Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Commentary

Association between self-reported signs and symptoms and SARS-CoV-2 antibody detection in UK key workers

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SUMMARY

Background: Screening for SARS-CoV-2 antibodies is under way in some key worker groups; how this adds to self-reported COVID-19 illness is unclear. In this study, we investigate the association between self-reported belief of COVID-19 illness and seropositivity.

Methods: Cross-sectional study of three key worker streams comprising (A) Police and Fire & Rescue (2 sites) (B) healthcare workers (1 site) and (C) healthcare workers with previously positive PCR result (5 sites). We collected self-reported signs and symptoms of COVID-19 and compared this with serology results from two SARS-CoV-2 immunoassays (Roche Elecsys® and EUROIMMUN).

Results: Between 01 and 26 June, we recruited 2847 individuals (Stream A: 1,247, Stream B: 1,546 and Stream C: 154). Amongst those without previous positive PCR tests, 687/2,579 (26%) reported belief they had COVID-19, having experienced compatible symptoms; however, only 208 (30.3%) of these were seropositive on both immunoassays. Both immunoassays had high sensitivities relative to previous PCR positivity (>93%); there was also limited decline in antibody titres up to 110 days post symptom onset. Symptomatic but seronegative individuals had differing symptom profiles and shorter illnesses than seropositive individuals.

Conclusion: Non-COVID-19 respiratory illness may have been mistaken for COVID-19 during the outbreak; laboratory testing is more specific than self-reported key worker beliefs in ascertaining past COVID-19 disease.

Introduction

After SARS-CoV-2 infection, most individuals mount antibody responses.[1–6] Serological tests aim to identify people who have

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previously been infected, through detection of anti-SARS-CoV-2 antibodies.[7] Currently, serological tests are important in helping us better understand how the disease has spread in the population, and can support pandemic planning and response.[8] In the future they may also be used for individual risk assessment; however at present, although antibody titre correlate with in vitro neutralisation[1,3] and clinical studies suggest an antibody-protection association,[9] it has not yet been proven whether presence of antibody indicates protection against future infection; as such, the usefulness of serology testing in clinical practice is currently unclear.

Early in the response to the epidemic, PCR testing was restricted to those needing hospital care. As testing provision rose, test availability was progressively extended to include health care workers, and (less extensively) to other key worker groups such as Police officers. Only in the latter stages of the first wave of infection was widespread community testing available to all who developed symptoms; initially this was restricted only to those with fever or cough. Collectively, this may have resulted in parts of the population believing they have had COVID-19 because of illness during the pandemic, some of whom may not have had the virus. However, the validity of this understandable assumption, and whether it is justified given particular symptom combinations, is poorly quantified. Although some COVID-19 symptoms, such as taste/smell disorders, are highly specific for COVID-19,[10] many others occur in other viral respiratory infections, and a recent Cochrane review has highlighted the substantial uncertainty about the value of clinical symptoms in the diagnosis of COVID-19.[11] Several studies have looked at the association between individual symptoms and seropositivity,[12,13] identifying associations with symptoms such as anosmia and ageusia and seropositivity; however, to our knowledge, no study has assessed the relationship between self-reported belief of previous COVID-19, combinations of signs and symptoms, and seropositivity.

The UK Government recently launched a mass antibody testing programme for staff in the National Health Service (NHS) and for care workers.[14] Large scale testing on historical sera has indicated a very high specificity of the assays used,[15] but concerns have been raised about a lack of data on assay performance >35 days post infection,[7] in community cases (particularly those that did not meet testing criteria early in the response), and serological responses which may be of short duration.[16]

Here we address existing uncertainties as to value of symptoms[11] in diagnosing historical COVID-19 infection. To do this, we (i) describe patterns of self reported symptoms in a cohort of key workers, (ii) describe serostatus in individuals >35 days post infection, in a cohort recruited about 2–3 months after the outbreak peak of the initial wave of COVID-19 infections in the UK, and (iii) analyse relationships between serostatus and self reported symptoms.

Materials and methods

Study population

The study population consists of various key workers, who are anticipated to be the initial users of UK government home antibody testing programme. Key workers were targeted for recruitment as part of the Evaluating Detection of SARS-CoV-2 AntiBodies at HOME (EDSAB-HOME) study (clinical trial registration: http://www.isrctn.com/ISRCTN56609224), a programme designed to evaluate the accuracy of point of care antibody tests.

Recruitment and data collection

We recruited three streams of key workers (A) Police and Fire & Rescue "Police and Fire" (B) healthcare workers "HCW" and; (C) healthcare workers who had a previous positive nasal or throat swab for SARS-CoV-2 (with or without symptoms) "HCW-PP" and therefore are confirmed to have had previous COVID-19. In this paper, our focus is on the relationship between prior signs and symptoms and seropositivity.

Prospective workplace based recruitment was conducted between 01 and 26 June 2020. Senior staff in the NHS, Police and Fire organisations were invited to a telephone conversation in which the study was described and invited to support the study. Those who agreed to take part in the study were sent an ethically approved advert by email to all staff. Individuals interested in the study were directed to an online study portal, where they completed an epidemiological questionnaire, and booked to attend a workplace clinic; availability was given on a first-come-first-served basis. At the clinic appointment, a venous blood sample was taken, which was linked back to their questionnaire via a unique study number. Further methodological details are in Supplementary Material.

Serological analysis

A volume of 6 mL EDTA anticoagulated blood was taken from each participant and sent to PHE Seroepidemiology Unit (SEU) in Manchester each day for plasma separation and sample banking. An aliquot of the samples was sent to PHE Porton Down for analysis. All samples were analysed using Roche Elecsys® Anti-SARS-CoV-2 (nucleocapsid (N))[15] and EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) assays (Spike (S) protein S1 domain).[17] Further details on sample receipt, storage and processing is available in Supplementary Material.

Manufacturer cut-offs were used to assess serological positivity (COI $\geq$ 1.0 for Roche Elecsys® and Ratio $>$ 0.8 for EUROIMMUN were considered positive) (Further details in Supplementary Material). For the purpose of assessing the relationship between clinical signs and symptoms and seropositivity and estimating seroprevalence amongst sub-cohorts, an individual was considered seropositive if they were positive on either immunoassay.

Questionnaire and categorisation of individuals

All data were collected via an online questionnaire (Snap Survey), and were stored and managed on a secure server using a relational database management system (SQL). Data were cleaned and analysed using R version 3.5.1. Participants were characterised and reported by an adapted version of the World Health Organization (WHO) criteria for confirmed, suspected and probable cases.[18] These definitions were pre-specified; further details are in Supplementary Material.

Statistical analysis

(i) To describe patterns of self reported symptoms in the cohort we: (a) performed univariable description of the cohort by age, gender, ethnicity, occupation, recent symptoms compatible with COVID-19 and previous known exposure to SARS-CoV-2 and by results on the two anti-SARS-CoV-2 immunoassays. Comparison was made with laboratory confirmed COVID-19 data for England obtained from data.coronavirus.gov.uk on 28 July 2020.

