

Protective effect of a second booster dose against long COVID among individuals infected with SARS-CoV-2 in southeastern Brazil

Ana Paula Bandeira Barboza ^{a,b,*}, Alessandra Luna-Muschi ^{a,b}, Debora Faffe ^{d,e}, Igor Carmo Borges ^{a,b}, Marina Farrel Côrtes ^{a,b}, Rafael Galliez ^{d,f}, Elisa Teixeira Mendes ^h, Fabio Eudes Leal ^{i,j}, Erika Manuli ^{c,i}, Fabio Ghilardi ^k, Helena Toledo Scheid ^f, Ana Beatriz Miranda Lourenço ^f, Mina Ozatha ^l, Vanderson Sampaio ^l, Orlando da Costa Ferreira Junior ^{d,g}, Amilcar Tanuri ^{d,g}, Ester Sabino ^{c,m}, Terezinha Marta Castiñeiras ^{d,f}, Silvia Figueiredo Costa ^{a,b,n}

^a Departamento de Moléstias Infecciosas e Parasitárias da Faculdade de Medicina da Universidade de São Paulo, Brazil

^b Departamento de Moléstias Infecciosas e Parasitárias da Faculdade de Medicina da Universidade de São Paulo, Laboratório de Investigação Médica (LIM-49), Instituto de Medicina Tropical, Brazil

^c Departamento de Moléstias Infecciosas e Parasitárias da Faculdade de Medicina da Universidade de São Paulo, Laboratório de Investigação Médica (LIM-46), Instituto de Medicina Tropical, Brazil

^d Núcleo de Enfrentamento e Estudos em Doenças Infecciosas Emergentes e Reemergentes (NEEDIER) da Universidade Federal do Rio de Janeiro, Brazil

^e Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil

^f Departamento de Doenças Infecciosas e Parasitárias, Faculdade de Medicina da Universidade Federal do Rio de Janeiro, Brazil

^g Laboratório de Biologia Molecular, Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Brazil

^h Pontifícia Universidade Católica de Campinas (PUC-Campinas), Centro de Ciências da Vida, Programa de Pós-Graduação Stricto Sensu em Ciências da Saúde, Brazil

ⁱ Faculdade de Medicina da Universidade Municipal de São Caetano do Sul, São Paulo, Brazil

^j Programa de Oncovirologia, Instituto Nacional do Câncer, Rio de Janeiro, Brazil

^k Hospital Alemão Oswaldo Cruz, São Paulo, Brazil

^l Instituto Todos pela Saúde, Brazil

^m Departamento de Patologia da Faculdade de Medicina da Universidade de São Paulo, Brazil

ⁿ Centres for Antimicrobial Optimisation Network (CAMO-Net-Brazil), Brazil

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ABSTRACT

Background: Long COVID is a complex condition with diverse symptoms, lacking specific diagnostic or therapeutic tools. Although vaccination reduces the risk of severe COVID-19, its role in preventing long COVID, particularly through booster doses, remains limited. This study assessed the impact of first and second booster doses and identified long COVID risk factors.

Methods: We conducted a multicenter observational study with a cross-sectional analysis of participants previously infected with SARS-CoV-2 during the pre-Omicron and Omicron epidemiological periods. Both periods included mixed populations of healthcare workers (HCWs) and non-HCWs, with HCWs representing 86% of all participants. The study included five medical centers in São Paulo and Rio de Janeiro. Clinical data and long COVID symptoms were collected through a single standardized electronic questionnaire sent to all eligible participants. Predictors of long COVID were evaluated using multivariable logistic regression. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

Results: A total of 2033 participants were included, and 67% (1370) reported long COVID symptoms. Independent risk factors included female sex (OR 2.25, 95% CI 1.81–2.79, $p < 0.001$), the presence of one, two, or three or more comorbidities (OR 1.74, 95% CI 1.36–2.23, $p < 0.001$; OR 2.14, 95% CI 1.43–3.20, $p < 0.001$; and OR 3.10, CI 1.55–6.19, $p = 0.001$, respectively); the occurrence of one or more reinfections (OR 2.35, 95% CI 1.84–3.01, $p < 0.001$; and OR 4.22, 95% CI 2.043–7.91, $p < 0.001$, respectively), and a severe acute illness (OR: 1.91; 95%CI 1.09–3.35; $p = 0.02$). Vaccination was protective, with the strongest effect observed among those

* Corresponding author at: Av. Dr. Enéas Carvalho de Aguiar, 470, Jardim América, São Paulo - SP, CEP 05403-000, Brazil.

E-mail addresses: anapaulabarboza@usp.br (A.P.B. Barboza), fabio.leal@online.uscs.edu.br (F.E. Leal), erika.manuli@online.uscs.edu.br (E. Manuli), vanderson.sampaio@itps.org.br (V. Sampaio), tmartapc@medicina.ufrj.br (T.M. Castiñeiras), silviacosta@usp.br (S.F. Costa).

