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COVID-19 PANDEMIC

Prevalence of Antibodies to COVID-19 Due to Infection or Vaccination in US Adults

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Objective.—Determine the seroprevalence of SARS-CoV-2 infection and vaccination in a population applying for life insurance.
Setting.—This is a cross-sectional study of 2584 US life insurance applicants, to determine the seroprevalence of antibodies to COVID-19. This convenience sample was selected on two consecutive days April 25-26, 2022.
Results.—For COVID-19, 97.3% are seropositive, and 63.9% have antibodies to nucleocapsid protein, a marker of prior infection. An additional, 33.7% have been vaccinated with no serologic evidence of infection.
Methodology.—Serum and urine samples from a nationwide group of insurance applicants for routine risk assessment were collected. The examination of applicants typically occurs, in their homes, their place of employment, or a clinic. The paramedic exam occurs 7-14 days after the insurance application. Before the exam, an office assistant calls the applicant and inquires if they have been in contact with a person with SARS-CoV-2, been ill within the last 2 weeks, felt sick, or recently had a fever. If the applicant answers yes, the exam is rescheduled.
Before sample collection, the applicant reads and signs a consent form to release medical information and testing. Next, the examiner records the applicant's blood pressure, height, and weight. Then, a blood and a urine sample are collected and sent with the consent form to our laboratory via Federal Express.
On April 25-26, 2022, we tested 2584 convenience samples from adult insurance applicants for the presence of antibodies to nucleocapsid and spike proteins from SARS-CoV-2. As a standard practice, we reported the client-specified test profile results to our life insurance carriers. In contrast, the COVID-19 test results were only available to the authors.
Patient and Public Involvement.—There was no patient involvement in study design, reporting of results, or journal publication selection. There was patient consent to publish de-identified study results. No public involvement occurred in the creation or completion of the study. The authors thank the participants in this study for approving the use of their blood samples to further society's understanding of the SARS-CoV-19 pandemic.

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Ethics Review.—Western Institutional Review Board reviewed the study design and determined it to be exempt under the Common Rule and applicable guidance. Therefore, it is exempt under 45 CFR § 46.104(d)(4) from using de-identified study samples for epidemiologic investigation, WIRB Work Order #1-1324846-1. In addition, all test subjects had signed a consent allowing research of their blood and urine samples with the removal of personally identifiable information.

Results.—The combined seroprevalence for antibodies to nucleocapsid, a marker of prior infection, and antibodies to spike protein, an indicator of either previous infection or vaccination, was 97.3%. Higher infection rates occur in younger vs older age groups, with a non-statistical difference for vaccinated and acquired natural immunity. For the age group 16-84, the total estimated seroprevalence of COVID-19 in the US is 249 million cases.

Conclusions.—The US population has widespread immune resistance to current variants of COVID-19 due to prior infection or vaccination. The infectivity of new variants and silent disease, independent of previous infection or vaccination, are the driving force behind the sporadic increase in clinical SARS-CoV-2 cases.

Since January 2020, the Centers for Disease Control and Prevention has reported 90[1] million cases of SARS-CoV-21. However, because a substantial proportion of infections may be asymptomatic or only mildly symptomatic, these reported cases substantially underestimate the true cumulative prevalence of the disease. Furthermore, except for severe cases, the symptoms of SARS-CoV-2 are easily confused with head colds, allergies, and influenza.[2] In addition, symptomatic patients may have chosen to avoid clinical testing, done testing at home, or had no access to testing, all resulting in an underestimation of the extent of the pandemic.

Antibodies against SARS-CoV-2 persist for months to years.[3,4] In addition, a natural infection produces antibodies to both nucleocapsid and spike proteins, while RNA vaccines produce antibodies against only spike. Thus, seroprevalence studies may provide a reliable estimate of the number of infections and the number vaccinated without evidence of prior disease.

METHODS

In April 2022, we tested 2584 individuals for antibodies to SARS-CoV-2 on a Roche 801 analyzer with Roche Elecsys Anti-SARS-CoV-2 and S reagents. They were part of an adult convenience sample from a pool of life insurance applicants who had blood tests performed at Clinical Reference Laboratories. All applicants had self-reported that they were well at the time of application and sample collection. The tests have a stated sensitivity of 100% and specificity of 99.5% and 99.8%, respectively.

We tested the differences in continuous variables between the antibody-positive and antibody-negative groups for significance with the Mann-Whitney U test. In addition, the chi-square X2 test was used for categorical variables. Our statistical analyses used R (version 3.6.1) and R-studio (version 1.2.1335) for all data.[5,6]

To estimate the total US burden of SARS-CoV-2 infections and vaccinations, we used the 2020 US Census data.[7] First, we multiplied the 2020 estimated census population by the proportion between the ages of 16 and 84 (77%).

