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SARS-CoV-2: Virology, epidemiology, diagnosis, pathogenesis, and control

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SARS-CoV-2: Virology, epidemiology, diagnosis, pathogenesis, and control

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Editorial: SARS-CoV-2: virology, epidemiology, diagnosis, pathogenesis, and control

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Editorial on the Research Topic
SARS-CoV-2: virology, epidemiology, diagnosis, pathogenesis, and control

Introduction

The rapid spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in the most severe public health challenge since the 1918 Spanish influenza pandemic (1). Consequently, the crisis caused by the coronavirus disease 2019 (COVID-19) has massively impacted global public health, which has rapidly called for public health authorities and scientists to improve our knowledge about this condition (2). In this Research Topic, we serve as editors, and our primary goal was to gather knowledge about the virology of SARS-CoV-2 and different aspects of COVID-19, including epidemiology, pathogenesis, diagnosis, and control. Below, we provide a brief context of the published studies, including 19 original articles, three narrative reviews, and one systematic review.

Virology: SARS-CoV-2 genomics and variants of concern

During the course of the COVID-19 pandemic, the emergence of SARS-CoV-2 variants has been associated with evasion of immunity from natural infection and vaccinations, reduced susceptibility to therapies, increased transmissibility, risk of reinfection, and disease severity (1, 3–7), resulting in a tremendous challenge to controlling the pandemic phase (8). Within this perspective, the rapid spread of SARS-CoV-2 variants worldwide has been associated with a series of waves, as reported in many countries worldwide. To understand this impact in Santiago (Chile), Acuña-Castillo et al. showed that the highest rate of reinfections described during the fourth and fifth COVID-19 waves

in Santiago was primarily driven by the omicron variant, where the interval between initial infection and reinfection was found to be 372 days.

In Brazil, Pinho et al. provided relevant insights into the transmission dynamics of SARS-CoV-2 variants in the Brazilian state of Pará by deep sequencing 1,003 SARS-CoV-2 genomes (from May 2020 to October 2022). They found the gamma variant as a predominant variant associated with 290 (28.91%) cases, followed by delta with 53 (5.28%) cases, omicron with 7 (0.69%) cases, and non-variants of concern (VOCs) with 651 (64.9%) cases. Similarly, Freitas et al. investigated the spatiotemporal dispersion of emerging SARS-CoV-2 variants in the first three years of the COVID-19 pandemic in Sergipe state, Northeast Brazil. The results revealed the presence of five predominant SARS-CoV-2 lineages (B.1, B.1.1, B.1.1.33, B.1.1.28, and B.1.212).

Epidemiology and pathogenesis of COVID-19

In the context of the epidemiology of COVID-19 cases, Zhang et al. described the epidemiological characteristics of overseas imported COVID-19 cases into China. The findings showed that most overseas imported COVID-19 cases occurred in young and middle-aged Chinese students and businessmen returning from Europe, the United States, and some neighboring countries.

Clinical and experimental advances have shown that individuals infected by SARS-CoV-2 demonstrate a wide range of disease severity ranging from asymptomatic cases to individuals who develop a severe respiratory disease that requires hospital admission or that leads to death (2, 9). In this Research Topic, Zhu et al. provided a solid and interesting review article highlighting the distinct phases of SARS-CoV-2 pathogenesis and critical points associated with the clinical management of patients with COVID-19. Moving forward, several original studies published on this topic also investigated parameters and mechanisms related to the SARS-CoV-2 pathophysiology, discussing potential mechanisms behind SARS-CoV-2-associated outcomes.

While SARS-CoV-2 infection is known to cause mainly respiratory disease in infected patients, extrapulmonary manifestations are also common, especially in severe cases (10). To address this question, Wu et al. conducted a cross-sectional descriptive study to evaluate the otologic symptoms in 2,247 patients with COVID-19. The most common otologic symptoms following SARS-CoV-2 infection were vertigo, tinnitus, otalgia, aural fullness, hearing loss, otorrhea, and facial paralysis.

To evaluate the epidemiological, clinical, and laboratory characteristics of patients with COVID-19 admitted to the intensive care unit (ICU), Sodré et al. conducted a cross-sectional study in a reference hospital for COVID-19 treatment in the Southern Region of Bahia State, Brazil. Briefly, they showed that the use of bladder catheters and central venous catheters were the main factors associated with death in patients with COVID-19 in the ICU. In another study, Canuti et al. evaluated the role of immune suppression in 1,727 hospitalized patients with COVID-19 in Milan (Lombardy, Northern Italy). The results demonstrated that immune suppression significantly predicted severe outcomes, while vaccination was a protective factor. In another context, Kaufman et al. investigated the association between SARS-CoV-2 spike-protein targeted antibody levels and clinically relevant outcomes. In summary, they showed that individuals with detectable SARS-CoV-2 spike-protein targeted antibody levels had less serious outcomes.

In general, young people and children with SARS-CoV-2 infection experience asymptomatic or mild disease, while patients with comorbidities are more likely to be susceptible to developing a severe respiratory disease that requires hospital admission (11). To address this question in Bangladesh, Sharif et al. investigated the prevalence and impact of long COVID-19 among patients with diabetes and cardiovascular diseases. They found that acute long COVID-19 was detected among 28.4% of patients and chronic long COVID-19 was detected among 71.6% of patients. In addition, they showed that the co-prevalence of cardiovascular diseases, diabetes, and COVID-19 was involved in most cases (95%). Within the same context, Malaeb et al. investigated the clinical features and the mortality outcomes of patients with COVID-19 admitted to the ICU during the first wave and two subsequent surges in Iraq, while Hu et al. evaluated the impact of the first wave of COVID-19 on Crohn's disease after the end of the zero-COVID policy in China.

Diagnosis of COVID-19

Accurate COVID-19 diagnosis and testing have shown to be key for disease control, especially before vaccines were widely available (2, 12). However, diagnostics also plays an important role in other contexts. Thus, Acuña-Castillo et al. did an ecological study on COVID-19 reinfection in Chile using RT-PCR data information from over 300,000 individuals tested between 2020 and 2022. They found that the highest rate of reinfections took place during the fourth and fifth COVID-19 waves and was primarily driven by the omicron variant. The reinfection rate was 1.52 per 100,000 inhabitants, and the interval between initial infection and reinfection was found to be close to one year.

Serological tests were of limited value for clinical decision-making and implementation of patient isolation and quarantine (12). However, SARS-CoV-2-specific antibodies are major players in the immune defense against COVID-19. To address this, Kaufman et al. conducted a retrospective study to estimate the association between SARS-CoV-2 spike-protein targeted antibody levels and clinical outcomes in a cohort of almost 200,000 patients. Individuals with detectable SARS-CoV-2 antibody levels were less prone to be infected by SARS-CoV-2 and had lower risks of developing serious diseases upon infection.

Vaccination against SARS-CoV-2 in different perspectives

In a rapidly moving field of study, several articles have evaluated the effectiveness of available vaccines against SARS-CoV-2 variants, especially within the emergence of omicron VOC. To address this

question, Song et al. conducted a systematic review to evaluate the effectiveness of SARS-CoV-2 vaccines against omicron infection. Using 42 articles for analysis, they concluded that one or two SARS-CoV-2 booster doses provide considerable protection against omicron infection and substantial protection against severe COVID-19-related events. Similarly, three other studies (Reyes et al., Paduano et al., and Liviero et al.) provided relevant insights into the protective effect of the COVID-19 vaccine in the general population, highlighting the importance of vaccination as an effective strategy to reduce the number of cases, hospitalizations, and deaths.