(b) To consider the relationships between different self reported symptoms, we coded symptoms as 1 (present) or 0 (not present). We computed a matrix of Pearson correlation coefficients reflecting co-existence of pairs of symptoms, both for personal symptoms and for those reported in household contacts. To illustrate patterns in this data, we performed hierarchical clustering of the correlation matrix using Euclidean distance metric and central cluster-

Table 1
Demographics and exposure characteristics, split by recruitment group

	Stream A: Police and Fire	Stream B: Healthcare workers (HCW)	Stream C: HCW Previously Positive (HCW-PP)	Total
Age				
18 – 25	37 (3.2%)	89 (5.8%)	13 (8.4%)	139 (4.9%)
25 – 40	420 (36.6%)	586 (37.9%)	62 (40.3%)	1068 (37.5%)
40 – 60	660 (57.5%)	747 (48.3%)	71 (46.1%)	1478 (51.9%)
60+	30 (2.6%)	124 (8.0%)	8 (5.2%)	162 (5.7%)
Sex				
Female	455 (39.7%)	1247 (80.7%)	126 (81.8%)	1828 (64.2%)
Male	692 (60.3%)	299 (19.3%)	28 (18.2%)	1019 (35.8%)
Ethnicity				
White	1085 (94.6%)	1128 (73.0%)	137 (89.0%)	2350 (82.5%)
Asian or British Asian	33 (2.9%)	237 (15.3%)	11 (7.1%)	281 (9.9%)
Black or Black British	2 (0.2%)	96 (6.2%)	1 (0.6%)	99 (3.5%)
Mixed	21 (1.8%)	42 (2.7%)	3 (1.9%)	66 (2.3%)
Other	6 (0.5%)	43 (2.8%)	2 (1.3%)	51 (1.8%)
Occupation				
First responder – police	528 (46.0%)	0 (0.0%)	0 (0.0%)	528 (18.5%)
First responder – fire & rescue	238 (20.7%)	0 (0.0%)	0 (0.0%)	238 (8.4%)
First responder – other (e.g. ambulance)	40 (3.5%)	1 (0.1%)	0 (0.0%)	41 (1.4%)
Hospital doctor	0 (0.0%)	245 (15.8%)	41 (26.6%)	286 (10.0%)
Hospital nurse	1 (0.1%)	471 (30.5%)	51 (33.1%)	523 (18.4%)
Hospital medical other	0 (0.0%)	249 (16.1%)	18 (11.7%)	267 (9.4%)
Hospital non-medical	0 (0.0%)	158 (10.2%)	9 (5.8%)	167 (5.9%)
Hospital lab based	0 (0.0%)	35 (2.3%)	1 (0.6%)	36 (1.3%)
GP doctor/nurse/other	0 (0.0%)	20 (1.3%)	4 (2.6%)	24 (0.8%)
Community nurse	0 (0.0%)	36 (2.3%)	9 (5.8%)	45 (1.6%)
NHS staff other	0 (0.0%)	309 (20.0%)	20 (13.0%)	329 (11.6%)
Other	340 (29.6%)	22 (1.4%)	1 (0.6%)	363 (12.8%)
Interacted face-to-face with patients and/or general public during lockdown (post-23 March)				
More frequently than before lockdown	24 (2.1%)	161 (10.4%)	13 (8.4%)	198 (7.0%)
Similar to before lockdown	494 (43.1%)	671 (43.4%)	91 (59.1%)	1256 (44.1%)
Less frequently than before lockdown	418 (36.4%)	564 (36.5%)	40 (26.0%)	1022 (35.9%)
No such interactions	39 (3.4%)	28 (1.8%)	2 (1.3%)	69 (2.4%)
Non-response	172 (15.0%)	122 (7.9%)	8 (5.2%)	302 (10.6%)
Do you think you have had previous COVID-19?				
Yes, I had symptoms but was not tested	225 (19.6%)	301 (19.5%)	0 (0.0%)	526 (18.5%)
Yes, I had symptoms but my test(s) were all negative	41 (3.6%)	112 (7.2%)	0 (0.0%)	153 (5.4%)
Yes, I had symptoms, and I had at least one positive test	24 (2.1%)	80 (5.2%)	152 (98.7%)	256 (9.0%)
Yes, I had symptoms, had a test, but it failed	5 (0.4%)	3 (0.2%)	0 (0.0%)	8 (0.3%)
I did not recognise that I had symptoms, but I tested positive when I was screened	0 (0.0%)	10 (0.6%)	2 (1.3%)	12 (0.4%)
No	557 (48.6%)	648 (41.9%)	0 (0.0%)	1205 (42.3%)
Unsure	295 (25.7%)	392 (25.4%)	0 (0.0%)	687 (24.1%)
Length of symptoms*				
Less than 7 days	115 (39.1%)	178 (36.1%)	30 (19.7%)	323 (34.4%)
7 -14 days	121 (41.2%)	185 (37.5%)	63 (41.4%)	369 (39.3%)
14 – 21 days	27 (9.2%)	66 (13.4%)	27 (17.8%)	120 (12.8%)
More than 21 days	28 (9.5%)	58 (11.8%)	30 (19.7%)	116 (12.4%)
Do not know	3 (1.0%)	6 (1.2%)	2 (1.3%)	11 (1.2%)
Unable to work (in workplace or at home) due to symptoms*				
Yes	191 (65.0%)	350 (71.0%)	144 (94.7%)	685 (72.9%)
No	103 (35.0%)	143 (29.0%)	8 (5.3%)	254 (27.1%)
Went to hospital due to suspected/confirmed COVID-19*				
Yes	4 (1.4%)	19 (3.9%)	15 (9.9%)	901 (96.0%)
No	290 (98.6%)	474 (96.1%)	137 (90.1%)	38 (4.0%)
Was in contact with a suspected or confirmed case in the 14-day prior to symptom onset*				
Yes, confirmed	23 (7.8%)	153 (31.0%)	92 (60.5%)	485 (51.7%)
Yes, suspected	47 (16.0%)	113 (22.9%)	26 (17.1%)	268 (28.5%)
No/Unsure	224 (76.2%)	227 (46.0%)	34 (22.4%)	186 (19.8%)
Has had a SARS-CoV-2 antibody test, and been informed of the result prior to recruitment				
Yes	4 (0.3%)	20 (1.3%)	68 (44.2%)	92 (3.2%)
No	1143 (99.7%)	1523 (98.5%)	86 (55.8%)	2752 (96.7%)
Did not answer	0 (0.0%)	3 (0.2%)	0 (0.0%)	3 (0.1%)
Had a household member who had COVID-19 compatible symptoms				
Yes	289 (25.2%)	396 (25.6%)	83 (53.9%)	768 (27.0%)
No	858 (74.8%)	1150 (74.4%)	71 (46.1%)	2079 (73.0%)
Had a household member who had SARS-CoV-2 positive nasal or throat swab				
Yes	21 (1.8%)	44 (2.8%)	32 (20.8%)	97 (3.4%)
No	1126 (98.2%)	1502 (97.2%)	122 (79.2%)	2750 (96.6%)
WHO criteria				
Confirmed (had a positive nasal or throat swab)	24 (2.1%)	90 (5.8%)	154 (100.0%)	268 (9.4%)
Suspected	158 (13.8%)	238 (15.4%)	0 (0.0%)	396 (13.9%)
Early-probable	72 (6.3%)	73 (4.7%)	0 (0.0%)	145 (5.1%)
Uncertain	40 (3.5%)	105 (6.8%)	0 (0.0%)	145 (5.1%)
No	853 (74.4%)	1040 (67.3%)	0 (0.0%)	1893 (66.5%)
Total	**1147**	**1546**	**154**	**2847**

* for only those who self-reported COVID-19 compatible symptoms. In addition, variables where the participant did not respond (NAs) amongst those who reported symptoms were not included (1 in Police and Fire and 3 in HCW). Final total was 939, split across Police and Fire (n=295), HCW (n=496) and HCW-PP (n=152).

