Clinical and functional assessment of SARS-CoV-2 sequelae among young marines – a panel study

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Summary

Background Long-term SARS-CoV-2 adverse health outcomes are of significant concern, especially among young adults with the potential for the greatest long-term morbidity. We sought to assess and characterize these outcomes in a cohort of Marines.

Methods We used a cohort of US Marines from a previous longitudinal, prospective observational study of acute SARS-CoV-2, most of whom were enrolled prior to infection. A panel study was established to assess for post-acute sequelae of COVID-19 (PASC), defined as symptoms at least 4 weeks after symptom onset or diagnosis. Symptoms were assessed through questionnaires and validated quality of health metrics. Periodic US Marine Corps fitness testing metrics provided an additional standardized functional assessment and were compared to a pre-pandemic cohort.

Findings Globally dispersed Marine participants (n = 899) seen an average of 330 days following initial enrollment were predominately male (n = 825, 91.7%), White (n = 613, 71.6%) or Black (n = 149, 17.4%) with a median age of 18 years (interquartile range: 18–19). Among 798 SARS-CoV-2 infected participants, 197 (24.7%) developed PASC. The most prevalent symptoms were loss of taste and/or smell (n = 82; 41.6%), shortness of breath (n = 74; 37.6%), and cough (n = 45; 22.8%). Those with PASC had higher rates and severity of somatic (p < 0.0001), general depressive (p < 0.0001), and anxiety (p = 0.005) symptoms. Compared to a historic cohort of Marines, participants with PASC scored worse on their physical fitness assessments due to slower run times (p = 0.002). Those with PASC continued to have decreased physical performance one year after completing initial training.

Interpretation In this population of healthy young adult US Marines with mostly either asymptomatic or mild acute COVID-19, one fourth reported physical, cognitive, or psychiatric long-term sequelae of infection. The Marines affected with PASC showed evidence of long-term decrease in functional performance suggesting that SARS-CoV-2 infection may negatively affect health for a significant proportion of young adults.

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Research in context

Evidence before this study

We searched PubMed for longitudinal studies among young, healthy adults evaluating the risk and effects of post-acute symptoms of COVID-19 (PASC). We used the following search terms: "military" AND ("long covid" OR "PASC"), ("PASC" OR "long COVID") AND "young" AND "adult". We identified several cohorts established to evaluate PASC in various populations. These included the RECOVER Cohort, the Johns Hopkins COVID Long Study, the Arizona CoVHORT, the Dallas Fort-Worth (DFW) COVID-19 Prevalence Study, as well as population-based cohort studies in the Ukraine, Denmark, Norway, and Costa Rica, and in military populations in France, Switzerland, Belgium, and the UK. Most of these studies highlighted a risk of PASC even following symptomatically mild infections. Furthermore, chronic symptoms were heterogeneous with the potential to negatively affect quality of life months after initial infection. In military populations, long-term sequelae were reported in comparable frequencies to the general population with negative effects on exercise performance.

Added value of this study

The findings of this COVID-19 Health Action for Marines (CHARM) 2.0 study add to the expanding literature on the chronic sequelae of acute infection with SARS-CoV-2. Importantly, our population was initially enrolled in the CHARM study at the very early stages of the SARS-CoV-2 pandemic and followed prospectively throughout the early stages of their recruit training. After completing recruit training, our population departed to their subsequent duty stations for additional training and/or assignment where they were located and assessed for PASC. Because our population was limited to active-duty US Marines, the population is a unique young and healthy subset of the general population with little to no comorbid conditions. As such, the initial acute SARS-CoV-2 infections were predominately asymptomatic or pauci-symptomatic. Regardless, even in this population, approximately a quarter developed PASC which was associated with higher rates of somatic symptoms, depression, and anxiety. Additionally, PASC had a measurable effect on physical fitness exemplified by an increase in the time needed to complete a standard run as part of the Marine Corps physical fitness assessment in those with PASC compared to uninfected Marines and a reference cohort of pre-pandemic Marines. These objective and subjective data highlight the significant negative effects of acute SARS-CoV-2 infection on long-term health and point to an important disease burden.

Implications of all the available evidence

The risk of long-term sequelae secondary to acute SARS-CoV-2 infection varies across studies; however, even in young healthy populations with very mild acute illness a proportion of infected individuals develop long-lasting symptoms. These symptoms are associated with physiological changes, measurable reductions in quality of life, and reduced physical fitness. The heterogenous nature of these chronic sequelae confound efforts to identify consistent risk factors and limit available tools for risk mitigation. More work is needed to better understand the host–pathogen interaction to inform these mitigation and treatment strategies.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), is associated with long-term clinical post-infection sequalae.[1] Despite advances in our understanding of pathogenesis,[2,3] the need for studies on post-acute sequelae of COVID-19 (PASC) is widely recognized.[4–6]

PASC is associated with decreased functional capacity[7] and reduced exercise capacity,[8] particularly concerning for those[9] whose jobs or hobbies require physical fitness[10] and who are unable to return to full participation following infection.[11] PASC can lead to decreased performance or absenteeism from work, impaired daily functioning, and poor quality of life.[12] Mental health concerns including depression, persistent cognitive and psycho-emotional deficits, and decreased inhibition, is seen even among young adults following mild COVID-19.[13]

Studying PASC in young adults who often experienced mild or asymptomatic infection[14] is particularly difficult due to their infrequent interaction with or distrust of the medical system.[15] However, younger adults have the potential to have the most long-lasting sequelae with extended disability and decreased functional status.[16,17] Longitudinal studies with proper controls are needed to determine the burden of PASC on young adults.[18]

The COVID-19 Health Action Response for Marines (CHARM) Study was a longitudinal, prospective, observational study of United States (US) Marine Corps recruits with scheduled visits and PCR testing for SARS-CoV-2 from Spring-Fall 2020.[14] More than 90% of the CHARM participants were serologically naïve at enrollment.[14,19] A follow-on study, CHARM 2.0, sought to extend the observation period among CHARM participants as they transitioned from recruit training into military service. The CHARM 2.0 study sought to assess

the risk of long-term SARS-CoV-2 complications in this otherwise young, healthy cohort.

