SARS-CoV-2 seropositivity and subsequent infection risk in healthy young adults: a prospective cohort study

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Summary

Background Whether young adults who are infected with SARS-CoV-2 are at risk of subsequent infection is uncertain. We investigated the risk of subsequent SARS-CoV-2 infection among young adults seropositive for a previous infection.

Methods This analysis was performed as part of the prospective COVID-19 Health Action Response for Marines study (CHARM). CHARM included predominantly male US Marine recruits, aged 18–20 years, following a 2-week unsupervised quarantine at home. After the home quarantine period, upon arrival at a Marine-supervised 2-week quarantine facility (college campus or hotel), participants were enrolled and were assessed for baseline SARS-CoV-2 IgG seropositivity, defined as a dilution of 1:150 or more on receptor-binding domain and full-length spike protein ELISA. Participants also completed a questionnaire consisting of demographic information, risk factors, reporting of 14 specific COVID-19-related symptoms or any other unspecified symptom, and brief medical history. SARS-CoV-2 infection was assessed by PCR at weeks 0, 1, and 2 of quarantine and participants completed a follow-up questionnaire, which included questions about the same COVID-19-related symptoms since the last study visit. Participants were excluded at this stage if they had a positive PCR test during quarantine. Participants who had three negative swab PCR results during quarantine and a baseline serum serology test at the beginning of the supervised quarantine that identified them as seronegative or seropositive for SARS-CoV-2 then went on to basic training at Marine Corps Recruit Depot—Parris Island. Three PCR tests were done at weeks 2, 4, and 6 in both seropositive and seronegative groups, along with the follow-up symptom questionnaire and baseline neutralising antibody titres on all subsequently infected seropositive and selected seropositive uninfected participants (prospective study period).

Findings Between May 11, 2020, and Nov 2, 2020, we enrolled 3249 participants, of whom 3168 (98%) continued into the 2-week quarantine period. 3076 (95%) participants, 2825 (92%) of whom were men, were then followed up during the prospective study period after quarantine for 6 weeks. Among 189 seropositive participants, 19 (10%) had at least one positive PCR test for SARS-CoV-2 during the 6-week follow-up (1·1 cases per person-year). In contrast, 1079 (48%) of 2247 seronegative participants tested positive (6·2 cases per person-year). The incidence rate ratio was 0·18 (95% CI 0·11–0·28; p<0·001). Among seropositive recruits, infection was more likely with lower baseline full-length spike protein IgG titres than in those with higher baseline full-length spike protein IgG titres (hazard ratio 0·45 [95% CI 0·32–0·65]; p<0·001). Infected seropositive participants had viral loads that were about 10-times lower than those of infected seronegative participants (ORF1ab gene cycle threshold difference 3·95 [95% CI 1·23–6·67]; p=0·004). Among seropositive participants, baseline neutralising titres were detected in 45 (83%) of 54 uninfected and in six (32%) of 19 infected participants during the 6 weeks of observation (ID50 difference p<0·0001).

Interpretation Seropositive young adults had about one-fifth the risk of subsequent infection compared with seronegative individuals. Although antibodies induced by initial infection are largely protective, they do not guarantee effective SARS-CoV-2 neutralisation activity or immunity against subsequent infection. These findings might be relevant for optimisation of mass vaccination strategies.

Funding Defense Health Agency and Defense Advanced Research Projects Agency.

Introduction

As of mid-December, 2020, more than 72 million SARS-CoV-2 infections have been diagnosed worldwide.[1] Serological surveys indicate that the actual number of infections has been many times higher than the cumulative incidence of diagnosed cases, with seropositivity rates approaching 10% in some countries and more than 40% in the Brazilian Amazon.[2–4] With the onset of mass SARS-CoV-2 vaccination programmes and the increasing proportion of previously infected individuals, the risk of

Lancet Respir Med 2021

Published Online
April 15, 2021
https://doi.org/10.1016/S2213-2600(21)00158-2

See Online/Comment
https://doi.org/10.1016/S2213-2600(21)00187-9

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Research in context

Evidence before this study

The number of people with antibodies against SARS-CoV-2 is estimated from seroprevalence studies to be many times higher than the rapidly growing number of diagnosed infections. Previous infection and seropositivity does not always prevent subsequent infection. SARS-CoV-2 reinfection has been reported after previous infection, as well as in some individuals who have antibodies against SARS-CoV-2. The reinfection risk in young adults has not been studied. We searched PubMed from database inception through to Nov 15, 2020, for the terms "SARS-CoV-2", "antibody", and "reinfection" without additional filters. Case reports described two individuals who tested seropositive and subsequently became infected with SARS-CoV-2. The risk of reinfection in seropositive individuals relative to seronegative individuals cannot be estimated from the previously available evidence.

Added value of this study

In this prospective cohort study of new Marine recruits without active infection, a lower proportion of participants who had baseline serum antibodies against SARS-CoV-2 became infected during the 6-week study period than of those without detectable antibodies. The risk of subsequent infection in seropositive individuals was associated with lower IgG antibody titres and absent or lower neutralising antibody activity. Our data highlight the disparity between seropositivity and complete protection from infection.

