

Original research

# Serological study of healthcare workers in four different hospitals in Madrid (Spain) with no previous history of COVID-19

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## ABSTRACT

**Objectives** Healthcare workers (HCWs) have been one of the most severely affected groups during the COVID-19 pandemic, though few studies have sought to determine the rate of undiagnosed cases among this population. In this study, we aim to determine the rate of undetected infection in HCWs, a potential source of nosocomial infection.

**Methods** Serological screening for IgG and IgM antibodies against SARS-CoV-2 was carried out among HCWs from four different hospitals in Madrid, Spain, from 6 April to 25 April 2020; HCWs with a previous diagnosis of infection based on real-time reverse transcriptase-PCR assay performed after presenting compatible symptoms were excluded. Prevalence of IgG and IgM antibodies was calculated among HCWs to obtain the rate of COVID-19 presence of antibodies in each hospital.

**Results** Of the 7121 HCWs studied, 6344 (89.09%) had not been previously diagnosed with COVID-19. A total of 5995 HCWs finally participated in the study, resulting in a participation rate of 94.49%. A positive IgM or IgG test against COVID-19 was revealed in 16.21% of the HCWs studied (n=972).

**Conclusion** This study reveals the importance of early detection of SARS-CoV-2 infection among HCWs to prevent nosocomial infection and exposure of patients, visitors and workers and the spread of COVID-19 in the overall community.

## BACKGROUND

COVID-19, a novel illness caused by SARS-CoV-2 or nCoV-2019, was first detected in Wuhan (province of Hubei, China) in December 2019.<sup>1</sup> Mild symptoms such as cough, olfactory and gustatory dysfunction<sup>2</sup> or fever, and severe symptoms such as shortness of breath and pneumonia have been described.<sup>3</sup> However, many cases of patients with asymptomatic COVID-19 have been reported. These patients pose a high risk of infection,<sup>4</sup> which has substantial implications for efforts to control the pandemic,<sup>5</sup> as it has been shown that viral loads in nasal swabs did not differ significantly between asymptomatic and symptomatic patients.<sup>6</sup>

Aiming to identify asymptomatic carriers,<sup>7 8</sup> massive testing programmes have been introduced in countries such as South Korea<sup>9</sup> and Iceland<sup>10</sup> in order to isolate the infected population and avoid rapid spread of the disease. However, the scarcity of rapid testing systems in the global market and delays in the

## Key messages

### What is already known about this subject?

- ▶ Asymptomatic and paucisymptomatic patients with COVID-19 are a significant source of disease transmission.
- ▶ Few studies have been published during the COVID-19 pandemic on the seroprevalence of SARS-CoV-2-specific antibodies among healthcare workers with no previous diagnosis.

### What are the new findings?

- ▶ Of the 7121 HCWs studied, 777 (10.91%) had previous diagnosis of COVID-19, testing positive for reverse transcriptase-PCR.
- ▶ Among the 5995 HCWs with no previous COVID-19 diagnosis actively employed in the four participating hospitals, who participated in the study, a positive IgM or IgG test against the SARS-CoV-2 virus was found in 16.21%.

### How might this impact on policy or clinical practice in the foreseeable future?

- ▶ Prevention efforts aimed at early detection of asymptomatic and paucisymptomatic carriers of COVID-19 among HCWs could help diminish the rate of transmission.
- ▶ Further research is needed to inform policy measures regarding COVID-19 prevention among HCWs.

decision to institute confinement policies in Europe<sup>11</sup> facilitated the spread of COVID-19 throughout Europe in the first wave. It is estimated that about 15% of the Spanish population could already be infected.<sup>12</sup>

Healthcare workers (HCWs) have been one of the most severely affected groups during the current pandemic, as shown in a systematic review,<sup>13</sup> with an estimated overall seroprevalence of SARS-CoV-2 antibodies among HCWs of 8.7%. A study carried out in a large hospital in Madrid estimated that around 12% of the HCWs had been infected, based on reverse transcriptase-PCR (RT-PCR) testing in 30% of staff members with compatible symptoms.<sup>14</sup> Beyond detectable cases, however, it is also important to ascertain the number of asymptomatic and undiagnosed HCWs



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who could be infected with SARS-CoV-2, as these employees are a potential source of nosocomial infection.