Methods

Study design

CHARM 2.0 was a panel study, a type of longitudinal study with data collected at specific intervals,[20] in which a cross-section of CHARM participants[14] were longitudinally sampled approximately every 6 months after completing recruit training. Visits were asynchronous and depended on a participant's availability at the site on the specific date (Fig. 1A and B). Remaining on active duty was an inclusion criterion for this study. CHARM 2.0 participants were enrolled at their duty stations throughout the continental United States, Hawaii, and Okinawa, Japan. Participants were contacted by the study team from February 2021 until April 2022. All participants provided written informed consent prior to the initiation of any study procedures. This study was approved by the ethical review committee at the Naval Medical Research Command, Silver Spring, MD (NMRC.2021.0004) in compliance with all Federal regulations governing the protection of human subjects. This study followed the STROBE guidelines.

Questionnaire

Participants completed a detailed questionnaire (see Supplementary Methods) that captured medical history since participation in the original CHARM study, including information on SARS-CoV-2 infections and acute and long-term effects of SARS-CoV-2 infection. The questionnaire also incorporated validated questionnaires to assess well-being (Patient Health Questionnaire-15, PHQ-15[21]), depression (PHQ-8),[22] anxiety (Generalized Anxiety Disorder-7, GAD-7[23]), and sleep (Epworth Sleep Scale, ESS[24–26]).

COVID-19 symptom severity was categorized using a 10-point continuous scale as mild (2–4), moderate (5–6) or severe (7–10) based on participant self-report severity. Participants also listed, using free text, the COVID-19 symptoms that lasted at least one month. Additionally, participants self-reported functional impairments due to COVID-19, including lost workdays, inability to perform one's duty, and reduced ability during Marine-specific fitness testing.

Fitness metrics

US Marine Corps official fitness data were also obtained from US Marine Corps Manpower and Reserve Affairs for all study participants.[27,28] Data from a reference
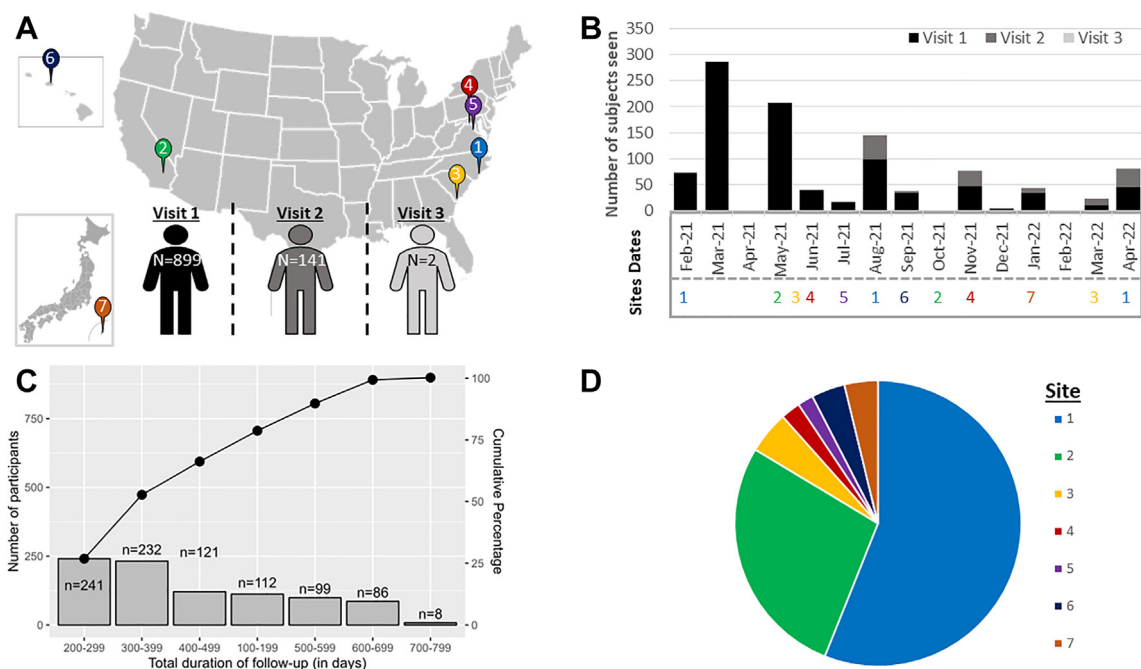

Fig. 1: Enrollment and follow-up of participants in CHARM 2.0 across research sites and over time. A: Locations of research sites included: 1) Camp Lejeune (Jacksonville, NC) and surrounding bases; 2) Twentynine Palms, CA and Camp Pendleton (Oceanside, CA); 3) Beaufort, SC and Marines Corps Recruit Depot Parris Island (Parris Island, SC); 4) Washington, DC; 5) Quantico, VA and Dam Neck, VA; 6) Marine Corps Base Hawaii MCBH (Kaneohe Bay, Honolulu, HI); 7) Okinawa, Japan. A total of 899 participants were seen once, 141 seen twice, and 2 seen three times. B: The number of participants completing the research survey and clinical assessments during each site visit by site over the study's duration. C: Total duration of participant follow-up from their initial enrollment into the CHARM 1.0 study (May–September 2020) and their last assessment in this study. D: The number of enrolled participants by research site.

cohort (years 2016–2019) were obtained for comparison. Data included results of each participant's physical fitness test (PFT), combat fitness test (CFT), and rifle range (or marksmanship) assessment, mandatory tests each Marine performs during recruit training and at least annually thereafter. The timing of these fitness metrics, which was asynchronous, relative to enrollment in CHARM 1.0 is shown in Supplementary Table S1.

