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SARS-CoV-2 seroprevalence and asymptomatic viral carriage in healthcare workers: a cross-sectional study

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ABSTRACT

Objective To determine the rates of asymptomatic viral carriage and seroprevalence of SARS-CoV-2 antibodies in healthcare workers.

Design A cross-sectional study of asymptomatic healthcare workers undertaken on 24/25 April 2020.

Setting University Hospitals Birmingham NHS Foundation Trust (UHBFT), UK.

Participants 545 asymptomatic healthcare workers were recruited while at work. Participants were invited to participate via the UHBFT social media. Exclusion criteria included current symptoms consistent with COVID-19. No potential participants were excluded.

Intervention Participants volunteered a nasopharyngeal swab and a venous blood sample that were tested for SARS-CoV-2 RNA and anti-SARS-CoV-2 spike glycoprotein antibodies, respectively. Results were interpreted in the context of prior illnesses and the hospital departments in which participants worked.

Main outcome measure Proportion of participants demonstrating infection and positive SARS-CoV-2 serology.

Results The point prevalence of SARS-CoV-2 viral carriage was 2.4% (n=13/545). The overall seroprevalence of SARS-CoV-2 antibodies was 24.4% (n=126/516). Participants who reported prior symptomatic illness had higher seroprevalence (37.5% vs 17.1%, χ^2=21.1034, p<0.0001) and quantitatively greater antibody responses than those who had remained asymptomatic. Seroprevalence was greatest among those working in housekeeping (34.5%), acute medicine (33.3%) and general internal medicine (30.3%), with lower rates observed in participants working in intensive care (14.8%). BAME (Black, Asian and minority ethnic) ethnicity was associated with a significantly increased risk of seropositivity (OR: 1.92, 95% CI 1.14 to 3.23, p=0.01). Working on the intensive care unit was associated with a significantly lower risk of seropositivity compared with working in other areas of the hospital (OR: 0.28, 95% CI 0.09 to 0.78, p=0.02).

Conclusions and relevance We identify differences in the occupational risk of exposure to SARS-CoV-2 between hospital departments and confirm asymptomatic seroconversion occurs in healthcare

Key messages

What is the key question?
► What are the rates of asymptomatic viral carriage and the seroprevalence of SARS-CoV-2 antibodies in UK healthcare workers?

What is the bottom line?
► In this study, the point prevalence of SARS-CoV-2 viral carriage was 2.4% and the overall seroprevalence of SARS-CoV-2 antibodies was 24.4%.

Why read on?
► This study identifies differences in the risk of exposure of healthcare workers to SARS-CoV-2 between ethnic groups and between hospital departments; these findings may inform future infection control and occupational health policy.

workers. Further investigation of these observations is required to inform future infection control and occupational health practices.

INTRODUCTION

Healthcare workers are critical to the ongoing response to the SARS-CoV-2 pandemic. During the course of their work, they are exposed to hazards that place them at risk of infection.[1] Previous studies have shown infection rates of up to 14% in symptomatic and 7.1% in asymptomatic healthcare workers,[2 3] which are higher than general population studies reported to date and suggest an occupational risk.

Antibody responses have been demonstrated post infection with SARS-CoV-2, but it is not yet known whether these correlate with immunity, or how long antibody titres will be maintained. The magnitude of antibody responses appears proportional to age and severity of infection suffered.[4] Asymptomatic seroconversion following exposure to SARS-CoV

and SARS-CoV-2 have been documented in small cohorts; again the quality and longevity of these immunological responses are unknown.[1 5]

Understanding the relationship between infection, symptomatology and the subsequent serological responses is critical to understanding herd immunity, vaccine deployment and safeguarding the workforce. Seroprevalence studies provide the foundation to inform this understanding.

University Hospitals Birmingham NHS Foundation Trust (UHBFT) is one of the largest hospital trusts in the UK with over 20 000 employees delivering care to 2.2 million people per annum. We conducted a cross-sectional study of 554 staff at UHBFT to determine the point prevalence of infection and seroprevalence of SARS-CoV-2 antibodies in healthcare workers and their relationship to prior symptoms of COVID-19 and the hospital departments in which participants worked.

