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### ► To cite this version:

R Garlantezec, E Tadié, C Heslan, P Gary-Bobo, S Oumari, et al.. SARS-CoV-2 seroprevalence and antibodies persistence among health care workers after the first COVID-19 wave in nine hospitals in Western France. *Infectious Diseases Now*, 2022, 52 (8), pp.447-452. 10.1016/j.idnow.2022.09.004 . hal-03789879

**HAL Id: hal-03789879**

**<https://hal.science/hal-03789879>**

Submitted on 14 Oct 2022

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**SARS-CoV-2 seroprevalence and antibodies persistence among health care workers after the first COVID-19 wave in nine hospitals in Western France**

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### **Funding**

This study was funded by a grant from the Nominoe Fund and the Rennes CHU.

### **Declaration of competing interests**

The authors declare no conflicts of interest.

### **Ethics Approval Statement**

The study obtained the agreement of the Lyon Institutional Review Board (May 28, 2020). All HCWs signed an informed consent form, and the study was recorded on ClinicalTrials.gov (#35RC20\_9716).

**Keywords:** SARS-CoV-2, seroprevalence survey, occupational exposure, health care workers

### **Highlights**

- Among healthcare workers (HCWs) in France after the first COVID-19 wave, SARS-CoV-2 IgG rate was 1.06% [0.86%-1.27%].
- Contact with HCWs or household members with COVID-19 was associated with IgG presence.
- Total SARS-CoV-2 antibodies decreased between day 0 and day 90 and with anosmia or ageusia.
- Total SARS-CoV-2 antibodies were higher in HCWs older than 50 years.

### **Abstract**

**Objectives.** To estimate the SARS-CoV-2 IgG seroprevalence rate in healthcare workers (HCWs) from Western France after the first 2020 wave, its determinants and the kinetics of total SARS-CoV-2 antibodies.

**Patients and Methods.** Overall, 9,453 HCWs responded to a self-questionnaire and underwent a lateral flow immunoassay to assess SARS-CoV-2 IgG presence. For 72 HCWs who tested positive, total anti-nucleocapsid antibodies were assessed at day 0, 30, and 90.

**Results.** SARS-CoV-2 IgG seroprevalence rate was 1.06% [0.86%-1.27%]. Factors associated with IgG presence were gender, performing upper respiratory tract samples, contact with HCWs or household members diagnosed with COVID-19. Total antibodies decreased between day 0 and day 90, with anosmia or ageusia, and were higher in HCWs older than 50 years.

**Conclusion.** We reported a low prevalence rate of IgG and identified several risk factors associated with its presence and persistence of total antibodies. Additional studies are needed to confirm these observations.

## Introduction

Since the 2019 coronavirus disease (COVID-19) pandemic onset, sero-epidemiological surveys have been of particular interest to better understand its characteristics and the risk factors associated with contamination [1–4]. Although numerous studies were conducted among particularly exposed population such as health care workers (HCWs), few studies were conducted with a large sample size in several hospitals, and data on the persistence of the SARS-CoV-2 antibodies in these populations remain scarce [3–6].

The objectives of the present study were therefore to estimate the seroprevalence of SARS-CoV-2 IgG in HCWs in France after the first 2020 wave, its determinants and the kinetics of total SARS-CoV-2 antibodies in those who tested positive.

## Methods

We conducted a seroprevalence survey between May 29 and July 10, 2020 in nine public hospitals (one University Hospital in Rennes and eight general hospitals) from Haute-Bretagne Public Hospital Network (Groupement Hospitalier de territoire [GHT] Haute-Bretagne),