Implications of all the available evidence

Despite seropositivity, some individuals can be reinfected by SARS-CoV-2. In young, healthy, seropositive adults, the risk of subsequent infection is about one-fifth that in seronegative individuals. Infection in seropositive individuals might be associated with lower IgG antibody titres or with a failure to generate or sustain neutralising antibodies, leaving them susceptible to reinfection and potential transmission. Despite previous SARS-CoV-2 infection or documented seropositivity, vaccination might still be necessary to boost the natural immune response and prevent reinfection and reduce transmission.

reinfection after natural infection is an important question for modelling the pandemic, estimating herd immunity, and guiding vaccination strategies.[5,6]

Most individuals mount a sustained serological response after initial infection.[7–10] Similar to the response to other coronaviruses, the production of IgG antibodies against SARS-CoV-2 peaks several weeks after infection, goes through a decline phase, and then stabilises. The overall humoral response to SARS-CoV-2 is highly variable among individuals.[8] SARS-CoV-2-specific IgG antibodies can be detected in serum from most individuals several months after infection.[10] However, a proportion of infected patients, ranging from 2·5% to 28% in different studies, do not maintain detectable circulating antibodies[8] or neutralising activity[9–11] at later time points. About 10% of individuals who developed antibodies to SARS-CoV-2, all of whom had full-length spike protein-specific IgG antibody titres lower than 1:320, failed to develop measurable neutralising activity.[10]

Reports have established that SARS-CoV-2 reinfection occurs after previous infection, including in seropositive individuals.[12–21] Several studies have reported that SARS-CoV-2 IgG antibodies[21,22] and neutralising antibodies[23] provide protection against subsequent infection. The initial trial results of the adenoviral vector-based SARS-CoV-2 vaccine, ChAdOx1 nCoV-19 (AZD1222), noted that among the 373 participants who were seropositive at baseline, three (0·8%) had subsequent positive swab PCR tests; in comparison, among 11 263 baseline seronegative participants in all groups of the same study, 218 (1·9%) developed a positive test.[20] A study of SARS-CoV-2 serological status and infection among health-care workers identified two (0·16%) infected participants of 1265 seropositive participants and 223 (2·0%) infected participants among 11 364 who were seronegative.[21] Young adults, of whom a high proportion are asymptomatically infected and become seropositive in the absence of known infection,[24,25] can be an important source of transmission to more vulnerable populations.[26] Evaluating the protection against subsequent SARS-CoV-2 infection conferred by seropositivity in young adults is important for determining the need for vaccinating previously infected individuals in this age group.

We utilised the COVID-19 Health Action Response for Marines (CHARM) study,[25] a longitudinal prospective cohort study, to examine the effect of SARS-CoV-2 seropositivity on the risk of developing SARS-CoV-2 infection in young (18–20 years), healthy, adult Marine recruits.

Methods

Study design and participants

The CHARM study was a prospective longitudinal study designed to identify SARS-CoV-2 infection regardless of the presence or absence of symptoms and to assess the host immune response around the time of acute infection. As part of the CHARM study, we did a prospective cohort study with an observation period beginning 2 weeks after enrolment when Marine recruits arrived at Marine Corps Recruit Depot—Parris Island (MCRDPI) to commence basic training. To mitigate the spread of SARS-CoV-2, just before transferring to MCRDPI, the United States Marine Corps (USMC) implemented two separate quarantine protocols. The first was a 2-week home quarantine. After that, the recruits travelled, while wearing face masks and adhering

to physical distancing, to a second USMC-supervised quarantine situated at either a college campus from May 11 to July 29, 2020, or a hotel from Aug 11 to Sept 21, 2020. The supervised quarantine facility followed extensive public health measures, as previously described,[25] that were strictly enforced by US Marine instructors at all times. The recruits and staff were forbidden to leave, and no visitors, other than deliveries of supplies and food along with local essential workers and the study staff, were allowed onto the premises. At the end of this quarantine period, the USMC required all recruits to test negative for SARS-CoV-2 by PCR before proceeding to MCRDPI to initiate basic training.

Within 48 h of arriving at the supervised quarantine location, recruits were offered the opportunity to volunteer for CHARM. Recruits were eligible if they were 18 years or older. Since recruits are a vulnerable population and at risk for coercion, special measures were undertaken, including having study briefers who were active-duty Navy personnel who wore civilian clothes, did not disclose military ranks, did not have members in the recruit's chain of command present, and ensured that participation would not affect a recruit's medical care or influence the grading of a recruit's military performance by superiors.

At enrolment, participants completed a questionnaire consisting of demographic information, risk factors, reporting of 14 specific COVID-19-related symptoms or any other unspecified symptom, and brief medical history. At weeks 0, 1, and 2 of quarantine, a mid-turbinate nares swab for SARS-CoV-2 PCR testing and sera were obtained, and participants completed a follow-up questionnaire, which included questions about the same COVID-19-related symptoms since the last study visit.

Participants who had three negative swab PCR results at weeks 0, 1, and 2 of quarantine and a baseline serum serology test at the beginning of the supervised quarantine that identified them as seronegative or seropositive for SARS-CoV-2, according to criteria described below, were followed prospectively for 6 weeks after transfer from the quarantine location to MCRDPI. At weeks 2, 4, and 6 after transfer, a mid-turbinate nares swab for SARS-CoV-2 PCR testing and sera were obtained and the follow-up symptom questionnaire administered. When clinically indicated—ie, due to the development of COVID-19 symptoms—some participants were evaluated at the MCRDPI clinic and diagnosed by rapid testing. If positive, they went to the isolation barracks, where the study team was able to follow up and repeat PCR testing outside of the scheduled longitudinal follow-up encounters. Participants without PCR results obtained during the MCRDPI study period were excluded from analysis.