METHODS

A cross-sectional study of asymptomatic healthcare workers at UHBFT was undertaken, recruiting 545 individuals who were at work over the course of 24 hours between 24 and 25 April 2020. Initial invitation to participate in the study was made via social media. There was no predefined sample size; participants self-reported for enrolment. Individuals were excluded if they reported symptoms of COVID-19 on the day. Individuals self-isolating at home due to personal symptomatic illnesses or illnesses in household contacts in the previous 2 weeks were indirectly excluded from the study.

All individuals volunteered a nasopharyngeal swab for SARS-CoV-2 RNA detection and a venous blood sample for anti-SARS-CoV-2 spike glycoprotein serology, tested using an ELISA developed inhouse by the University of Birmingham Clinical Immunology Service. Detection of SARS-CoV-2 RNA was performed using real-time PCR (Viasure, CerTest Biotec) directed against the ORF1ab and N genes following guanidine isothiocyanate inactivation of nasopharyngeal swabs.[6] Serological analysis was performed using a high-sensitivity ELISA developed inhouse by the University of Birmingham Clinical Immunology Service. Serological analysis was performed at biological containment level 2. High-binding plates (Greiner Bio-One) were coated with trimeric SARS-CoV-2 spike glycoprotein[7 8] and blocked with StabilCoat solution (Sigma-Aldrich). Serum was prediluted 1:40 prior to analysis. A combined secondary layer containing horseradish peroxidase conjugated ovine polyclonal antibodies against IgG, IgA and IgM followed by 3,3′,5,5′-tetramethylbenzidine development was used to detect the presence of antibodies. The cut-off for positivity on the ELISA was set at 2 SD above the mean OD450 of eight pre-2019 negative sera run independently across seven separate plates.

Prior validation of this assay has shown it demonstrates 100% sensitivity in individuals with PCR-proven disease 7 days post symptom onset (n=59 hospitalised, n=31 community) and 97.8% specificity based on 270 individual negative pre-2019 samples. Intra-assay coefficient of variation (CV%) is 1.58% and interassay CV% is 7.5% for negative controls and 17.3% for positive controls and 7.2% for controls running at the cut-off of positivity on the assay.

Participants were asked to retrospectively report any illnesses consistent with COVID-19 that they had suffered in the previous 4 months. Ethnodemographic data and their department of work were also recorded. UHBFT patient mortality data were sourced from NHS England and information on the total number of PCR-positive inpatients from the UHBFT infection control team. Indices of deprivation in participants' postcodes were sourced from 2019 UK Ministry of Housing, Communities and Local Government statistics.[9]

Data were analysed using Graph Pad Prism V.8.4.2. Categorical data were compared using the χ^2 test and optical density distributions using the Kruskal-Wallis test with Dunn's post-test comparison for symptomatic and asymptomatic groups. Seroprevalence data are expressed as a percentage, with binomial CI calculated using Wilson's method. Indices of deprivation were transformed using the function [log(R)/(32 844−R)], where R represented the individual rank of a participant's postcode within the national data; these parameters were specified as deprivation scores; numerically lower values represent more deprived postcodes. Unpaired, two-tailed t-tests were used to compare the means of the seropositive and seronegative populations of these data. Using intensive care as a reference population, the relative risk (RR) of seropositivity for individuals working in other specific departments was determined, and the 95% CI for this RR was determined using Koopman's asymptotic score method.

Univariate analysis and multiple logistic regression were performed using seropositivity as the outcome variable. Age, sex, ethnicity, Index of Multiple Deprivation score and the departments in which individuals worked were included as independent variables. In univariate analysis, categorical variables were compared using two-sided χ^2 tests, and OR was calculated using the Baptista-Pike method. In this analysis, the OR represents the odds of seropositivity for an individual working in that department compared with not working in that department. In multiple logistic regression involving continuous variables, the OR represents change in odds of seropositivity changes per each increasing year of age or increasing unit of deprivation score.