Outcome definitions

We defined post-acute sequelae of COVID-19 (PASC) as persistent symptoms and/or delayed or long-term complications of SARS-CoV-2 infection beyond 4 weeks from symptom onset or first PCR positive test.[29,30] Participants' free-text reports of COVID-19 symptoms lasting at least 4 weeks were examined to identify clearly relevant keywords, which were grouped into 14 categories by two study team physicians. Keyword occurrences flagged by text search and vetted by the physicians were then used to construct a category-based symptom experience profile for each participant. SARS-CoV-2 infection was determined based on documented infection using PCR during the original CHARM prospective cohort study,[19] seropositivity (as defined below) or self-reported SARS-CoV-2 infection.

Serum IgG SARS-CoV-2-specific antibodies were assessed using an enzyme-linked immunosorbent assay (ELISA) as previously described (see Supplementary Methods).[19,31] Samples with a S-specific IgG titer higher than 150 were considered seropositive. Pre-vaccination, presence of S-specific IgG antibodies was used to assess probable antecedent infection. After vaccination, IgG antibodies to the N protein were used to determine SARS-CoV-2 infection. In this case, serum samples were evaluated at a 1:50 dilution in plates coated with N protein, and those with an OD 492 nm value higher than the average of the 8 negative controls plus three times their SD were considered positive.

SARS-CoV-2 vaccination data

SARS-CoV-2 vaccination data were obtained from the Department of Defense (DoD) electronic health records. Participants were characterized as vaccinated against SARS-CoV-2 if they completed a two-dose vaccination series of an mRNA vaccine or single dose of the Johnson & Johnson vaccine prior to the assessment time point.

Statistical analysis

Comparisons between groups based on demographics, vaccination status, presence of PASC, or other measures were made using Pearson's chi-square for categorical data or Students' t-tests or analysis of variance (ANOVA) for continuous measures, unless appropriate assumptions were not met in which case Fisher's Exact and Kruskal–Wallis rank sum tests were utilized. Missing data were assumed to be missing completely at random.

For linear mixed modeling, outliers were detected by Bonferroni Outlier Test using t distribution to test larger studentized residual in the models as being statistically different from other observations. Outlier observations with Bonferroni p-value < 0.05 were conservatively removed from data for further analysis.[32,33] Robust linear mixed-effect models (see Supplementary Methods) with the R package *robustlmm* were used to evaluate the effect of PASC and vaccination on fitness metrics to account for non-independence, random variability, unbalanced groups, and a within-subjects design. Fitness metrics were non-normally distributed (identified by Shapiro–Wilk test) and were heteroskedastic (Bartlett and Breusch Pagan test). All statistical analyses were performed using R Statistical Software (v4.1.2; R Core Team 2021) and interpreted using a two-tailed *alpha* = 0.05.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript.

Results

A total of 899 (25.9%) of the 3472 CHARM 1.0 participants consented for at least one visit in CHARM 2.0. Participants were predominately male (91.8%), white (71.6%) with a median age of 18 years (interquartile range: 18, 19) (Table 1). Among the 899 participants evaluated at 7 different sites, 141 (15.7%) were also evaluated at a second CHARM 2.0 visit (two participants were also evaluated at a third visit) (Fig. 1A and B). The mean total duration of follow-up from initial CHARM 1.0 study enrollment was 367 days (standard deviation, SD: 145) (Fig. 1C). The average number of days between enrollment in the prospective, longitudinal assessment of CHARM 1.0 to the first panel visit was 330 days (SD: 131.4) with an additional average of 234 days (SD: 90.1) between the first and second CHARM 2.0 visits and second and third CHARM 2.0 visits, respectively. Over half of the participants were enrolled at site 1 (Fig. 1D).

Of the 899 participants, most (n = 798; 88.8%) were previously infected with SARS-CoV-2 as determined by at least one of i.) PCR testing during the active surveillance period of the original CHARM 1.0 study (n = 367, 46.0%); ii.) 4-fold increase in serum IgG titers at the CHARM 2.0 follow-up visits (n = 406, 50.9%); or iii.) self-reported diagnosis (n = 25, 3.1%). A total of 307 participants had an asymptomatic infection, ascertained either by active longitudinal surveillance in CHARM 1.0 or based on self-report at follow-up and 491 had a symptomatic infection. Among the 141 participants evaluated at more than one CHARM 2.0 visit, 3 (2.1%) had a documented seroconversion between the first and second CHARM 2.0 visit. Among the 790 participants with SARS-CoV-2 who answered questions regarding

	CHARM 2.0 participants (N = 899)	CHARM 1.0 participants not participating in CHARM 2.0 (N = 2573)	p-value
Age, median (25%,75%)	18 (18, 19)	18 (18, 19)	0.7
Sex, n (%)			0.1
Female	74 (8.2)	260 (10.1)	
Male	825 (91.7)	2313 (89.9)	
Ethnicity, n (%)			0.1
Hispanic	238 (35.7)	603 (32.3)	
Non-Hispanic	429 (64.3)	1268 (67.7)	
(Missing)	232	702	
Race, n (%)			0.01
White	613 (71.6)	1870 (76.9)	
Black	149 (17.4)	366 (15.0)	
Asian	29 (3.4)	52 (2.1)	
American Indian/ Alaska Native	10 (1.2)	29 (1.2)	
Hawaiian/other Pacific Islander	3 (0.4)	12 (0.5)	
Multi-racial	27 (3.2)	67 (2.8)	
Other	25 (2.9)	37 (1.5)	
(Missing)	43	140	