Institutional Review Board approval was obtained from the Naval Medical Research Center (protocol number NMRC.2020.0006) in compliance with all applicable US federal regulations governing the protection of human subjects. All participants provided written informed consent.

Procedures

For SARS-CoV-2 quantitative PCR testing, all swabs in viral transport media were kept at 4°C. All assays were performed within 48 h of sample collection at high complexity Clinical Laboratory Improvement Amendments-certified laboratories using the US Food and Drug Administration-authorised Thermo Fisher TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, Waltham, MA, USA). Lab24Inc (Boca Raton, FL, USA) performed PCR testing from study initiation (May 11, 2020) until Aug 24, 2020, and the Naval Medical Research Center (Silver Spring, MD, USA) from Aug 24, 2020, until the conclusion of the study (Nov 2, 2020).

The presence and concentrations of IgG SARS-CoV-2-specific antibodies in serum were determined using an ELISA as previously described.[25] Briefly, 384-well Immulon 4 HBX plates (Thermo Fisher Scientific, Waltham, MA, UA), or 96-well half area Microlon plates (Greiner Bio-One, Monroe, NC, USA), were coated overnight at 4°C with recombinant His-tagged spike receptor-binding domain (S-RBD) (Sino Biological, Beijing, China) or full-length spike protein (LakePharma, Irving, TX, USA) at a concentration of 2 µg/mL in phosphate-buffered saline (PBS). Plates were washed three times with 0·1% Tween-20 (Thermo Fisher Scientific) in PBS (PBS-T) using an automated ELISA plate washer (AquaMax 4000, Molecular Devices, San Jose, CA, USA), and blocked for 1 h at room temperature with 3% milk (Bio-Rad, Hercules, CA, USA) PBS-T. Blocking solution was removed and serum samples diluted in 1% milk PBS-T were dispensed in the wells. At least two positive controls (sera with known IgG presence), eight negative controls (sera collected before July 14, 2019), and four blanks (no serum) were included in every assay. Plates were incubated for 2 h at room temperature, and then washed three times with PBS-T. Next, peroxidase conjugated goat F(ab´)2 Anti-Human IgG (Abcam, Cambridge, UK) was added at 1:5000–1:10 000 dilutions (determined after optimisation for each antibody lot) in 1% milk PBS-T, and plates were incubated for 1 h at room temperature. Plates were washed six times with PBS-T, developed using o-phenylenediamine (Sigma-Aldrich, St Louis, MO, USA), and the reaction was stopped after 10 min with 3M HCl. Optical density (OD) at 492 nm was measured using a microplate reader (SpectraMax M2, Molecular Devices). All serum samples were screened at a 1:50 dilution with S-RBD. Those samples with an OD 492 nm value higher than the average of the negative controls plus three times their SD in the screening assay underwent titration assay (six serial 1:3 serum dilutions starting at 1:50) using both S-RBD and full-length spike protein. Serum samples were considered positive for each assay when at least two consecutive dilutions showed higher OD 492 nm than the average of the negative controls plus three times their SD at the correspondent dilution or 0·15 OD 492 nm. Specificity was 100% on both S-RBD and full-length spike protein ELISA using 70 control sera

