Lack of antibodies to SARS-CoV-2 in a large cohort of previously infected persons

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Summary: Our data suggest that a sizable proportion of persons do not have IgG antibodies following SARS-CoV-2 infection, which independently relates to illness severity, race/ethnicity, obesity, and immunosuppressive medication. These factors should be considered when interpreting results of serologic testing.

Abstract

Background Reports suggest that some persons previously infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lack detectable IgG antibodies. We aimed to determine the proportion IgG seronegative and predictors for seronegativity among persons previously infected with SARS-CoV-2.

Methods We analyzed serologic data collected from health care workers and first responders in New York City and the Detroit metropolitan area with history of a positive SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) test result and who were tested for IgG antibodies to SARS-CoV-2 spike protein at least 2 weeks after symptom onset.

Results Of 2,547 persons with previous confirmed SARS-CoV-2 infection, 160 (6.3%) were seronegative. Of 2,112 previously symptomatic persons, the proportion seronegative slightly increased from 14 to 90 days post symptom onset (p=0.06). The proportion seronegative ranged from 0% among 79 persons previously hospitalized to 11.0% among 308 persons with asymptomatic infections. In a multivariable model, persons taking immunosuppressive medications were more likely to be seronegative (31.9%, 95% confidence interval [CI] 10.7%-64.7%), while participants of non-Hispanic Black race/ethnicity (versus non-Hispanic White) (2.7%, 95% CI 1.5%-4.8%), with severe obesity (versus under/normal weight) (3.9%, 95% CI 1.7%-8.6%), or with more symptoms were less likely to be seronegative.

Conclusions In our population with previous RT-PCR confirmed infection, approximately one in 16 persons lacked IgG antibodies. Absence of antibodies varied independently by illness severity, race/ethnicity, obesity, and immunosuppressive drug therapy. The proportion seronegative remained relatively stable among persons tested up to 90 days post symptom onset.

Introduction

Many published studies using in-house and commercial assays have examined development of antibodies to SARS-CoV-2 following infection. These studies demonstrate that IgG, IgM, and IgA antibodies targeted to the virally encoded surface spike (S) and nucleocapsid (N) proteins develop nearly simultaneously in a high proportion of patients within 2 weeks after symptom onset.[1-8]

However, accumulating data indicate that IgG antibodies may not be detected in some persons following infection, particularly among those with milder illnesses. In one study of 20 persons with mild disease subsequently tested with five commercial assays 28-54 days after symptom onset, 10-30% tested negative depending on the assay, with no obvious differences whether the assays tested antibodies targeted to the S or N proteins.[9] In another study of 27 patients with mild disease tested with two commercial assays 8-12 weeks post symptom onset, 22% were seronegative.[10] A third study of 24 patients with mild disease tested with a commercial assay detecting total antibody, 13% were seronegative 21-24 days post symptom onset.[11] However, these results contrast with another study of 624 patients with mild disease tested with an in-house assay, of whom 99% tested positive.[12] Unlike patients with mild disease, studies consistently show that all or nearly all hospitalized patients develop antibodies to the S and N proteins.[11, 13-17]

Fewer studies have examined longer-term persistence of anti-SARS-CoV-2 antibodies. Serum IgA and IgM antibodies appear to rapidly wane over weeks or a few months;[18, 19] whereas, evidence suggests longer-term persistence of IgG antibodies.[17-21] Nevertheless, in one study 40% and 13% of asymptomatic and symptomatic persons, respectively, lacked IgG antibodies approximately 10 weeks after the initial positive RT-PCR result or symptom onset.[22] The lack of antibody in that study resulted both from persons not developing

antibody as well as from waning antibody among those who did. In another study, IgG antibodies targeting the receptor binding domain (RBD) decreased with an estimated half-life of 36 days.[23] In yet another study of seven asymptomatic patients, two (29%) lacked IgG antibodies approximately 8 weeks after initial infection.[24]

To further determine the proportion of persons without detectable IgG antibodies after SARS-CoV-2 infection and predictors for lack of antibody, we studied serologic data from 2,547 persons with previous confirmed SARS-CoV-2 infection. We identified these persons during a large-scale serologic survey of healthcare personnel and first responders in two large U.S. metropolitan areas.