All participants provided written, informed consent prior to enrolment in the study.

RESULTS

The point prevalence of PCR positivity in asymptomatic healthcare workers was 2.4% (n=13/545). Of these individuals, 15.4% (n=2/13) had detectable anti-SARS-CoV-2 antibodies in their serum and 38.4% (n=5/13) subsequently became unwell with symptoms consistent with COVID-19. Serum was available for analysis on 516 individuals, and 26.3% (n=136/516) of participants reported a prior illness consistent with COVID-19 (table 1). The overall seroprevalence across the cohort was 24.4% (n=126/516); individuals reporting a prior symptomatic illness had significantly greater seroprevalence than those who had remained asymptomatic throughout the time period assessed (36.8% vs 17.1%, χ^2=19.75, p<0.0001) (figure 1A). Antibody responses in individuals who had experienced a prior symptomatic illness were quantitatively greater than those who remained asymptomatic (Kruskal-Wallis statistics 7.159, p=0.02, Dunn's post-test comparison of symptomatic vs asymptomatic individuals: mean rank difference 17.02, adjusted p=0.02) (figure 1B).

We explored the relationship between the timing of healthcare worker illness associated with seropositivity and weekly trust-wide COVID-19 mortality, as a surrogate of overall patient burden (figure 1C). Illnesses associated with positive serology were occurring for over 3 weeks prior to UK lockdown. The temporal pattern of reported symptomatic illnesses associated with seropositivity in healthcare workers preceded that of trust-wide deaths by approximately 1 week. The highest incidence of symptomatic illness associated with seropositivity (77.8%, n=14/18) was observed in the week beginning 28 March 2020, 1 week before peak weekly mortality was reached within UHBFT.

Table 1 Demographics of study participants

	All participants, n (%)	Seropositive, n (%)	Seronegative, n (%)	Seroprevalence (%)	P value
n	516	126	390	24.4	
Age (years)	42 (30–51)	41 (30–51)	42 (31–51)		0.48
Sex					
Male	128 (24.8)	24 (19.0)	104 (26.7)	18.8	0.09*
Female	388 (75.2)	102 (81.0)	286 (73.3)	26.3	
Ethnicity					
Asian British Bangladeshi	2 (0.4)	2 (1.6)	0 (0.0)	100.0	0.18†
Asian British Indian	25 (4.8)	6 (4.8)	19 (4.9)	24.0	
Asian British other/not stated	18 (3.5)	3 (2.4)	15 (3.8)	16.7	
Asian British Pakistani	11 (2.1)	4 (3.2)	7 (1.8)	36.4	
Black African	10 (1.9)	5 (4.0)	5 (1.3)	50.0	
Black British	17 (3.3)	5 (4.0)	12 (3.1)	29.4	
Filipino	21 (4.1)	6 (4.8)	15 (3.8)	28.6	
Mixed heritage	10 (1.9)	4 (3.2)	6 (1.5)	40.0	
Not disclosed	74 (14.3)	15 (11.9)	59 (15.1)	20.3	
Other	13 (2.5)	5 (4.0)	8 (2.1)	38.5	
White Irish	5 (1.0)	1 (0.8)	4 (1.0)	20.0	
White other	25 (4.8)	4 (3.2)	21 (5.4)	16.0	
White British	285 (55.2)	66 (52.3)	219 (56.2)	23.2	

P values pertain to analysis of differences between seropositive and seronegative groups.
Median and IQR are provided.
*χ^2=16.25, df=12.
†χ^2=2.964, df=1, Z=1.722.