To estimate the portion of the population previously vaccinated, we selected a value of 1000 ng/ml for spike antibodies. The cut-off was chosen from prior work by Yunkai et al on antibody inhibition of receptor binding

Table 1. Characteristics of the Study Population by SARS-CoV-2 Nucleocapsid Antibody Status

Nucleocapsid Antibodies	Negative n = 932	Positive n = 1648	p
TOTALS	36%(932/2580)	64%(1648/2580)	
Age (yr), median[IQR]	44.9 [35.9-55.8]	41 [32.7-51.6]	$<10^{-11}$
<40	31.4%(349)	68.6%(763)	
40.1-60	38.3%(436)	61.7%(702)	
60.1-85	44.5%(147)	55.5%(183)	
Sex (% male)	55.9%(521)	55.5%(915)	0.83[1]

[1] p-value by Pearson Chi-square test

domain (RBD) to the ACE-2 receptor.[8] The Roche anti-spike test and the work by Yunkai Yu, Dominic Esposito, Zhigang Kang, et al are each calibrated with equivalent cut-offs of 1 ng/ml of mouse monoclonal anti-COVID-19 allowing a direct comparison of results.[8] The amount we chose is more than double the IC50 (450 ng/ml), the amount of antibody that inhibits 50% COVID-19 spike binding to the ACE-2 receptor.

RESULTS

The overall rate of SARS-CoV-2 antibody positivity, including nucleocapsid and spike, was 97.3%. Sixty-four percent were positive for antibodies to nucleocapsid. In addition, infection rates trended downward as age increased, from 68.6% to 55.5%. The opposite occurred for the seronegative group, with the older group having the highest prevalence (Table 1).

There is a significant statistical difference in age between the infected and non-infected groups. But, in contrast, there is a non-statistical difference in the distribution by age for the spike test (Table 2).

The sex difference between negative and positive test results was not statistically significant.

We multiplied our determined prevalence by the age-adjusted adult 2020 Census to generalize our findings. As a result, the calculated number of infections (63.9%) was 163 million with a (95% CI of 161-165 million), or about two times the number of cases reported to the CDC as of August 1, 2022.[9] In our study, the estimated number of infected or vaccinated is 248 million (95% CI 247-249 million). Using a cut-off of 1000 ng/ml for spike antibodies, 76% of those previously infected have levels of anti-spike consistent with vaccination (Table 3).

Our estimate is statistically identical to CDC-reported rates for COVID-19 vaccination; the rates are age-dependent and vary between (68.9%–91.6%).[9]

DISCUSSION

We studied the seroprevalence of SARS-CoV-2 antibodies in a geographically diverse sample of US adults in April 2022. The rate of prior infection ranged from 55.5% to 68.6% and inversely correlated with age. This trend may coincide with a higher rate of asymptomatic infection in the younger age group, possibly associated with a healthier immune response and higher rates of vaccination in the older age groups

Our results suggest that more infections have occurred than reported, consistent with prior reported data.[10,11] The difference is likely due to asymptomatic or minimally symptomatic infections for which the patient did not seek care or symptomatic disease for

Table 2. Characteristics of the Study Population by SARS-CoV-2 Spike Antibody Status

Spike Antibodies	Negative n = 69	Positive n = 2511	P[1]
TOTALS	2.7% (69/2580)	97.3% (2511/2580)	
Age (ys), median[IQR]	44.0 [35.7-54.1]	42.1 [33.9-53.5]	0.45
<40	2.2% (24)	97.8% (1088)	
40.1-60	3.0% (34)	97.0 (1104)	
60.1-85	3.3% (11)	96.7% (319)	
Sex (% male)	53.6% (37)	55.7% (1399)	0.84[1]

[1] p-value by Pearson Chi-square test

which clinical evaluation was not obtained or reported.

In a CDC-funded study, Clarke, Jones, Deng, et al reported the prevalence of nucleocapsid antibodies as 57.7% (95% CI = 57.1-58.3).[11] These results from a clinical cohort were similar to those reported here for a non-clinical population of 63.9% (95% CI = 62.1-66.8), possibly increasing its portability to the general US population.

Early studies that included age showed a higher prevalence in older patients. Older age is associated with more co-morbidities and more severe diseases with a higher probability of being identified as infected. We find the opposite trend, with an increasing prevalence in the younger population with an almost linear decrease in seroprevalence with increasing age. The lowest frequency is in the elderly, in agreement with the CDC-funded study.[11] Several possible factors may contribute to this. First, the elderly are the most at risk of complications from COVID-19 and the most likely to reduce unnecessary exposure. Second, they were among the first group targeted for vaccination.

The future mortality and morbidity risk to the insurance industry is currently undetermined. These include but are not limited to delays in identifying a current disease, patient avoidance of physician office visits, and delays in identifying or treating existing disease. In addition, "long-COVID," the potential of multiorgan involvement, including cardiac remodeling or cardiovascular, renal, and neurologic disease, will require additional study.

CONCLUSION

The rate of SARS-CoV-2 seropositivity in adult life insurance applicants is 97.3%, representing a combination of infection and vaccination that provides protection against se-

Table 3. Previously Infected Population by SARS-CoV-2 Spike Antibody Level

	Spike Antibody		
Prior Infection Status	≤1,000 ng/ml	>1,000ng/ml	P[1]
TOTAL	618 (23.9%)	1966 (76.1%)	
COVID-19 Positive	370 (22.4%)	1282 (77.6%)	0.016
Negative	248 (26.6%)	684 (73.4%)	

[1] p-value by Pearson Chi-square test

vere clinical disease for the current variants of COVID-19. The infection burden is approximately twice that of CDC-reported clinical cases, with a significantly higher rate of prior infection among younger individuals.

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