On the other hand, Asefa et al. investigated the adverse reactions to COVID-19 vaccines among Ethiopian healthcare professionals. Among the 277 study participants, the most reported short-term adverse reactions were injection site pain, headache, fever, fatigue, chills, and muscle pain, and there was no detectable association between adverse reactions and the types of COVID-19 vaccine (Oxford AstraZeneca, Johnson & Johnson, Sinopharm, and Pfizer) subjects received. Subsequently, Xie et al. described relevant insights into the COVID-19 vaccination among groups of cancer patients, while Giudice et al. reported the factors involved in parents' hesitancy to vaccinate their children against COVID-19 in Italy. In the same direction, Kotronia et al. investigated willingness to vaccinate and the associated factors in samples of unvaccinated and vaccinated adults in Poland. These findings suggest that although COVID-19 vaccines have shown to be safe and effective, some individuals were reluctant to take the vaccine during the pandemic course, negatively impacting the establishment of effective vaccination programs and therapeutic interventions. Branch-Elliman et al. explained how we can improve our response to infectious diseases using COVID-19 as a study model.

Final considerations

Despite the passage of 4 years since the beginning of the pandemic, there are still many gaps that we need to address about this devastating disease that will certainly be recorded as one of the greatest public health problems in the history of humanity. The COVID-19 pandemic has highlighted both our ability to respond and our resilience to face biothreats of this magnitude. Most importantly, the lessons acquired during the COVID-19 pandemic will be essential for dealing with future public health threats, particularly for the response against new pathogens. Through this Research Topic, we contributed to the advancement of knowledge related to COVID-19 in several aspects, including epidemiology, genomic surveillance, diagnosis, pathogenesis, and control.

Author contributions

SS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. LP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors SS, SK, and LP declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Epidemiological characteristics of overseas imported COVID-19 cases into China: A scoping literature review

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Previous studies investigating the characteristics of imported cases were mostly limited to a certain province/city or a specific sub-group during a certain period with a small sample size, which may not provide an overall picture of the characteristics of imported cases. In this scoping literature review, we comprehensively synthesized the epidemiological characteristics of overseas imported COVID-19 cases into China by retrieving six literature databases, with aims to provide implications for more targeted control, prevention, and medical treatment of this disease. After dropping duplicates and reviewing titles, abstracts, and full-texts, 50 articles were included in the review finally, including 26 (52%) articles in English and 24 (48%) articles in Chinese. According to the type of data sources, the 50 studies were divided into three categories: 13 (26%) articles using data sourced from the Chinese Infectious Diseases Online Reporting System, 15 (30%) articles using data from the websites of national/local health departments, and 22 (44%) articles using hospital admission data. Most of the overseas imported COVID-19 cases were young and middle-aged Chinese students and businessmen returning from the United States, Europe, and some neighboring countries. Airport routine health screening measures could not identify COVID-cases effectively, although scheduled multiple nucleic acid tests were required before boarding. Almost all imported cases were identified during the hotel quarantine period. Although a large proportion of imported cases were asymptomatic or with mild symptoms in the published literature, they may be due to participant selection bias. The exact proportion of asymptomatic cases may need to be further investigated especially through population-based large-scale studies.

KEYWORDS

imported, COVID-19, epidemiological characteristics, review, China

1. Introduction

Since the first case of COVID-19 was identified in late-December in Wuhan, Hubei Province of central China, the epidemic spread rapidly and was declared a global pandemic by WHO on 11 March 2020 (1). COVID-19 is one of the most widespread epidemics in human history, not only posing a huge threat to the health of vulnerable populations (e.g., older adults) but also severely impacting global economic development (2). Looking back at China's tremendous

efforts in fighting against COVID-19 in the past 3 years, the whole process could be roughly divided into four stages (3). The first stage is the formation of the COVID-19 epicenter in Wuhan and its spread to other provinces from 31st December 2019 to 29th February 2020; The second stage lasted from 1st to 21st March 2020, characterized with the containment of COVID-19 outbreaks and the number of cases was reduced to less than 10 in most provinces; The third stage is the sporadic outbreak mostly triggered by overseas imported cases from March 2021 to June 2022. In this stage, the priority of precautionary measures has gradually shifted from domestic infected cases to overseas imported cases. To prevent overseas imported cases, since 29th March 2022 Civil Aviation Administration of China introduced the "Five-One" policy to limit the number of international flights, namely each airline can only operate one flight per week to travel to and out of China (4). Moreover, a flight would be suspended for 1–2 weeks when confirmed cases accounted for a certain percentage (e.g., 4%) of inbound passengers. The duration of hotel/home quarantine for incoming passengers was updated correspondingly (Figure 1), according to the domestic and international COVID-19 epidemic situations. The fourth stage is the relaxation of strict COVID-19 restrictions (e.g., case tracing) since 7th December 2022. COVID-19 was initially classified as a B-category notifiable infectious disease in China but managed under A-category protocols. From 8th January 2023, control measures against COVID-19 have been downgraded from A-category to B-category (5).

The infectivity and virulence of SARS-CoV-2 evolve rapidly, and the corresponding prevention guidelines have been updated to the 10th edition in China (6). Under the new policy, the control strategy has shifted from the hard-line "zero-COVID" measures such as strict lockdown and large-scale all-staff COVID-19 testing to co-exist with the virus. The current priorities include the protection and medical treatment of infected vulnerable populations, increasing the vaccination rate of older adults, strengthening surveillance, and optimizing border control. International flights are anticipated to restore gradually. The duration of hotel quarantine has been reduced from at least 2–3 weeks in a certain period to 5 days in November 2022. From 8th January 2023, quarantine requirements on inbound travelers have been canceled. Overseas imported cases into China are likely to increase in the following several months because of the relaxed international travel restrictions (e.g., the cancelation of hotel quarantine), which may burden the already overloaded healthcare system, especially in the under-developed rural areas.

Most of previous studies focused on the spatial and temporal distribution of overseas imported COVID-19 cases, and they were published at an early stage with a small sample size (7, 8). In this scoping literature review, we aimed to comprehensively synthesize the epidemiological characteristics of overseas imported COVID-19 cases into China, to provide implications for more targeted control, prevention, and medical treatment of this disease.

2. Methods

2.1. Search strategy

Articles involving epidemiological characteristics of overseas imported COVID-19 cases into China were searched using the combination of keywords: imported COVID-19 AND (China OR mainland China OR Taiwan OR Hongkong OR Macao), including studies published from the time of database creation to November 10, 2022. Literature databases used for this review included PubMed, Embase, and Scopus. Three Chinese literature databases, China National Knowledge Infrastructure (CNKI), Wanfang, and Weipu, were also retrieved to avoid language bias, as the Ministry of Science and Technology of China released a notice on 29 January 2020 to encourage Chinese researchers to publish COVID-19 related studies in domestic journals (9). The initial search results were imported into an Endnote library. Duplicate records were removed using the EndNote function of "find duplicates." Appropriate peer-reviewed studies were identified by a three-step process (Figure 2): screening titles, reviewing abstracts of articles that were difficult to judge by screening their titles, and reviewing the full-texts. Studies were independently appraised by two investigators (ZZH and JX). Where consensus could not be reached, there was a group discussion to determine the final articles included for reviewing. Reference lists and similar articles recommended by PubMed were also scanned for

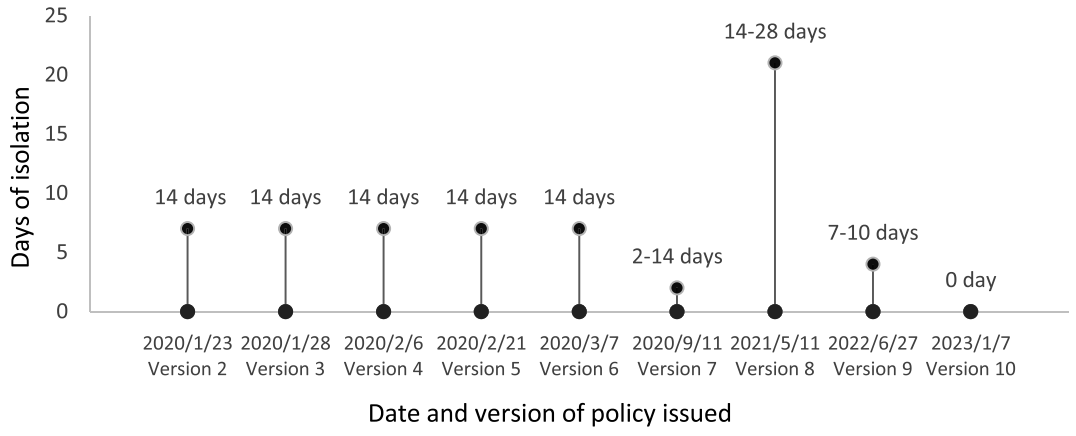

FIGURE 1
Duration of mandatory hotel/home quarantine for incoming passengers according to the 2nd–9th editions of the COVID-19 prevention and control guidelines.