Methods

Overall approach

Most published studies of SARS-CoV-2 antibody development have been limited by sample size and enrollment of symptomatic persons who have sought health care. To obtain a large sample across a broad spectrum of illness severities ranging from asymptomatic to hospitalization, we conducted a serosurvey for anti-SARS-CoV-2 IgG antibodies among healthcare personnel and first responders and determined those who had had a previous positive SARS-CoV-2 RT-PCR test result as described below. These persons comprised the final study population from which comparisons of antibody prevalence by demographic and clinical variables permitted evaluation of their contribution to antibody development and persistence.

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Population tested

In the Detroit metropolitan area, healthcare personnel at 27 hospitals who worked in emergency departments, intensive care units, general inpatient units, surgical units, and who provided support services to those areas, such as radiology, were offered antibody testing.[25] In addition, first responders and public safety personnel in emergency medical services agencies overseen by seven local Medical Control Authorities were invited to participate. In New York City, all police, firemen, medical examiner and corrections staff, and staff at 11 hospitals and seven outpatient facilities were offered testing.

Data collection

The study period was from May 18 through June 13 in the Detroit metropolitan area and through June 19, 2020 in New York City. Eligibility included age at least 18 years, completion of an on-line, self-administered survey, and consent for phlebotomy and storage of a serum sample for confirmation of test results, if necessary. To exclude persons with very recent infection without sufficient time for antibody development, persons were not eligible to participate if, during the previous 2 weeks before survey completion, they reported new onset of worsening cough, shortness of breath, or change in sense of taste or smell, or if they had tested positive for SARS-CoV-2 by nasal or throat swab or saliva sample. The survey gathered information on demographics, occupation, underlying medical conditions, symptoms since March 1, 2020 (listed in Table 2), whether they sought health care because of symptoms, hospitalization, and previous SARS-CoV-2 test date and result from samples obtained by nasal or throat swab or saliva sample. At the time of the survey, RT-PCR was the only testing available for these samples. Body mass index (kg/m^2) was calculated from self-reported weight and height, and weight status was categorized as underweight/normal (BMI <25), overweight (25 to <30), obese (30 to <40) and severely obese ($\geq$40).

This activity was determined by CDC to be public health surveillance in accordance with 45 C.F.R. Part 4. The Region 2 South Healthcare Coalition deferred to CDC's determination and did not require IRB review. The New York City Department of Health IRB reviewed and approved the survey protocol.

Confirmation of previous SARS-CoV-2 infection

Participants were considered to have had a previous confirmed SARS-CoV-2 infection if they 1) self-reported a positive RT-PCR test and were IgG antibody positive, or 2) self-reported a positive RT-PCR test, were IgG antibody negative, and there was a record of a positive RT-PCR test in health department records. Reporting of RT-PCR test results to health departments is mandatory nationwide; however, reporting may be incomplete, and records may not be accessible for those who lived outside the study jurisdiction. Only persons with confirmed SARS-CoV-2 infection were included in the final analysis.

Sample testing and processing

After blood collection, samples were centrifuged and transferred to a central testing laboratory for testing with the ORTHO Clinical Diagnostics VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Test. This assay detects IgG antibodies directed at the SARS-CoV-2 S1 protein domain, which contains the receptor binding domain responsible for virus binding to the angiotensin converting enzyme-2 (ACE-2) receptor on target cells. Two independent evaluations of this assay indicated specificities of 99.7% (95% CI 98.6-100%) and 100% (95% CI 95.4-100%).[26, 27] Participants received test results within 72 hours of collection. Aliquots of residual sera were transferred and maintained at -70°C for long-term storage at CDC.

To help assess the generalizability of the serosurvey results with other SARS-CoV-2 IgG antibody tests, a sample of 126, 39, and 80 specimens with signal-to-cutoff ratios (S/CO) ratios of <0.05 (low negative), 1.0-1.5 (low positive), and >1.5 (higher positive), respectively, were selected from the overall serosurvey population for testing with the Abbott Architect SARS-CoV-2 IgG assay, which detects IgG antibodies targeted at viral nucleoprotein; the ORTHO Clinical Diagnostics VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Antibody Test, which detects total Ig antibodies targeted at the S1 protein domain; and an ELISA test developed at CDC that detects total Ig antibodies directed at spike protein.[28]

Statistical analysis

Differences in categorical variables were assessed by the chi-square or Fisher's exact test with a 2-sided probability, or Cochran-Armitage trend test for variables with ordered categories. Comparisons for continuous variables were assessed with the t-test. Multivariable logistic regression was used to assess independent risk factors for the absence of SARS-CoV-2 antibodies, controlling for sex, age, race/ethnicity, obesity, number of symptoms, immunosuppression, immunosuppressant medications, and medical care seeking. SAS 9.4 software (Research Triangle Institute) was used for descriptive analyses and SAS-callable SUDAAN 11.0.1 was used to estimate model-adjusted proportions (no clustering design or sample weights were used).