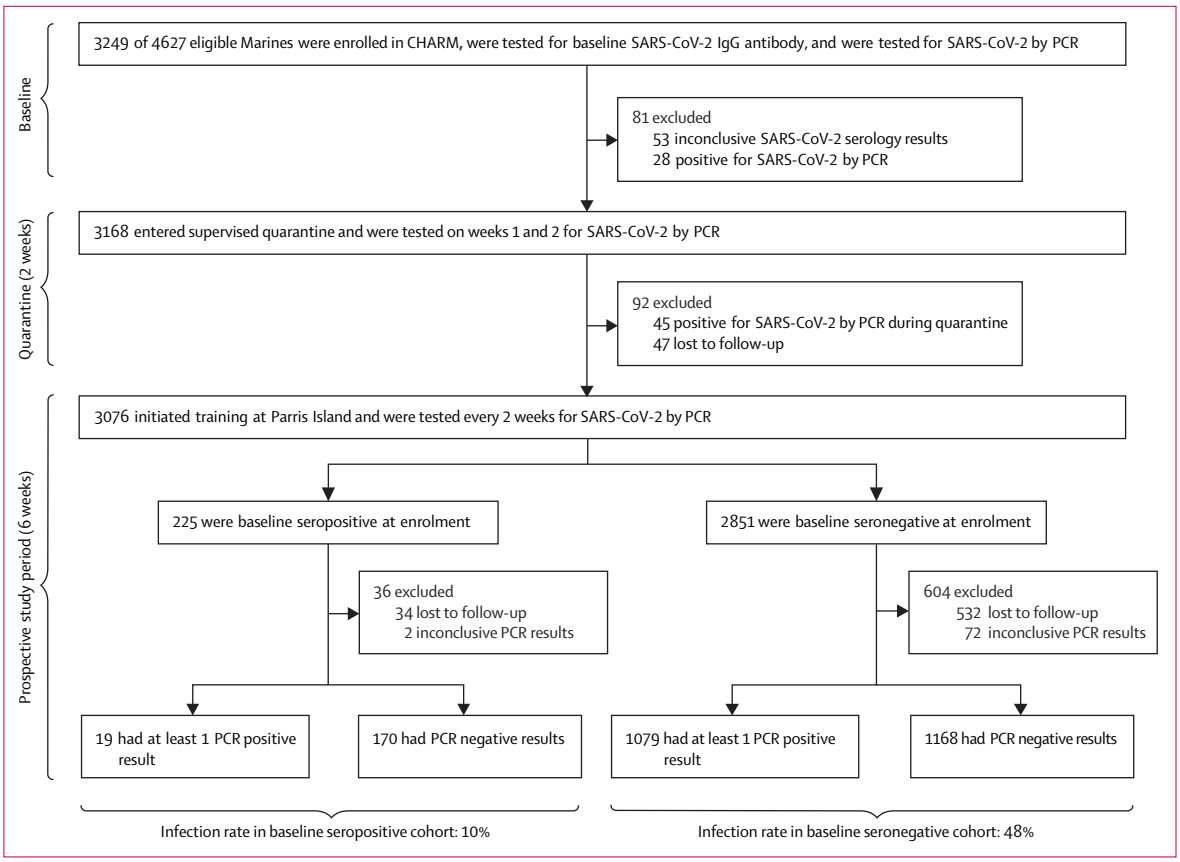

Figure 1: **Study profile**
Participants lost to follow-up either dropped out of the study, were separated from the Marine Corps, or were removed from the base for medical or administrative reasons. The study team did not know the reason for participants missing study visits.

obtained before July 14, 2019. Participants were only considered seropositive to SARS-CoV-2 if IgG titrations for both ELISA gave a positive result at a minimum of 1:150 dilution.

To determine serum virus neutralising activity, two-fold serial dilutions of heat-inactivated serum at an initial dilution of 1:20 were prepared in serum-free media (Minimum Essential Medium; Thermo Fisher Scientific, Cat No. 11095080 containing 25 mM HEPES and 0·05 g/L gentamicin sulfate) and incubated with an equal volume of mNeonGreen SARS-CoV-2[27] for 1 h at 37°C at a final concentration of 200 plaque-forming units in humidified 5% CO_2. Virus–serum mixtures were then added to Vero-E6 monolayers in 96-well optical black plates and incubated at 37°C. Plates were read using the BioTek Cytation 5 plate reader (BioTek, Winooski, VT, USA; EX 485 nm, EM 528 nm) at 24 h post-infection. Following background signal correction, neutralisation titres at a fluorescent end point of 50% virus reduction (ID_{50}) were determined.

Statistical analysis

Race was categorised as non-Hispanic White, non-Hispanic Black, non-Hispanic other, and Hispanic. Cochran-Armitage χ^2 for trend was used to compare proportions testing positive for SARS-CoV-2 by increasing titres, determined as described in the procedures. Cumulative incidence rates computed by Kaplan–Meier method were used to estimate the risk of SARS-CoV-2 infection between seropositive and seronegative participants and also between different titres among the seropositive participants. Observational follow-up began upon arrival at MCRDPI and participants were censored at the first observed positive PCR, at the latest time with valid PCR assay, or at the termination of the study (6 weeks of observation). The Cox proportional hazards model controlled for age, sex, and race. The p values from the cumulative incidence curves were determined by the log-rank test and the p value and 95% CIs for Cox proportional hazard model was computed by the R function coxph. The cycle threshold (Ct) values of viral genes were quantile normalised to remove the batch effects between Lab24Inc and Naval Medical Research Center laboratory assays. The 95% CIs and p values for comparing the mean of the Ct values for two groups are computed based on the two-sample *t* test. Analyses, figures, and tables were generated using R 3.6.3.

Role of the funding source
The funders had no role in the design of the protocol, data collection, data management, data analysis, data interpretation, or writing of the report.

Results
From May 11, 2020, to Nov 2, 2020, 3249 (70%) of 4657 eligible participants were enrolled in CHARM. After excluding 28 participants who were SARS-CoV-2 PCR positive at baseline and 53 who lacked baseline serology results, 3168 (98%) underwent a supervised 2-week quarantine. 45 participants who were SARS-CoV-2 PCR positive on at least one of two PCR tests performed during quarantine, and 47 who were lost to follow-up, were further excluded from the study prior to the prospective study period (figure 1).

Of the remaining 3076 participants, 225 (7%) were baseline seropositive, having SARS-CoV-2 IgG titres in serum samples obtained at the beginning of quarantine that were greater than 1:150 both with S-RBD and with full-length spike protein ELISA, and 2851 (93%) were baseline seronegative. Among the seropositive participants, 36 (16%) were excluded from analysis because they were lost to follow-up (n=34) or had inconclusive PCR results during the study period (n=2). In the seronegative group, 604 (21%) participants were excluded because they were lost to follow-up (n=532) or had inconclusive PCR results (n=72; figure 1). Participants were lost to follow-up for specific reasons unknown to the study team, including dropping out of the study, being separated from the Marines, or being removed from the base for medical or administrative reasons. Most participants were 18–20 years old and male (table 1). The two groups were well balanced except that of a higher proportion of participants self-identified as Hispanic or as Black in the seropositive group. A total of 19 (10%) of 189 seropositive participants (1·1 cases per person-year) and 1079 (48%) of 2247 seronegative participants (6·2 cases per person-year) had at least one positive SARS-CoV-2 PCR result during the 6-week study period, representing an incidence rate ratio of 0·18 (95% CI 0·11–0·28; p<0.001) for SARS-CoV-2 infections in the seropositive group (table 2). The temporal incidence of infection in the seronegative group was much higher than that in the seropositive group (log-rank p<0·001; figure 2A). After adjusting the effects of race, age, and sex on the SARS-CoV-2 infections, the hazard ratio (HR) comparing seropositive participants and seronegative participants was 0·16 (95% CI 0·10–0·25; p<0·001; appendix p 2).