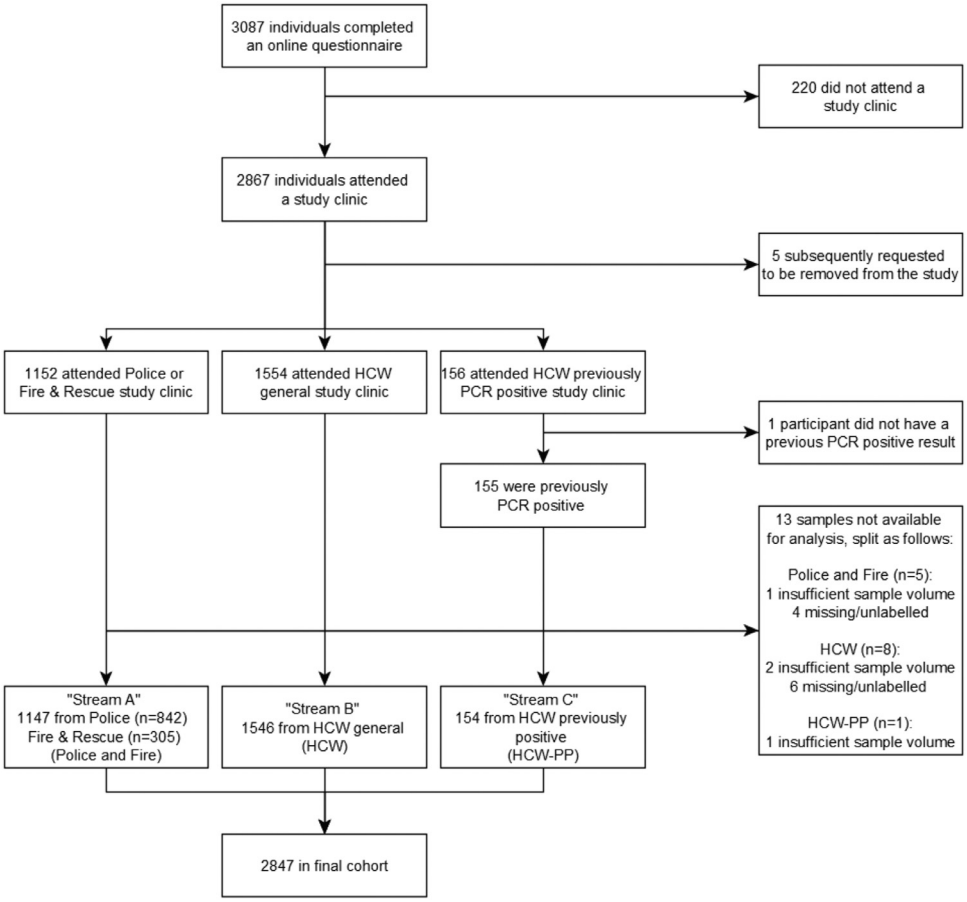

Figure 1. Recruitment and sample analysis flow diagram.

ing.[19] and depicted the reassorted matrix as a heatmap. We used the R *hclust* and *corrplot* functions to achieve this.

(ii) To describe the serostatus of individuals, we (a) tabulated associations with single clinical risk factors and serostatus; (b) used Spearman's rank correlation coefficient to assess the concordance between the two laboratory immunoassay performed; (c) explored the relationship between symptom onset and quantitative serological response graphically and modelled relationships using linear quantile regression (implemented in the R *quantreg* package) to test for evidence for a change over time; and (d) assessed the relationship between duration of symptoms (described as a categorical variable) and antibody assay signal (a continuous variable) using Kendall's correlation coefficient.

(iii) To describe relationships between serostatus and self reported symptoms, we (a) computed unadjusted odds ratios (ORs) with 95% confidence intervals (95% CIs) favouring seropositivity given various clinical risk factors for those in Police and Fire and HCW groups using binary logistic regression, and (b) estimated sensitivity of the immunoassays as the proportion of confirmed cases (individuals who had a previously PCR-positive nasal or throat swab) with detectable SARS-CoV-2 antibodies.

Results

Recruitment

Recruitment opened on 27 May 2020, and study clinics ran from 1 June to 26 June 2020 in two non-healthcare worker sites (one Police and one Fire & Rescue) and in six NHS acute hospitals in England (Supplementary Figure 1). 3087 individuals completed a questionnaire, of whom 2867 (93%) attended a clinic appointment and were successfully enrolled into the study. We excluded individuals due to non-eligibility ($n = 1$, who was recruited in "Stream C" however did not have a previously positive PCR result), technical issues (e.g. insufficient sample) preventing blood samples being analysed ($n = 14$), and withdrawals from the study after recruitment ($n = 5$). There were no test failures on either immunoassay. The final cohort contained 2847 individuals: 1147 from Police and Fire (Stream A); 1546 health care workers (HCW) (Stream B); and 154 from the healthcare worker previously COVID-19 positive test group (HCW-PP) (Stream C) (Figure 1).

Cohort description

Of the 2847 individuals, 36% were male and 64% were female. Their ages ranged from 19 to 73 years with a median age of 43 (Table 1, Supplementary Figure 2). Ethnicity was majority white (83%), followed by 10% Asian, 3% Black, 2% Mixed and 2% Other. Overall 44% reported face to face interactions during lockdown with clients/patients at a similar frequency to pre-lockdown. At the time the questionnaire was administered, only 3% had a COVID-19 antibody test and had been informed of the result. All the HCW-PP group had (by definition) a previously positive SARS-CoV-2 PCR result; by contrast, only 2% of the Police and Fire group and 6% of the HCW group had a prior PCR positive result.

Amongst the 2579 individuals without any prior PCR positivity, 27% ($n = 687$) reported believing they had COVID-19 due to compatible symptoms (Table S1). By group, 29% (416/1456) in HCW and 24% (271/1123) in Police and Fire groups reported such a belief.