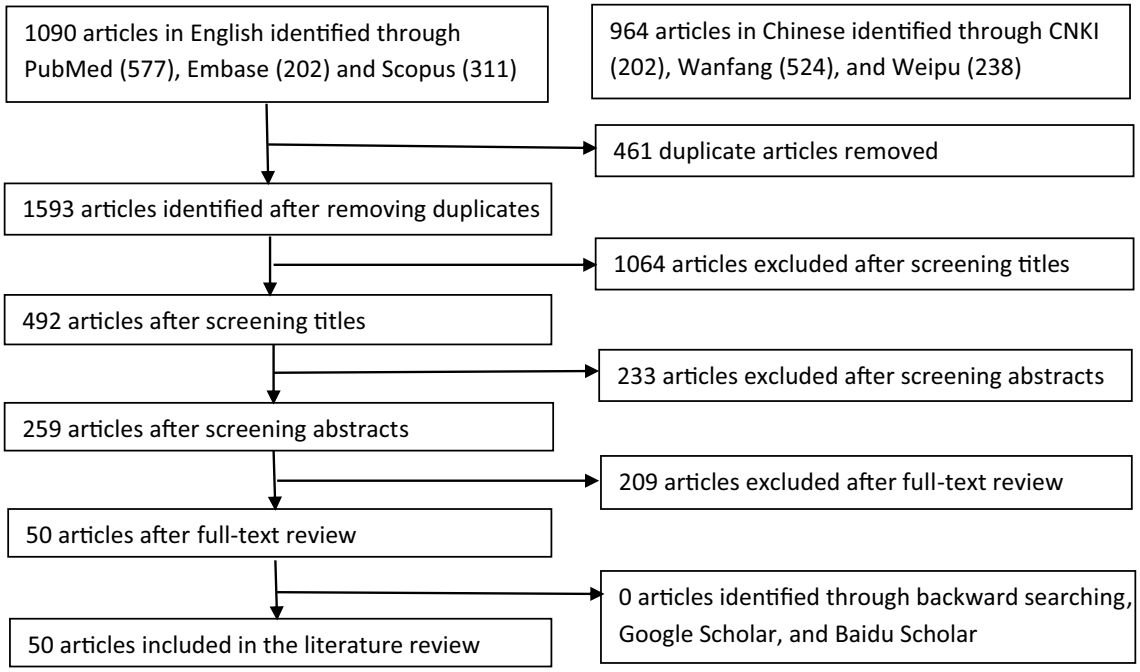

FIGURE 2
Flow chart of literature search process.

additional articles not previously identified. The 'Google Scholar' and 'Baidu Scholar' search engines were also used to retrieve relevant literature.

This scoping review of overseas imported cases includes articles from three data sources: China Infectious Disease Reporting System, national/local health department websites, and hospital inpatient data. Due to the different emphases of the source data, we divided the collected literature data into three tables to facilitate the statistics of useful information.

2.2. Inclusion and exclusion criteria

The studies selected in this review met the following criteria:

- Investigated the epidemiological characteristics of overseas imported COVID-19 cased into China.
- Peer-reviewed studies published from database inception to 10 November 2022.
- Conference abstracts, letters, editorials, field investigations, reports and unrefereed preprints on medRxiv and bioRxiv were excluded.

3. Results

Figure 1 shows the process of selection of articles for inclusion in the review. An initial search generated 2054 articles, with 53.1% being published in English and the rest in Chinese. After dropping duplicates and reviewing titles, abstracts, and full-texts, 50 articles were finally included in the review, including 26 (52%) articles in English and 24 (48%) articles in Chinese. According to the type of data sources, the 50 studies were divided into three categories: 13 (26%) articles using data sourced from the Chinese Infectious Diseases Online Reporting System, 15 (30%) articles using data from the websites of national/local health departments, and 22 (44%) articles using hospital admission data. They were summarized in Tables 1–3, respectively.

Table 1 summarized the key characteristics of 13 articles analyzing imported cases reported through the online Chinese Infectious Diseases Reporting System (CIDRS). The number of source countries ranged from 5 to 53 and the most frequent top-three countries were United States, United Kingdom, and Philippines. The ranges of median and mean ages were 23–36 and 34–36 years, respectively. Most imported cases were males, with the proportion reaching as high as 90.1%. Study, work, and business were the most frequent reasons for traveling. The vast majority of cases were Chinese citizens (71.3–96.6%). The number of days from entry to diagnosis ranged from 1.5 to 6.3, while it should be noted that there was a special case taking 14 days to be diagnosed. The proportion of asymptomatic cases varied largely from zero to 87%. The proportion of cases identified through airport health screening measures ranged from zero to 74%.

Table 2 showed the characteristics of 15 articles analyzing COVID-19 cases sourced from the websites of national or local health departments. Basically, the characteristics of imported cases in Table 2 were similar with that in Table 1 in the aspects of reasons for traveling and days from entry to diagnosis, because COVID-19 data released on the official websites were also sourced from the CIDRS. By contrast, characteristics of imported cases based on hospital admission data (Table 3) were slightly different with cases sourced from the CIDRSA and governmental websites in Tables 1, 2. The number of source countries reached 177 by May 2021. Moreover, they were relatively more diverse compared to that in Tables 1, 2. The proportion of asymptomatic cases ranged from zero to 71.4%, which was lower than

TABLE 1 Summary of 13 studies using data sourced from the Chinese Infectious Diseases Reporting System.