Results

Overall antibody prevalence

Of the 36,918 persons surveyed, 2,613 (7.1%) self-reported having tested positive for SARS-CoV-2 by RT-PCR in a nasal or throat swab or saliva sample (Figure 1). Of these 2,613 persons, samples from four persons were not tested for antibody due to lipemia, two persons

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presented twice for testing, 2,387 tested antibody positive, and 220 tested antibody negative. Of the 220 persons who tested antibody negative, 32 with only a record of a negative RT-PCR result and 28 with no testing record at the health department were excluded from further analysis. Thus, the final data set consisted of 2,547 persons with confirmed previous SARS-CoV-2 infection, of whom 160 (6.3%) were seronegative (Figure 1). A total of 2,164 persons were from New York and 383 were from Detroit, and the average age was 40.1 years (standard deviation [SD] 10.4 years).

Factors related to seronegativity

Of 2,547 persons in the final data set, 1,995 (78.3%) reported at least one symptom listed in Table 2 and an exact date of symptom onset. Antibody testing occurred a mean of 63.6 days (SD 16.0 days) after symptom onset. The mean time since symptom onset was slightly longer among those who tested antibody negative (66.5 days, SD 17.3 days; p=0.06) than those who tested positive (63.4 days, SD 15.9 days; p=0.06) and the proportion testing negative increased slightly as the duration from symptom onset to antibody testing date increased (Table 1). Among the 265 persons who reported no symptoms and who could recall the RT-PCR testing date, a mean of 53.5 days (SD=19.7 days) elapsed between RT-PCR and antibody testing. For this group, seroprevalence did not differ significantly by the interval between RT-PCR and antibody testing dates (supplementary Table 1).

Although the absence of antibodies did not differ significantly by age and sex, non-Hispanic Black participants were less likely than non-Hispanic White participants to lack antibodies (3.2% versus 6.1%, respectively; Table 1, crude risk ratio 0.53 (95% CI 0.29-0.97, Supplementary Table 2). The proportion without antibodies decreased with increasing weight status (10.0% below or normal weight, 5.6% overweight, 5.4% obesity, 3.6% severe obesity, p=<0.001; Table 1).

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Persons who reported immunosuppressive therapy or medications, such as cancer treatment, were much more likely to be seronegative; approximately one in four lacked antibodies (26.7% versus 6.2% not taking immunosuppressive therapy or medications, p=0.001; Table 1). However, antibody prevalence did not differ according to the presence of immunosuppressing conditions, such as human immunodeficiency virus infection or autoimmune disease. Persons who sought medical care for COVID-19 symptoms were less likely to lack antibodies compared to those who didn't seek care (5.2% versus 8.0%, p=0.005). Seroprevalence was not related to the presence of diabetes, hypertension, chronic heart disease, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, or hypertension (data not reported).

Several measures of previous illness severity were related to absence of antibodies. Asymptomatic persons were more likely to lack antibodies (11.0%) than those previously symptomatic (5.6%, p=<0.001), with seronegativity decreasing as the number of symptoms increased (Table 2). Among individual symptoms, history of fever and loss of taste or smell had the strongest relationship with the presence of antibody. None of 79 persons who reported previous COVID-19-related hospitalization were seronegative compared to 6.5% of those not hospitalized (p=0.02; Table 1).

In a multivariable model, characteristics that remained significantly associated with lacking antibodies included taking immunosuppressive medications (31.9% versus 6.2% for persons not taking such medications), non-Hispanic White and Hispanic race/ethnicity (6.4% and 8.6%, respectively versus 2.7% for non-Hispanic Black race/ethnicity), under/normal weight status (9.4% versus 5.4% for obesity) and fewer symptoms (persons with 0-2 symptoms had higher risk of lacking antibodies compared with persons with 6-9 symptoms)(Figure 2, supplementary Table 2). Seeking medical care for COVID-19 symptoms was no longer

associated with serologic status after adjustment for covariates. Hospitalization could not be included in the model since no hospitalized persons were seronegative.