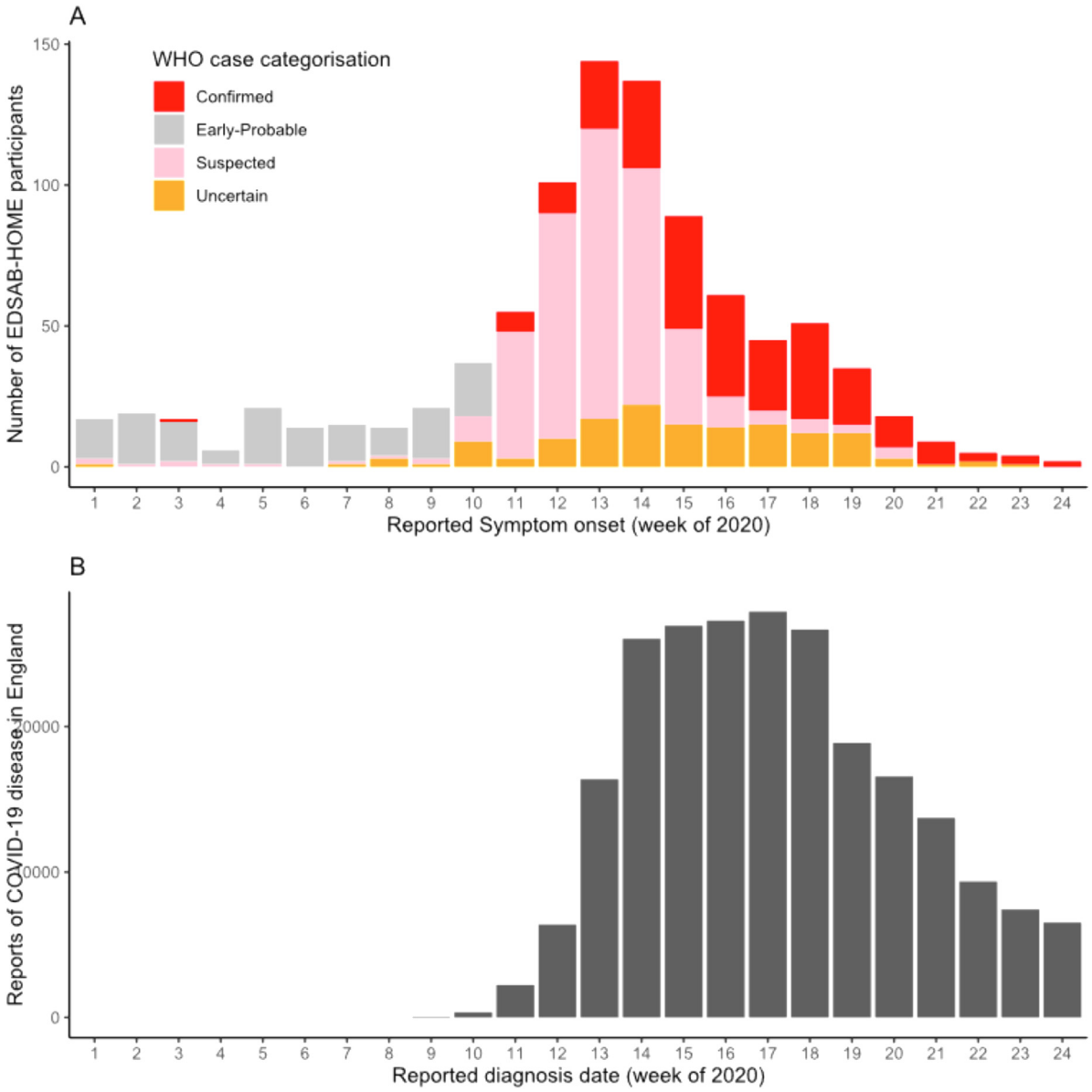

Figure 2. Epidemiological curve for participants reporting symptoms.

Amongst individuals who reported symptoms, the median time since symptoms onset to the EDSAB-HOME recruitment visit was 75 days (IQR 63 – 92 days), which was on average 12 days longer than the time since symptoms amongst the known previous PCR positives (median 63 days). In this cohort, hospitalisation due to COVID-19 like illness was unusual, being reported in 1% and 4% of Fire & Police and HCW groups, respectively (Table 1).

Symptom correlations

Some symptoms were commonly reported together (Figure 3). For example, altered sense of taste (ageusia) and smell (anosmia) were commonly co-reported; a symptom cluster of breathlessness, cough, muscle aching, fever and fatigue was also evident. Reporting symptoms in both these clusters was strongly associated with both belief that one had previously had COVID-19, and with seropositivity. Much weaker associations with seropositivity and with belief in prior COVID-19 infection were observed with other symptom clusters identified, and with clusters of symptoms reported in household members.

Seropositivity and its relationship to belief in past COVID-19 infection

Seropositivity was more common amongst those who reported having had COVID-19 compatible symptoms: amongst those who self-reported COVID-19 compatible symptoms, 28% in Police and Fire and 50% of HCW were seropositive compared with 5% and 11% of those who did not report such symptoms (Table 2). Therefore, self-belief of having had COVID-19 was associated with seropositivity, in both the Police and Fire (OR 7.5, 95% CI 5.0–11.3) and in HCW (OR 7.9, 95% 6.1–10.3). However, of those seropositive across both groups, only 68% thought they had COVID-19 (equating to 32% of the seropositives having had asymptomatic COVID-19).

Seropositivity and reported symptoms

Our study detected seropositivity as associated with several known clinical risk factors (Table 2, Figure 3, Supplementary Figure 3). The association varied for individual symptoms and seropositivity, such as altered sense of smell (OR 19.5, 95% CI 14.3–26.9) and

