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

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Brief Communication

Keywords: Antibody Responses, SARS-CoV-2, Primary Health Care Workers, Reinfection

Posted Date: January 12th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-142984/v1

Abstract

We determined the duration and baseline determinants of antibody responses to SARS-CoV-2 up to nine months after COVID-19 symptoms onset in 173 primary health care worker patients from Spain. Seropositivity to SARS-CoV-2 spike and RBD antigens was 92.49% (60.69% IgM, 76.3% IgA, 90.17% IgG), with four suspected reinfection cases. Antibody levels significantly correlated with fever, hospitalization, anosmia/hypogeusia, allergies, smoking and occupation, and persisted 149-270 days in this cohort of patients

Main Text

One of the key questions to understand the evolution of the COVID-19 pandemic is the duration of the immune response generated upon infection with SARS-CoV-2. Studies have shown that 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. Although a number of cases of SARS-CoV-2 reinfection have been reported, antibodies were recently associated with protective immunity over a 30-week follow up[2]. The spike (S) protein on the virus surface is considered the main target of protective antibodies, and the component of leading vaccine candidates[3]. 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[4], and non-neutralizing antibodies with Fc receptor-mediated functions are likely important for protective immunity.

Despite an increasing understanding of the nature of antibody responses, their longevity remains to be defined as the pandemic evolves. This is a critical question to predict whether the frequency of reinfection may increase if immunity wanes. As massive global immunization campaigns advance, this knowledge will give clues as to how long vaccine immunity may last and how preexisting antibodies and other baseline variables could affect vaccine effectiveness. Although some initial reports indicated a marked decline in antibodies after 3 months[5], subsequent studies have shown relatively stable antibody levels, mostly IgG, over a period of up to 6-8 months in small sets of patients[1,6–10].

We aimed to evaluate the seroprevalence and levels of antibodies 149-270 days after the onset of symptoms in a cohort of 173 primary health care workers (HCW) from 5 counties in Barcelona province, Spain, with COVID-19 at the first peak of the pandemic (March-April 2020) and being followed up for 1.5 years. HCW are at the forefront of this medical emergency and thus considered a high-risk population. 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.

Demographic and clinical data were collected to characterize the factors associated with disease presentation, presence of sequelae, long COVID-19 and reinfection in this cohort. Most cases were mild-moderate COVID-19, with 24 hospitalized, and 64 presenting with sequelae. Median age was 49 years (IQR 41-58) and 137 were females. Clinical information included history of previous allergies and smoking (13 smokers, 31 ex-smokers). Baseline symptoms recorded were 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 (diarrhea, abdominal pain, nausea and/or vomiting, anorexia), otolaryngology (sneezing, rhinorrhea, nasal

obstruction, epistaxis, tinnitus, hearing loss), neurological (impaired consciousness, ataxia, acute cerebrovascular disease, seizures, neuralgia), ophthalmology (conjunctiva hyperemia, tearing, dry eyes, blurred vision), and skin disorders (rash, vesicular lesions, maculopapular lesions, itchy skin, pseudo perniosis).

Three cross-sectional surveys were done between September and November 2020 to obtain venous blood for assessing maintenance of anti-SARS-CoV-2 seropositivity and analyze baseline factors associated with antibody levels. 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[11]. The cutoff for seropositivity was calculated with 55 prepandemic samples as 10 to the mean plus 3 standard deviations of $\log_{10}$-transformed mean fluorescence intensity values.

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 considerably sustained also for IgM and IgA, considered of shorter duration. Computing all immunoglobulin isotypes, seroprevalence 5-9 months after the initial COVID-19 episode was as high as 92.49%, indicating highly stable persistence of responses.

There were four suspected reinfections (Table S1). Before the second positive PCR, two symptomatic cases were seronegative, one asymptomatic was seropositive with low antibodies, and one had unknown serostatus. Unfortunately, viral RNA was not available from the first episodes for genome sequencing and demonstration of different strains.

The baseline factors most consistently and significantly associated with higher levels of antibodies 5-9 months later in stepwise multivariable regression analyses were having been admitted to hospital, presenting with fever (n=131), anosmia and/or hypogeusia (n=106), and previous allergies (n=24) (Table 1). 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 occupations (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 1). 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 1). 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[12]. 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[13] 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[14]. Smoking had previously been associated with lower antibody responses[15] 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, the majority of HCW patients remained seropositive for anti-S antibodies up to nine 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[2]. Data suggest 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.

Declarations

Acknowledgements: We thank the HCW for their participation in the study. We are grateful to Chenjerai Jairoce and Robert Mitchell for assistance with sample processing.

Funding: The study received support from the Fundació Privada Daniel Bravo Andreu. ISGlobal receives support from the Spanish Ministry of Science and Innovation through the “Centro de Excelencia Severo Ochoa 2019-2023” Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program.