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receiving two booster doses (OR: 0.18; 95% CI 0.07–0.46; $p < 0.001$). In the Omicron period sub-analysis, only the second booster dose was associated with reduced risk compared with a complete primary series (OR 0.50, CI 0.34–0.74, $p < 0.001$), whereas one booster dose showed no significant effect.

Conclusion: COVID-19 vaccination, especially two booster doses, reduced long COVID risk. Female sex, comorbidities, reinfections, and severe acute illness were independent risk factors for developing long-lasting symptoms.

1. Introduction

Despite the transition of the COVID-9 pandemic into an endemic phase, SARS-CoV-2 continues to circulate globally, posing an ongoing risk of recurrent exposure. The World Health Organization registered 925,700 cases of COVID-19 in 2025 as of June 15th [1]. Emerging evidence suggests that repeated SARS-CoV-2 infections may increase the risk of developing long-term covid conditions, commonly referred to as long COVID [2,3]. This syndrome is characterized by the onset of new, recurrent, or persistent symptoms lasting over one month, or even years, beyond the initial infection [4–7]. Current estimates suggest that approximately 42% of infected individuals experience long COVID symptoms, although prevalence varies based on factors such as viral variant, vaccination status, and characteristics of the studied population [6,8,9]. Applying these estimates on a global scale implies that over 77 million people worldwide may have experienced persistent symptoms related to long COVID in the past five years [1].

Long COVID can affect individuals of all ages, sexes, and health statuses, including those who experienced mild or asymptomatic initial infections [10]. More than 200 symptoms have been identified, affecting multiple organ systems. Common manifestations include fatigue, post-exertional malaise, neurocognitive complaints such as brain fog, as well as cardiovascular, gastrointestinal, dermatological, and hormonal disturbances [11]. This wide-ranging symptomatology leads to a heterogeneous clinical presentation with varying degrees of functional impairment, posing significant challenges for clinicians and representing a growing global public health concern [12].

Several risk factors for long COVID have been consistently identified, including pre-existing comorbidities, severe acute COVID-19 infection, reinfections, female sex, and socioeconomic deprivation [13–15]. Vaccination has been shown to substantially reduce the risk of severe acute disease and, consequently, the likelihood of developing long COVID [16–18]. However, the optimal vaccination regimen, particularly the number and timing of booster doses to prevent long COVID remains incompletely understood [19,20]. Despite widespread administration of primary vaccine series, booster uptake has been lower in many regions due to issues such as limited availability, inequitable distribution, and vaccine hesitancy [21–23].

In this context, our study aimed to evaluate risk factors associated with long COVID among healthcare professionals in a middle-income country. We assessed the impact of both primary vaccine series and booster doses and estimated the prevalence of long COVID across infections caused by different SARS-CoV-2 variants of concern.

2. Methods

We conducted a multicenter, cross-sectional observational study including individuals infected with SARS-CoV-2 between February 2020 and May 2024, with varying degrees of COVID-19 severity. Participants were recruited from five medical centers in three cities in the state of São Paulo and one center in Rio de Janeiro, Brazil (Supplementary material 1).

The study population comprised two groups of infected individuals: participants identified from records of previous COVID-19 studies and healthcare workers (HCWs) identified through Occupational Health Services and diagnostic centers at each participating institution. All participants were evaluated at a single time point after SARS-CoV-2

infection.

For analytical purposes, participants were categorized according to the predominant SARS-CoV-2 variant at the time of infection, defined as the pre-Omicron period and the Omicron period, based on epidemiological surveillance data.

All participants were invited through e-mail, telephone calls or text message. A telephone line was provided for eventual queries. An electronic questionnaire was used to assess sociodemographic data, self-reported pre-existing comorbidities, the number, dates and type of COVID-19 vaccine doses received prior to infection, the number and dates of COVID-19 episodes, the severity of the acute disease, and the presence of a wide range of symptoms, such as fatigue, memory loss, headache, poor concentration, dyspnea, insomnia, loss of smell, loss of taste, chest pain, persistent cough, myalgia/arthritis, and diarrhea (Supplementary material 2). An internal validation process of the questionnaire was conducted involving the research team and external volunteers, none of whom participated in the study. This process aimed to assess clarity, comprehension, and consistency of the questions before deployment. The questionnaire was revised four months after the beginning of the research, with the inclusion of cough, diarrhea, and memory loss as additional symptoms. However, the first 572 volunteers were not questioned about these three symptoms. The median time from SARS-CoV-2 infection to questionnaire administration was 16 months (IQR, 12–21) in the pre-Omicron period and 3 months (IQR, 2.1–4.5) in the Omicron period.

The immunization status was classified according to the number of vaccine doses received at least 14 days or more prior to the acute infection, which evolved into five categories (unvaccinated, one vaccine dose, two vaccine doses, primary vaccine series with one booster dose, and primary vaccine series with two booster doses). Inconsistencies in vaccination data, including discordant or implausible vaccination dates, were addressed by consulting the state-level platform VacíVida (São Paulo) and the national SI-PNI/DataSUS registry as October 9th, 2024 [24,25].