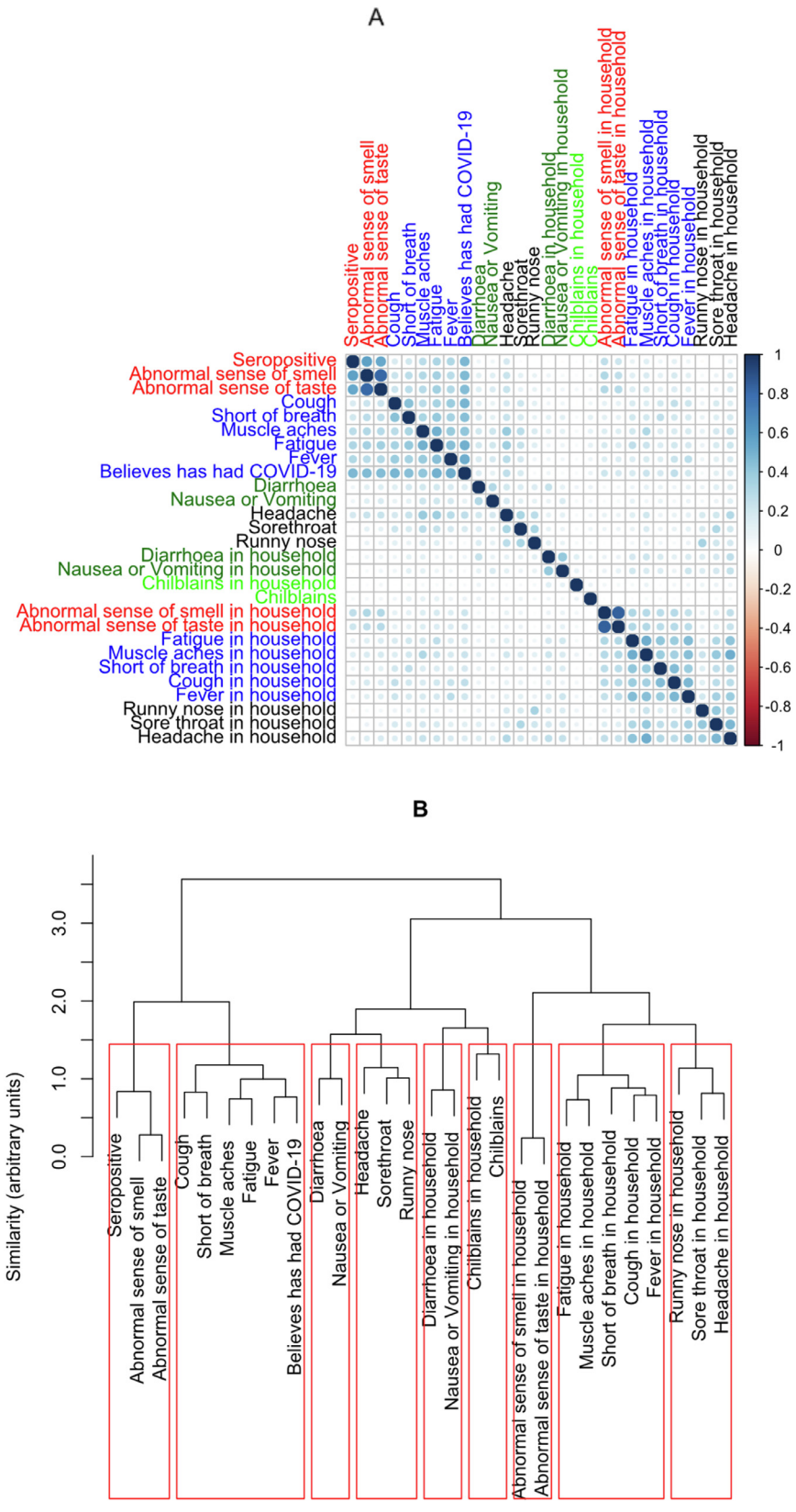

Figure 3. Symptoms and symptom clusters as reported by participants.

Table 2
Seropositivity by recruitment group for clinical risk factors*

	Police and Fire Number and percentage of individuals who are seropositive N (%)	OR	HCW Number and percentage of individuals who are seropositive N (%)	OR
Belief of previously having had COVID-19				
Yes	81 (27.5%)	7.5 (5.0-11.3)	245 (49.4%)	7.9 (6.1-10.3)
No	41 (4.8%)	ref	115 (11.0%)	ref
Length of symptoms				
Less than 7 days	42 (9.5%)	ref	113 (19.4%)	ref
7 -14 days	47 (21.9%)	2.7 (1.7-4.2)	118 (40.1%)	2.8 (2.0-3.8)
14 – 21 days	12 (22.2%)	2.7 (1.3-5.4)	37 (41.1%)	2.9 (1.8-4.6)
More than 21 days	12 (19.4%)	2.3 (1.1-4.5)	41 (39.4%)	2.7 (1.7-4.2)
Unable to work (in workplace or at home) due to symptoms				
Yes	64 (23.3%)	3.0 (2.0-4.5)	208 (41.7%)	3.0 (2.3-3.9)
No	52 (9.2%)	ref	125 (19.2%)	ref
Went to hospital due to suspected/confirmed COVID-19				
Yes	3 (75.0%)	19.2 (2.4-390)	16 (76.2%)	8.2 (3.2-25.2)
No	113 (13.5%)	ref	317 (28.1%)	ref
Was in contact with a suspected or confirmed case in the 14-day prior to symptom onset				
Yes	26 (24.5%)	2.3 (1.4-3.8)	200 (47.4%)	4.0 (3.1-5.3)
No/Unsure	90 (12.2%)	ref	133 (18.3%)	ref
Had a household member who had COVID-19 compatible symptoms				
Yes	62 (21.5%)	3.6 (2.5-5.3)	140 (35.4%)	2.3 (1.8-3.0)
No	60 (7.0%)	ref	220 (19.1%)	ref
Had a household member who had SARS-CoV-2 positive nasal or throat swab				
Yes	8 (38.1%)	5.5 (2.1-13.2)	26 (59.1%)	5.1 (2.8-9.5)
No	114 (10.1%)	ref	334 (22.2%)	ref
WHO criteria				
Confirmed (had a positive nasal or throat swab)	23 (95.8%)	456 (92.5-8250)	87 (96.7%)	253 (92.7-1042)
Suspected	52 (32.9%)	9.8 (6.2-15.4)	130 (54.6%)	10.5 (7.6-14.6)
Early-probable	3 (4.2%)	0.9 (0.2-2.5)	12 (16.4%)	1.8 (0.9-3.2)
Uncertain	3 (7.5%)	1.6 (0.4-4.7)	24 (22.9%)	2.6 (1.6-4.2)
No	41 (4.8%)	ref	107 (10.3%)	ref
Symptom				
A new continuous cough Yes	52 (16.4%)	2.1 (1.4-3.1)	144 (34.5%)	2.2 (1.7-2.9)
No	70 (8.4%)		216 (19.1%)	
Fever (or high temperature) Yes	53 (22.3%)	3.5 (2.4-5.2)	191 (45.6%)	4.8 (3.7-6.2)
No	69 (7.6%)		169 (15.0%)	
Shortness of breath Yes	45 (18.0%)	2.3 (1.6-3.5)	115 (35.2%)	2.2 (1.7-2.8)
No	77 (8.6%)		245 (20.1%)	
Sore throat Yes	45 (10.4%)	1.0 (0.6-1.4)	139 (22.8%)	1.0 (0.8-1.2)
No	77 (10.8%)		221 (23.6%)	
Runny nose Yes	28 (8.3%)	0.7 (0.4-1.1)	101 (25.2%)	1.2 (0.9-1.5)
No	94 (11.6%)		259 (22.6%)	
Headache Yes	64 (13.4%)	1.6 (1.1-2.4)	220 (30.6%)	2.2 (1.7-2.8)
No	58 (8.7%)		140 (16.9%)	
Muscle aches Yes	66 (19.4%)	3.2 (2.2-4.7)	209 (36.3%)	3.1 (2.4-3.9)
No	56 (6.9%)		151 (15.6%)	
Altered sense of smell Yes	67 (50.4%)	17.7 (11.5-27.5)	202 (73.5%)	19.5 (14.3-26.9)
No	55 (5.4%)		158 (12.4%)	
Altered sense of taste Yes	62 (43.4%)	12.0 (7.9-18.4)	206 (70.1%)	16.7 (12.4-22.7)
No	60 (6.0%)		154 (12.3%)	
Extreme fatigue Yes	63 (21.4%)	3.7 (2.5-5.4)	196 (38.7%)	3.4 (2.6-4.3)
No	59 (6.9%)		164 (15.8%)	
Diarrhoea Yes	16 (9.8%)	0.9 (0.5-1.5)	66 (33.3%)	1.8 (1.3-2.5)
No	106 (10.8%)		294 (21.8%)	
Nausea/Vomiting Yes	6 (6.9%)	0.6 (0.2-1.3)	46 (35.1%)	1.9 (1.3-2.8)
No	116 (10.9%)		314 (22.2%)	
Small itchy red patches on fingers/toes Yes	3 (9.7%)	0.9 (0.2-2.6)	10 (23.8%)	1.0 (0.5-2.0)
No	119 (10.7%)		350 (23.3%)	
Total	**1147**		**1546**	