Author	Data period	Number of imported cases	Number of source countries and top 3	Age	Gender (% of males)	Reason for traveling (top3)	Chinese nationality (%)	Days from arrival to diagnosis	Asymptomatic case (%)	Clinical classification (Mild: Moderate: Severe: Critically)	Cases infected by imported	Identified through airport screening (%)	Province/ municipality of entry
Fang et al. (10).	5 Mar. to 30 Mar. 2020	171	24 and United Kingdom (37.3%), United States (18.6%), and France (11%)	Median: 23 years 18–40 years: (77.7%)	56.7	Study (56.6%) Work (24.6%) Business (5.3%)	71.3	2.9	0.6	69:96:4:1	–	18.7	Shanghai
Zhen et al. (11)	3 Mar. to 1 Apr. 2020	103	22 and United Kingdom (26.2%), Philippine (12.6%), and United States (12.6%)	Median: 31 years Range: 11–63 years	61.2	Business/work (39.8%) Study (35.0%) Unemployed (12.6%)	75.7	3.0	14.1	38:54:0:0	36	47.6	Guangzhou
Liu et al. (12).	1 Mar. to 7 Apr. 2020	91	19 and Italy (25.3%), United Kingdom (18.7%), and Spain (17.6%)	Mean: 33.7 years Range: 7–73 years	52.8	Study (34.1%) Business/work (23.1%)	86.8	3.0	47.3	16:32:0:0	–	–	Zhejiang
Dong et al. (13).	1 Jun.to 30 Sep.2021	171	23 and Philippines (24%), U.A.E (22.8%), and United Kingdom (14.6%)	Median: 28 years Range: 10 ms–64 years	66.7	Work (33.3%) Study (24.6%) Unemployed (18.71%)	80.1	More than 14 days: 10. %	77.8	132:37:1:1	–	13.5	Beijing
Zhao et al. (14).	Up to 18 Jun.2021	207	Africa	18–40 years (72.9%)	69.6	Business (62.3%) Study (18.4%) Unemployed (15%)	26.1	4.0	87.0	5:22:0:0	66	0.0	Guangzhou
Yu et al. (15).	15 Mar.2020 to 31Aug. 2021	552	53 and Spain (14.1%), France (13.2%), and United States (13.0%)	20–40 years (59.8%) Range: 3-77 years	63.9	Unemployed (18.8%) Business (14.7%)	91.8	–	44.2	106:139:0:0	–	45.5	Tianjin
Chen et al. (16).	29 Jan. to 12 Jul. 2020	72	18 and United States (22.2%), Russia (20.8%), and United Kingdom (15.3%)	Mean: 35.9 years Range: 14–74 years	51.5	Study (43.1%) Business/work (13.9%)	87.5	–	54.2	5:28:0:0	0	–	Liaoning
Chen et al. (17).	19 Mar.2020 to 31 May.2021	325	44 and Philippines (19.7%), United States (15.4%), and Russia (8.9%)	Median: 36.1 years Range: 1 ms–71 years	71.0	Work (25.2%) Business (15.4%) Study (15.1%)	–	1.5	0	76:249:0:0	0	40.9	Fujian
Li et al. (18).	1 Jan.2020 to 31 Jul.2021	77	15 and Russian (19.5%), Japan (19.5%), and Philippines (13%)	21-40 years: (59.7%)	90.1	Fisherman (66.2%) Work (6.5%) Study (5.6%)	–	–	58.4	1:10:0:0	–	–	Dalian
Qi et al. (19).	1 Sept. 2020 to 28 Jan. 2021	136	32 and Philippines (19.1%), India (10.3%), and Nigeria (8.8%)	Mean: 34.9 years Range: 6 ms -6 years	67.7	Business/work (33.1%)	–	–	72.1	7:30:1:0	–	–	Zhejiang
Hu et al. (20).	28 Feb. to 30 Nov. 2020	450	9 and Iraq (8.4%), Egypt (4.0%), and Ethiopia (4.0%)	Median: 34 years Range: 2 ms -70 years	82.9	Business/work (40.9%)	87.3	–	40.9	54:212:0:0	193	73.9	Chengdu
Zhang et al. (21).	11 Mar. to 6 Jul. 2020	268	32 and United Kingdom (20.1%), Bangladesh (16.8%), and United States (15.3%)	Median: 32 years Range: 3–70years	59.0	Study (34.9%) Business/work (32.6%)	89.2	–	31.7	–	36	73.5	Guangzhou
Liu et al. (22).	21 Jan. to 6 Apr. 2020	321	37 and United States (25.2%), United Kingdom (22.7%), and France (6.5%)	20–39 years: (61.1%)	47.0	Tourism (32.4%) Business/work (27.4%) Study (26.5%)	96.6	6.3	3.4	–	52/16	32.7	Taiwan

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TABLE 2 Summary of 15 studies using data sourced from the websites of national/local health departments.

Author	Data period	Number of imported cases	Number of source countries and top 3	Age	Gender (% of males)	Reason for traveling (%)	Chinese nationality (%)	Days from arrival to diagnosis	Asymptomatic case (%)	Cases infected by imported	Identified through airport screening (%)	Province of entry
Lam et al. (23).	1 Jan. to 31 May 2020	657	NA and United Kingdom (61.6%), United States (13.4%), and France (7.2%)	15–24 years: (40%)	28.7	Study (37.3%)	–	2.6	20.8	250	25.6	Hong Kong
Chen et al. (24).	1 Mar. to 2 Jun.2020	200	31 and United Kingdom (28.5%), United States (17.5%), and Philippines (8.5%)	Median: 31.6 years Range: 2–70 years	63	Living abroad (11.5%)	81	4.3	79.5	–	95.0	Guangdong
Wang et al. (25).	13 Feb.2021	31	1 and Kenya	Mean: 45.8 years Range: 27–61 years	96.8	Work (74.2%)	97.2	–	20.0	–	100.0	Guangdong
Yang et al. (26).	1 Jan. 2020 to 28 Feb.2021	1,585	9 and Philippines (48.9%), Indonesia (19%), and Singapore (17.6%)	Median: 33 years Mean: 35.2 years Range: 1–70 years	79.8	Work (21.3%) Unemployed (13.6%) Business (12.9%)	–	Median1 Range0-27	58.1	–	–	Mainland China
Li (27).	24 Mar. to 15 Sep.2020	184	-	Median: 32 years Range: 13–72 years	54.5	Study (36.41%) Business (45.7%) Unemployed (4.3%)	–	–	0.0	0	–	Inner Mongolia
Cao (28).	5 Mar. to 31 Dec.2020	90	9 and Iran (41.1%), Saudi Arabia (22.2%), and Russia (12.2%)	Mean: 27.4 years Range: 4 ms to 62 years	78.9	Study (53.3%) Work (11.1%) Unemployed (8.9%)	100%	–	25.6	–	–	Gansu
Tian et al. (29).	18 Mar.2020	5	1 and United Kingdom	Median: 38 years Range: 1–69 years	40	Visit	–	1.8	20.0	1	40.0	Beijing
Ma et al. (30).	24 Mar. to 16 May.2020	19	5 and Russia (57.9%), United States (21.0%), and Thailand (10.5%)	–	6/10	–	–	–	–	–	–	Jilin
Shen et al. (31).	27 Feb. to 15 Aug. 2020	2,278	66 and Russia (33.2%), United Kingdom (14.4%), and United States (10.1%)	Range: 2 ms to 76 years	65.0	–	–	–	7.7	–	–	Mainland China
Chen et al. (32).	1 Mar. to 10 Apr. 2020	179	28 and United Kingdom (29.6%), United States (17.9%), and Philippines (9.5%)	Mean: 31.6 years Range: 1–70 years	62.0	–	78.8	4.2	81.0	–	91.6	Guangdong
Li et al. (33).	26 Feb. to 18 Mar. 2020	188	18 and Iran (25.0%), Italy (22.3%), and Spain (15.4%)	Mean: 33.5 years Range: 1–70 years	57.5	Business/work (45.4%) Study (25.9%)	94.2	–	0.5	80	–	Mainland China
Li et al. (34).	23 Jan. to 8 Aug.2020	1,074	-	15–24 years most	–	Study/tourism	–	–	–	2,198	–	Hong Kong
Yang et al. (35).	1 Apr. to 31 Jul.2020	187	8 and Philippines (45%), United Kingdom (35%), and United States (14%)	–	–	–	–	–	–	–	–	Hong Kong
Zhao et al. (36).	1 Jan. to 19 Feb. 2020	45	-	20–59 years: (80.4%)	57.7	–	–	–	0.2	–	–	Jilin
Guo et al. (37).	29 Feb. to 20 May 2020	1,709	50 and Russia (40.1%), United Kingdom (18.0%), and United States (10.7%)	Mean: 35.4 years Range: 2 ms to 72 years	58.9	Study (40.0%)	–	–	–	–	–	Mainland China

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TABLE 3 Summary of 22 studies using hospital admission data.