Tables

Table 1. Baseline variables associated with the levels of antibodies to SARS-CoV-2 spike antigens 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
IgM[2]	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[5]	-0.265	-0.443	-0.086	0.004	-0.223	-0.357	-0.090	0.001
	Physicians[5]	-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

[1]Estimate of the model (beta coefficient). A transformed beta value (%) of the log-linear model can be calculated with the formula: ((10^beta)-1)*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 (see text for interpretation). [2]log_{10}MFI: logarithm 10 median fluorescent intensity (antibody levels). [3]CI: confidence interval of

the model estimate (beta). [4]ns: not significant (not retained in the stepwise forward/backward multivariable model). [5]Nurses (n=68) include 8 auxiliary nurses, and physicians (n=70) include 1 dentist. Both are compared to other occupational categories working in primary health care centers including customer and social services staff (n=35).

Supplementary Information

Table S1. Characteristics of the suspected SARS-CoV-2 reinfection cases.

	First COVID-19 episode		Second COVID-19 episode		Serology
Socio demographics	**Symptoms**[1]	**PCR**	**Symptoms**	**PCR**	
Female 29 yr nurse	March 15th - May 14th	Positive: April 2nd Negative: April 22nd	October 13th – December 23rd	Positive: October 13th	Seronegative September / Seroconverted October
Female 41 yr physician	March 24th - May 25th	Positive: March 27th Negative: April 21st, May 4th	August – Present (Jan 2021)	Positive: August 25th September 8th Negative: October 9th	Seronegative May & August / Seroconverted September
Female 58 yr administrative	March 23rd – March 25th	Positive: March 23rd Negative: April 6th	May 20th – May 22nd	Positive: May 21st, June 4th, 11st, 18th Negative: June 25th	Unknown April / Seropositive November
Female 44 yr physician	March 23rd – April 3rd	Positive: March 25rd Negative: April 4th	No symptoms	Positive[2]: November 19th	Seropositive[3] September

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

[2]PCR was done prior to a surgical procedure and not as part of a routine screening, the participant had no symptoms.

[3]Low level antibody responses above the seropositivity threshold.

References

1. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Science (80-)*. 2020;370(6521):1227 LP - 1230. doi:10.1126/science.abd7728
2. Lumley SF, O'Donnell D, Stoesser NE, et al. Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers. *N Engl J Med*. December 2020. doi:10.1056/NEJMoa2034545
3. Krammer F. SARS-CoV-2 vaccines in development. *Nature*. 2020;586(7830):516-527. doi:10.1038/s41586-020-2798-3
4. Klingler J, Weiss S, Itri V, et al. Role of IgM and IgA Antibodies in the Neutralization of SARS-CoV-2. *medRxiv*. January 2020:2020.08.18.20177303. doi:10.1101/2020.08.18.20177303
5. Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat Microbiol*. 2020;5(12):1598-1607. doi:10.1038/s41564-020-00813-8
6. Ripperger TJ, Uhrlaub JL, Watanabe M, et al. Orthogonal SARS-CoV-2 Serological Assays Enable Surveillance of Low-Prevalence Communities and Reveal Durable Humoral Immunity. *Immunity*. 2020;53(5):925-933.e4. doi:10.1016/j.immuni.2020.10.004
7. Liu C, Yu X, Gao C, et al. Characterization of antibody responses to SARS-CoV-2 in convalescent COVID-19 patients. *J Med Virol*. November 2020. doi:10.1002/jmv.26646
8. Chen Y, Zuiani A, Fischinger S, et al. Quick COVID-19 Healers Sustain Anti-SARS-CoV-2 Antibody Production. *Cell*. November 2020. doi:10.1016/j.cell.2020.10.051
9. Choe PG, Kim K-H, Kang CK, et al. Antibody Responses 8 Months after Asymptomatic or Mild SARS-CoV-2 Infection. *Emerg Infect Dis*. 2020;27(3). doi:10.3201/eid2703.204543
10. Figueiredo-Campos P, Blankenhaus B, Mota C, et al. Seroprevalence of anti-SARS-CoV-2 antibodies in COVID-19 patients and healthy volunteers up to 6 months post disease onset. *Eur J Immunol*. 2020;50(12):2025-2040. doi:10.1002/eji.202048970
11. Dobaño C, Vidal M, Santano R, et al. Highly sensitive and specific multiplex antibody assays to quantify immunoglobulins M, A and G against SARS-CoV-2 antigens. *J Clin Microbiol*. January 2020:DOI: 10.1101/2020.06.11.147363. doi:10.1101/2020.06.11.147363
12. Hartley GE, Edwards ESJ, Aui PM, et al. Rapid generation of durable B cell memory to SARS-CoV-2 spike and nucleocapsid proteins in COVID-19 and convalescence. *Sci Immunol*. 2020;5(54):eabf8891. doi:10.1126/sciimmunol.abf8891
13. Yang JM, Koh HY, Moon SY, et al. Allergic disorders and susceptibility to and severity of COVID-19: A nationwide cohort study. *J Allergy Clin Immunol*. October 2020. doi: 10.1016/j.jaci.2020.08.008
14. Moncunill G, Mayor A, Santano R, et al. SARS-CoV-2 Seroprevalence and Antibody Kinetics Among Health Care Workers in a Spanish Hospital After 3 Months of Follow-up. *J Infect Dis*. 2021 Jan 4;223(1):62-71. doi: 10.1093/infdis/jiaa696
15. Schaffner A, Risch L, Aeschbacher S, et al. Characterization of a Pan-Immunoglobulin Assay Quantifying Antibodies Directed against the Receptor Binding Domain of the SARS-CoV-2 S1-Subunit of the Spike Protein: A Population-Based Study. *J Clin Med*. 2020;9(12). doi:10.3390/jcm9123989