Long COVID was defined according to the Centers for Disease Control and Prevention (CDC) definition available in use until August 2024 as a “wide range of physical and mental health consequences experienced by some patients that persist four or more weeks after SARS-CoV-2 infection, including patients who had initial mild or asymptomatic acute infection” [5].

2.1. Data collection

Data were collected using a secure structured electronic questionnaire developed in Google Forms and administered once to each participant. The questionnaire was self-administered and completed remotely by participants using a personalized invitation link sent via e-mail, or text message. Data collection occurred between March 2022 and May 2024. Of the 8709 eligible individuals invited, 2234 questionnaires were returned. After exclusion of incomplete questionnaires and those not meeting inclusion criteria, 2033 questionnaires were analyzed. Responses were automatically recorded in a protected database and exported for analysis. Mandatory fields were used to minimize missing data, and logical checks were embedded to reduce inconsistent responses.

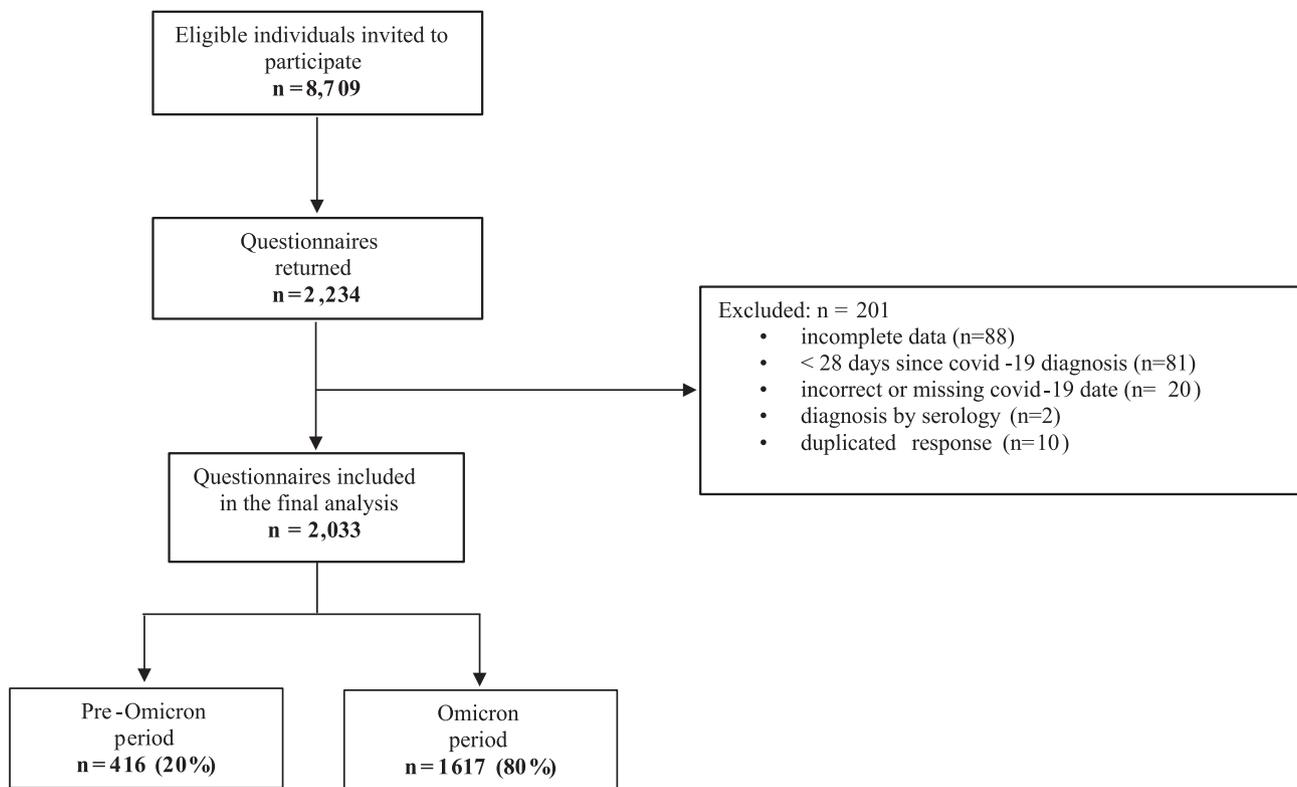


Fig. 1. Flowchart of participant recruitment and inclusion in the study.

2.2. Sample size calculation

The sample size was calculated to estimate the incidence of long COVID in the cohort of exposed individuals with adequate precision. Assuming an expected prevalence of 20%, a 95% confidence level, and a margin of error of 5%, the minimum required sample size was approximately 246 participants.