All 3472 participants in the CHARM 1.0 study were eligible for enrollment in the CHARM 2.0 long-term follow-up panel study. While efforts were made to contact all CHARM 1.0 participants, not all participants were able to be contacted or remained on active duty, and among those, only a proportion reported for follow-up visits n = 899. The demographics of the CHARM 1.0 participants who did and did not participate in CHARM 2.0 are compared using Wilcoxon rank sum test (for age) and Pearson's Chi–Square test for categorical variables (sex, ethnicity, race).

Table 1: Demographics of CHARM participants who participated in the CHARM 2.0 panel study compared to non-participants.

the impact of their infection on their work activities, 18.8% (137/790) reported that they missed work or were unable to fulfill their duties or normal activities due to COVID-19 and 5.8% (46/792) presented for medical care.

When asked about the overall impact of the illness on their health, only 195 reported an effect with participants predominantly self-reporting a mild illness (151, 77.4%), while 20.0% (n = 39) and 2.6% (n = 5) reported moderate or severe illnesses, respectively. No participants in our study were hospitalized. The most frequently reported acute symptoms were tiredness (n = 339; 42.5%), difficulty breathing/shortness of breath (n = 338; 41.4%), nasal congestion (n = 336; 42.1%), and dry cough (n = 327; 41.0%) (Fig. 2).

Almost a third (197/620; 31.8%) of SARS-CoV-2 infected participants who responded to the question about current health status reported not having returned to full health at the time of their first CHARM 2.0 encounter. Among the 798 participants with a SARS-CoV-2 infection, 197 (24.7%) met the definition for PASC during at least one of their CHARM 2.0 visits,

most 56% (110/197) following a PCR-confirmed infection. Of note, 10/110 (9%) of those with PASC following a PCR-confirmed infection were asymptomatic during their infection. Participants with PASC reported a constellation of overlapping symptoms (Fig. 3). The most common long-term symptom (lasting ≥4 weeks) was loss of taste and/or smell (82/197; 41.6%) followed by shortness of breath (74/197; 37.6%), and cough (45/197; 22.8%). Participants with PASC were more likely to report not having returned to full health during their first CHARM 2.0 visit (78/169; 46.1%) than were infected participants without PASC (119/451; 26.4%) (p < 0.0001). Interestingly, 15 (10.6%) of the 141 participants with multiple CHARM 2.0 visits met the definition for PASC (reported symptoms that lasted at least 1 month) at CHARM 2.0 Visit 2 but not CHARM 2.0 Visit 1.

Among 197 participants with PASC, 33 met the PASC definition at their first CHARM 2.0 visit and had a second visit as well (mean of 242 days between encounters). Among these, 48.5% (16/33) reported loss of taste/smell at visit 1 and 31.3% (5/16) reported the symptom continuing at the second visit (Supplementary Table S2). Similarly, 39.4% (13/33) reported shortness of breath at the initial CHARM 2.0 visit with 30.8% (4/13) continuing to report it at the second. None of the participants reported loss of taste or smell or shortness of breath only during the second CHARM 2.0 visit.

PASC was associated with an increased severity of somatic symptoms at each CHARM 2.0 visit as measured by the PHQ-15 (Table 2). Specifically, 13.2% of participants with PASC at panel visit 1 had a medium or high level of somatic symptom severity compared to 5.9% of participants with a SARS-CoV-2 infection but not meeting the definition of PASC and 6.7% of participants without a documented infection (p = 0.005). The proportion of participants with depressive symptoms as measured by the PHQ-8 (Table 2) was similar (p = 0.2) in participants with PASC, those uninfected without PASC and in uninfected participants in the first CHARM 2.0 visit. Mean PHQ-8 (current depression) scores were higher (p < 0.0001) in those with PASC (mean = 3.2) than uninfected participants (mean = 1.9) or those infected without PASC (mean = 1.9).

Participants with PASC more commonly reported anxiety symptoms than those without PASC. At the first CHARM 2.0 visit, 9.3% of participants with PASC had an anxiety level greater than mild; higher than in those without PASC and uninfected participants (p = 0.05) (6.3% and 1.9%, respectively). PASC participants also documented greater daytime sleepiness compared to other participants with mean ESS scores significantly higher in those with PASC seen at the initial CHARM 2.0 visit (p = 0.0002) and those assessed at a second CHARM 2.0 visit (p = 0.04).

SARS-CoV-2 vaccinations were initiated before and during the CHARM 2.0 follow-up with variable

Fig. 2: Self-reported severity of solicited acute symptoms of COVID-19 among CHARM 2.0 participants Footnote: These are the symptoms reported by participants as part of their survey completion during their initial CHARM 2.0 visits. The severity indicated is based on the maximum severity reported at any of the panels.

vaccination proportions. During the first CHARM 2.0 visit, 31.8% (275/866) of participants had completed the vaccination series (data missing on 33 participants). By the second visit, that increased to 82.9% (116/140) (data missing on 1 participant). Stratification by vaccination status demonstrated no significant differences in the prevalence or severity of somatic, or depressive symptoms, anxiety, or daytime sleepiness at panel visit 1. An increase in somatic symptoms among unvaccinated participants was identified at the second CHARM visit (p = 0.03, Supplementary Table S3).

