare no conflict of interest

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Tables

Table 1. Characteristics of the suspected SARS-CoV-2 reinfection cases.

Socio demographics	First COVID-19 episode			Second COVID-19 episode		Serology
	Symptoms ¹	PCR	Symptoms	PCR		
Female 29 yr nurse	March	Positive: April 2 nd	October	Positive: October 13 th		Seronegative September /
	15 th -	Negative: April	13 th -			Seroconverted October
	May 14 th	22 nd	December			
			23 rd			
Female 41 yr physician	March	Positive: March	August	Positive: August 25 th		Seronegative May & August
	24 th -	27 th	2020- Jan	September 8 th		/ Seroconverted September
	May 25 th	Negative: April	2021	Negative: October 9 th		
		21 st , May 4 th				
Female 58 yr administrative	March	Positive: March	May 20 th -	Positive: May 21 st ,		Unknown April /
	23 rd -	23 rd	May 22 nd	June 4 th , 11 st , 18 th		Seropositive November
	March	Negative: April 6 th		Negative: June 25 th		
	25 th					
Female 44 yr physician	March	Positive: March	None	Positive ² : November		Seropositive ³ September
	23 rd -	25 th		19 th		
	April 3 rd	Negative: April 4 th				

¹Date of start and end of the first and last symptoms. All dates are 2020 unless otherwise indicated.

²PCR was done prior to an unrelated surgical procedure and not as part of any routine COVID-19 screening, the participant had no symptoms.

³Low level antibody responses above the seropositivity threshold.

Table 2. Baseline variables associated with SARS-CoV-2 spike antibody levels 5-9 months after COVID-19 symptoms onset by multivariable stepwise regression models.

		Spike				Receptor binding domain			
	Predictors	Beta ¹	95%CI ³		p-value	Beta	95%CI		p-value
IgM²	Hospitalization	0.187	0.025	0.348	0.024	0.324	0.150	0.498	<0.001
	Previous allergies	0.157	0.000	0.314	0.051	ns	ns	ns	ns
	Sputum and/or hemoptysis	0.156	-0.050	0.363	0.137	0.268	0.047	0.489	0.018
	Anosmia/hypogeusia	0.108	-0.003	0.220	0.057	0.091	-0.028	0.210	0.133
	Fever	0.091	-0.038	0.219	0.165	0.112	-0.027	0.250	0.113
	Digestive alterations	ns	ns	ns	ns	-0.089	-0.210	0.033	0.152
IgA	Fever	0.250	0.094	0.406	0.002	0.178	0.060	0.296	0.003
	Previous allergies	ns ⁴	ns	ns	ns	0.157	0.016	0.298	0.029
	Hospitalization	ns	ns	ns	ns	0.156	0.013	0.299	0.033
	Shivers	0.160	0.024	0.296	0.022	0.087	-0.014	0.188	0.091
	Anosmia/hypogeusia	0.139	0.004	0.273	0.043	ns	ns	ns	ns
	Smoking	-0.269	-0.524	-0.015	0.038	-0.222	-0.411	-0.032	0.022
	Nurses	-0.265	-0.443	-0.086	0.004	-0.223	-0.357	-0.090	0.001
	Physicians	-0.186	-0.360	-0.003	0.046	-0.219	-0.352	-0.087	0.001
IgG	Anosmia/hypogeusia	0.413	0.258	0.568	<0.001	0.189	0.077	0.301	0.001
	Fever	0.398	0.218	0.578	<0.001	0.301	0.169	0.432	<0.001
	Previous allergies	0.269	0.053	0.485	0.015	0.137	-0.021	0.295	0.090
	Hospitalization	0.187	-0.024	0.398	0.082	0.236	0.068	0.404	0.006
	Age	0.007	0.000	0.014	0.050	0.006	0.001	0.011	0.023
	Cough	0.124	-0.034	0.283	0.123	ns	ns	ns	ns
	Digestive alterations	ns	ns	ns	ns	0.088	-0.025	0.202	0.126
	Smoking	-0.295	-0.580	-0.009	0.043	ns	ns	ns	ns
	Nurses	ns	ns	ns	ns	-0.187	-0.335	-0.039	0.014
	Physicians	ns	ns	ns	ns	-0.105	-0.253	0.042	0.159

¹Estimate of the model (beta coefficient), see text for interpretation.

²log₁₀MFI: logarithm 10 median fluorescent intensity (antibody levels).

³CI: confidence interval of the model estimate (beta).

⁴ns: not significant (not retained in the stepwise forward/backward multivariable model)

Figures

Figure 1

SARS-CoV-2 antibody levels by days since COVID-19 symptoms onset. IgA, IgG and IgM levels are represented in log10 median fluorescence intensity (log10 MFI). Black dots represent seropositive individuals and grey dots represent seronegative individuals. Paired samples are joined by grey lines. The blue solid line represents the fitted curve calculated using the linear model method. Shaded areas represent 95% confidence intervals.