Seroprevalence was mapped to the departments where individuals work within UHBFT (figure 1D). Seroprevalence was highest in those working in housekeeping (34.5%, n=10/29), acute medicine (33.3%, n=10/30) and general internal medicine (30.3%, n=30/99) and lowest in participants working in intensive care (14.8%, n=9/61), emergency medicine (13.3%, n=2/15) and general surgery (13.0%, n=3/23). Using intensive care as a reference population, an increased RR of seropositivity was observed for those working in housekeeping (RR 2.34, CI 1.07 to 5.01, p=0.03), acute medicine (RR 2.25, CI 1.04 to 4.86, p=0.04) and general internal medicine (RR 2.05, CI 1.08 to 4.05, p=0.03).

Univariate and multivariate analyses were undertaken using serostatus as the dependent variable and incorporating participant age, sex, ethnicity, Index of Multiple Deprivation score of participants' postcodes and the hospital departments where participants worked as independent variables (table 2). Women had a higher seroprevalence than men (26.3% vs 18.8%), but this difference was not statistically significant in univariate or multivariate analysis (adjusted OR: 1.49, 95% CI 0.81 to 2.83, p=0.07). Univariate and multivariate analyses both demonstrated individuals of BAME (Black, Asian and minority ethnic) ethnicity were at significantly greater risk of seropositivity than individuals of white ethnicity (adjusted OR: 1.92, 95% CI 1.14 to 3.23, p=0.01). Working in intensive care medicine was associated with significantly reduced risk of seropositivity in multivariate analysis (adjusted OR: 0.28, 95% CI 0.09 to 0.78, p=0.02).

On average, the Index of Multiple Deprivation score was significantly lower in the home postcodes of BAME participants compared with white participants (−0.570 vs −0.232, t=3.747, p=0.0002). However, no significant differences between any individual government indices of deprivation were observed

between individuals who were seropositive and seronegative in this study (table 3). Furthermore, in multiple logistic regression analysis, the Index of Multiple Deprivation did not influence serostatus in this study (OR 0.99, 95% CI 0.7387 to 1.323, p=0.9503). This supports an interpretation that the observed difference in seroprevalence rates in this cohort is more likely due to occupational risk, rather than external factors.