PASC was associated with a significantly increased 3-mile run time on the standard Marine fitness test

(Table 3). Specifically, after controlling for gender and the timing of the physical fitness assessment, PASC participants ran 25.1 s (95% CI: 9.0–41.2) slower than a pre-pandemic reference cohort composed of 22,612 Marine recruits from 2016 to 19. However, uninfected CHARM participants had run times comparable to the reference cohort. The longer run time yielded a significant reduction (–5.2 points; 95% CI: –9.3 to –1.1) in the overall physical fitness score only among those with PASC (Supplementary Table S4). The CHARM population in general (regardless of infection or PASC) performed better than the reference population on their rifle range assessments (Supplementary Table S5).

Fig. 3: Upset plot of post-acute sequelae of COVID-19 lasting at least 4 weeks among 197 CHARM 2.0 participants with post-acute symptoms of COVID-19. Footnote: The upset plot enables the visualization of the overlap of numerous nominal variables. Within each vertical 'column', the number of individuals with each symptom (one darkened circle) or combination of symptoms (multiple darkened circles) is shown in the above column chart. Across each horizontal row, the number of individuals with a symptom with or without other symptoms is shown in the bar chart on the left side of the figure.

	CHARM 2.0 Visit 1				CHARM 2.0 Visit 2			
	PASC (N = 182)	Infected no PASC (N = 613)	Uninfected (N = 104)	p-value	PASC (N = 48)	Infected no PASC (N = 80)	Uninfected (N = 13)	p-value
PHQ-15								
Minimal (0–4), n (%)	111 (61%)	484 (80%)	86 (84%)	<0.001	30 (62%)	59 (76%)	10 (77%)	0.09
Low (5–9), n (%)	47 (26%)	88 (14%)	9 (8.8%)		9 (19%)	16 (21%)	2 (15%)	
Medium (10–14), n (%)	14 (7.7%)	28 (4.6%)	1 (1.0%)		6 (12%)	3 (3.8%)	0 (0%)	
High (15–30), n (%)	10 (5.5%)	8 (1.3%)	6 (5.9%)		3 (6.2%)	0 (0%)	1 (7.7%)	
Mean (standard deviation)	4.3 (4.9)	2.4 (3.6)	2.4 (4.2)	<0.001	4.3 (5.0)	2.6 (3.2)	3.1 (4.8)	0.3
PHQ-8								
None-minimal (0–4), n (%)	138 (76%)	514 (85%)	83 (81%)	0.2	34 (71%)	64 (82%)	11 (85%)	0.04
Mild (5–9), n (%)	26 (14%)	65 (11%)	14 (14%)		11 (23%)	7 (9.0%)	0 (0%)	
Moderate (10–14), n (%)	9 (4.9%)	16 (2.6%)	3 (2.9%)		1 (2.1%)	5 (6.4%)	0 (0%)	
Moderately Severe (15–19), n (%)	6 (3.3%)	8 (1.3%)	2 (2.0%)		2 (4.2%)	1 (1.3%)	2 (15%)	
Severe (20–24), n (%)	3 (1.6%)	5 (0.8%)	0 (0%)		0 (0%)	1 (1.3%)	0 (0%)	
Mean (standard deviation)	3.2 (4.9)	1.9 (3.6)	1.9 (3.6)	<0.001	2.9 (4.2)	2.4 (4.1)	2.6 (6.4)	0.12
GAD-7								
None-minimal (0–4), n (%)	146 (80%)	532 (88%)	90 (88%)	0.05	37 (77%)	66 (85%)	12 (92%)	0.3
Mild (5–9), n (%)	19 (10%)	53 (8.7%)	10 (9.8%)		7 (15%)	6 (7.7%)	0 (0%)	
Moderate (10–14), n (%)	9 (4.9%)	10 (1.6%)	1 (1.0%)		1 (2.1%)	4 (5.1%)	0 (0%)	
Severe (15–21), n (%)	8 (4.4%)	12 (2.0%)	1 (1.0%)		3 (6.2%)	2 (2.6%)	1 (7.7%)	
Mean (standard deviation)	2.52 (4.6)	1.52 (3.4)	1.29 (2.9)	0.005	2.7 (4.7)	1.9 (4.0)	1.4 (4.1)	0.11
ESS								
0–10, n (%)	142 (78%)	501 (83%)	84 (83%)	0.4	38 (79%)	66 (85%)	11 (85%)	0.8
>10, n (%)	40 (22%)	106 (17%)	17 (17%)		10 (21%)	12 (16%)	2 (15%)	
Mean (standard deviation)	7.0 (5.3)	5.6 (5.0)	4.7 (5.0)	<0.001	7.5 (5.1)	5.3 (5.3)	5.2 (5.8)	0.04

Patient Health Questionnaire-15, PHQ-15; Patient Health Questionnaire-8, PHQ-8; Generalized Anxiety Disorder-7, GAD-7; Epworth Sleep Scale, ESS; post-acute sequelae of COVID-19, PASC. The proportion of individuals in each severity category based on PASC, no PASC and uninfected status was compared using Fisher's Exact Test for count data with a simulated p-value (based on 2000 replicates) and the overall scores were compared using a Kruskal–Wallis rank sum test. CHARM 2.0 visit 3 not assessed due to the small number of participants.

Table 2: Self-assessment of somatic symptoms (PHQ-15), depression (PHQ-8), anxiety (GAD-7), and daytime sleepiness (ESS) among panel 1 and 2 participants stratified by the presence of PASC.