Western France. All HCWs older than 18 years were eligible to participate except those with legal protection (guardianship, curatorship). After providing a written consent, participants answered a short standardized self-questionnaire including information on their socio-professional category, their ward, symptoms reported since the start of the epidemic in France (February 2020), SARS-CoV-2 RT-PCR test results and contact with patients, and HCWs or household members diagnosed with COVID-19. After responding to this questionnaire, HCWs underwent a lateral flow immunoassay (LFIA) finger-prick test (NG-Test<sup>®</sup>, NG Biotech Laboratoires, Guipry-Messac, France), that allows for the detection of IgG and IgM anti-SARS-CoV-2 antibodies in 20 minutes. In addition, for a sample of HCWs from the Rennes university hospital who were tested positive (n=72), total SARS-CoV-2 anti-nucleocapsid antibodies were assessed using Roche Elecsys<sup>®</sup> anti SARS-CoV-2 assay (Roche Diagnostics, Meylan, France) at day 0, 30, and 90 after inclusion. The cut-off index (COI) delivered by the system was taken as representative of the amount of circulating anti-nucleocapsid IgG (Figure 1). This study was recorded on ClinicalTrials.gov (#35RC20\_9716) and obtained the ethical agreement of the Lyon Institutional Review Board (CPP- May 28 2020).

Descriptive analyses were presented as a percentage for qualitative variables and as median and interquartile range for quantitative variables. Based on a validation study of the NG-Test<sup>®</sup> in the same population [7] which reported excellent interobserver concordance (100%) and a good validity for IgG detection (sensitivity of 82.5% [71.9%-92.3%] and specificity of 98.3% [95.0%-100.0%]), only IgG results are presented since IgM NG-Test<sup>®</sup> results showed poor agreement with ELISA Wb Wantai associated with a low sensitivity [7,8]. Crude and adjusted seroprevalence rate taking into account the characteristics of the test (sensitivity and specificity) were estimated with their 95% confidence intervals [9]. The NG-Test<sup>®</sup> IgG positivity factors were studied as a function of socio-professional characteristics and contacts with

COVID-19 cases. We performed multivariate logistic regression by systematically adjusting on gender and age in addition to variables with a p-value  $<0.2$  in univariate analyses. Finally, a description of symptoms in relation with IgG NG-Test results was reported.

For total SARS-CoV-2 antibodies, the median levels (COI) with interquartile range at day 0, day 30, and day 90 were reported. In addition, factors associated with these levels were assessed using a mixed linear model with total SARS-CoV-2 antibodies log transformed as a response variable, and age, sex, and symptoms with individual levels were taken into account with an autocorrelated matrix. Before this multivariate analysis, symptoms were individually tested in univariate analysis and only symptoms associated with total SARS-CoV-2 antibodies detection were included. In addition, as a sensitivity analysis, the final model was rerun by including only HCWs with a history of RT-PCR positivity and taking into account the time between each serological assay and RT-PCR positivity.

A p-value  $<0.05$  was considered significant. The results are presented as odd ratios (OR) with their 95% confidence intervals for multivariate logistic regression and as beta coefficient and their 95% confidence intervals for multivariate mixed linear regression.

## Results

A total of 9,453 HCWs were included in the survey among 12,000 eligible HCWs (76% participation rate). The characteristics of HCWs participating are presented in Table 1. The majority of participants were women (78.9%) and aged under 50 years (76%). Most represented occupations were nurses and related occupations. For Rennes University Hospital, no significant difference in participants and non-participants regarding age, gender and occupation (data not shown) was observed. The adjusted anti-SARS-CoV-2 seroprevalence

was equal to 1.06% [0.86% -1.27%] for the GHT and to 1.76% [1.45% -2.06%] for Rennes University Hospital.

Univariate analysis indicated significant associations between the presence of anti-SARS-CoV-2 IgG and gender ( $p=0.003$ ), contact with COVID-19 patients ( $p=0.003$ ), performing upper respiratory tract samples from a COVID-19 patient ( $p<0.001$ ), contact with HCWs or household members diagnosed with COVID-19 ( $p<0.001$ ) (Table 1). All reported symptoms were associated with anti-SARS-CoV-2 IgG presence, except for odynophagia and abdominal pain. HCWs who tested positive for SARS-CoV-2 IgG were more likely to report clinical presentation of COVID-19, with a higher number of symptoms (Table 1).