DISCUSSION
In this cross-sectional study of asymptomatic healthcare workers, the point prevalence of SARS-CoV-2 nasopharyngeal carriage (2.4%) was concordant with a contemporaneous UK study[2] but less than an earlier study performed during the peak of the pandemic (cumulative total 14.0%).[3] The relatively low prevalence of viral RNA carriage in our cohort appears to be in keeping with the national epidemiology of the first wave of the UK SARS-CoV-2 epidemic. In contrast, we report a higher overall SARS-CoV-2 seroprevalence of 24.4%. This suggests the cumulative infection rate determined using molecular testing should have been far higher than was reported in previous studies.[2 3] This is consistent with data demonstrating the relative insensitivity of nasopharyngeal swabs in determining viral carriage,[5 10] but may also reflect access to testing. With respect to the assay used to determine seropositivity, the coefficient of variance of internal quality control material designed to run close to the clinical cut-off of the assay was 7.2%, suggesting that true seroprevalence lies between 23.8% and 26.0% based on the data from our cohort. Thus, the overall seroprevalence of SARS-CoV-2 antibodies in healthcare workers in this study is significantly greater than the 6% seroprevalence in the general population of the Midlands region determined by Public Health
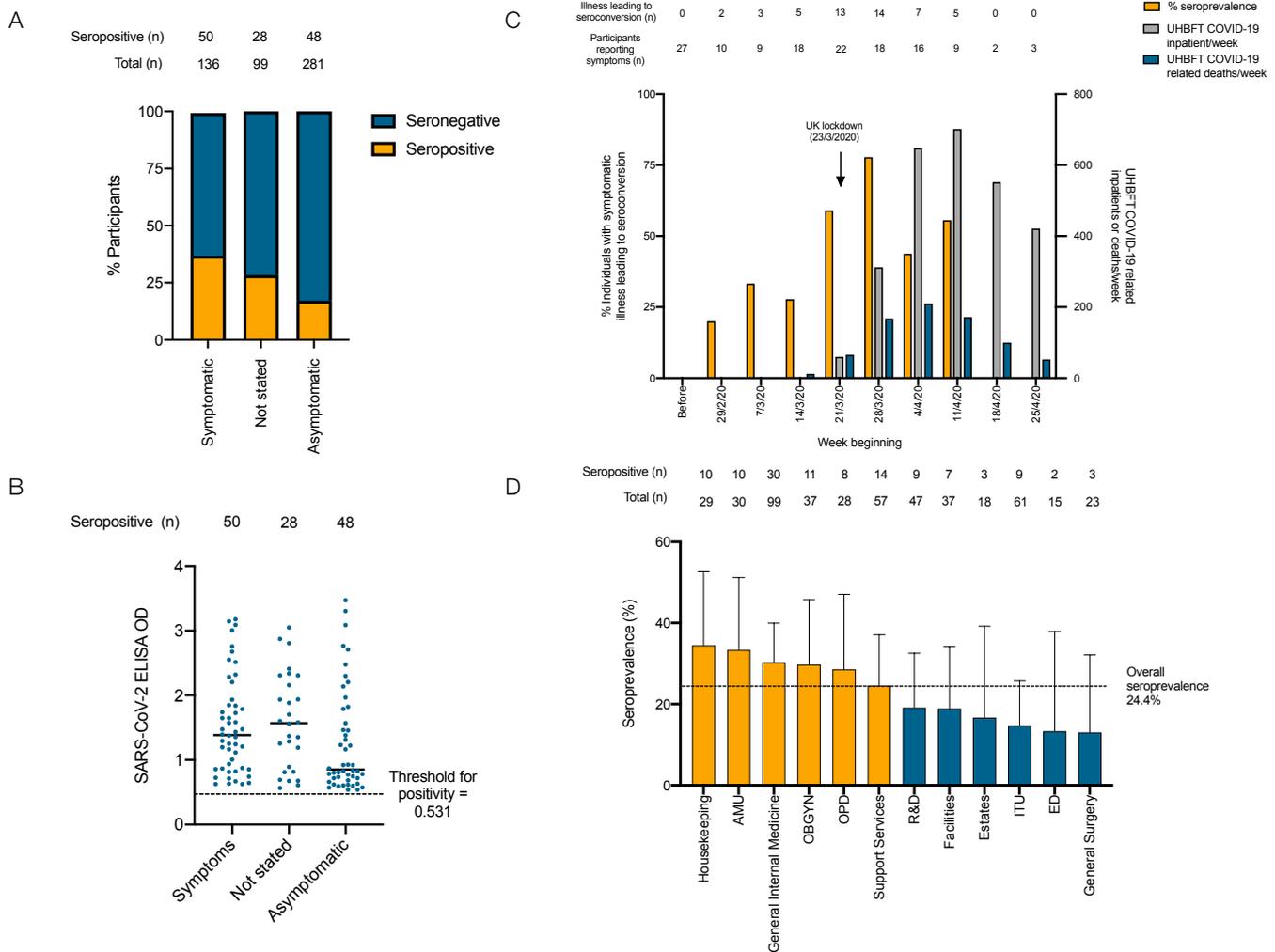
Figure 1 (A) Seroprevalence rates in study participants self-reporting prior symptomatic illnesses consistent with COVID-19 compared with asymptomatic individuals. (B) Optical density (OD) of anti-SARS-CoV-2 antibodies in individuals with positive serology classified by self-reported prior symptomatic illness (n=126). Line shows the median value of each group. (C) Timing of prior symptomatic illness in study participants and their relationship with seroprevalence of SARS-CoV-2 antibodies, total inpatients at UHBFT who had tested positive for SARS-CoV-2 by PCR and overall UHBFT-wide deaths in the weeks of March and April 2020. (D) Seroprevalence of SARS-CoV-2 antibody in study participants by department in which they work. AMU, acute medical unit; ED, emergency department; ITU, intensive care unit; OBGYN, obstetrics and gynaecology; OPD, outpatient department; R&D, research and development; UHBFT, University Hospitals Birmingham NHS Foundation Trust.