Discussion

We describe subjective and objective results of a panel study assessing PASC among a young population of US Marines. The strength of this study is that participants were followed longitudinally prior to SARS-CoV-2 infection, through initial infection, and subsequently assessed for PASC reducing the risk of selection bias and other confounders. Additionally, we analyzed self-

	Run time		Pullups		Crunch		Combined Score	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Intercept	1508.45	1506.14–1510.75	6.67	6.54–6.80	98.14	97.84–98.43	240.3	239.71–240.88
Reference cohort	Reference	–	Reference	–	Reference	–	Reference	–
Uninfected	−12.12	−33.32 to 9.09	−0.63	−1.68 to 0.41	5.28	2.56–8.00	2.48	−2.95 to 7.91
Infected no PASC	8.87	0.17–17.56	−0.28	−0.71 to 0.15	3.36	2.22–4.49	−0.16	−2.38 to 2.06
Infected + PASC	25.08	9.00–41.16	−0.69	−1.50 to 0.11	0.92	−1.17 to 3.01	−5.17	−9.28 to −1.06
Female	Reference	–	Reference	–	Reference	–	Reference	–
Male	−202.52	−204.91 to −200.14	8.40	8.26–8.53	11.11	10.81–11.41	7.24	6.64–7.85
Timepoint 1	Reference	–	Reference	–	Reference	–	Reference	–
Timepoint 2	67.98	67.13–68.83	−0.10	−0.13 to −0.06	−2.68	−2.81 to −2.56	−7.9	−8.10 to −7.70
Timepoint 3	85.95	84.91–86.98	0.68	0.64–0.72	−0.45	−0.60 to −0.31	−5.17	−5.41 to −4.93

PASC, post-acute sequelae of COVID-19; CI, confidence interval. Run times are assessed in seconds to complete a three-mile run. Pull-ups and crunches are assessed as the number of repetitions possible within 2 min. Each parameter is scored based on pre-specific metrics and a total score is calculated with a maximum score of 300 points. The physical fitness timepoints were based on each participant's individual schedule, the timing of which was asynchronous and occurred as shown on Table S1.

Table 3: The association between PASC and physical fitness test scores among 899 CHARM 2.0 participants and a reference cohort of 22,612 Marines as assessed by a robust linear mixed-effect models after controlling for potentially important covariates.

reported symptoms and standardized, structured physical performance assessments to evaluate the effect of SARS-CoV-2 infection and PASC.

Among the 899 participants, 88.8% had a SARS-CoV-2 infection. Almost a quarter (24.7%) of these individuals had at least one COVID-19 symptom that lasted for at least 4 weeks meeting the *a priori* definition of PASC established for this study. Among those with PASC, 10 had no acute SARS-CoV-2 symptoms after PCR-confirmed infection suggesting that PASC can occur among asymptomatic individuals. Many participants reported that lingering symptoms impaired their productivity at work, caused them to miss work, and/or limited their ability to perform normal duty/activities.

Marines with PASC had significantly decreased physical fitness test scores up to approximately one year post-infection with a three-mile run time that averaged in the 65th percentile of the reference cohort. Scores for events evaluating upper body (pull-ups, crunches, and ammo can lift) were not significantly reduced by PASC; however, overall physical fitness scores were reduced. The poorer run times and overall scores among PASC participants are indicative of ongoing functional effects.

Although Marine fitness metrics might be an atypical objective measure of physical performance for the general population, they can provide insight into how PASC affects the musculoskeletal, neurologic, and cardiopulmonary systems. For example, a three-mile run evaluates aerobic exercise, overhead lifting of an ammunition can and pull-ups evaluate strength, and shooting a rifle evaluates fine-motor skills. Each event is regulated and standardized.[27,28] Although effort cannot be directly measured, the United States Marine Corps creates a competitive environment to maximize performance and uses these scores as a criterion for rank promotion and subsequent pay increases, incentivizing maximal effort.

Standardized health-based assessments for somatization, depression, and anxiety further highlighted the detrimental health effects of PASC. Almost 10% of participants with PASC had PHQ-8 scores ≥10. Increased somatization has been associated with increased stress, depression, and problems with emotions.[22] Additionally, PASC participants had higher GAD-7 scores suggesting increased anxiety in a population with unique inherent occupational stressors associated with higher rates of anxiety, depression, and post-traumatic stress disorder.[34] Increased severity of anxiety among those with PASC combined with greater rates of mental health disorders in general could portend an ominous combination and should be closely followed.

Like others, we identified cardiopulmonary symptoms as some of the most prevalent.[35–37] The high prevalence of symptoms like shortness of breath, difficulty breathing, cough, and fatigue is particularly notable when combined with decreased objective measures of aerobic performance such as running. These results suggest pathology in the cardiopulmonary system. In contrast we observed no reduction in scores assessing strength and marksmanship suggesting the lack of detectable pathology in the neuro-musculoskeletal system. We have previously found in this same cohort that SARS-CoV-2 infection causes prolonged dysregulation of immune cell epigenetic patterns like auto-immune diseases.[38] Further studies into the pathophysiology and epigenetic changes due to PASC and the potential ongoing detrimental effects on aerobic activity in this and similar populations is warranted.

Importantly, the definition utilized for PASC in our study of at least 4 weeks, although within the timeframe and consistent with other studies, is less than the 3 months post-infection proposed in a consensus definition.[39] Our study was designed and implemented during a time (early 2021) when the definitions of PASC and/or long-COVID were evolving. It is unknown whether our observed PASC rate would be the same using updated definitions and criteria. Regardless, the proportion of participants we have observed with symptoms lasting at least a month is concerning, particularly in the context of this young, otherwise healthy population. Furthermore, the decreased run times in those with PASC suggest that these predominantly mild infections caused a long-term, and significant functional deficit among a large proportion of this previously healthy young adult cohort.