Variables remaining significantly associated with IgG positivity in the multivariate analysis were gender (OR=1.37 [95% confidence intervals: 1.03;1.82]), performing upper respiratory tract samples from a COVID-19 patient (OR=1.39 [1.02;1.91]), contact with HCWs (OR=2.03 [1.55;2.67]) or household member diagnosed with COVID-19 (OR=1.52 [1.06;2.19]) (Table 2). Median total anti-nucleocapsid antibodies were 58.6 COI [IQR: 26.6;100.4] at day 0, 60.7 COI [27.5;113.7] at day 30, and 27.5 COI [11.8;114.0] at day 90 (Figure 2). The only symptoms associated with total antibodies levels in univariate analysis were anosmia, and/or ageusia (data not shown). Using a multivariate linear mixed model, variables associated with total SARS-CoV-2 antibodies were age >50 years (Beta=0.92 [0.19–1.65]), self-reported anosmia/ageusia (-0.83 [-1.35 – -0.31]) and sampling at day 90 (Beta=-0.41 [-0.56 – -0.26]) (Table 3). Sensitivity analysis restricted to HCWs with reported previous positive RT-PCR ( $n=47$ , median time between RT-PCR and day 0: 62.5 days [IQR: 54.5-72.0]) and considering the time between this positive RT-PCR and each total SARS-CoV-2 antibody determination, showed similar associations (data not shown).

## Discussion

In this study performed after the first COVID-19 wave and before any vaccine strategy implementation, we reported a low prevalence rate of SARS-CoV-2 IgG among HCWs. Factors associated with seroconversion were gender, performing upper respiratory tract samples from a COVID-19 patient, and contact with HCWs or household members diagnosed with COVID-19. Total SARS-CoV-2 antibodies levels were lower at day 90 and among those who reported anosmia or ageusia but higher in HCWs older than 50 years.

These results on SARS-CoV-2 IgG seroprevalence rate among HCWs after the first COVID-19 wave in Western France are in line with those reported in the general population in this area [2]. In two systematic reviews with meta-analyses of seroprevalence surveys among HCWs [3,4], authors reported a non-significant excess risk of seroconversion among males and an excess risk in those with household members diagnosed with COVID-19. Our results are in accordance with these studies. In addition, these meta-analyses did not report any increased risk in frontline workers as in our study. Moreover, consistently with other large scale studies [5,10], we report an association between seroconversion and self-reported contact with HCWs diagnosed with COVID-19. Finally, the association that was observed with performing upper respiratory tract samples performed from a COVID-19 patient could be explained as a result of exposure to aerosolization [11]. Levels of SARS-CoV-2 antibodies decreased between day 0 and day 90, in line with previous reports [12–16]. Although several factors were associated with higher levels of total neutralizing antibodies such as symptom severity [12,13,15], older age, and gender, results concerning factors associated with the level of total anti-nucleocapsid antibodies raised little interest to date, and may be different. We observed higher levels of total antibodies in HCWs older than 50 years, and lower levels in those who reported anosmia. However, Pallett *et al.* [6] found no association between anti-nucleocapsid IgG antibodies



levels and age or anosmia. This discrepancy may be explained by the more heterogeneous characteristics of their population that included both HCWs and residents of long-term care facilities, and by its cross-sectional design.