The primary aim of this study is to evaluate the presence of antibodies against COVID-19 based on the rate of virus-specific IgM and IgG antibodies in a large sample of HCWs from four hospitals in Madrid, Spain.

## METHODS

### Subjects

HCWs from four different hospitals from Quironsalud Group in the region of Madrid were evaluated, including Fundación Jiménez Díaz University Hospital (FJD), a tertiary facility located in central Madrid with a staff of 3696 and capacity for more than 600 inpatients. Additionally, three secondary hospitals throughout the Madrid region participated: Rey Juan Carlos University Hospital in Móstoles, with 1693 employees and 358 beds, Hospital General de Villalba in Collado Villalba, with 859 employees and 164 beds, and Hospital Infanta Elena (HUIE) in Valdemoro, with 873 employees and 144 beds.

Each facility provides medical care for a particular area of the Madrid region. In all four, the rate of bed occupation by patients with COVID-19 surpassed 80% during the peak of the pandemic, beginning on late February.

The recruitment procedure was as follows: all employees of the four hospitals received at their corporate e-mail an invitation to undergo a rapid antibody IgG and IgM COVID-19 test, regardless of their occupation or risk of exposure to the disease. The main inclusion criterion for this study was active staff members of the hospital during the COVID-19 crisis who did not test positive for SARS-CoV-2.

HCWs with no prior COVID-19 diagnosis (positive RT-PCR) were identified based on the protocol used to identify, assess and test symptomatic HCWs. The protocol was applied in the four participating hospitals by the occupational health departments and hospital administrators during the entire period that SARS-CoV-2 was present in the community.

All HCWs were contacted via e-mail at the start of epidemic to request that they inform the institution's occupational health department in case of symptoms compatible with COVID-19. We also received information of the human resources department of the HCWs who were diagnosed with COVID-19 in other healthcare facilities and were on sick leave, so we could offer them follow-up in our occupational health department and register them in our database as COVID-19 RT-PCR positive.

The symptoms taken into account were the following: fever  $\geq 37.7^{\circ}\text{C}$ , sudden onset of persistent cough, chest pain, shortness of breath or other respiratory symptoms and diarrhoea. All patients with suspected infection based on the presence of clinical symptoms were subsequently examined (including laboratory testing and chest X-rays where necessary) and with RT-PCR tested using a nasopharyngeal swab (preferably 48–72 hours after the onset of symptoms). If RT-PCR determination was negative,

the assay was repeated 48–72 hours later. Finally, if the result was negative, a sample of venous blood was collected to determine presence of IgM and IgG antibodies against COVID-19 (preferably 10–11 days after symptom onset). This procedure was based on the most recent studies and protocols.<sup>15–17</sup>

Among a total of 7121 HCWs, 6344 (89.09%) were classified as HCWs with no prior diagnosis of COVID-19. A total of 5995 HCWs with no prior diagnosis of COVID-19 voluntarily participated in the study, with a rate of participation of 94.49% (range, 90.19%–95.61%) (table 1).

### Methodology

Blood samples were taken by specialised nurses and analysed in the department of microbiology of each hospital. The rapid test used to determine the disease status of HCWs without a prior diagnosis is a membrane-based immunochromatography lateral flow assay from Biozek Medical. The test was taken using venous blood. Positive IgM or IgG antibodies are indicated by a coloured band. The assay measures IgM and IgG qualitatively, providing a positive or negative result with a manufacturer-reported sensitivity of 100% (95% CI: 86% to 100%) and specificity of 98% (95% CI: 89.4% to 99.9%) for IgG and a sensitivity of 85% (95% CI: 62.1% to 96.8%) and specificity of 96% (95% CI: 86.3% to 99.5%) for IgM<sup>18</sup>; the specificity and sensitivity of this test are comparable to other rapid test kits reviewed.<sup>19</sup> The validation analysis performed in a Microbiology Laboratory of the Autonomous Community of Madrid showed a sensitivity of 79.4% and a specificity of 74%.