2.3. SARS-CoV-2 diagnosis

The diagnosis of infection by SARS-CoV-2 was defined as a positive real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay or antigen rapid test on specimens obtained from nasopharyngeal swabs.

2.4. SARS-CoV-2 variant determination

We used sequencing data from three different sources (genomes generated from samples collected in Brazil and deposited on network Global Initiative on Sharing All Influenza Data by the Fiocruz Genomic Network [26], Instituto Todos pela Saúde [27], and whole genome sequencing data from other cohort studies of our research group) to infer the most likely viral variant infecting a participant at time of the positive swab. The variant was determined to be predominant based on its presence in 75% of the isolates: wild-type infections (February 26th, 2020, to February 28th, 2021); Gamma variant infections (March 5th to August 5th, 2021); Delta variant infections (August 20th to December 18th, 2021), and Omicron variant infections (December 25th, 2021 – May 11th, 2024). The Omicron variant wave unfolded into distinct waves represented by different sublineages, some of which co-circulated and could not be specified.

2.5. Statistical analysis

Categorical variables were described as absolute and relative

frequencies. Depending on their distribution, continuous variables were presented as mean \pm standard deviation or median and interquartile range. Associations between predictors and long COVID were evaluated using the Chi-squared test. Variables with a p -value < 0.05 were considered statistically significant and included in the multivariate logistic regression model. The odds ratio (OR) and its corresponding 95% confidence interval (CI) were calculated.

We conducted two stepwise logistic regression models to assess the impact of vaccination. In the first model, unvaccinated individuals served as the reference category. In the second model, individuals who had completed the primary vaccine series and received two booster doses were used as the reference group. A sub-analysis was conducted among Omicron-period infections to minimize potential recall bias. The number of vaccine doses at the time of infection was treated as an ordinal variable. The presence of comorbidities was grouped as none, one, two, and three or more, reflecting increasing clinical risk. Number of SARS-CoV-2 infections were categorized as one, two, or three or more, based on frequency distribution. IBM® SPSS® software version 29.0 was used for statistical analyses.

This study was approved by FMUSP's Ethics Committee (CAAE: 42708721.0.0000.0068) and by the National Committee of Research Ethics (CAAE: 30161620.0.1001.5257). All enrolled participants were over 18 years old and declared informed consent. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cross-sectional studies.

3. Results

A total of 2234 participants were enrolled, and 2033 questionnaires analyzed. Responses with missing information or elapsed time after infection less than 28 days were excluded. Among the analyzed responses, 20% (416) were from participants infected during the pre-Omicron period, and 80% (1617) during the Omicron period, with response rates of 12% and 36%, respectively (Fig. 1). The median number of days between infection and entry into the study was 110 days

Table 1
Sociodemographic and clinical characteristics of the 2033 study participants, overall and by pre-Omicron and Omicron periods.

Variable	All participants (n = 2033) N (%)	Pre-Omicron period (n = 416)	Omicron period (n = 1617)
Female	1512 (74%)	298 (72%)	1214 (75%)
Age (median, IQR)	41 (29–53)	44 (32–56)	39 (28–52)
Ethnicity			
White	1269 (62%)	258 (70%)	1011 (63%)
Mixed race	504 (25%)	112 (27%)	393 (24%)
Black	201 (10%)	40 (10%)	161 (10%)
Asian	48 (2%)	5 (1%)	43 (3%)
Healthcare worker*	1638 (86%)	268 (68%)	1370 (91%)
Severe COVID-19	106 (5%)	68 (16%)	38 (2%)
Immunization status			
Unvaccinated	270 (13%)	262 (63%)	8 (0,5%)
1 dose	62 (3%)	47 (11%)	15 (0,9%)
2 doses	315 (15%)	98 (24%)	217 (13%)
2 doses +1 booster	1070 (52%)	9 (2%)	1061 (66%)
2 doses +2 boosters	316 (15%)	0	316 (20%)
Primary vaccination series platform			
Inactivated virus vaccine	1064 (54%)	97 (26%)	967 (60%)
Viral vector vaccine	420 (24%)	10 (9%)	410 (26%)
mRNA-based vaccine	179 (9%)	0	179 (11%)
Mixed platforms	38 (2%)	0	38 (2%)
Number of mRNA vaccine doses			
None	674 (33%)	401 (96%)	273 (17%)
One	1072 (53%)	15 (4%)	1057 (65%)
Two	242 (12%)	0	242 (15%)
Three	41 (2%)	0	41 (2%)
Four	4 (0,2%)	0	4 (0,2%)
Number of mRNA OR viral vector vaccine doses			
None	512 (25%)	378 (90%)	134 (8%)
One	693 (34%)	28 (7%)	665 (41%)
Two	329 (16%)	10 (2%)	319 (20%)
Three	393 (19%)	0	393 (24%)
Four	106 (5%)	0	106 (7%)
Number of infections			
One	1425 (70%)	386 (93%)	1039 (64%)
Two	538 (26%)	28 (7%)	510 (31%)
Three or more	70 (4%)	2 (0,5%)	68 (4%)
Number of comorbidities			
None	1334 (66%)	295 (70%)	1039 (64%)
One	473 (23%)	89 (21%)	384 (24%)
Two	166 (8%)	25 (6%)	141 (9%)
Three or more	60 (3%)	7 (2%)	53 (3%)
Variant of concern			
Ancestral strain	255 (13%)	255 (61%)	NA
Gamma	107 (5%)	107 (28%)	NA
Gamma-Delta	8 (0,8%)	8 (2%)	NA
Delta	43 (2%)	43 (10%)	NA
Delta-Omicron	3 (0,1%)	3 (0,7%)	NA
Omicron	1617 (78%)	NA	1617 (100%)
Days from COVID-19 to study enrollment (median, IQR)	110 (72–325)	490 (385–657)	93 (63–137)