* variables where the participant did not respond (NAs) have been excludedRef = reference group.

taste (OR 16.7, 95% CI 12.4–22.7), and new continuous cough (OR 2.2, 95% CI 1.7–2.9).

We also noted that date of symptom onset (which related to WHO classification) was associated with likelihood of seropositivity: while 46% of the "suspected" (symptoms after 5 March) group were seropositive, only 10% of "early-probable" (symptoms up to 5 March, which predate the national outbreak) were seropositive (Figure S3). Of the "uncertain" group, 19% were seropositive, while 8% were seropositive in those reporting no symptoms (Table 2).

Overall, amongst those who reported a belief of having had COVID-19, characteristics of symptoms reported by Police and Fire and HCW differed compared to HCW-PP. For example, a higher proportion reported symptoms fewer than 7 days (39%, 36%, 20% respectively) or had a household member with COVID-19 compatible symptoms (25%, 26%, 53% respectively) (Table 1). Thus, we observed that those reporting symptoms who were seronegative differed in multiple respects from those who reported symptoms and were seropositive.

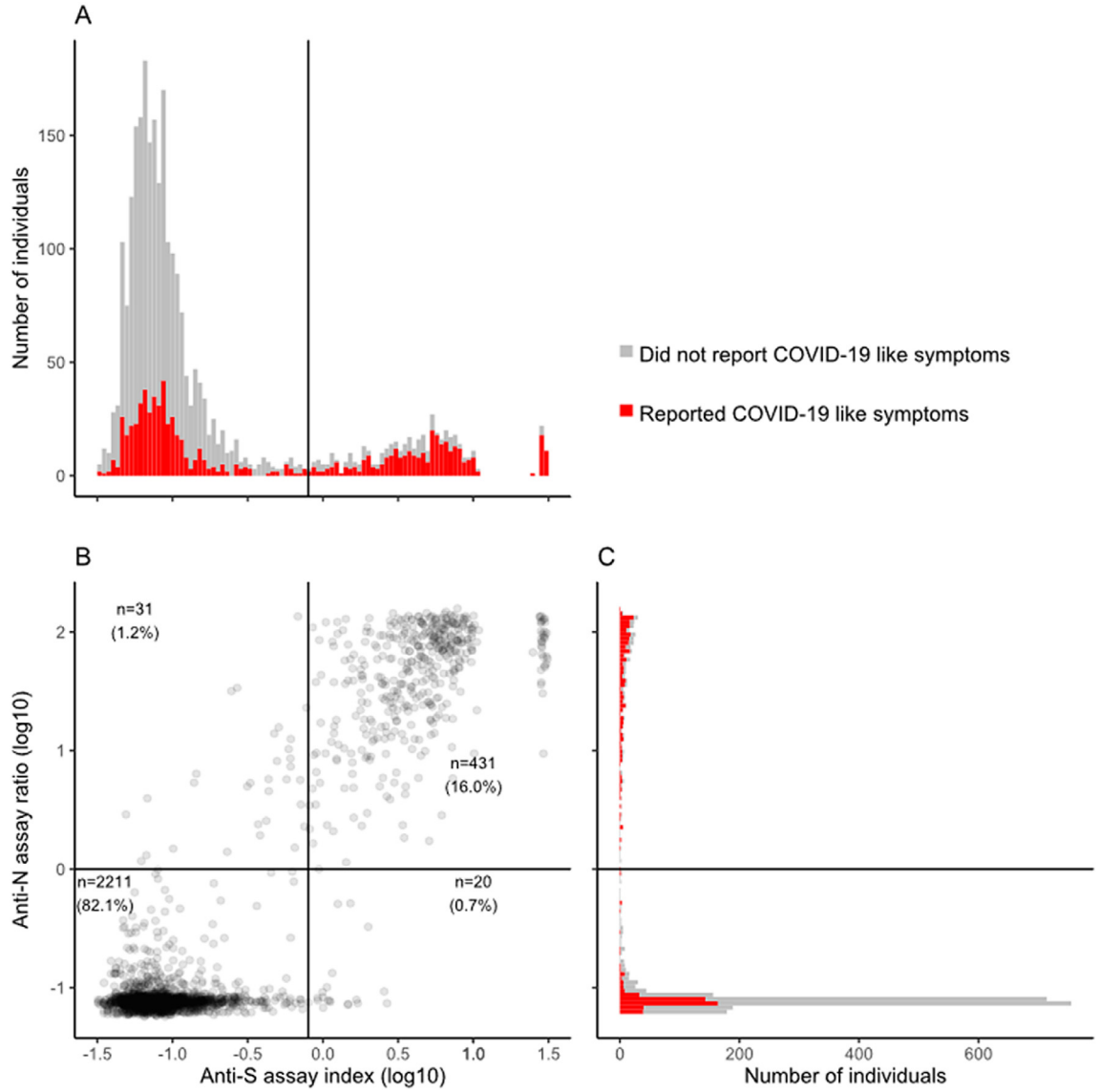

Figure 4. Serological distributions results of two different SARS-CoV-2 immunoassays in individuals from Fire and Police and Healthcare worker cohorts.

Amongst individuals not reporting any previous COVID-19 compatible symptoms (or any previous PCR test), 158 (8%) were seropositive. Amongst these, 81% reported that no household member (or did not know) had any COVID-19 compatible symptoms; together, these data highlight the potential of serology to identify (albeit to an unknown degree), previously unidentified asymptomatic SARS-CoV-2 infection.

Serological assay performance

One explanation for the large numbers of symptomatic individuals with negative serology might be the sensitivity of the assays.[7] In our study, anti-Spike (EUROIMMUN) (Anti-S1) indices and Anti-Nucleocapsid (Roche Elecsys®) (Anti-N) protein assay ratios showed a strong positive correlation ($r = 0.93$, $p < 0.001$) and each had a clear bimodal distribution within streams A and B (Figure 4).