Samples for serological tests were obtained from 6 April to 25 April 2020 in the four participating hospitals. Results were reported to HCWs through the institutional smart app of Quironsalud and reviewed by the microbiology and occupational health department.

At the end of the testing stage, data processing started, and those HCWs previously diagnosed with COVID-19 based on positive RT-PCR and registered in our database were excluded. Those who presented compatible symptoms and had negative RT-PCR result were included.

We calculated the prevalence of virus-specific IgG and IgM antibodies among undiagnosed HCWs in each hospital. Serological test results were classified according to four different categories: IgM positive and IgG positive, IgM positive and IgG negative, IgM negative and IgG positive and IgM negative and IgG negative (indicating no immune response to SARS-CoV-2 infection or absence of infection). Those who had an IgM positive result were asked to perform an RT-PCR in order to dismiss active infection.

FJD HCWs were classified depending of their professional categories in administrative assistants, orderly, nursing, investigation staff, cleaning staff, doctors, nursing assistants, technicians, admission and patients support and other staff. Every category was classified depending on the presence or absence of antibodies response. Means comparison was calculated.

**Table 1** HCWs undergoing COVID-19 serological testing in the four participating hospitals

	FJD	HURJC	HUIE	HGV	All hospitals
	n (%)	n (%)	n (%)	n (%)	N (%)
Total HCWs	3696 (51.90)	1693 (23.77)	873 (12.26)	859 (12.06)	7121 (100)
HCWs with prior RT-PCR COVID-19 diagnosis	463 (12.53)	137 (8.09)	93 (10.65)	84 (9.78)	777 (10.91)
HCWs eligible for the study	3233 (87.47)	1556 (91.91)	780 (89.35)	775 (90.22)	6344 (89.09)
Participation in screening of eligible HCWs	3091 (95.61)	1482 (95.24)	723 (92.69)	699 (90.19)	5995 (94.50)

FJD, Fundación Jiménez Díaz; HCW, healthcare worker; HGV, Hospital General de Villalba; HUIE, Infanta Elena University Hospital; HURJC, Rey Juan Carlos University Hospital; RT-PCR, reverse transcriptase-PCR.

**Table 2** COVID-19 IgG and IgM values among previously undiagnosed HCWs working in four hospitals

	FJD	HGV	HUIE	HURJC	Total
	n (%)	n (%)	n (%)	n (%)	N (%)
Total	3091	699	723	1482	5995
IgM (+) IgG (+)	103 (3.33)	7 (1.00)	32 (4.43)	30 (2.02)	172 (2.86)
IgM (+) IgG (-)	51 (1.65)	2 (0.29)	17 (2.35)	19 (1.28)	89 (1.48)
IgM (-) IgG (+)	363 (11.64)	64 (9.16)	103 (14.25)	181 (12.21)	711 (11.86)
IgM (-) IgG (-)	2574 (83.27)	626 (89.56)	571 (78.98)	1252 (84.48)	5023 (83.79)

FJD, Fundación Jiménez Díaz; HGV, Hospital General de Villalba; HUIE, Infanta Elena University Hospital; HURJC, Rey Juan Carlos University Hospital.