NA = not applicable

* Healthcare workers status was missing for missing 132 participants

(IQR 72–325). The median number of days from last vaccine dose to SARS-CoV-2 infection was 130 days (87–204).

The baseline characteristics, sociodemographic features of study participants are described in Table 1. Overall, the median age was 41 (29–53), and most of the volunteers self-reported as females (1512; 74%) based on sex assigned at birth. The majority of the participants self-identified as White (1269; 62%), followed by Mixed race (504;

25%), Black (201; 10%), and Asian (48; 2%). Ethnicity was not declared in 0.5% of persons. Out of 1901 persons who declared their activity, 1638 (86%) were identified as HCWs, with a higher proportion observed during the Omicron period than in the pre-Omicron period. This information was missing for 132 (6%) participants.

Most of the study population reported no pre-existing comorbidities (1334; 66%). Among the 34% who reported previous comorbidities, the most common were chronic arterial hypertension (321; 16%); obesity (188; 9%), chronic lung disease (129; 6%), and diabetes (111; 5%). Depression and anxiety disorders were spontaneously reported by 32 (1,5%) and 24 (1%) participants, respectively. Out of the 106 persons (5%) who required hospitalization, 26 (25%) were admitted to general wards, and 80 (75%) were admitted to the intensive care unit. The distribution of severe cases showed a higher proportion among males (36/521; 7%) compared to females (70/1512; 5%) with statistical significance ($p = 0.04$).

Most of the subjects were vaccinated as follows: 62 (3%) received one dose, 315 (16%) received two doses, 1070 (52%) received two doses and one booster dose, and 316 (16%) received two doses and two booster doses. Among the 270 (13%) unvaccinated persons, 80% (217) were infected before the vaccine became available on January 17th, 2021. Of the 1386 persons who completed the primary vaccination series and received one or two booster doses, 92% (1277) were vaccinated with a heterologous booster. The most frequent vaccination regimen (811, 63%) in this group was two doses of inactivated virus vaccine (CoronaVac®) followed by at least one booster dose of an mRNA-based vaccine (Pfizer, BioNTech BNT162b2). Overall, the primary vaccination series was predominantly completed with an inactivated virus vaccine (1064, 54%), followed by viral vector vaccines (420, 24%), and mRNA vaccines (179, 9%).

A total of 1425 (70%) individuals experienced a single SARS-CoV-2 infection, while 538 (26%) had one reinfection, 70 (3,4%) had two or more reinfections. According to temporal inference of the SARS-CoV-2 variant of concern, the most recent infection experienced by each subject occurred during the Ancestral period in 13% ($n = 255$), the Gamma wave in 5% ($n = 107$), the Delta wave 2% ($n = 43$), and the Omicron wave in 79% ($n = 1617$). A small number of infections occurred between the Gamma-Delta ($n = 8$), and the Delta-Omicron ($n = 3$) transitions. Eighty-six infections occurred between the end of the primary vaccine series and the first booster dose, and 39 infections occurred between the first and second booster doses.

Long COVID was identified in 1370 individuals (67%), with a prevalence of 70% in the pre-Omicron and 66% in the Omicron period. The most prevalent symptoms, as listed in Fig. 2, were fatigue (812, 60%), poor concentration (748, 55%), memory loss (451, 48%), and myalgia/arthritis (435, 32%). The majority of those with long COVID had from 1 to 3 symptoms (61%).

In bivariate analyses, female sex, number of SARS-CoV-2 infections, severe COVID-19, the VOC, and the presence of pre-existing comorbidities were significantly associated with an increased risk of developing long COVID. Conversely, being immunized at the time of infection and having completed the primary vaccination series with an mRNA-based platform were independently associated with a lower risk of long COVID. Neither the total number of mRNA vaccine doses nor the total number of combined mRNA and viral vector vaccine doses was significantly associated with long COVID risk. The association with VOCs was largely driven by the Ancestral strain ($n = 255$), in which 193 individuals (75%) developed long COVID.