Supplemental laboratory testing

Of 126 low-negative ($\leq$0.05 S/CO on the ORTHO IgG antibody assay) samples, one was positive only on the CDC assay; all other samples were negative on all assays. Of 39 low-positive (1.0-1.5 S/CO) samples, all tested positive on the CDC and ORTHO total antibody assays, while on the Abbott assay, 17 (44%) tested negative. For these 39 persons, the intervals between the previous positive PCR test and antibody testing were similar among those who tested positive (mean 61.2 days, SD 15.2) or negative (59.2 days, SD 23.8). Of the 80 higher positive (>1.5 S/CO) samples, 79 (99%) tested positive on the CDC assay, 76 (95%) tested positive on the Abbott assay, and 79 (99%) tested positive on the ORTHO total antibody assay.

Discussion

Among 2,547 persons with confirmed previous SARS-CoV-2 infection, 6.3% lacked anti-SARS-CoV-2 IgG antibodies. This result can be compared with an Icelandic study in which 1,215 persons with qPCR-diagnosed infection were tested approximately 3 months after recovery using pan-Ig assays measuring anti-N or anti-S1-RBD antibodies.[19] Negative antibody results were obtained in either one or both assays in 9.0% and 4.9%, respectively. In another seroprevalence study of New York City healthcare personnel, 6.5% of 2,044 persons with a history of a PCR-positive infection were seronegative.[29] However, the interval between symptom onset and antibody testing was not reported and seven different assays were used at various time points.

In our study population, the proportion lacking antibodies increased slightly as the time between symptom onset and antibody testing increased, although this trend was on the margins of statistical significance (p=0.06). This finding along with the fact that fewer than 10% were seronegative up to 90 days after symptom onset suggested a minimal temporal rate of seroreversion over the time period studied. This is consistent with another study that showed that IgG antibodies to the receptor binding domain and N protein slightly decreased over 6-7 months post illness onset.[20] In the Icelandic study of 1,215 patients, the proportion positive testing positive with two pan-Ig antibody levels remained stable over 3 months and IgG anti-N and anti-S1 antibody levels decreased slightly after 6 weeks from diagnosis.[19]

Our data affirm previous observations that IgG antibodies are less likely to develop in persons with milder disease.[9-11, 17, 24, 30, 31] In our study, 11% of those asymptomatically infected lacked antibodies; whereas, none of 79 persons previously hospitalized lacked IgG antibodies. Between these two extremes, those with fewer symptoms were less likely to have antibodies. Our large sample also enabled identification of other factors presumptively related to the presence of antibodies following infection. In a multivariable model controlling for potential confounding factors such as symptom frequency and seeking medical care, non-Hispanic Black race/ethnicity and obesity were associated independently with higher prevalence of antibodies following infection; taking immunosuppressive medication was inversely associated with the presence of antibodies.

While serologic surveys have often found that persons of non-Hispanic Black race/ethnicity have higher seroprevalence than non-Hispanic whites, we are not aware of other studies that have examined racial and ethnic differences in development of antibodies following infection. Our finding that non-Hispanic Whites were over twice as likely to lack antibody (adjusted 6.4%) than non-Hispanic Blacks (adjusted 2.7%) following infection requires

confirmation by other studies. In addition, we are also not aware of other studies showing that persons with lower body mass index are less likely to have detectable IgG antibodies following infection. However, this finding is consist with another large study that found lower pan-Ig antibody levels in persons with lower body mass index.[19] While it is plausible that immunosuppressive drugs would inhibit antibody development, it is not clear why race/ethnicity or obesity would independently predict the presence or absence of IgG antibodies following infection.

We observed a high concordance of ORTHO IgG antibody assay results with the ORTHO total antibody and CDC assays, each of which measures antibody to spike protein. In contrast, the Abbott assay, which measures IgG antibody to nucleoprotein, appeared to have lower sensitivity, particularly among the low positives on the ORTHO IgG assay. It should be noted that the 39 low positives represented only 1.6% of the 2,387 positive samples. Among these samples, the similar intervals between PCR and antibody testing among those testing positive and negative on the Abbott assay suggested that the lower sensitivity was not due to waning antibody. Our results suggested that if an orthogonal testing algorithm were employed (Ortho IgG antibody assay followed by the Abbott assay) approximately 5% of the positive results would have been excluded; however, our study design did not permit assessment of potential improvements in positive predictive value with this approach.