**RESULTS**

The seroprevalence analysis (table 2) revealed that among all of the HCWs tested across the four hospitals, 2.86% had IgM positive and IgG positive virus-specific antibodies (range per hospital, 1.00%–4.43%). IgM positive and IgG negative test results were obtained in 1.48% (range, 0.29%–2.35%). Positive IgG and negative IgM test results were obtained in 11.86% (n=711) of the HCWs, the lowest hospital having a rate of 9.16%, and the highest a rate of 14.25%. Absence of antibodies (IgG negative and IgM negative) was the result in 83.79% tests (n=5023); these percentages ranged from 78.98% to 89.56% depending on the hospital studied.

Considering a positive test for IgM and/or IgG antibodies to indicate an immune response against COVID-19, an average of 16.21% (n=972) of previously undiagnosed HCWs presented an immune response (figure 1).

Mean comparison was made with the 16.21% of antibodies response in every professional category in FJD. Results can be seen in table 3. There was a higher incidence of antibody response in administrative assistants with 22 employees (24.18%, p<0.095), as well as in nursing assistants with 98 (20.99%, p<0.095) of employees with positive immune response. Orderly and investigation staff had a lower incidence with 9.73% and 9.45%, respectively (p<0.095).

No differences were found in nursing, doctors, cleaning staff, technicians, admission and patients support and other staff.

**DISCUSSION**

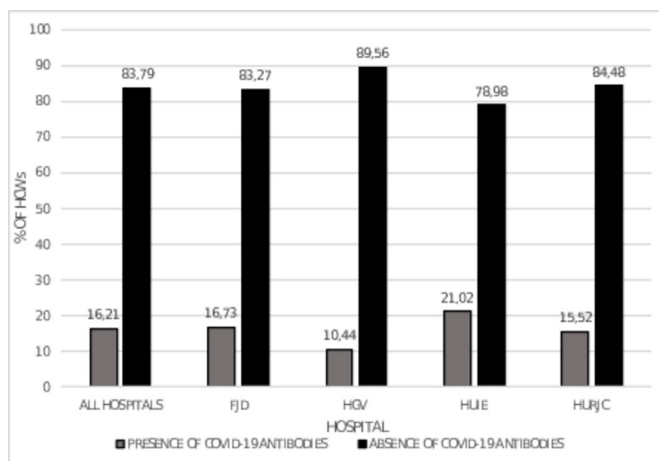
The main objective of this study was to determine the rate of presence of antibodies against COVID-19 among HCWs with no prior diagnosis. We found that 16.2% (n=972) had a disease-specific

immune response 1 month after the first HCW was diagnosed. Since the start of the epidemic, 777 RT-PCR-confirmed symptomatic HCWs had been diagnosed. Comparing results, this means that more than a double of HCWs passed COVID-19 without being diagnosed with the disease.

No statistical differences were found in nursing, doctors, cleaning staff, technicians, admission and patients support and other staff, which would mean that it is a representative sample of the HCWs who had direct interaction with patients. The statistical difference with the average of the total 16.2% who had a disease-specific immune response shown in nursing assistants with a 20.99% with antibodies against COVID-19 might be explained by the closer interaction with patients, given the fact that these HCWs are the ones in charge of daily hygiene of patients and mobilising them. The lower incidence in orderly and investigation staff could be explained by the absence of close contact with patients. The higher incidence in administrative assistants could be explained by non-nosocomial infections, due to the absence of close contact with patients.

The viral load in asymptomatic patients and the potential for transmission by asymptomatic carriers of the disease has been described previously.<sup>20</sup> The results of our study underscore the importance of identifying asymptomatic carriers in order to control the transmission of the disease in the community. HCWs are at particularly high risk of exposure to the new coronavirus, especially where there is a scarcity of personal protection equipment (PPE).<sup>21</sup> Preventive measures such as proper identification of asymptomatic HCWs and adequate PPE provision should be a priority for infection control in healthcare facilities.