In the two multivariate logistic regression analysis (Table 2), female sex, presence of comorbidities, severe COVID-19, and number of reinfections remained significantly associated with persistent long-term symptoms. All vaccination regimens were associated with reduced odds of long COVID. Although a significant association with the variant of concern (VOC) was observed in the bivariate analysis, this was not retained after adjustment for other covariates. In model 1, with the unvaccinated as the reference group, immunization demonstrated a

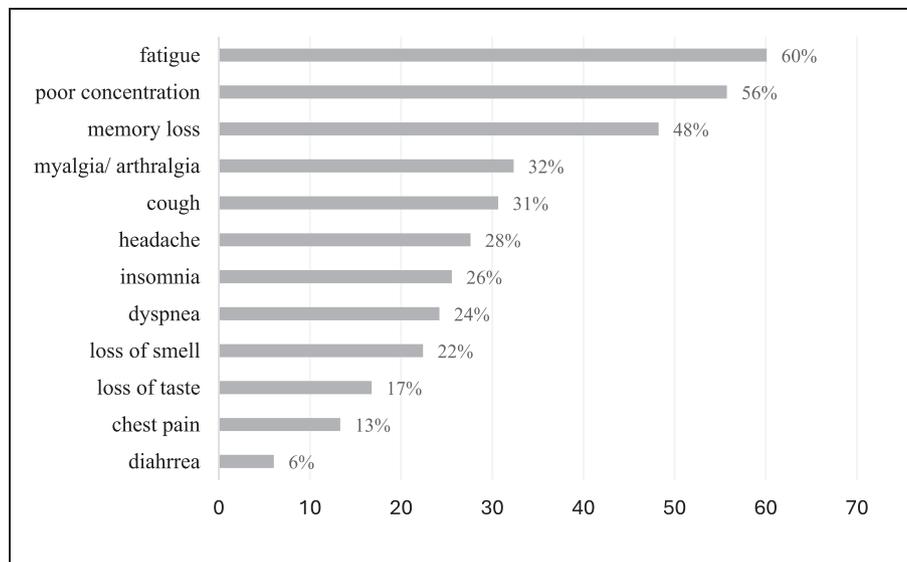


Fig. 2. Frequency of reported symptoms among 1370 individuals with long COVID.

protective effect, with higher numbers of vaccine doses corresponding to progressively reduced odds of long COVID. In model 2, using two booster doses as the reference group, receiving fewer doses was associated with increased odds of long COVID.

In the sub-analysis restricted to the Omicron period (Table 3), including individuals who had received two or more vaccine doses, severe COVID-19, and completion of the primary vaccination series with an mRNA-based vaccine were no longer significantly associated with long COVID. Due to the small number of unvaccinated individuals ($n = 8$) and those who had received only one vaccine dose ($n = 15$), these groups were excluded from the analysis. Individuals with two vaccine doses served as the reference group. Only the receipt of a second booster dose was associated with a reduced risk of long COVID (OR 0.50; 95% CI: 0.33–0.74; $p < 0.001$). The variable for the variant of concern (VOC) was not included, as all infections occurred during the Omicron period.

4. Discussion

In this multicentric study we observed a high prevalence of long COVID in a population that predominantly consisted of non-hospitalized HCWs who experienced mild cases of COVID-19. The study period encompassed infections caused by the Ancestral strain to the Omicron variant. We demonstrated that reinfections, presence of comorbidities, severe COVID-19, and female sex were independent risk factors for long COVID, while vaccination conferred a protective effect, with a higher benefit observed with the second booster dose.

It is noteworthy that two thirds of our study sample were diagnosed with long COVID during a follow-up of about four months, which demonstrated a higher frequency of this condition compared to most previous studies. A systematic review of 194 studies estimated a prevalence of 44.8% (95% C.I. 38.6%–51.2%) for long COVID at a mean follow-up of about four months, regardless of hospitalization status and excluding studies involving HCWs [28]. However, other studies have also demonstrated a detection rate of long COVID as high as 84% [29]. We believe that these discrepancies in the detection rate of long COVID is probably explained by differences in study population characteristics, such as the frequency of female sex and the number of previous SARS-CoV-2 infections, which is expected to be higher in HCWs [30,31]. HCWs may be more likely to recognize symptoms and seek medical evaluation, potentially contributing to earlier diagnosis of long COVID, as reflected by the higher response rate observed during the Omicron period.

Growing evidence highlights the role of COVID-19 vaccination not

only in preventing severe acute illness but also in reducing the risk of developing long COVID [16,19,32]. In our study, vaccination was associated with a lower likelihood of persistent symptoms. In sub-analyses conducted during the Omicron period, receiving two booster doses emerged as an independent protective factor among individuals who had completed the primary vaccine series. These findings align with previous studies, including a case-control study involving Brazilian healthcare workers that demonstrated a protective effect with two booster doses [20], and two U.S. cohort studies in which receiving at least one booster dose was associated with a greater degree of protection against long COVID compared to being unvaccinated [33] or having completed only the primary vaccine series [34]. While completion of the primary vaccine series with an mRNA platform was associated with a lower risk of long COVID in unadjusted analyses, this association was not sustained after multivariable adjustment. In this study population, where most participants completed their primary series with an inactivated virus vaccine and subsequently received heterologous booster doses, including viral vector or mRNA vaccines, our findings suggest a beneficial effect of revaccination independent of vaccine platform. From a pathophysiological perspective, vaccination may mitigate long COVID by modulating the host immune response, limiting viral persistence, and reducing systemic inflammation [10], though further research is needed to clarify these mechanisms.