There were in total 268 individuals who had previous PCR test positive infection (all 154 from HCW-PP C, plus 24 from Police and Fire and 90 from HCW) (Figure 5, Supplementary Table 1). The majority were white ($n = 212$, 79%) and female ($n = 188$, 70%). 4% ($n = 12$) had been identified through screening and were asymptomatic at time of swab. 11% had been hospitalised, and all were on average 63 days (IQR 52 – 75 days) post symptom onset. 65% had illness lasting 2 weeks or less (Supplementary Material). Based on these 268 individuals, the sensitivity of the anti-S1 and anti-N assays was 93.3% (95% CI 89.6% - 95.7%) and 96.6% (95% CI 93.7% - 98.2%), respectively. The composite sensitivity (i.e. proportion of positive on at least one assay) was 98.1% (95% CI 95.7% - 99.2%). Of the 12 individuals who had been asymptomatic when they had their positive PCR test, 8 were seropositive across both assays (Additionally, 1 was positive on EUROIMMUN only, while 3 were negative across both).

Do antibody assay signals decline?

There is weak statistical evidence for higher anti-S antibody signals in individuals with longer illness duration ($p = 0.06$; for anti-N, $p = 0.16$, Figure 6A, C). There is also evidence for a decline over time for anti-S antibody signals with symptom onset to blood sample intervals of between 35 and 110 days, intervals chosen as they include 87% of symptomatic known previous PCR positive cases ($n = 233$). Regression models estimate a decline per month of 29.1% (95% CI 3.1% to 36.7%) (Figure 6B), with similar results in models adjusting for initial illness duration (not shown). There was a

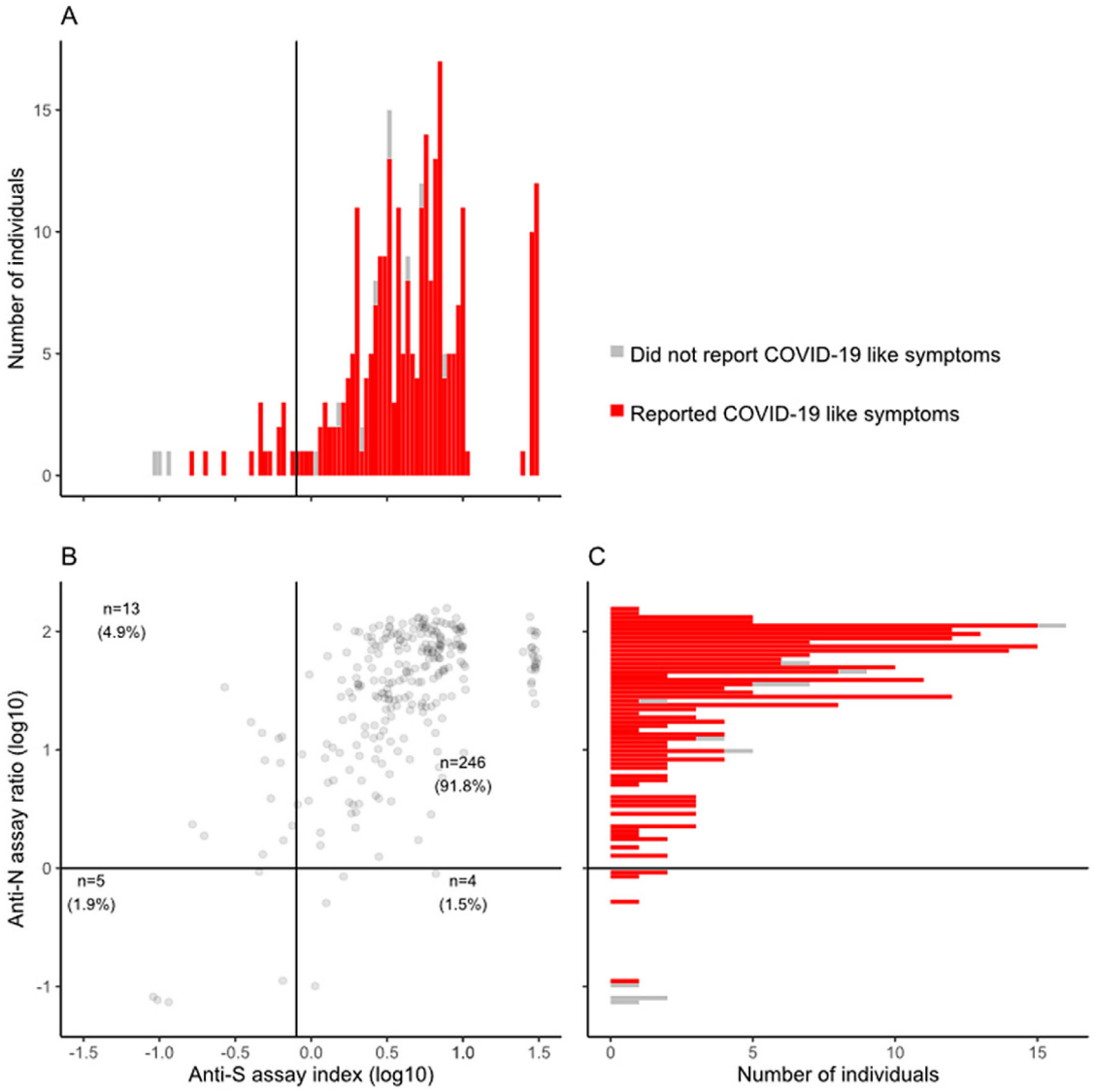

Figure 5. Serological distributions results of two different SARS-CoV-2 immunoassays in individuals with previous PCR positivity only (WHO defined "confirmed" cases).

similar trend in anti-N signals (with an estimated 18.7% decline per month, 95% CI −26.2% to 36.9%), however this was not statistically significant (Figure 6D). Over the time period studied, this decline has minimal impact on the serological detection of individuals with microbiologically confirmed COVID-19 disease (Supplementary Table 2). In summary, neither serological assay sensitivity, nor declining antibody titres, appear to provide an explanation for approximately half of symptomatic individuals who are antibody negative in our cohort.

Discussion

In summary, in our study of key workers, amongst those without prior positive PCR tests, 27% believed they had previously had COVID-19 due to compatible symptoms, but of these, around half lacked any serological evidence of having had the infection. Amongst individuals who had previously had symptoms, those who were seronegative reported earlier dates of symptom onset, shorter duration of symptoms and were less likely to report high specificity COVID-19 symptoms of ageusia and anosmia.

The high proportion of individuals reporting belief they had had COVID-19 but who were seronegative did not appear to be readily explicable by low serological assay sensitivity or rapidly declining antibody titres. We estimated that both immunoassays used had high sensitivity (93.3%, CI 89.6% - 95.7%; and 96.7%, CI 93.7% - 98.2%, for EUROIMMUN and Roche Elecsys® assays, respectively) in the key worker populations studied, who were on average 63 days post PCR-confirmed infection. This is despite many of these individuals having had relatively mild disease: amongst the 268 previously PCR positive individuals studied, only about 10% had been hospitalised.