England.[11] Data from two other studies also found elevated infection or seroprevalence in healthcare workers compared with the general population.[12] [13] Collectively, these studies suggest a marked occupational risk of exposure to SARS-CoV-2 associated with healthcare work during the COVID-19 pandemic.

We identify variation in the seroprevalence of SARS-CoV-2 antibodies among different groups of healthcare workers. The highest seroprevalence was observed in housekeepers (34.5%) and those working in acute medicine (33%) or general internal medicine (30.3%), with lower seroprevalence among participants working in intensive care medicine (14.8%). Multiple logistic regression confirmed a significantly lower risk of seropositivity in individuals working in intensive care medicine. This strongly supports the conclusion that differential risk of SARS-CoV-2 exposure exists within the hospital environment. The reasons underlying this are likely to be multifactorial: in accordance with national guidelines, intensive care units were designated high-risk environments and the use of enhanced personal protective equipment (PPE) including filtered face piece (class 3) respirators mandated. In contrast, fluid-resistant

surgical masks were recommended in other clinical areas. The contribution of enhanced PPE in protecting staff from infection with SARS-CoV-2 should be studied further, including the availability of training, space and supervision to use PPE effectively. Differential occupational exposure to severe respiratory viruses was previously observed during the 2003 SARS-CoV outbreak.[14]

We demonstrate that BAME ethnicity confers a significantly increased risk of seropositivity in this study. Although individuals of BAME ethnicity within this study, on average, lived in significantly more deprived areas, the Index of Multiple Deprivation score of participants' home postcode did not significantly influence serostatus within our multiple logistic regression model. It is not clear from our study whether this increased risk of seropositivity arises from a greater risk of exposure to the virus, or a greater risk of infection if exposed to the SARS-CoV-2 virus. Regardless of the cause, this finding demands urgent further investigation, particularly in view of the ethnic disparities in the outcome from COVID-19.[15]

We demonstrate viral carriage in 2.4% of asymptomatic participants and positive SARS-CoV-2 serology in the absence

Table 2 Multiple logistic regression model incorporating seropositivity at time of study as the dependent variable and age, sex, ethnicity, Index of Multiple Deprivation score and department in which participants worked at the time of the study as independent variables

Variable	Unadjusted OR	95% CI	P value	Z	Adjusted OR	95% CI	Z	P value
Age	–	–		–	0.98	0.96 to 1.00	0.60	0.55
Sex (female)	1.54	0.94 to 2.56	0.09	1.72	1.49	0.81 to 2.83	1.79	0.07
Ethnicity (BAME)	**1.58**	**1.01 to 2.49**	**0.05**	**1.97**	**1.92**	**1.14 to 3.23**	**1.26**	**0.01**
Index of Multiple Deprivation score	–	–	–		0.99	0.74 to 1.32	2.46	0.95
Acute medicine	1.60	0.76 to 3.37	0.24	1.17	0.99	0.34 to 2.86	0.01	0.99
Emergency department	0.47	0.10 to 1.81	0.31	1.01	0.36	0.05 to 1.69	1.19	0.23
Estates	0.61	0.18 to 2.00	0.43	0.78	0.57	0.11 to 2.29	0.75	0.45
General internal medicine	1.45	0.89 to 2.32	0.13	1.52	0.93	0.42 to 2.12	0.17	0.86
General surgery	0.45	0.14 to 1.37	0.19	1.30	0.24	0.03 to 1.05	1.71	0.09
Facilities	0.71	0.29 to 1.63	0.41	0.81	0.52	0.15 to 1.60	1.10	0.45
Housekeeping	1.68	0.79 to 3.62	0.19	1.30	1.01	0.31 to 3.09	0.02	0.99
Intensive care	0.50	0.24 to 1.01	0.06	1.87	**0.28**	**0.09 to 0.78**	**2.37**	**0.02**
Obstetrics and gynaecology	1.34	0.63 to 2.71	0.44	0.78	0.85	0.30 to 2.39	0.30	0.77
Research and development	0.71	0.33 to 1.50	0.38	0.88	0.44	0.15 to 1.22	1.54	0.12