One of the main strengths of our study was the large sample size of HCWs included with a high participation rate. Although we could not exclude a selection bias, participants' characteristics were not different from other HCWs in Rennes University Hospital, which represented the majority of HCWs included in our study. In addition, even if we used a rapid LFIA to assess IgG, its use was validated in the same population [7] and the seroprevalence rate was corrected for validation characteristics, as recommended [9]. The chosen time period, before the initiation of vaccination, was the unique opportunity to conduct this study without being biased by such external factor. Several limitations of our study must be highlighted such as the lack of seroneutralization assays and the absence of IgM determination for the seroprevalence study. However, considering the timing of our seroprevalence survey and the short time between production of SARS-CoV-2 IgM and IgG, it is improbable that we underestimated the seroprevalence rate in our study by not measuring IgM. One may also pinpoint the lack of international standardization for anti-nucleocapsid IgG level determination. Although the method we used could only be considered as semi-quantitative to determine anti-nucleocapsid antibodies, we performed a pilot study (Figure 1) that reported a good dose response between COI values and dilutions. The observed decrease of COI values over time, clearly indicates anti-nucleocapsid clearance some time after the infection.

## **Conclusion**

In a large sample of HCWs from different hospitals in Western France, we reported a low prevalence rate of IgG and identified several risk factors, including contact with HCWs or household members diagnosed with COVID-19. In addition, total anti-NC antibodies were shown to decrease between day 0 and day 90, to be lower in those who reported anosmia or ageusia but higher in HCWs older than 50 years. Additional studies are needed to confirm these observations and to understand the underlying mechanisms.

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**Figure 1. Relation between anti-NC (COI) and anti-S (IU/mL) for four health care worker (A, B, C and D) samples with 2-fold dilutions (from 1/2 to 1/16) in Roche universal diluent.**

**Figure 2. Box plot of the COI distribution at day 0, 30 and 90 (n=72 health care workers).****Table 1.** Description and univariate analysis of risk factors for anti-SARS-CoV2 IgG detection among 9,453 HCWs included in the SARS-CoV2 seroprevalence survey of the GHT Haute Bretagne (May-July 2020)

	Total		IgG NG-Test negative (N=9,211, 97.44%)		IgG NG-Test positive (N=242, 2.56%)		p-value
	N	(%)	N	(%)	N	(%)	
<b>Gender</b>							
	Women	7,456 (78.87%)	7,283 (97.68%)	173 (2.32%)			0.003
	Men	1,997 (21.13%)	1,928 (96.54%)	69 (3.46%)			
<b>Age</b>							
	<30 years	2,231 (23.60%)	2,169 (97.22%)	62 (2.78%)			0.44
	30-39 years	2,543 (26.90%)	2,485 (97.72%)	58 (2.28%)			
	40-49 years	2,413 (25.53%)	2,344 (97.14%)	69 (2.86%)			
	50-59 years	1,970 (20.84%)	1,926 (97.77%)	44 (2.23%)			
	>60 years	296 (3.13%)	287 (96.96%)	9 (3.04%)			
<b>Hospital</b>							
	Rennes University Hospital	6,990 (74.05%)	6,772 (96.88%)	218 (3.12%)			-
	Redon	661 (6.99%)	650 (98.34%)	11 (1.66%)			
	Vitré	628 (6.64%)	620 (98.73%)	8 (1.27%)			
	Fougères	326 (3.44%)	323 (99.08%)	3 (0.92%)			
	Guerche de Bretagne	207 (2.18%)	206 (99.52%)	1 (0.48%)			
	St Méen Le Grand	199 (2.10%)	199 (100.00%)	0 (0.00%)			
	Montfort sur Meu	190 (2.00%)	190 (100.00%)	0 (0.00%)			
	Janzé	179 (1.89%)	178 (99.44%)	1 (0.56%)			
	Grand Fougeray	73 (0.77%)	73 (100.00%)	0 (0.00%)			
<b>Occupations</b>							
	Auxiliary nurses	1,912 (20.26%)	1,870 (97.80%)	42 (2.20%)			0.4
	Cleaners/Stretchers-bearers	655 (6.94%)	641 (97.86%)	14 (2.14%)			
	Nurses/Midwives	2,294 (24.31%)	2,240 (97.65%)	54 (2.35%)			
	Residents	333 (3.53%)	320 (96.10%)	13 (3.90%)			
	Medical staff	735 (7.79%)	716 (96.73%)	19 (2.59%)			
	Students	311 (3.30%)	306 (98.39%)	5 (1.61%)			
	Other HCWs with patient contact	429 (4.55%)	415 (96.74%)	14 (3.26%)			
	Other HCWs without patient contact	1,819 (19.28%)	1,764 (96.98%)	55 (3.02%)			
	Administrative staff	947 (10.04%)	924 (97.57%)	23 (2.43%)			
<b>Smoking status</b>							
	No	7,168 (75.83%)	6,977 (97.34%)	191 (2.66%)			0.43
	Yes, but not every day	1,488 (15.74%)	1,457 (97.92%)	31 (2.08%)			
	Yes, every day	797 (8.43%)	777 (97.49%)	20 (2.51%)			
<b>Immunodepression</b>							
	No	9,154 (96.84%)	8,921 (97.45%)	233 (2.55%)			0.62
	Yes	299 (3.16%)	290 (96.99%)	9 (3.01%)			
<b>Contact with COVID-19 patients</b>							