Author	Data period	Number of imported cases	Number of source countries and top 3	Age	Gender (% of males)	Reason for traveling (%)	Days from arrival to diagnosis	Asymptomatic case (%)	Clinical classification (Mild: Moderate: Severe: Critically)	Clinical symptoms (top 2)	Comorbidities (top 2)	Province of entry
Chen et al. (38)	25 Jan. to 20 Feb.2020	29	–	Median: 39 years	69	–	2.4	3.4	–	Fever (17.6%) Cough (12.5%)	-	Chongqing
Liu et al. (39)	5 Mar. to 22 Mar.2020	58	9 and United Kingdom (32.8%), Italy (15.5%), and United States (15.5%)	Median: 29 years	53.4	–	3	8.6	–	Fever (50.0%) Cough (41.4%)	Hypertension (12.1%) Diabetes (6.9%)	Shanghai
Zhai et al. (40).	15 Mar. to 30 Apr.2020	53	6 and United Kingdom (49%)	Median: 27 years Moderate: 23 years	39.6	–	–	0.0	4:6:0:0	Cough (52.8%) Sore throat (50.9%)	In total (13.2%)	Beijing
Zhang et al. (41).	29 Mar. to 31 Aug.2020	79	10 and Singapore (34.18%), Russia (22.78%), and Kazakhstan (11.46%)	Mean: 38 years Range: 19–57 years	88.7	Business/work mostly	6	24.2	2:4:1:0	Cough (22.8%) Fever (11.4%)	Hypertension (67.6%) Diabetes (2.5%)	Xi'an
Liu et al. (42).	29 Feb. to 27 Mar.2020	109	–	Median: 24 years Mean: 27.3 years	58.7	Work/study mostly	1	–	44:62:3:0	Fever (39.1%) Cough (33.3%)	Diabetes (1.8%) Hypertension (2.8%)	Beijing
Hu et al. (43).	1 Jul.2020 to 15 Jan. 2021	23	–	Mean: 45.1 Range: 23–72 years	56.5	–	–	–	Severe:0	Fever (87%) Cough (65.2%)	-	Hubei
Yong et al. (44).	1 Dec.2020 to 15 Ap.2021	75	7 and Nigeria (38.6%), Egypt (38.7%), and Ethiopia (16%)	–	90.7	–	–	20.0	53:7:0:0	Cough (9.3%) Sore throat (6.7%)	-	Sichuan
Du et al. (45).	23 Jan. to 19 Feb. 2020	33	–	30–59 years (69.7%)	60.6	–	4	0.0	–	Fever (78.8%) Cough (48.5%)	-	Inner Mongolia
Li et al. (46).	29 Feb. to 20 Mar.2020	71	11 and Spain (31.0%), United Kingdom (23.9%), and Italy (22.5%)	Median: 24 years	38.0	–	4	2.8	–	Cough (49.3%) Fever (42.3%)		Beijing
Li et al. (47).	22 Mar. to 17 May.2021	46	8 and Pakistan (39.1%), Sudan (21.7%), and United Arab Emirates (19.6%)	Mean: 40.5 years	89.1	–	–	67.4	7:3:0:0	Fever (6.6%) Cough (6.6%)	Hypertension (13%) Diabetes (6.6%)	Shanxi
Yan et al. (48).	28 Jul.2020 to 31 Dec.2021	137	22 and Uzbekistan (20.4%), Singapore (12.4%), and Germany (10.9%)	Median: 38 years Mean: 37.8 years Range: 8–68 years	89.8	–	<3d 67.8% >3d 32.2%	48.9	5:5:0:0	Fever (22.6%) Cough (18.9%)	Hypertension (7.3%) Diabetes (2.2%)	Xian
Liu et al. (49).	16 Dec. to 31 Dec. 2021	17	–	30-40 years: (52.9%)	94.1	–	–	11.8	2:1:0:0	Sore throat (58.8%) Cough (47%)	In total 17%	Hunan
Qiu et al. (50).	19 Mar. to 1 Sept. 2020	10	10 and Italy (50.0%), Spain (30.0%)	Median: 45 years Range: 18–73 years	30.0	–	4.0	20.0	1:9:0:0	Cough (60%) Fever (50%)	In total 20%	Zhejiang
Cai et al. (51).	14 Mar. to 8 Apr. 2020	38	10 and United Kingdom (26.3%), Italy (13.2%), and France (10.5%)	Median: 14.5 years Range: 2.3–17 years	57.9	Study 91.4%	–	42.1	8:14:0:0	Cough (28.9%) Fever (18.4%)	–	Shanghai
Chen et al. (52).	1 Feb. to 31 Mar. 2020	90	na and Africa (20.0%), Europe (4.4%)	Mean: 38.7 years	64.4	–	3.2	31.1	28:52:2:0	Cough (40.0%) Fever (31.0%)	Diabetes (5.6%) Hypertension (5.6%)	Guangdong
Luo et al. (53).	20 Jan. to 31 Oct. 2020	78	Mainly United Kingdom, Philippines, and United States	Mean: 35 years 14–40 years: (66.7%)	76.9	Study mostly	1	50.0	39:38:1:0	Cough (16.7%) Sore throat (11.5%)	Hypertension (5.1%) Diabetes (1.3%)	Fujian
Zhang et al. (54).	20 Jan. to 20 Mar. 2020	69	11 and United Kingdom (26.1%), Spain (24.6%), and Italy (24.6%)	Median: 27 years Range: 6–69 years	40.6	Study 44.9% Business/work 17.4%	4	2.9	29:37:2:1	Cough (43.5%) Fever (39.1%)	In total 21.7%	Beijing
Dan et al. (55).	Oct.2020 to May 2021	177	na and United States (11.11%), Zambia (6.84%), and Nigeria (6.84%)	Median: 34 years Range: 16–85 years	76.8	–	1.7	–	24.3	–	–	Guangdong
Yuan et al. (56).	14 Mar. to 3 Apr. 2020	41	5 and United Kingdom (68.3%), United States (14.6%), and Spain (7.31%)	Mean: 27.4 years	31.7	–	–	–	24:17:0:0	Cough (31.7%) Fever (26.8%)	0	Beijing
Zhao et al. (57).	21 Jan. to 5 Apr. 2020	7	3 and Russia (42.9%), United Kingdom (42.9%), and Denmark (14.3%)	Mean: 23.6 years	71.4	–	–	71.4	0:6:1:0	Fever (14.3%) Diarrhea (14.3%)	In total 28.6%	Hebei
Bi et al. (58).	19 Mar. to 24 Apr.2020	56	8 and United Kingdom (32.1%), United States (26.8%), and France (19.6%)	Mean: 29.3 years	50	–	–	0.0	0:51:3:2	Cough (42.9%) Fever (37.5%)	Hepatitis B (8.9%) Diabetes (7.1%)	Tianjin
Bao et al. (59).	23 Jan. to 3 Sep.2020	79	–	Mean: 28 years	74.7	Work/study mostly	–	1.3	37:39:2:0	Cough (24.1%) Fever (16.5%)	Hypertension (3.8%) Coronary heart disease (1.3%)	Gansu

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that in Tables 1, 2. The most frequent clinical symptoms of imported cases were fever, coughing, and sore throat, with the highest percentages reaching 87, 65, and 51%, respectively. The most common comorbidities were hypertension and diabetes.

4. Discussion

Imported COVID-19 cases are an important source triggering local sporadic outbreaks. One imported case could reportedly result in more than 2,000 infections in a short period of time. Thus, strict border control measures had been taken by the government to reduce the risk, such as scheduled multiple nucleic acid tests before boarding, a "Five-One" flight policy, closed-loop management, and hotel quarantine. Previous studies investigating the characteristics of imported cases were mostly limited to a certain province/city or a specific sub-group during a certain period, which may not provide an overall picture of the characteristics of imported cases. In this study, we comprehensively reviewed the epidemiological characteristics of overseas imported COVID-19 cases into China, retrieving six literature databases. Findings of this literature review may not only provide evidence for the development of current control measures against COVID-19 but also facilitate the management of imported cases.