Almost all (94.5%) of the HCWs employed in the four participating hospitals have been tested for COVID-19 antibodies. Infection-control strategies based on general screening, such as the one described here, take on additional significance given that asymptomatic personnel with unknown infectious status should be considered potential carriers of the disease, possibly increasing



**Figure 1** COVID-19 antibody response in HCWs with no previous diagnosis in the four participating hospitals. FJD, Fundación Jiménez Díaz; HCW, healthcare worker; HGV, Hospital General de Villalba; HUIE, Infanta Elena University Hospital; HURJC, Rey Juan Carlos University Hospital.

**Table 3** COVID-19 antibody response in the different professional categories of HCWs in FJD

Professional category	Antibody response N (%)	No antibody response N (%)	P values (p<0.095)
Administrative assistants	22 (24.18)	69 (75.82)	0.084
Orderly	11 (9.73)	102 (90.27)	0.066
Nursing	141 (16.63)	707 (83.37)	0.987
Investigation	12 (9.45)	115 (90.55)	0.041
Cleaning staff	21 (16.80)	104 (83.20)	1.000
Doctors	121 (16.33)	620 (83.67)	0.837
Other	18 (16.07)	94 (83.93)	0.957
Nursing assistance	98 (20.99)	369 (79.01)	0.028
Technicians	45 (15.25)	250 (84.75)	0.571
Admission and patients support	28 (16.28)	144 (83.72)	0.962

nosocomial transmission.<sup>6,22</sup> Arons *et al.*<sup>23</sup> described rapid transmission of COVID-19 in a skilled nursing facility after the infection of an HCW. The facility contained a substantial number of asymptomatic residents, which may have contributed to disease transmission.

A seroprevalence study<sup>24</sup> of 578 HCWs reports the presence of antibodies or past or current positive RT-PCR in 11.2% HCWs, with a 40.0% of HCWs not been previously diagnosed with COVID-19, which would not differ much from our study. Another study has shown a proportion of 31.9% of infected workers as asymptomatic.<sup>25</sup>

Results from the weekly epidemiological report issued by the Madrid regional government on 28 April 2020<sup>26</sup> suggest a possible correlation between the incidence of cases in a particular geographic area and the number of HCWs immunised in the care facilities serving the area. According to the document, Madrid and Móstoles are among the municipalities with the highest number of COVID-19 cases, which may explain the amount of immunised HCWs in the FJD (located in central Madrid) and HUIE (located in Valdemoro). Specifically, as of 24 April, the municipality of Madrid had the highest rate of infection, with 31 469 RT-PCR-confirmed cases and a cumulative incidence of 963.50 per 100 000 inhabitants; for its part, Valdemoro is one of the most highly affected areas in the region of Madrid (cumulative incidence of 901.52 per 100 000 inhabitants), which could explain the high prevalence of IgM positive and IgG positive and isolated IgM positive cases among HCWs in both hospitals. The low cumulative incidence (714.52 per 100 000 inhabitants) and the low total number of cases in Collado Villalba (455) may explain the fact that this regional hospital has the lowest levels of seroprevalence of IgG and IgM antibodies among the facilities studied.

This investigation has certain limitations due to the dynamic viral load of SARS-CoV-2 and the immune response to infection, given that our data only reflect the presence of antibodies within a certain period of time. This should be taken into account when measuring antibodies response in HCWs over time. Specificity of IgM antibodies would also be a limitation of the study, since most of the IgM positive HCWs did not show active infection when RT-PCR tested. Another matter to be borne in mind is the role played by the sensitivity and specificity of the serological tests and the fact that this study does not account for different occupational and non-occupational disease exposure among HCWs.

This study provides data from different areas within the same geographic region with varying incidence levels of COVID-19 in the respective patient populations and how HCWs have been affected differently by the new coronavirus. In conclusion, it reveals the importance of early detection of infections among HCWs in order to prevent nosocomial infection and exposure of patients, visitors and workers and disease spread within the greater community.

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**Data availability statement** All data relevant to the study are included in the article or uploaded as supplementary information (<https://orcid.org/0000-0002-2253-8173>).

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