Our study did have some limitations. The intent was to serially follow 3472 Marines who participated in the CHARM 1.0 study; however, by the CHARM 2.0 study initiation 32.1% had left active duty likely related to normal attrition from the grueling military lifestyle. Additionally, the number of Marines who were able to be identified and enrolled decreased over time due to their geographic dispersion following recruit training. Additionally, our recruitment predominately occurred in larger, more centralized bases. This could have led to an oversampling of Marines with medical concerns inconsistent with the general active-duty Marines who were garrisoned at these larger facilities where transition from the military to the civilian sector occurs. Conversely, Marines with debilitating symptoms leading to medical discharge might not have continued in the study; however, this is unlikely given the that the process for leaving the military often takes well over a year and occurs at the larger facilities. Furthermore, because our population was active-duty Marines, all required to receive SARS-CoV-2 vaccinations upon their availability (Department of Defense policy at the time of this study), we were unable to fully analyze the potential role and timing of vaccination in understanding PASC risk and the effect on other signs and symptoms.

We are unable to assess the chronicity of PASC in our population given our study design. Depending on the symptom, approximately 10–15% of participants

with specific symptoms at their first CHARM 2.0 visit continued to have those same symptoms at their second CHARM 2.0 visit. Regardless, based on the reported PASC symptoms, the potential current and future public health implications in this population could be substantial. Chronic health complications from PASC especially in a young and previously healthy population with a long-life expectancy could decrease work productivity and increase healthcare costs. Significant changes in the Years-of-Life lived with a disability can disproportionally increase disability-adjusted life years and should be considered when allocating resources and designing policy.[40]

In this longitudinal CHARM 2.0 study we identified that PASC is common in young, healthy adults and causes prolonged physical and mental health symptoms and decreases in subjective and objective performance measures. Additional research is needed to further characterize PASC among cohorts of various ages in relation to timing of infection and vaccination, investigate both physical and mental health symptoms, and explore host-related changes related to PASC to inform mitigation strategies and treatment guidelines.

Contributors
The study design and protocol were developed by CKP, CGB, CG, SCS, and AGL. EC, LW, DW, JM, AM, EONH collaboratively collected data in the field. SV, CM, IR, PS, MS collaboratively perform microbiological and immunological research assays. DB, YG, CKP were responsible for preparing the data for analysis. SJ and CKP led the data analysis. CKP and AGL drafted the manuscript, and all authors contributed to data interpretation and provided critical revisions. All authors have reviewed and given their approval of the final version of the manuscript and accept responsibility for the decision to submit for publication.

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

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Human subjects protection
This study was approved by the ethical review committees of the Naval Medical Research Command, Silver Spring, MD (NMRC.2021.0004) in compliance with all Federal regulations governing the protection of human subjects. All participants provided written informed consent.

Disclaimer
The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government. There are no restrictions on its use. There are no conflicts of interest among any of the authors.

Appendix A. Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.lana.2024.100909.

References
1 Carfi A, Bernabei R, Landi F, for the Gemelli Against Covid Post Acute Care Study Group. Persistent symptoms in patients after acute COVID-19. *JAMA*. 2020;324(6):603–605.
2 Klein J, Wood J, Jaycox JR, et al. Distinguishing features of long COVID identified through immune profiling. *Nature*. 2023;623(7985):139–148.
3 Cervia-Hasler C, Bruningk SC, Hoch T, et al. Persistent complement dysregulation with signs of thromboinflammation in active Long Covid. *Science*. 2024;383(6680):eadg7942. https://doi.org/10.1126/science.adg7942.
4 Mahase E. Covid-19: what do we know about "long covid"? *BMJ*. 2020;370:m2815. https://doi.org/10.1136/bmj.m2815.
5 Davis HE, McCorkell L, Vogel JM, Topol EJ. Long COVID: major findings, mechanisms and recommendations. *Nat Rev Microbiol*. 2023;21(3):133–146.
6 The Lancet. Long COVID: 3 years in. *Lancet*. 2023;401(10379):795. https://doi.org/10.1016/S0140-6736(23)00493-2.
7 Yang C, Zhao H, Tebbutt SJ. A glimpse into long COVID and symptoms. *Lancet Respir Med*. 2022;10(9):e81. https://doi.org/10.1016/S2213-600(22)00217-X.
8 Durstenfeld MS, Peluso MJ, Kaveti P, et al. Inflammation during early post-acute COVID-19 is associated with reduced exercise capacity and Long COVID symptoms after 1 year. *medRxiv*. 2022. https://doi.org/10.1101/2022.05.17.22275235.
9 Ledford H. How common is long COVID? Why studies give different answers. *Nature*. 2022;606(7916):852–853.
10 Rao P, Peritz DC, Systrom D, Lewine K, Cornwell WK 3rd, Hsu JJ. Orthostatic and exercise intolerance in recreational and competitive athletes with long COVID. *JACC Case Rep*. 2022;4(17):1119–1123.
11 Hull JH, Wootten M, Moghal M, et al. Clinical patterns, recovery time and prolonged impact of COVID-19 illness in international athletes: the UK experience. *Br J Sports Med*. 2022;56(1):4–11.
12 Chudzik M, Babicki M, Kapusta J, et al. Long-COVID clinical features and risk factors: a retrospective analysis of patients from the STOP-COVID registry of the PoLoCOV study. *Viruses*. 2022;14(8):1755.
13 Manukyan P, Deviaterikova A, Velichkovsky BB, Kasatkin V. The impact of mild COVID-19 on executive functioning and mental health outcomes in young adults. *Healthcare (Basel)*. 2022;10(10):1891.
14 Lizewski RA, Sealfon RSG, Park SW, et al. SARS-CoV-2 outbreak dynamics in an isolated US military recruit training center with rigorous prevention measures. *Epidemiology*. 2022;33(6):797–807.
15 Investing in the health and well-being of young adults committee on improving the health, safety, and well-being of young adults. Washington, DC: Institute of Medicine; National Research Council; 2015.