A limitation is the retrospective collection of symptoms histories, and the potential for recall bias. However, about 97% of the cohort provided symptom data naïve to their serological status, which may have reduced the potential for ascertainment bias. Additionally, we note that (perhaps due to interest in COVID-19 during the ongoing pandemic, and suspected COVID-19 being a memorable event) retrospective symptom collection yielded symptom onsets which closely mirror the UK outbreak amongst seropositive (but not seronegative) cases. We also noted that the reporting of symptoms known to be strongly predictive of COVID-19, such as combinations of altered taste and smell,[10] is strongly associated with serostatus, supporting the validity of symptom self reporting. In addition, several other self reported factors which are known to

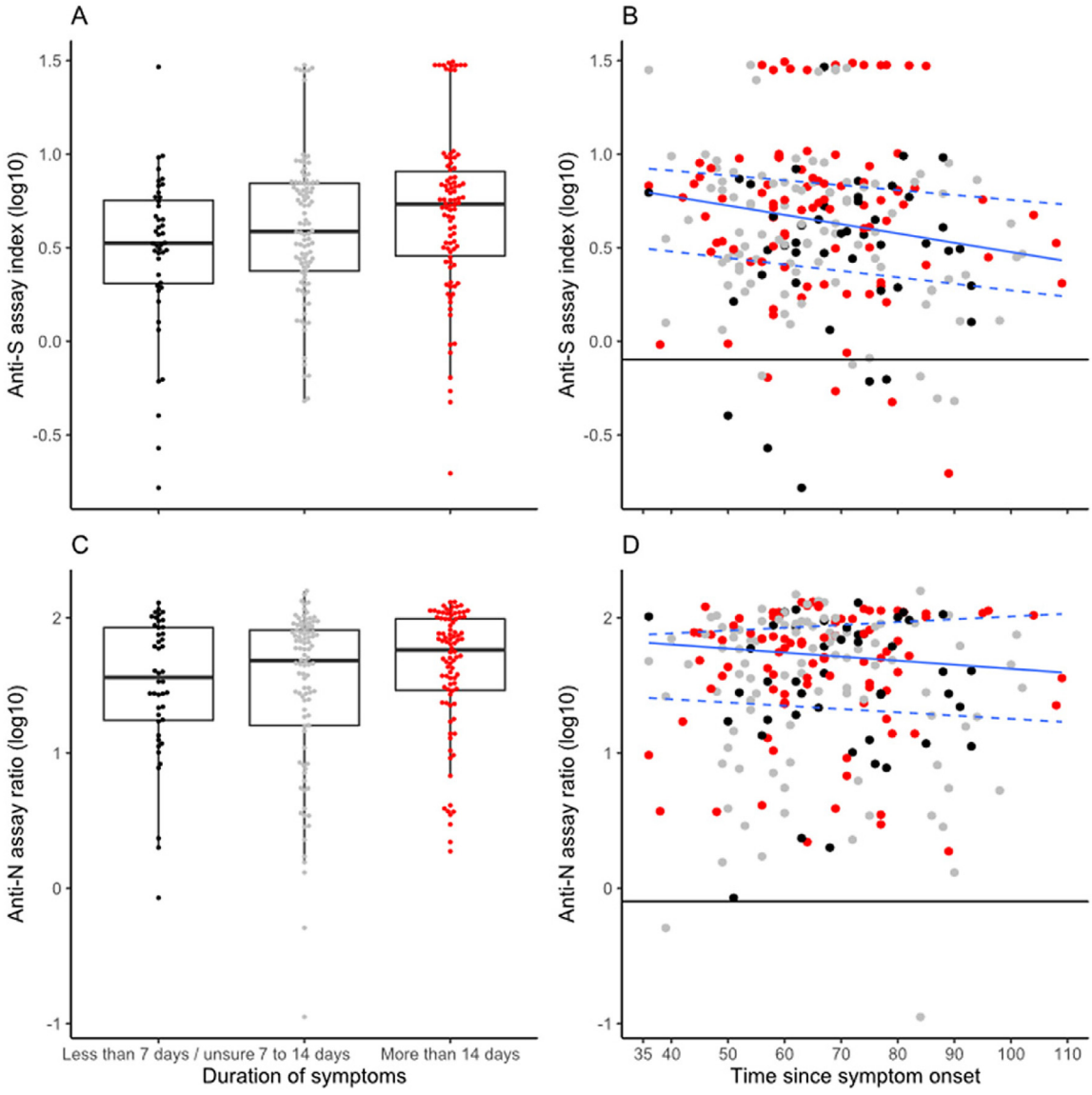

Figure 6. Antibody responses, illness duration and interval post illness.

increase likelihood of infection, such as having had contact with a confirmed/suspected COVID-19 case and having a household member who had a positive swab were also associated with seropositivity.

Comparing the data obtained with published data, the test sensitivity estimates we derive are compatible with published results for individuals with mild disease (and who had met the threshold for testing),[20,5] but are higher than those reported by laboratory assessments using smaller panels with few individuals > 21 days post infection,[15,17] likely due to the increased time since symptom onset (median 63 days in this cohort) relative to that in previous evaluations. This extended interval has allowed, both antibody concentrations, and perhaps also affinity of antibodies for SARS-CoV-2[21,22] to rise over time. Our results are also congruent with reports from anti-Spike S1 Receptor binding domain assays,[23] and with neutralisation assays,[16] in that we observed anti-S titres decline over time post infection. We did not observe such significant declines in anti-N immunoassay signals over the time interval studied, but cannot exclude this happening; nevertheless, we can extend the existing literature by showing that these declines have minimal impact on assay performance up to 110 days post infection in symptomatic key workers.

We caution against generalisation to antibody kinetics in other populations, however: the front line key workers we studied, all of whom worked during the pandemic, may have been re-exposed to SARS-CoV-2 during their work, and may have been serially boosted by re-exposure to virus leading to sustained antibody responses, as noted with other viral pathogens.[24]

In conclusion, this study indicates that a substantial proportion of key workers believe that they have had COVID-19 based on symptoms experienced during the first wave of the pandemic in the UK, but had no antibodies detectable a median of 63 days since symptom onset, despite high immunoassay specificity and limited reduction of antibody titres over the study period.

Ethical statement

The study was approved by NHS Research Ethics Committee (Health Research Authority, IRAS 284,980) on 02/06/2020 and PHE Research Ethics and Governance Group (REGG, NR0198) on 21/05/2020.

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Author contributions

Study design - RM, STP, HEJ, AEA, TB, AC, MH, IO, DW
Data collection – RM, DW, PDK
Study site coordination - RS, PM, JB, AH, NT, AC, IR
Processed and analysed immunoassay results - RB, EL, TB
Statistical analysis and expertise – RM, DW, STP, HEJ, TA
Drafted manuscript – RM, DW
Data interpretation – RM, STP, HEJ, AEA, MH, IO, DW
All authors critically reviewed the final paper and agree to be accountable for all aspects of the work and approved the final version for publication.

Declaration of Competing Interest

The authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.03.019.

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