This study had several limitations. The sample was a working population that may have been healthier than the population-at-large, had few adults aged over 65 years, and did not include children. There may have been inaccuracies in self-reporting of information, such as the dates of symptom onset and RT-PCR specimen collection. We could not ascertain whether specific immunocompromising conditions or medications influenced antibody development since our survey did not gather this level of detail. Persons who died would not be included in this survey, although this number was expected to be small. Our observation that 6.3% had no

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detectable antibody should likely be considered a minimum estimate because some of the 60 seronegative persons excluded because of no health department record of a positive RT-PCR result or only a negative recorded negative RT-PCR result may have actually had a positive result that was not reported. In addition, compared to infected persons in the population-at-large, symptomatic persons were likely overrepresented in our study population (87.9% [2,239/2,547] reported at least one symptom) since a previous positive RT-PCR test was required for inclusion. This would have decreased the observed proportion seronegative since symptomatic persons were more likely to have antibody. The presence of antibodies might have also been influenced by other unmeasured factors.

Our results have several important implications. We found that that approximately one in 16 persons lacked IgG antibodies following infection; however, repeat clinically apparent SARS-CoV-2 infections in the population-at-large appear exceedingly rare despite tens of millions of infections worldwide. While certainly not definitive, this observation suggests short-term immunity from clinically apparent reinfection despite lack of demonstrable antibody in some persons. Our finding that race/ethnicity, weight status, and illness severity were independent predictors of IgG antibody presence after SARS-CoV-2 infection may provide immunological insights for future study and stress the importance of using similar populations when comparing antibody assay performance. Our results suggesting minimal IgG antibody seroreversion provides reassurance in interpreting population seroprevalence studies. However, the absence of IgG antibodies cannot rule out previous infection in some individuals, which raises social, ethical, and practical concerns for development of public health policies based on serologic testing.

NOTES

Declarations of interest: None of the authors have any conflicts of interest to declare.

The findings and conclusions in this paper are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

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Table 1. Presence and absence of IgG antibody and among persons with previous SARS-CoV-2 infection, by personal characteristics.

	Antibody positive N (%)	Antibody negative N (%)	p-value[a]
Total (n=2547)	2387 (93.7)	160 (6.3)	
Mean age (SD)	40.1 (10.4)	40.6 (10.4)	0.50
Age group (yrs)			
18-24 yrs	68 (94.4)	4 (5.6)	0.47
25-34 yrs	772 (93.8)	51 (6.2)	
35-44 yrs	775 (94.1)	49 (6.0)	
45-59 yrs	661 (93.4)	47 (6.6)	
60-64 yrs	88 (93.6)	6 (6.4)	
65+ years	23 (88.5)	3 (11.5)	
Sex			
Male	1566 (94.2)	96 (5.8)	0.15
Female	821 (92.8)	64 (7.2)	
Race/ethnicity			
Non-Hispanic White	1037 (93.9)	67 (6.1)	0.07
Non-Hispanic Black	361 (96.8)	12 (3.2)	
Non-Hispanic Asian	184 (93.4)	13 (6.6)	

Hispanic	603 (91.8)	54 (8.2)	
Other Non-Hispanic race	64 (92.8)	5 (7.3)	
Not stated	138 (93.9)	9 (6.1)	
Days since symptom onset[b]			
14-29 days	39 (95.1)	2(4.9)	0.06
30-39 days	107 (95.5)	5 (4.5)	
40-49 days	227 (95.8)	10 (4.2)	
50-59 days	387 (94.4)	23 (5.6)	
60-69 days	557 (95.4)	27 (4.6)	
70-79 days	349 (93.6)	24 (6.4)	
80-89 days	253 (93.7)	17 (6.3)	
90-118 days	76 (89.4)	9 (10.6)	
Weight category[c]			
Under/normal	488 (90.0)	50 (10.0)	<0.001
Overweight	1012 (94.4)	60 (5.6)	
Obesity	794 (94.6)	45 (5.4)	
Severe obesity	132 (96.4)	5 (3.6)	
Immunosuppressed (e.g., HIV, autoimmune disease)			
Yes	29 (93.6)	2 (6.5)	0.97
No	2358 (93.7)	158 (6.3)	
Immune-weakening therapy or medications			
Yes	11 (73.3)	4 (26.7)	0.001
No	2376 (93.8)	156 (6.2)	
Sought medical care			
Yes	1504 (94.8)	83 (5.2)	0.005