We found that the importing countries were mainly high-income countries (e.g., United States and United Kingdom) or neighboring countries (e.g., Russia and Malaysia) with close trade links with China. United States and United Kingdom are the top two destinations for Chinese students. In 2021, many overseas Chinese students were selected to return to China due to the following reasons. First, many western countries abandoned case tracking, early detection, and case isolation and selected coexistence with the virus, leading to the surge of COVID-19 related morbidity and mortality (60). By contrast, China took a strict zero-case policy at that time and the epidemic was well-contained. Second, some universities transferred to online teaching temporarily to avoid campus outbreaks. Third, evidence has shown that the well-being of Chinese international students deteriorated in the early stage of COVID-19 pandemic. Over the debate of COVID-19 origin, a high prevalence of mental health issues (e.g., depression, anxiety, and feeling of discrimination) was observed among them (61, 62). In addition, work and business activities were also the most common reasons for traveling to China, while most incoming travelers were Chinese nationals. International air flights are the major way for imported cases (63), however, some travelers sought to enter *via* land or port when most international flights were suspended at the early stage of the global pandemic. For example, most cases imported from Russia were through land border ports, especially the Suifen River Estuary in Mudanjiang city. The proportion of male travelers was higher than their female counterparts. Our result is supported by the findings of a global study of population mobility networks (61), which utilized the characteristics of travelers and geographical factors to predict the COVID-19 cross-border transmission.

According to the level of severity, COVID-19 was initially divided into four types: mild, moderate, severe, and critical cases. However, published literature shows that many infections of COVID-19 are asymptomatic (64). It has been reported that viral loads of asymptomatic patients were similar with those of symptomatic individuals (65), suggesting that asymptomatic patients have a similar capacity in infectivity for transmission. The potential transmission of asymptomatic infections poses a significant challenge to the control and prevention of COVID-19. Two prerequisites must be met for the diagnosis of asymptomatic COVID-19 infection: the absence of self-perceived or clinically recognizable symptoms; and a positive reverse transcription-PCR (RT-PCR) test. In this review, the proportion of asymptomatic cases was highly variable with a range from 0 to 87%. Not only in the imported cases, a highly varied proportion of asymptomatic infections was also reported in the general population (8). This may be due to participant selection bias. For example, in this review we found the average proportion of asymptomatic infections based on hospital admission data (22%) was lower than the proportion based on data sourced from CDC (41%) and the websites of local health departments (26%). Most of the imported cases were young and middle-aged who were less likely to have clinical manifestations than the vulnerable sub-groups (e.g., children, pregnant women, and older adults with chronic diseases). Moreover, symptomatic cases may opt to postpone their trips. Relative fewer cases from low- and middle-income countries due to the soaring airfares and the limited number of flights may also contribute to the participant selection bias. Another factor associated with clinical manifestations was the course of disease. Evidence has shown that the proportion of asymptomatic infections ranged from approximately 20–75% at initial testing, however, only 4% remained asymptomatic throughout the disease finally (66). In addition, vaccination status, the type of vaccines, and virus strain also affect the presentation of clinical symptoms. The exact proportion of asymptomatic cases needs to be further investigated especially through population-based large-scale studies.

We observed a high heterogeneity in sample size, patients' age, COVID-19 symptoms, and comorbidities, although most of the included studies were conducted in China. Fever and coughing were the most common symptoms. Regarding comorbidities for patients with COVID-19, the highest severity factors were hypertension, diabetes, obesity, chronic obstructive pulmonary disease, and cardiovascular disease. This is consistent with a recent literature review focused on the general population (8). Pustahija et al. found there were no significant differences between travel-associated cases and cases identified in the general population in terms of the epidemiological and clinical characteristics in Serbia (67). Another study from Bolivia found similar epidemiological characteristics of imported COVID-19 cases with this study (68). It should be noted that the differences between studies in symptoms and comorbidities cannot be compared directly without taking demographical factors, virus strain, healthcare systems, selection criteria, the course of disease, and border control measures into account.

In response to the COVID-19 pandemic, many countries have imposed international travel restrictions to prevent the importation of COVID-19 cases. In this review, we found the proportion of imported cases identified by airport health screening measures varied considerably from zero to 100% with an average of 49.9%, indicating that entry screening alone may not detect imported cases effectively at borders. Although more than 90% of COVID-19 patients had a fever, body temperature might not be an adequate screening method as it may miss travelers in the incubation period or travelers concealing fever during travel (69). Despite the ineffectiveness of entry screening measures, travel restrictions may delay the transmission of COVID-19 between countries and have concomitant positive effects such as discoursing the travel of ill persons and raising the awareness of

infectious disease control (63, 70). In this review, the number of days from entry to diagnosis ranged from 1.5 to 6.3 days. To prevent the importation of COVID-19 cases, 1–3 weeks' hotel quarantine was mandatorily required by the Chinese government for all incoming passengers before 8th January 2023. Moreover, multiple RT-PCT tests were required before boarding and during the quarantine period to minimize the risk of importation as much as possible. However, it should be noted that quarantine hotels are not designed with infection control and there is a risk of within hotel transmission between guests and/or staff. It has been estimated that 8–11 per 1,000 cases identified during hotel quarantine may be infected by another unlinked case during quarantine (71). Therefore, its impact on the characteristics of imported cases should be minimal.

To maintain international personnel and economic exchanges, quarantine strategies have been adjusted timely according to the infectivity, virulence, and incubation period of different variants. A recent systematic review suggested that the incubation periods of Alpha, Beta, Delta, and Omicron variants were 5.0, 4.5, 4.4, and 3.4 days, respectively (72). With the shortening of the incubation periods of new variants, the quarantine period has been reduced accordingly. Now hotel quarantine requirements on inbound travelers are no longer required. Nevertheless, negative RT-PCR test 48 h before departure and online self-declaration of health status are still in place to prevent importation. These targeted measures significantly reduced the importation of COVID-10 cases into China and the pressure on the healthcare system (73). China will keep monitoring the characteristics of imported cases to adjust prevention policies to lessen the impact on economic and social development.

5. Conclusion

Most of the overseas imported COVID-19 cases were young and middle-aged Chinese students and businessmen returning from the United States, Europe, and some neighboring countries. Airport routine health screening measures could not identify COVID-cases effectively although scheduled multiple nucleic acid tests were required before boarding. Almost all imported cases were identified during the hotel quarantine period. Although a large proportion of imported cases were asymptomatic or with mild symptoms in the published literature, they may be due to participant selection bias. The exact proportion of asymptomatic cases needs to be further investigated especially through population-based large-scale studies.

Author contributions

JX, RX, BL, and ZZ conceived the review. ZZ, JX, YC, QL, YY, JC, YL, and ZX participated in the literature search and selection. ZZ and JX drafted the manuscript and made the tables. ZZ, MM, CW, BL, RX, and JX revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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An online survey on clinical characteristics of otologic symptoms linked to COVID-19 infection

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Objective: To report the otologic symptoms that present in patients with COVID-19 infection and investigate the pathogenic characteristics during the period of the pandemic.

Materials and methods: This cross-sectional descriptive study included participants with COVID-19 infection. COVID-19 infection was verified in these patients by nucleic acid test or antigen test. An online questionnaire was developed to analyze the association between the COVID-19 pandemic and the characteristics of otologic symptoms.

Results: This study included 2,247 participants, of which nearly half had one or more otologic symptoms. The presents of otologic symptoms were associated with gender (OR = 1.575, $p < 0.0001$), age (OR = 0.972, $p < 0.0001$), and occupation (healthcare worker: $p < 0.0001$; personnel of enterprises or institutions: OR = 1.792, $p < 0.0001$; student: OR = 0.712, $p < 0.044$). The otologic symptoms following COVID-19 infection in order were vertigo (25.95%), tinnitus (19.05%), otalgia (19.00%), aural fullness (17.18%), hearing loss (11.62%), otorrhea (1.25%), and facial paralysis (0.27%).

Conclusion: The present study shows that otologic symptoms are common among the COVID-19 infected participants and that these symptoms mostly recover spontaneously. During the corona-virus pandemic, the involvement of the cochleovestibular system and facial nerve should not be overlooked while treating the COVID-19 infected individuals.

KEYWORDS

COVID-19, otologic symptoms, cross-sectional survey, clinical characteristics, pathological mechanisms

1. Introduction

Almost 3 years have passed since the World Health Organization declared the coronavirus infection (COVID-19) a pandemic. Enormous progress has been made in the impact and response to life-threatening symptoms of COVID-19 across the lifespan (1). Current studies focus on clinical features following COVID-19 infection more in major organs such as lung and heart. However, the concern over otologic manifestations following COVID-19 infection is relatively limited (2–5).