Figures

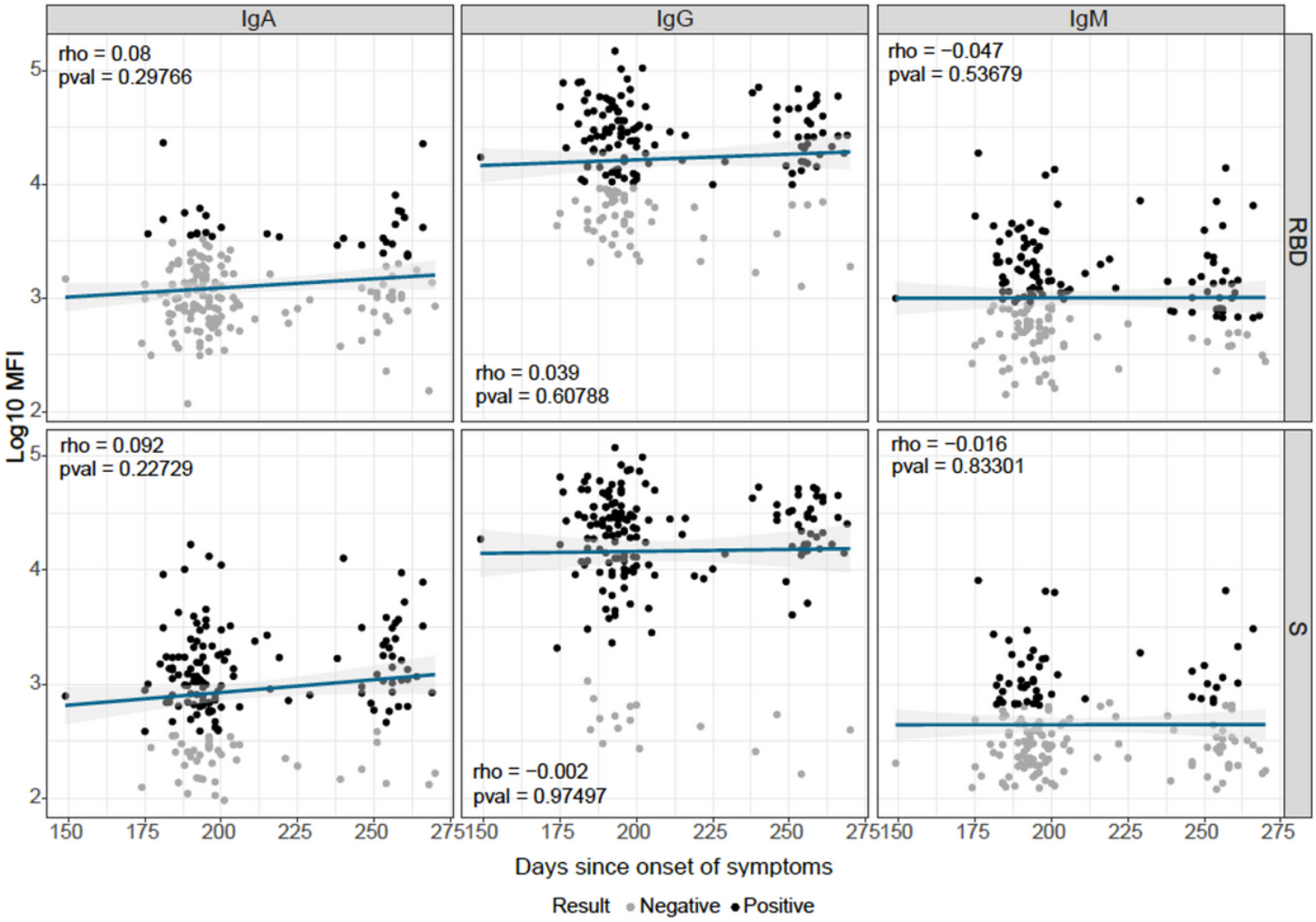

Figure 1

Antibody levels by days since COVID-19 symptoms onset.