Compared with non-Hispanic White participants, non-Hispanic Black participants appeared to be protected against SARS-CoV-2 infections in a univariate analysis of race (HR 0·75 [95% CI 0·62–0·91]; p=0·004; appendix p 2). However, this effect was not significant when adjusted for the baseline seropositivity in the multivariate analysis (HR 0·86 [0·70–1·04]; p=0·12). This strong effect in the univariate analysis is mostly due to a higher proportion of the non-Hispanic Black participants being seropositive. To further examine the relationship of race and ethnicity to risk of infection, we analysed the data with stratification of the baseline seropositivity to remove its confounding effects. In the stratified analysis for the Cox regression model, non-Hispanic participants who identified their race as other and Hispanic participants had a higher rate of PCR positive tests (43% and 41%, respectively) compared with non-Hispanic White participants (37%), but the hazard ratios were not significant (appendix p 3).

Within the seropositive group, we assessed the association between the SARS-CoV-2 IgG baseline titres and the risk of infection. We found a strong association between subsequent PCR positive infection and lower titres of IgG antibodies directed to full-length spike

See Online for appendix

	Seropositive group (n=225)	Seronegative group (n=2851)
Mean age, years	19·0 (1·8)	19·1 (1·9)
Sex		
Female	22 (10%)	229 (8%)
Male	203 (90%)	2622 (92%)
Race		
Non-Hispanic White	56 (25%)	1698 (60%)
Non-Hispanic Black	50 (22%)	349 (12%)
Non-Hispanic Other	7 (3%)	183 (6%)
Hispanic	112 (50%)	621 (22%)
Non-US residence		
No	216 (96%)	2749 (96%)
Yes	4 (2%)	19 (1%)
N/A*	5 (2%)	83 (3%)
Non-US birth		
No	189 (84%)	2626 (92%)
Yes	32 (14%)	192 (7%)
N/A	4 (2%)	33 (1%)

Data are n (%) or mean (SD). A total of 173 participants were excluded because they were lost to follow-up, did not have a valid baseline IgG, or became PCR positive during the quarantine period. The table includes all 3076 participants who entered training and were followed prospectively, including the 640 participants who were later excluded from further analysis (figure 1). N/A=not applicable.*If a participant answered unknown or left a question blank, then the value is grouped into N/A.

Table 1: **Participant demographics**

	Seropositive group (n=189)	Seronegative group (n=2247)	Proportion difference (95% CI), p value	Incidence rate ratio (95% CI), p value
PCR positive	19 (10%)	1079 (48%)	−38% (−45 to −31), p<0·001	..
Observed person year	17·1	175·2	..	..
Incidence rate per year	1·11	6·16	..	0·18 (0·11 to 0·28), p<0·001

The analysis is based on the 2436 participants who had valid PCR data obtained during the prospective follow-up period. MCRDPI=Marine Corps Recruit Depot—Parris Island.

Table 2: **Comparison of SARS-CoV-2 infection (PCR positive) at MCRDPI between the seropositive and seronegative groups**