No	883 (92.0)	77 (8.0)	
Hospitalized			
Yes	79 (100.0)	0 (0.0)	0.02
No	2308 (93.5)	160 (6.5)	

[a]t-test mean age; Cochran-Armitage trend test age group and duration since symptom onset; chi-square remaining categories

[b]Excludes 308 persons without symptoms and 127 persons who did not report a date of symptom onset. The interval between symptom onset and antibody testing ranged from 4-118 days.

[c]One participant was excluded due to implausible body mass index value

Table 2 Presence and absence of IgG antibody among persons with previous SARS-CoV-2 infection, by symptom history

Symptoms	Antibody positive N (%)	Antibody negative N (%)	p-value[a]
Any symptoms			
Yes	2113 (94.4)	126 (5.6)	<0.001
No	274 (89.0)	34 (11.0)	
Fever			
Yes	1521 (96.6)	54 (3.4)	<0.001
No	866 (89.1)	106 (10.9)	
Chills			
Yes	1318 (96.4)	50 (3.7)	<0.01
No	1069 (90.7)	110 (9.3)	
Cough			
Yes	1435 (95.5)	68 (4.5)	<0.001
No	952 (91.1)	92 (8.8)	
Sore throat			
Yes	902 (93.9)	59 (6.1)	0.82
No	1485 (93.6)	101 (6.4)	
Shortness of breath			
Yes	1067 (95.5)	50 (4.5)	<0.001
No	1320 (92.3)	110 (7.7)	
Diarrhea			
Yes	861 (96.6)	30 (3.4)	<0.001

No	1526 (92.2)	130 (7.9)	
Muscle aches			
Yes	1483 (95.7)	66 (4.3)	<0.001
No	904 (90.6)	94 (9.4)	
Loss of taste or smell			
Yes	1406 (97.0)	44 (3.0)	<0.001
No	981 (89.4)	116 (10.6)	
Headache			
Yes	1403 (94.2)	87 (5.8)	0.27
No	984 (93.1)	73 (6.9)	
Number of symptoms			Trend p-value
0	274 (89.0)	34 (11.0)	<0.001
1	132 (89.6)	20 (13.2)	
2	124 (87.9)	17 (12.1)	
3	204 (91.9)	18 (8.1)	
4	265 (92.7)	21 (7.3)	
5	301 (94.1)	19 (5.9)	
6	351 (97.0)	11 (3.0)	
7	320 (97.6)	8 (2.4)	
8	251 (96.5)	9 (3.5)	
9	165 (98.2)	3 (1.8)	

[a]Cochran-Armitage trend test for symptoms; chi-square remaining categories

Figure Legends

Figure 1: Participant selection.