Evidence suggests that hearing loss, tinnitus, vertigo, and facial palsy may work as a potential long-term sequela of COVID-19 reducing the quality of life and negatively affecting interpersonal communication and social life (6–8). Meanwhile, current findings raise the value of unexplained cochleovestibular symptoms during the pandemic, as these may be the only presenting symptoms indicating COVID-19 or partial (1). Therefore, identifying otologic symptoms is very critical.

Though many papers have reported audiovestibular symptoms or facial palsy associated with COVID-19 infection, the underlying pathological mechanisms of otologic symptoms are still unclear (9–11).

To investigate the otologic manifestations thoroughly during the COVID-19 pandemic and to analyze the potential predictive variables, this study investigates the onset, duration, and clinical outcomes of otologic symptoms in patients with COVID-19 infection during the pandemic period in China. To the best of our knowledge, this is an epidemiological survey on this issue with the largest sample size to date.

2. Materials and methods

2.1. Participants

This study included 2247 COVID-positive participants comprising 1,138 without otologic symptoms and 1,109 with otologic symptoms. The patients with COVID-19 infection verified with nucleic acid test or antigen test were recruited in the pandemic period from December 20, 2022 to January 10, 2023. Potential COVID-19-positive participants were approached through a social media application (WeChat). All participants were from 30 provinces in China. Table 1 shows the demographic characteristics of all participants with COVID-19.

2.2. Study design

To investigate the characteristics of otologic symptoms in individuals with COVID-19 infection during the pandemic period, we conducted a descriptive and analytical cross-sectional study using an online anonymous questionnaire through Questionnaire Star (https://www.wjx.cn/) survey platform.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (K2023-059). Data was managed anonymously.

Briefly, the questionnaire contained an introduction detailing the aim of the study and a statement of participant confidentiality and anonymity. Participants were required to complete the questionnaire consisting of three sections. Section 1 aimed to collect sociodemographic data (age, gender, occupation, education) and general health condition (vaccination, smoking history, drinking history, pre-existing chronic comorbidities). Then the questionnaire put forward a critical question as to whether they ever had any of the following otologic symptoms: otalgia, hearing loss, tinnitus, aural fullness, vertigo, dizziness, disequilibrium, otorrhea, and facial paralysis following COVID-19 infection. If the response was NOT, then the survey was over. If the response was YES, then continue to complete Section 2 designed to investigate general COVID-19 symptoms, including fever/chill, respiratory symptoms (nasal congestion, runny nose, cough, sore throat), systematic symptoms (asthenia, ache, diarrhea, poor appetite), and others (anosmia, dysgeusia). Otologic symptoms following COVID-19 infection were assessed in Section 3. Specifically, we asked participants if they had new otologic symptoms and the onset, duration, and clinical outcomes of these symptoms. Furthermore, participants with pre-existing otologic symptoms were asked if their symptoms deteriorated after contracting COVID-19. In addition, participants were asked if they took any medicine following the COVID-19 infection.

In our study, we included participants if they met the following inclusion criteria: (1) COVID-19 infection was verified by nucleic acid test or antigen test; (2) Participation in the study was voluntary. The incorrect and uncompleted questionnaires have been excluded.

2.3. Statistical analysis

SPSS 26.0 for Windows software (SPSS Inc., Chicago, Il, USA) was used for statistical analysis. Descriptive statistics included total numbers (N), percentages (%), median (Mean) and interquartile range (IQR). Single factor analysis was used with the chi-square test, Fisher's exact test, or the Mann–Whitney U test for predictor variables (demographics, comorbidities, clinical characteristics, and presentation). Logistic regression analysis was subsequently used to assess the associations between each significantly different variable and the outcome. Odds ratio (OR), p-value ($\leq$0.05), and 95% confidence interval (CI) were used to identify any significant relationships among variables.

3. Results

3.1. Participant characteristics

We recruited 2,247 COVID-positive participants comprising 1,138 without otologic symptoms and 1,109 with otologic symptoms. Table 1 shows the demographic characteristics of all participants with COVID-19. The study included 34.27% males and 65.73% females with a median age of 36.0 years (IQR, 28.0 to 46.0 years). Among the individuals, healthcare workers accounted for the largest proportion (47.89%), and soldiers the least (1.34%). The vast majority of the participants were highly educated [with an undergraduate degree (66.98%), with a graduate degree or above (21.63%)], a few smoked (10.5%), more than half had no drinking history (59.15%), and almost all were vaccinated with the COVID-19 vaccine (97.73%). The most frequent comorbidities were hypertension (5.7%) and diabetes (1.8%).

Among these demographic and comorbidities variables, we found significant differences in age, gender, occupation, and incidence rate of hypertension between the participants without otologic symptoms and those with otologic symptoms. A subsequent binary logistic regression revealed that the COVID-19 participates with otologic symptoms were associated with age (OR = 0.972, 95%CI:0.963–0.981, $p < 0.0001$), gender (OR = 1.575,

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TABLE 1 General statistics.

Variables	Population	Without otologic symptoms	With otologic symptoms	*P
	N (%)	*N* (%)	*N* (%)	
	2,247	1,138 (50.65)	1,109 (49.35)	
Age (years), Mean (P_{25}, P_{75})	36.0 (28.0,46.0)	38.0 (30.0,48.0)	35.0 (28.0,42.0)	**0.001**
Gender				
Males	770 (34.27)	443 (38.93)	327 (29.49)	**0.001**
Females	1477 (65.73)	695 (61.07)	782 (70.51)	
Occupation				
Health care worker	1076 (47.89)	573 (50.35)	503 (45.36)	**0.003**
Teacher	133 (5.92)	66 (5.80)	67 (6.04)	
Personnel of enterprises or institutions	306 (13.62)	122 (10.72)	184 (16.59)	
Student	207 (9.21)	105 (9.23)	102 (9.2)	
Farmer	35 (1.56)	20 (1.76)	15 (1.35)	
Soldier	30 (1.34)	11 (0.97)	19 (1.71)	
Freelance	185 (8.23)	103 (9.05)	82 (7.39)	
Others	275 (12.24)	138 (12.13)	137 (12.35)	
Education level				
Junior high school or below	99 (4.41)	53 (4.66)	46 (4.15)	0.121
High school	157 (6.99)	81 (7.12)	76 (6.85)	
Undergraduate degree	1505 (66.98)	734 (64.5)	771 (69.52)	
Graduate degree or above	486 (21.63)	270 (23.73)	216 (19.48)	
Smoking history				
No	2011 (89.5)	1016 (89.28)	995 (89.72)	0.733
Yes	236 (10.5)	122 (10.72)	114 (10.28)	
Drinking history				
No	1329 (59.15)	656 (57.64)	673 (60.69)	0.143
Yes	918 (40.85)	482 (42.36)	436 (39.31)	
COVID-19 vaccination history				
No	51 (2.27)	29 (2.55)	22 (1.98)	0.369
Yes	2196 (97.73)	1109 (97.45)	1087 (98.02)	
Comorbidities				
No	1922 (85.5)	974 (85.6)	948 (85.5)	0.943
Yes	325 (14.5)	164 (14.4)	161 (14.5)	
Hypertension				
No	2120 (94.3)	1059 (93.1)	1061 (95.7)	**0.007**
Yes	127 (5.7)	79 (6.9)	48 (4.3)	
Diabetes				
No	2206 (98.2)	1116 (98.1)	1090 (98.3)	0.697
Yes	41 (1.8)	22 (1.9)	19 (1.7)	

*P, Participants with otologic symptoms group vs. those without otologic symptoms group; The bold values indicate the $p < 0.05$ meaning statistically significant.

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TABLE 2 Multivariable regression analysis for otologic symptoms.