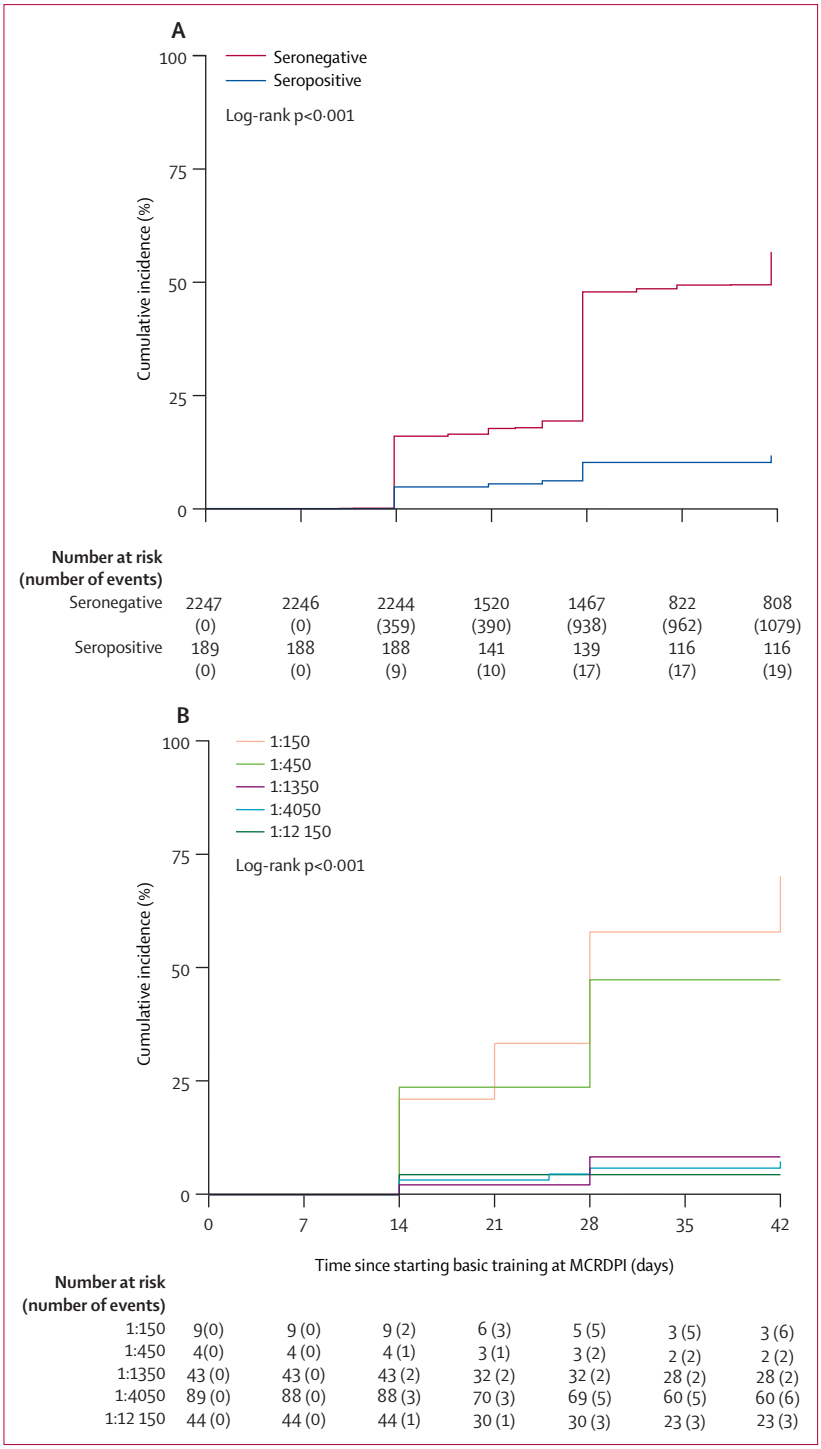

Figure 2: **SARS-CoV-2 PCR positive incidence curves during the 6-week follow-up period**
(A) Kaplan–Meier graph of overall cumulative incidence for testing PCR positive in the baseline seropositive and seronegative groups. (B) Kaplan–Meier graph of cumulative incidence for testing PCR positive in the seropositive group at different baseline full-length spike protein IgG titres, which ranged from 1:150 to 1:12 150. MCRDPI=Marine Corps Recruit Depot—Parris Island.

protein (log-rank p<0·001) as well as to S-RBD (log-rank p=0·0019; figure 2B; table 3; appendix p 5). The detailed Cox proportional hazard analysis gives a HR of 0·45 (95% CI 0·32–0·65; p<0·001) and 0·67 (0·47–0·96; p=0·028) for the full-length spike protein titre and S-RBD titre, both log-transformed, respectively (appendix p 2).

We examined baseline SARS-CoV-2 IgG neutralising antibody activity in all seropositive participants who became PCR positive during the observation period and in the first 54 participants who were seropositive but remained PCR negative. Neutralising activity was above the limit of detection in 45 (83%) of 54 seropositive participants who never became PCR positive, and in six (32%) of 19 participants infected during the 6 weeks of observation. The neutralising activity assessed as 50% inhibitory dose (ID50) was significantly higher in the participants who did not become PCR positive during the study (trend p<0·0001; table 3; appendix p 6).

We also compared viral load, estimated by PCR Ct values, between the seronegative and seropositive PCR infected groups and found that seronegative individuals had on average 3·95 lower cycle values for ORF1ab gene (95% CI 1·23 to 6·67; p=0·004), 2·60 lower cycles for S gene (–0·58 to 5·77; p=0·11), and 3·30 lower cycles for N gene (0·27 to 6·33; p=0·033) than seropositive individuals. The lower Ct values suggest an approximately 10-times higher viral load in the samples from seronegative participants (table 4). PCR positivity for more than 7 days was observed in six (32%) of 19 seropositive and subsequently infected participants compared with 510 (47%) of 1079 seronegative and subsequently infected participants (difference –0·16 [–0·38 to 0·07]; p=0·18). Symptomatic infection occurred in three (16%) of 19 participants versus 347 (32%) of 1079 participants (difference –0·16 [–0·38 to 0·05]; p=0·13), respectively. The specific symptoms reported by subsequently infected seropositive and seronegative participants are presented in the appendix (p 4).

Discussion

This study of primarily young, male Marine recruits found that the presence of antibodies to SARS-CoV-2 conferred an 82% reduced incidence rate of SARS-CoV-2 infection. The percentage of symptomatic infections in seropositive participants was half that in those who were seronegative, but the difference was not statistically significant. Among the seropositive group, the participants who became infected had lower antibody titres than those who were uninfected, and they were more likely to lack detectable baseline neutralising antibody activity. Our results indicate that although antibodies induced by infection are largely protective, they do not guarantee effective immunity against subsequent infection.

This study leveraged a 2-week USMC-mandated quarantine period during which baseline SARS-CoV-2 antibody status was established on arrival. Only baseline seronegative or seropositive participants who had multiple negative SARS-CoV-2 PCR tests during the

	Participants	1:150 titre	1:450 titre	1:1350 titre	1:4050 titre	1: 12 150 titre	Mean (SEM)*	p value†
S-RBD								
PCR positive	19	5 (26%)	5 (26%)	6 (32%)	3 (16%)	0	674·5 (181·1)	..
PCR negative	170	6 (4%)	49 (29%)	81 (48%)	27 (16%)	7 (4%)	1186·3 (86·2)	0·017
Full-length spike protein								
PCR positive	19	6 (32%)	2 (11%)	2 (11%)	6 (32%)	3 (16%)	1202·6 (472·7)	
PCR negative	170	3 (2%)	2 (1%)	41 (24%)	83 (49%)	41 (24%)	3723·7 (259·9)	p<0·0001
ID50 range‡	..	<20	(20–40)	(40–80)	(80–160)	(160–320)	..	..
Neutralisation								
PCR positive	19	13 (68%)	2 (11%)	3 (16%)	1 (5%)	0	16·8 (3·1)	..
PCR negative	54	9 (17%)	10 (19%)	19 (35%)	15 (28%)	1 (2%)	48·2 (5·7)	p<0·0001