Figure 2: Adjusted percent with absence of SARS-CoV-2 antibodies among persons with previous SARS-CoV-2 infection.
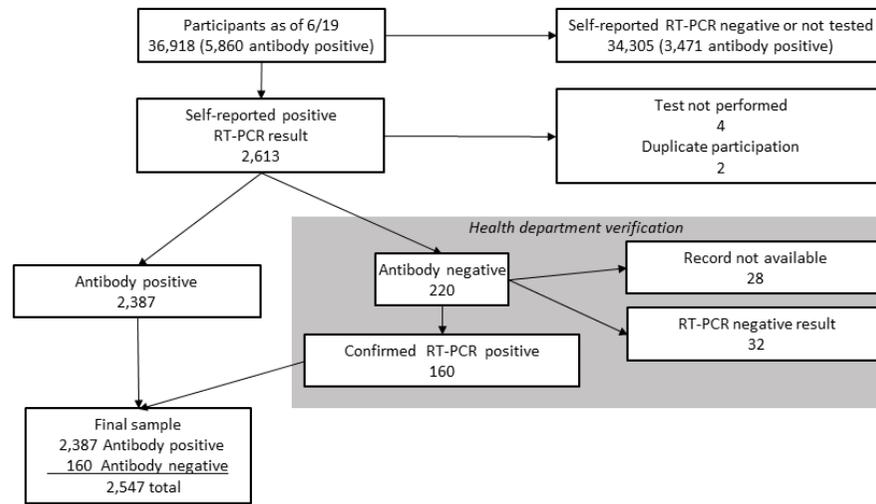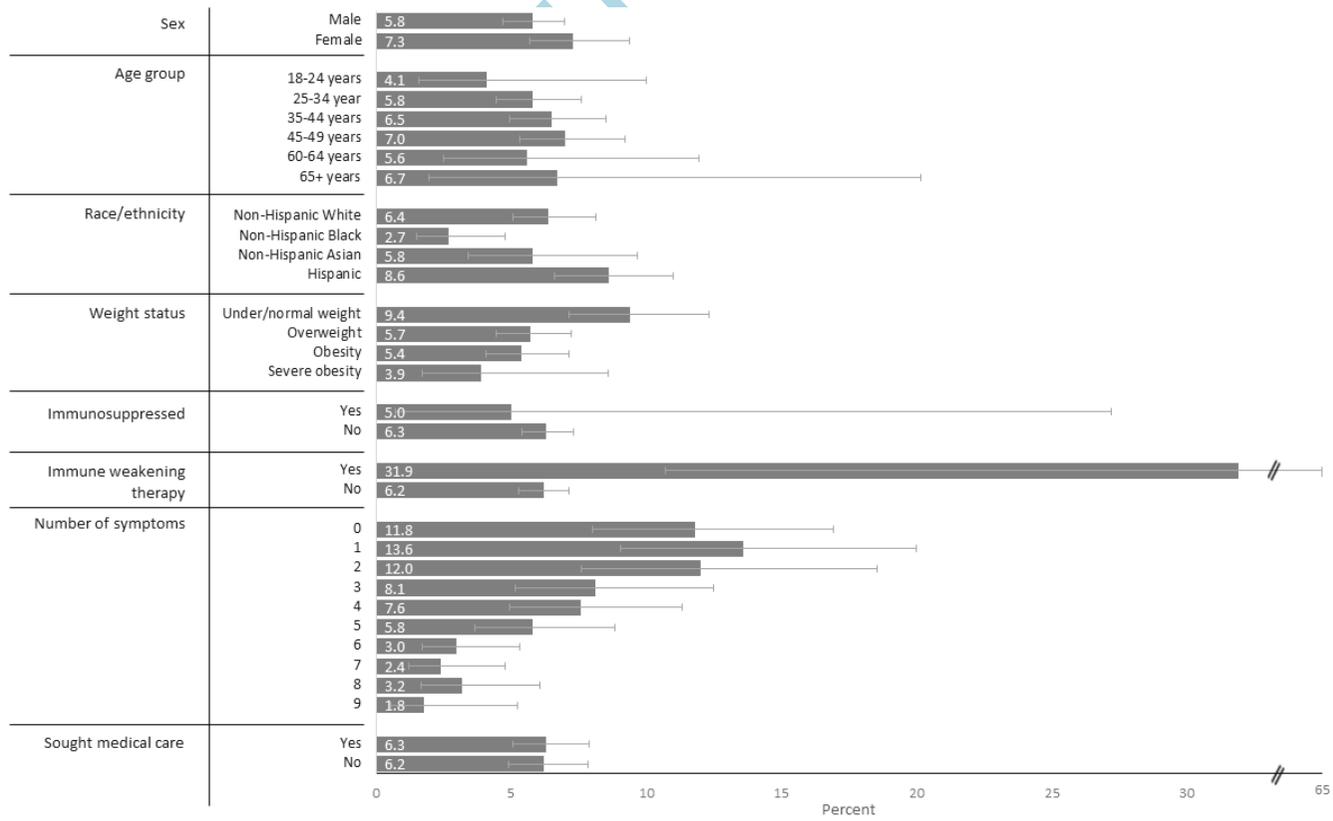
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Figure 1

| Participants as of 6/19 36,918 (5,860 antibody positive) | → | Self-reported RT-PCR negative or not tested 34,305 (3,471 antibody positive) |

| Self-reported positive RT-PCR result 2,613 | → | Test not performed 4 Duplicate participation 2 |

Health department verification

| Antibody positive 2,387 |

| Antibody negative 220 | → | Record not available 28 |
| | → | RT-PCR negative result 32 |

| Confirmed RT-PCR positive 160 |

| Final sample 2,387 Antibody positive 160 Antibody negative 2,547 total |

Figure 2