	No	5,450 (57.65%)	5,332 (97.83%)	118 (2.17%)	0.003
	Yes	4,003 (42.35%)	3,879 (96.90%)	124 (3.10%)	
<b>Performing upper respiratory tract samples from COVID-19 patients</b>					
	No	7,800 (82.51%)	7,621 (97.71%)	179 (2.29%)	<0.001
	Yes	1,653 (17.49%)	1,590 (96.19%)	63 (3.81%)	
<b>Contact with HCWs diagnosed with COVID-19</b>					
	No	6,194 (65.52%)	6,083 (98.21%)	111 (1.79%)	<0.001
	Yes	3,259 (34.48%)	3,128 (95.98%)	131 (4.02%)	
<b>Contact with household members diagnosed with COVID-19</b>					
	No	8,558 (90.53%)	8,354 (97.62%)	204 (2.38%)	<0.001
	Yes	895 (9.47%)	857 (95.75%)	38 (4.25%)	
<b>Self-reported symptoms</b>					
	Fever	1,400 (14.81%)	1,311 (93.64%)	89 (6.36%)	<0.001
	Headache	3,425 (36.36%)	3,300 (96.35%)	125 (3.65%)	<0.001
	Myalgia	1,387 (14.67%)	1,303 (93.94%)	84 (6.06%)	<0.001
	Cough	1,900 (20.10%)	1,819 (97.10%)	81 (4.26%)	<0.001
	Sore throat	1,825 (19.31%)	1,772 (96.87%)	53 (2.90%)	0.3
	Rhinitis	2,717 (28.74%)	2,632 (94.14%)	85 (3.13%)	0.03
	Dyspnea	802 (8.48%)	755 (94.24%)	47 (5.86%)	<0.001
	Asthenia	1,701 (17.99%)	1,603 (74.61%)	98 (5.76%)	<0.001
	Anosmia	256 (2.71%)	191 (74.61%)	65 (25.39%)	<0.001
	Diarrhea	878 (9.29%)	843 (96.01%)	35 (3.99%)	0.005
	Abdominal pain	1,217 (12.87%)	1,182 (97.12%)	35 (2.88%)	0.45
<b>Number of symptoms</b>					
	median [IQR]	1 [0 ; 3]	1 [0 ; 3]	3 [1 ; 5]	<0.001
<b>COVID-19 (clinical status)</b>					
	No	7,505 (80.08%)	7,434 (98.20%)	136 (1.80%)	<0.001
	Possible	1,360 (14.39%)	1,330 (97.79%)	30 (2.21%)	
	Probable	523 (5.53%)	447 (85.47%)	76 (14.53%)	

HCWs: health care workers.