16 Kim Y, Bae S, Chang HH, Kim SW. Long COVID prevalence and impact on quality of life 2 years after acute COVID-19. *Sci Rep.* 2023;13(1):11207. https://doi.org/10.1038/s41598-023-36995-4.

17 Mogensen I, Ekstrom S, Hallberg J, et al. Post COVID-19 symptoms are common, also among young adults in the general population. *Sci Rep.* 2023;13(1):11300. https://doi.org/10.1038/s41598-023-38315-2.

18 Subramanian A, Nirantharakumar K, Hughes S, et al. Symptoms and risk factors for long COVID in non-hospitalized adults. *Nat Med.* 2022;28(8):1706–1714.

19 Letizia AG, Ge Y, Vangeti S, et al. SARS-CoV-2 seropositivity and subsequent infection risk in healthy young adults: a prospective cohort study. *Lancet Respir Med.* 2021;9(7):712–720.

20 Menard S. *Handbook of longitudinal research: design, measurement, and analysis.* Amsterdam: Elsevier; 2008:30–31.

21 Kroenke K, Spitzer RL, Williams JB. The PHQ-15: validity of a new measure for evaluating the severity of somatic symptoms. *Psychosom Med.* 2002;64(2):258–266.

22 Kroenke K, Strine TW, Spitzer RL, Williams JB, Berry JT, Mokdad AH. The PHQ-8 as a measure of current depression in the general population. *J Affect Disord.* 2009;114(1-3):163–173.

23 Plummer F, Manea L, Trepel D, McMillan D. Screening for anxiety disorders with the GAD-7 and GAD-2: a systematic review and diagnostic metaanalysis. *Gen Hosp Psychiatry.* 2016;39:24–31.

24 Lapin BR, Bena JF, Walia HK, Moul DE. The Epworth sleepiness scale: validation of one-dimensional factor structure in a large clinical sample. *J Clin Sleep Med.* 2018;14(8):1293–1301.

25 Shattuck NL, Matsangas P. Psychomotor vigilance performance predicted by Epworth Sleepiness Scale scores in an operational setting with the United States Navy. *J Sleep Res.* 2015;24(2):174–180.

26 Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep.* 1991;14(6):540–545.

27 Marine Corps Order 6100.13A. Marine corps physical fitness and combat fitness tests (CFT/PFT). Department of the Navy. https://www.marines.mil/News/Publications/MCPEL/Electronic-Library-Display/Article/2524537/mco-610013a-wch-4/. Accessed March 15, 2024.

28 Marine Corps Reference Publication 3-01A, Rifle marksmanship. *Publication control number: 144 000091 00.* Department of the Navy; 2012. Available at: https://www.trngcmd.marines.mil/Portals/207/Docs/wtbn/MCRP%203-01A.pdf. Accessed March 15, 2024.

29 Nalbandian A, Sehgal K, Gupta A, et al. Post-acute COVID-19 syndrome. *Nat Med.* 2021;27(4):601–615.

30 Thaweethai T, Jolley SE, Karlson EW, et al. Development of a definition of postacute sequelae of SARS-CoV-2 infection. *JAMA.* 2023;329(22):1934–1946.

31 Soares-Schanoski A, Sauerwald N, Goforth CW, et al. Asymptomatic SARS-CoV-2 infection is associated with higher levels of serum IL-17C, matrix metalloproteinase 10 and fibroblast growth factors than mild symptomatic COVID-19. *Front Immunol.* 2022;13:821730. https://doi.org/10.3389/fimmu.2022.

32 Fox J. *Applied regression analysis and generalized linear models.* 2nd ed. Sage; 2008.

33 Fox J, Weisberg S. *An R companion to applied regression.* 2nd ed. Sage; 2011.

34 Hruby A, Lieberman HR, Smith TJ. Symptoms of depression, anxiety, and post-traumatic stress disorder and their relationship to health-related behaviors in over 12,000 US military personnel: Bi-directional associations. *J Affect Disord.* 2021;283:84–93.

35 Singh I, Joseph P, Heerdt PM, et al. Persistent exertional intolerance after COVID-19: insights from invasive cardiopulmonary exercise testing. *Chest.* 2022;161(1):54–63.

36 van Voorthuizen EL, van Helvoort HAC, Peters JB, van den Heuvel MM, van den Borst B. Persistent exertional dyspnea and perceived exercise intolerance after mild COVID-19: a critical role for breathing dysregulation? *Phys Ther.* 2022;102(10):pzac105.

37 Baratto C, Caravita S, Faini A, et al. Impact of COVID-19 on exercise pathophysiology: a combined cardiopulmonary and echocardiographic exercise study. *J Appl Physiol (1985).* 2021;130(5):1470–1478.

38 Mao W, Miller CM, Nair VD, et al. A methylation clock model of mild SARS-CoV-2 infection provides insight into immune dysregulation. *Mol Syst Biol.* 2023;19(5):e11361. https://doi.org/10.5252/msb.202211361.

39 Soriano JB, Murthy S, Marshall JC, Relan P, Diaz JV, W HO. Clinical case definition working group on post-COVID-19 condition. A clinical case definition of post-COVID-19 condition by a Delphi consensus. *Lancet Infect Dis.* 2022;22(4):e102–e107.

40 Smith MP. Estimating total morbidity burden of COVID-19: relative importance of death and disability. *J Clin Epidemiol.* 2022;142:54–59.