Despite the benefits observed, vaccine uptake for booster doses has been suboptimal in many regions due to limited access, logistical barriers, and vaccine hesitancy [21,22]. This underlines the importance of reinforcing public health messaging and ensuring equitable distribution of vaccines. In the perspective of long COVID preventive strategies, this may support revisiting the current recommendations of the WHO's Strategic Advisory Group of Experts on Immunization (SAGE), which prioritize revaccination for older adults with multiple comorbidities, immunocompromised individuals, pregnant women, and healthcare workers with direct patient contact [35].

The increased risk of long COVID after SARS-CoV-2 reinfection is also a well-established association. It has been shown that reinfection was independently associated with a higher risk of all-cause mortality, hospitalization, and long COVID [2]. It probably reflects the burden of repetitive tissue damage, viral persistence, and immune priming by the virus [10]. Reinfections can exacerbate immune dysregulation, potentially leading to more severe and prolonged symptoms [2,3].

Comorbidities have also been shown to be a risk factor in other studies [10,13,30]. Conditions such as asthma, chronic kidney disease, diabetes, obesity can exacerbate immune response against the virus

Table 2
Clinical parameters associated with long COVID: logistic regression analysis (Models 1 and 2) presenting odds ratios and 95% confidence intervals.

Model 1 – Reference category for immunization status: unvaccinated individuals.				
Risk Factor	All participants* (n = 2022)	ODDS Ratio	Confidence Interval (95% C.I.)	p value
Female	1504	2.25	1.81–2.79	p < 0.001
Severe COVID-19	105	1.91	1.09–3.35	p = 0.02
Immunization status				
Unvaccinated	270	(Ref)		
1 dose	62	0.36	0.15–0.86	p = 0.02
2 doses	306	0.34	0.14–0.83	p = 0.02
2 doses +1 booster	1068	0.34	0.13–0.85	p = 0.02
2 doses +2 boosters	316	0.18	0.07–0.46	p < 0.001
Number of infections				
One	1416	(Ref)		
Two	536	2.35	1.84–3.01	p < 0.001
Three or more	70	4.02	2.04–7.91	p < 0.001
Number of comorbidities				
None	1327	(Ref)		
One	470	1.74	1.36–2.23	p < 0.001
Two	165	2.14	1.43–3.20	p < 0.001
Three or more	60	3.10	1.55–6.19	p = 0.001
Variant of concern				
Ancestral	255	(Ref)		
Gamma	107	1.14	0.49–2.65	p = 0.76
Delta	43	1.06	0.36–3.13	p = 0.90
Omicron	1617	1.52	0.61–3.77	p = 0.36
Model 2 – Reference category for immunization status: individuals who completed the primary vaccine series and received two booster doses.				
Risk Factor	All participants* (n = 2022)	ODDS Ratio	Confidence Interval (95% C.I.)	p value
Female	1504	2.25	1.81–2.79	p < 0.001
Severe COVID-19	105	1.91	1.09–3.35	p = 0.02
Immunization status				
2 doses +2 boosters	316	(Ref)		
Unvaccinated	270	5.55	2.17–14.18	p < 0.001
1 dose	62	2.02	0.99–4.15	p = 0.05
2 doses	306	1.90	1.30–2.78	p < 0.001
2 doses +1 booster	1068	1.90	1.43–2.52	p < 0.001
Number of infections				
One	1416	(Ref)		
Two	536	2.35	1.84–3.01	p < 0.001
Three or more	70	4.02	2.04–7.91	p < 0.001
Number of comorbidities				
None	1327	(Ref)		
One	470	1.74	1.36–2.23	p < 0.001
Two	165	2.14	1.43–3.20	p < 0.001

Table 2 (continued)

Model 2 – Reference category for immunization status: individuals who completed the primary vaccine series and received two booster doses.				
Risk Factor	All participants* (n = 2022)	ODDS Ratio	Confidence Interval (95% C.I.)	p value
Three or more	60	3.10	1.55–6.19	p = 0.001
Variant of concern				
Ancestral	255	(Ref)		
Gamma	107	1.14	0.49–2.65	p = 0.76
Delta	43	1.06	0.36–3.13	p = 0.90
Omicron	1617	1.52	0.61–3.77	p = 0.36

* Infections that occurred during the Gamma-Delta (n = 8) and Delta-Omicron (n = 3) transition periods were not included, yielding a lower number of cases in this analysis compared to the overall cohort.