Unadjusted OR and adjusted OR following multiple logistic regression are presented. OR presented for individual hospital departments represents the odds of seropositivity for individuals working in that department compared with not working in that department. Statistically significant OR are in bold (p<0.05)
The area under the receiver operating characteristic curve of this model was 0.675 (95% CI 0.619 to 0.732, p<0.0001).
BAME, Black, Asian and minority ethnic.

of prior symptomatology in 17.1%. Using similar immunological methods, Hains *et al*[5] reported seroconversion in 44.0% (n=11/25) of healthcare workers in a US dialysis unit, including asymptomatic seroconversion. It is not known whether asymptomatic viral carriage leads to transmission in the hospital setting and it is not possible to interrogate this retrospectively. However, our data would support the assessment of widespread healthcare worker testing, including track and trace, on viral transmission during future waves of a pandemic.[16]

Finally, in keeping with previous studies that have correlated the severity of COVID-19 with the magnitude of the consequent antibody response,[4] we demonstrate that antibody responses were, on average, significantly greater in individuals with prior symptomatic illness compared with those who remained asymptomatic. Further studies must determine the neutralising capacity of antibody responses associated with different severities of disease, the titres at which neutralising antibodies provide protection against infection and the duration of that protection.

Table 3 Indices of deprivation scores associated with home postcode of study participants

Index of deprivation	Seropositive	Seronegative	P value
Index of Multiple Deprivation	−0.395 (0.89)	−0.345 (0.79)	0.58
Income	−0.352 (0.99)	−0.316 (0.80)	0.69
Employment	−0.267 (1.00)	−0.312 (0.78)	0.61
Education and skills	−0.149 (0.87)	−0.160 (0.76)	0.90
Health and disability	−0.361 (0.73)	−0.347 (0.60)	0.84
Barriers to housing and services	−0.446 (0.57)	−0.333 (0.60)	0.07
Living environment	−0.433 (0.78)	−0.444 (0.71)	0.88
Income deprivation affecting children	−0.381 (0.93)	−0.333 (0.80)	0.59
Income deprivation affecting older adults	−0.369 (0.88)	−0.274 (0.75)	0.25

Mean and SD (in parentheses) are provided. Numerically lower values represent more deprived postcodes.
Means of seropositive and seronegative groups were compared using the unpaired, two-tailed Student's t-test.

There are a number of limitations to our cross-sectional study. Participants self-presented to enrol, which may introduce bias in the study cohort; however, the balance of participants working in intensive care, acute medicine and general internal medicine represents a fair reflection of front-line staff caring for patients with COVID-19. Both acute and non-acute, non-patient-facing occupational groups were recruited to enable comparison. Data were not available to determine how representative our sampling was through comparison of the numbers recruited to individual groups with the total number of staff at work on the day of the study. By failing to capture more recent infections leading to seroconversion, this may underestimate the true seroprevalence, although this study would have captured the peak of the pandemic. The relationship between symptomatic illness and antibody positivity requires confirmation in larger studies, particularly given that 19.2% (n=99/516) of participants did not provide information about whether they had suffered a prior symptomatic illness before serological analysis was undertaken. Further studies are necessary to consider whether the increased risk of seropositivity observed within individuals of BAME ethnicity is homogeneous throughout the individual ethnic populations that collectively constitute the BAME group. Finally, longitudinal studies will be required to demonstrate the persistence of current seropositivity and to directly attribute seroconversion events to PCR-proven SARS-CoV-2 infection.

In conclusion, we document the high seroprevalence of SARS-CoV-2 antibodies in healthcare workers with and without prior symptomatic illness and identify the groups of workers who have significantly different seroprevalence, suggesting differential occupational risk.

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