Data are number of participants (%), unless otherwise specified. S-RBD and ID50 titres were determined as described in procedures.S-RBD=spike receptor-binding domain. *Mean (SEM) of titre denominators or ID50 values was computed after converting undetectable titre <20 to be 10. †Cochran-Armitage test for the trend. ‡ID50 is the titre at which a 50% reduction in virus infection was observed.

Table 3: **SARS-CoV-2 S-RBD and full-length spike IgG titres and neutralising antibody activity in PCR positive and PCR negative seropositive participants**

supervised quarantine were included in the prospective study. The three negative PCR tests during quarantine helped ensure that infections diagnosed during basic training were not persistent infections but incident infection occurring during the prospective period. The 2-week home quarantine preceding the supervised quarantine, as well as the relatively low frequency of infections diagnosed on arrival and during quarantine, further indicate that only incident infections were included in our analyses. The aggregate infection rate in both groups during the 6 weeks of observation at MCRDPI was 1098 (45%) of 2436 participants. In contrast, only 28 (0·9%) of 3249 participants were PCR positive on arrival at the supervised quarantine and less than 2% of participants became PCR positive during the 2-week quarantine period. Recruits were not permitted to leave MCRDPI and visitors were not allowed. Infections might have been introduced by permanent staff who interacted with the recruits. In view of the consecutive two periods of quarantine, the relatively low rate of infection during quarantine, and the three consecutive negative PCR tests, it is unlikely that any participants with persistent infection preceding their arrival at MCRDPI were entered into the prospective study. Even if this occurred, such cases would be unlikely to affect the relative risk calculations comparing the seropositive and seronegative groups. This methodology allowed for the creation of two well defined groups that entered basic training without active infection and differed primarily by baseline serology.

The high rate of infection at MCRDPI can be attributed to the crowded living conditions, demanding regimen, and requirement for personal contact during basic training despite the pandemic leads, which is known to contribute to an increased risk for respiratory epidemics.[28] The close quarters and constant contact among recruits that are needed for team building allow a viral infection to rapidly proliferate within a unit. The physically and mentally demanding training environment might also suppress immunity. These factors are not typically present in the civilian community. Therefore, the study setting limits the generalisability of our findings to other settings where the frequency and intensity of exposure and the susceptibility of the host might differ.

	Seropositive group (n=19)	Seronegative group (n=1079)	Difference comparison (95% CI), p value
PCR positivity >7 days	6 (32%)	510 (47%)	–0·16 (–0·38 to 0·07), p=0·18*
Symptomatic	3 (16%)	347 (32%)	–0·16 (–0·38 to 0·05), p=0·13
N gene Ct	27·7 (7·6)	24·4 (5·5)	3·30 (0·27 to 6·33), p=0·033†
S gene Ct	26·9 (7·1)	24·3 (5·3)	2·60 (–0·58 to 5·77), p=0·11
ORF1ab Ct	28·0 (7·0)	24·0 (5·3)	3·95 (1·23 to 6·67), p=0·004

Data are n (%) or mean (SD), unless otherwise specified. Ct=cycle threshold. *Difference in proportion for binary variables. †Difference in mean for the Ct values.

Table 4: **Comparison of symptoms and Ct values between SARS-CoV-2 infected (PCR positive) seropositive and seronegative groups**

The two groups had similar demographic profiles, with the exception of a higher prevalence of self-identified Hispanic and non-Hispanic Black participants in the seropositive group. The seropositive group included almost 50% Hispanic and 22% non-Hispanic Black participants compared with 22% and 12%, respectively, in the seronegative group. This is probably due to minority populations having higher seroprevalence rates during the COVID-19 pandemic in general and among young adults specifically.[26]

Rates of infection and the risk reduction provided by seropositivity are important for understanding transmission dynamics for COVID-19, for epidemiological modelling, and for estimating and achieving herd immunity levels—a major goal of mass vaccination strategies. Herd immunity is difficult to predict if the infection risk after natural and vaccine-induced immunity is unknown. Since SARS-CoV-2 vaccines might not provide sterile immunity, it is possible that both previously infected

and vaccinated individuals might later become infected. It is not known whether either can contribute to transmission events. We found only a modest, approximately 10-times decrease in nares viral load as estimated by swab PCR Ct levels in the seropositive compared with the seronegative infected participants. This finding suggests that some reinfected individuals could have a similar capacity to transmit infection as those who are infected for the first time. The rate at which reinfection occurs after vaccines and natural immunity is important for estimating the proportion of the population that needs to be vaccinated to suppress the pandemic.

The clinical outcomes between seropositive and seronegative groups were similar, with the majority (84% and 68%, respectively) being asymptomatic. The two groups did not show a significant difference in the duration of PCR positivity. No participants in either group needed inpatient care. Although our findings are limited to healthy young adults, studying this population does have the advantage of reducing the confounding factors of age and comorbid illnesses.[29] Infection in seropositive participants was associated with lower SARS-CoV-2 IgG titres and absent or lower levels of neutralising antibody activity. Young adults have high rates of asymptomatic and pauci-symptomatic infection, which has been associated with lower levels of antibodies and potentially a less robust immune memory response.[30,31] This could lead to higher overall rates of reinfection among this population than in other populations. We did not examine the role of cell-mediated immunity or host, environment, and viral factors leading to reinfection.