Table 3

Clinical parameters associated with long COVID among participants in Omicron period (≥ 2 vaccine doses). Logistic regression model presenting odds ratios and 95% confidence intervals (CI).

Risk Factor	Omicron period* (n = 1594)	ODDS Ratio	Confidence Interval (95% C.I.)	p value
Female	1199	2.94	1.79–2.93	p < 0.001
Severe COVID-19	35	1.59	0.65–3.83	p = 0.30
Immunization status				
2 doses +1 booster	217	(Ref)		
2 doses +2 boosters	1060	0.97	0.70–1.35	p = 0.87
2 doses +2 boosters	317	0.50	0.34–0.74	p < 0.001
Number of infections				
One	1022	(Ref)		
Two	506	2.31	1.79–2.97	p < 0.001
Three or more	66	4.70	2.25–9.80	p < 0.001
Number of comorbidities				
None	1027	(Ref)		
One	375	1.74	1.32–2.29	p < 0.001
Two	139	2.26	1.46–3.51	p < 0.001
Three or more	53	2.75	1.35–5.58	p = 0.005
Primary vaccination series platform				
mRNA-based vaccine	179	0.81	0.58–1.14	p = 0.24

* Unvaccinated individuals (n = 8) and those with only one vaccine dose (n = 15) were excluded due to small sample sizes. The variant of concern was not included, as all infections in this cohort occurred during the Omicron period.

leading to prolonged symptoms and complications [36]. Severe COVID-19 and long COVID share a similar immune profile, including increased gene expression of the cGAS-STING pathway and elevated production of interferon-alpha, which promotes the maintenance of a systemic inflammatory state [37]. We observed a reduced number of severe COVID-19 cases during Omicron period, which may explain the loss of significance of association with long COVID.

While male individuals present a higher risk presenting a severe COVID-19 [38], female sex has been linked to a higher risk of long COVID, which might be explained by biological mechanisms and because women are in general more attentive to their body and related distress [13,39].

In our study, infections with the ancestral strain were associated with an increased risk of long COVID in the bivariate analysis, however this

association did not remain significant in the multivariate model. This may be partly explained by the proportionally small number of individuals infected with this strain in our sample. On the other hand, some studies have reported a decreased risk associated with later pandemic periods, particularly during the Omicron wave, which may reflect both changes in viral characteristics and the widespread introduction of vaccination, even though the risk of long COVID remains substantial [8,40,41]. These changes in viral virulence, occurring alongside increasing vaccination coverage and booster uptake among participants over the study period, may have contributed to the observed protective effects of vaccination.

Our study has some limitations. First, our findings were based on a self-reported survey, without in-person assessments, a context in which random or systematic misreporting is conceivable. In addition, because participation was voluntary, individuals with persistent symptoms may have been more likely to respond, potentially leading to an overestimation of long COVID prevalence. Moreover, participants infected in the pre-Omicron period may be more susceptible to recall bias, due to the longer period of observation. However, our findings were confirmed in the Omicron period sub-analysis, which was conducted closer to the time of infection and therefore partially mitigates concerns related to recall bias. We were able to record a chronological sequence of SARS-CoV-2 infections, vaccination dates, and vaccine platforms, allowing for the identification of breakthrough infections and supporting causal inferences. Finally, the study was conducted across five medical centers, enhancing the robustness and diversity of the dataset.

5. Conclusion

Our findings highlight the benefits of vaccination, particularly, the impact of a second booster dose in reducing the likelihood of long COVID.

In addition, we underscore the burden of repeated SARS-CoV-2 exposure on the risk of developing long COVID, as well as the influence of comorbidities, female sex, and severe acute COVID-19.

CRedit authorship contribution statement

Ana Paula Bandeira Barboza: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Alessandra Luna-Muschi:** Formal analysis, Data curation. **Debora Faffe:** Supervision, Formal analysis, Data curation. **Igor Carmo Borges:** Writing – review & editing, Formal analysis. **Marina Farrel Côrtes:** Methodology. **Rafael Galliez:** Supervision, Formal analysis, Data curation. **Elisa Teixeira Mendes:** Data curation. **Fabio Eudes Leal:** Data curation. **Erika Manuli:** Data curation. **Fabio Gilhardi:** Data curation. **Helena Toledo Scheid:** Data curation. **Ana Beatriz Miranda Lourenço:** Data curation. **Mina Ozatha:** Formal analysis. **Vanderson Sampaio:** Formal analysis. **Orlando da Costa Ferreira Junior:** Writing – review & editing, Data curation. **Amilcar Tanuri:** Writing – review & editing, Funding acquisition. **Ester Sabino:** Writing – review & editing, Funding acquisition. **Terezinha Marta Castiñeiras:** Writing – review & editing, Funding acquisition, Data curation. **Silvia Figueiredo Costa:** Writing – review & editing, Visualization, Supervision, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2026.128354>.

Data availability

Data will be made available on request.

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