(COVID-19 definition: Possible = at least one symptom. Probable = fever, dyspnea and at least one of the following symptoms : headache, myalgia, asthenia ; anosmia even isolated)

**Table 2.** Risk factors for anti-SARS-CoV2 IgG among HCWs from GHT Haute Bretagne hospitals: multivariate analysis (n=9,453 HCWs, May-July 2020).

	OR [95% CI ]
<b>Gender</b>	
Women	Ref
Men	1.37 [1.03 ; 1.82]
<b>Age</b>	
<30 years	Ref
[30-40 years[	0.84 [0.58 ; 1.23]
[40-50 years[	1.15 [0.81 ; 1.64]
≥50 years	0.97 [0.66 ;1.42]
<b>Contact with COVID-19 patients</b>	
No	Ref
Yes	1.05 [0.79 ; 1.40]
<b>Performing upper respiratory tract samples from COVID-19 patients</b>	
No	Ref
Yes	1.39 [1.02 ; 1.91]
<b>Contact with HCWs diagnosed with COVID-19</b>	
No	Ref
Yes	2.03 [1.55 ; 2.67]
<b>Contact with household members diagnosed with COVID-19</b>	
No	Ref
Yes	1.52 [1.06 ; 2.19]

OR=Odds Ratios. 95%CI :95% confidence intervals. HCWs: health care workers

**Table 3.** Factors associated with anti-N SARS-CoV-2 antibody levels (COI) during the follow up of 75 health care workers who tested positive after the first COVID-19 wave (France, 2020).

		<b>Median [Q1-Q3]* Total SARS-CoV-2 Antibodies (COI)</b>	<b>Beta coefficient [CI 95%] log Total anti-N SARS-CoV-2 Antibodies (COI)</b>	<b>p-value</b>
<b>Gender</b>	Women	53.3 [22.8 ; 103.5]	Ref	0.77
	Men	68.1 [42.8 ; 95.0]	0.09 [-0.69 ; 0.51]	
<b>Age</b>	<30 years	38.9 [26.6 ; 59.3]	Ref	0.43
	[30-40 years[	60.3 [19.6 ; 103.3]	0.28 [-0.40 ; 0.96]	
	[40-50 years[	89.6 [46.6 ; 110.1]	0.62 [-0.03 ; 1.28]	
	≥50 years	88.4 [64.7 ; 98.3]	0.92 [0.19 ; 1.65]	
<b>Ageusia</b>	No	85.9 [50 ; 114.3]	Ref	0.002
	Yes	42.8 [14.8 ; 82.9]	-0.83 [-1.35 ; -0.31]	
<b>Time</b>	Day 0	58.6 [26.6 ; 100.4]	Ref	0.23
	Day 30	60.7 [27.5 ; 113.7]	0.06 [-0.04 ; 0.17]	
	Day 90	27.5 [11.8 ; 114.0]	-0.41 [-0.56 ; -0.26]	

\*Median value and first (Q1) and third (Q3) quartile at Day 0 (except for the covariate time).

### Acknowledgements

We thank all participants, nurses and residents for inclusion of the participants. We thank Pr Boudjema, Pr Malledant, Nicolas Mevel, Sophie Huitorel, Agnes Gazzola, Anne-Sophie Jouault, and Valerie Turmel for their help in organizing this study.

### Author's contribution

RG and CP conceived the study. CP, RG, VT and PT contributed to the protocol and design of the study. ET, PGB, AS, SO and the AntiCOV-HB working group contributed to the implementation of the study or data collection. CH and VT conducted the total anti-N antibodies assays. RG, CP and AS conducted the statistical analysis. RG contributed to the preparation of the report. All authors critically reviewed and approved the final version. All authors had full access to all data in the study and had final responsibility for the decision to submit for publication.



### Ethics Approval Statement

The study obtained the agreement of the Lyon Institutional Review Board (“Comité de protection des personnes”, May, 28<sup>th</sup> 2020). All HCWs signed an informed consent form, and the study was recorded on ClinicalTrials.gov (#35RC20\_9716).