Since the study population is a fairly accurate representation of the races and ethnicities in the US population among 18–20-year-olds, the results are most applicable to young male adults. The relative risk of infection might be different in seropositive females and in adults of other ages or health status, who might differ in immunological responses to SARS-CoV-2 infection.[32] Notably, a study of subsequent SARS-CoV-2 infection in seropositive compared with seronegative British health-care workers found a nearly identical adjusted odds ratio of reinfection (0·17 [95% CI 0·13–0·24]) to that reported in Marine recruits.[32] The concurrence of these studies suggests that the risk of reinfection might be similar for young adults and the general population. Other limitations of our study include not being able to investigate the exposure event during a seropositive participant's initial infection before arrival at quarantine, the absence of information about the symptoms and severity of the first episode, the inability to confirm initial SARS-CoV-2 infection by PCR in the seropositive group, and potentially missing detectable infections that occurred between sampling every 2 weeks. A total of 18% of the study participants (15% of baseline seropositive participants and 19% of baseline seronegative participants) who began basic training at MCRDPI did not report for follow-up 2 weeks later (figure 1). This is not a usual loss to follow-up within a study, but is rather a composite of individuals who drop out of the study, who are transferred off the base for medical reasons, or who are separated from the USMC. The reason any recruit did not return for follow-up is unknown to the investigators.

Our investigation is likely to underestimate the risk of SARS-CoV-2 infection in previously infected individuals because the seronegative group included an unknown number of previously infected participants who did not have significant IgG titres in their baseline serum sample. Despite this underestimation, we found that previously infected participants identified by seropositivity are susceptible to repeat infection, with nearly one-fifth the incidence rate of those without evidence of previous infection. This suggests that COVID-19 vaccination might be necessary for control of the pandemic in previously infected young adults.

Contributors

AGL, IR, and SCS provided overall leadership and guidance to the investigation. YG performed statistical analysis and data curation. SV, CG, DLW, RAL, CKP, and IR contributed to data analysis. RAL, NM, JM, SM, EN, and ESA contributed to sample collection and management. CG, DLW, and WDG contributed to data collection. SV, NAK, CAB, DE, AS-S, PB, NM, CMM, SM, DMP, DS, PS, and IR performed serological assays. HWC and VAS performed PCR assays. AGL, DLW, and CKP had access to all deidentified data. YG, SV, DLW, and IR verified the data. AGL, YG, SV, DLW, NAK, AS-S, RAL, CMM, CKP, MT, IR, and SCS contributed to data interpretation. SEL collected and managed samples. AGL, CG, FJ, RAL, and EN supervised sample collection. AGL, CG, and RAL supervised data collection. RPT, FK, AB, and IR supervised serology assays. MS supervised PCR assays. MC-G and MT provided project administration and coordination. YG, SV, VDN, CKP, and IR prepared figures. AGL, YG, SV, IR, and SCS wrote and revised the original draft. All authors reviewed and approved the final manuscript. AGL and SCS had final responsibility for the decision to submit for publication.

Declaration of interests

DMP owned stock in Co-Diagnostics Inc during the conduct of the study. DS and FK have filed a patent regarding serological assays for SARS-CoV-2 and Icahn School of Medicine at Mount Sinai has founded a company to commercialise serological assays they developed. AGL, CG, DLW, HWC, DE, WDG, FJ, JM, EN, CKP, ESA, MS, VAS, RAL, SEL, PS, MT, and PS are military service members or government service employees. This work was prepared as part of their official duties. Title 17, US Code §105 provides that copyright protection under this title is not available for any work of the US Government. Title 17, US code §101 defines a US Government work as a work prepared by a military service member or employee of the US Government as part of that person's official duties. The views expressed in the article are those of the authors and do not necessarily express the official policy and position of the US Navy, the Department of Defense, the US Government or the institutions affiliated with the authors. AGL, YG, SV, CG, DLW, HWC, NAK, CAB, DE, M-CG, WDG, FJ, RAL, SEL, JM, NM, CMM, PB, SM, VDN, EN, CKP, ESA, AS-S, MS, VAS, MT, PS, RPT, AB, IR, and SCS declare no competing interests.

Data sharing

The data obtained from this project are restricted by US security laws or regulations since it involves Department of Defense Marine recruits. The individual participant data fall under the requirements required under applicable law or regulation, including without limitation of 15 US Code §3710a as applicable.

Acknowledgments

We thank Quanterix (Billerica, MA, USA) for providing beta-level serological assay kits used for ELISA validation studies; Mary Anne

Amper, Nitish Seenarine, Mital Vasoya, Illya Aronskyy, and Braian Qela for outstanding technical assistance; German Nudelman and Stas Rirak for database development; Celia Gelernter and Adam B Sealfon for editing assistance; Mitchel Rabinowitz for project management; Terri Brantley and Andrea Gates for administrative support; Adam Armstrong for strategic guidance; the many US Navy corpsmen who assisted in the logistics and sample acquisition; and the devoted Marine recruits who volunteered for this study. This work received funding from the Defense Health Agency through the Naval Medical Research Center (9700130) and from the Defense Advanced Research Projects Agency (contract number N6600119C4022).

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