Multivariable analysis	b	b standard error	Wald	*P*	OR	95%CI
Age (years)	−0.029	0.005	38.1	**0.0001**	0.972	0.963–0.981
Gender						
Females	0.454	0.094	23.531	**0.0001**	1.575	1.311–1.893
Occupation						
Health care worker*			30.98	**0.0001**		
Teacher	0.185	0.188	0.967	0.325	1.203	0.832–1.739
Personnel of enterprises or institutions	0.583	0.135	18.781	**0.0001**	1.792	1.376–2.333
Student	−0.34	0.169	4.052	**0.044**	0.712	0.511–0.991
Farmer	0.115	0.353	0.106	0.745	1.122	0.561–2.243
Soldier	0.604	0.395	2.334	0.127	1.829	0.843–3.969
Freelance	−0.095	0.163	0.338	0.561	0.91	0.661–1.252
Others	0.181	0.138	1.725	0.189	1.199	0.915–1.571
Hypertension	−0.02	0.202	0.009	0.923	0.981	0.660–1.457

*Each occupation vs. health care worker respectively. The bold values indicate the $p < 0.05$ meaning statistically significant.

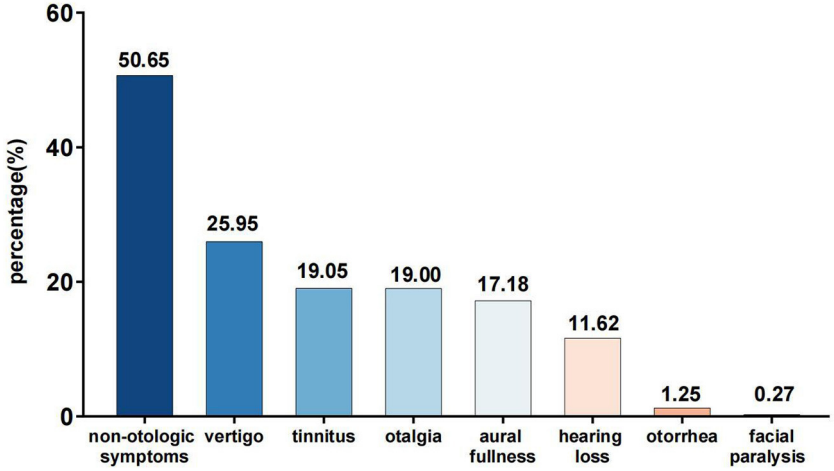

FIGURE 1
Otologic symptoms in the COVID-19 infected individuals.

95%CI:1.311–1.893, $p < 0.0001$), occupation (healthcare worker: $p < 0.0001$; personnel of enterprises or institutions: OR = 1.792, 95%CI:1.376–2.333, $p < 0.0001$; student: OR = 0.712,95%CI:0.511-0.991, $p < 0.044$) (Table 2).

3.2. Otologic symptoms

Among all the COVID-19 infected participants, the number of participants complaining of vertigo, tinnitus, otalgia, aural fullness, hearing loss, otorrhea, and facial paralysis were 583 (25.95%), 428 (19.05%), 427 (19.00%), 386 (17.18%), 261 (11.62%), 28 (1.25%), 6 (0.27%), respectively (Figure 1).

The most common otologic symptom reported in the course of COVID-19 infection was vertigo. Of the 583 participants with vertigo (dizziness, disequilibrium), 401 (68.78%) reported dizziness, 222 (38.08%) reported vertigo attacks with body position change, 161 (27.62%) reported experiencing unstable standing and walking, 68 (11.66%) reported vertigo who felt spinning around. More than half patients had nausea (52.66%), 38.59% had sweating, and 21.27% had vomiting during vertigo attacks. Nearly half of these participants had occasional vertigo attacks (48.54%). There were 83.19% experiencing vertigo for the first time and 16.81% with previous vertigo disease. In 48.71% of participants, vertigo recovered completely, and in 40.65% partially (Table 3).

Tinnitus was the second most common otologic symptom. Our study found that 173 (40.42%) patients described their tinnitus as occasional, 162 (37.85%) as intermittent and tolerable, 86 (20.09%) as continuous and tolerable, and only 7 (1.64%) as intolerable. The quantities of the COVID-19 infected participants

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TABLE 3 Questionnaire data of the COVID-19 infected patients with vertigo.

Survey question	Value(s)
	N (%)
	583
Characteristics	
Vertigo	68 (11.66)
Dizziness	401 (68.78)
Unstable standing and walking	161 (27.62)
Attack when a body position change	222 (38.08)
Others	32 (5.49)
Accompanying symptoms	
Nausea	307 (52.66)
Vomiting	124 (21.27)
Sweating	225 (38.59)
Others	146 (25.04)
Severity	
Occasionally	283 (48.54)
Intermittently, tolerable	197 (33.79)
Continuously, tolerable	81 (13.89)
Intolerable	22 (3.77)
Vertigo history	
No	485 (83.19)
Yes	98 (16.81)
Medication	
No	462 (79.25)
Yes	121 (20.75)
Prognosis	
Complete recovery	284 (48.71)
Partial recovery	237 (40.65)
Persistent	62 (10.63)

TABLE 4 Questionnaire data of the COVID-19 infected patients with tinnitus.

Survey question	Value(s)
	N (%)
	428
Side	
Unilateral	224 (52.34)
Bilateral	204 (47.66)
Severity	
Occasionally	173 (40.42)
Intermittently, tolerable	162 (37.85)
Continuously, tolerable	86 (20.09)
Intolerable	7 (1.64)
Characteristics	
Low-frequency	164 (38.32)
High-frequency	121 (28.27)
Hard to say	143 (33.41)
Tinnitus history	
No	241 (56.31)
Yes	187 (43.69)
Medication	
No	393 (91.82)
Yes	35 (8.18)
Prognosis	
Complete resolution	184 (42.99)
Partial recovery	179 (41.82)
Persistent	65 (15.19)

with tinnitus who described their tinnitus as low-frequency, high-frequency, and hard to say were similar. Most cases recovered completely or partially without taking medicines (Table 4).

In our study, participants with unilateral otalgia (56.67%) were a little more than those with bilateral otalgia (43.33%). Otalgia usually tended to be intermittent (82.90%). The median score of visual analog scale used to describe the severity of otalgia was 4.0 (IQR, 3.0 to 6.0). Most cases did not take any medicine (Table 5).

Aural fullness was found in more than one-third of all participants with otologic symptoms (34.81%). 155 (40.16%) described their aural fullness as occasional, 147 (38.08%) as intermittent and tolerable, 75 (19.43%) as continuous and tolerable, and only 9 (2.33%) as intolerable. Almost all cases recovered completely or partially without taking any medicine (Table 6).

Hearing loss was reported by 11.62% of participants. The severity of hearing loss was estimated by patients based on their subjective feeling as mild (78.16%), medium (17.62%), and severe (4.21%). Most participants could not determine how long exactly this symptom had persisted. Furthermore, one unanticipated finding was that participants with bilateral hearing loss were more than those with unilateral hearing loss. 83.14% of participants didn't take any medication during the course of hearing loss. 26.82% of participants recovered completely, and 55.17% recovered partially (Table 7).

It was shown that 28 out of 2247 participants (1.25%) had otorrhea. 13 (46.43%) described their otorrhea as occasional, 7 (25.00%) as intermittent, and 8 (28.57%) as continuous. The proportion of the COVID-19 infected participants with unilateral otorrhea was much higher than those with bilateral otorrhea. A majority of these cases did not take medication and over half of the participants recovered completely or partially (Table 8).

The least common otologic symptom was facial paralysis (0.27%). Five out of 6 had previous history, but only 1 took medication. All of them recovered completely or partially (Table 9).

TABLE 5 Questionnaire data of the COVID-19 infected patients with otalgia.

Survey question	Value(s) *N* (%) 427
Side	
Unilateral	242 (56.67)
Bilateral	185 (43.33)
Severity (0–10)	
Mean (P25, P75)	4.0 (3.0, 6.0)
Characteristics	
Intermittently	354 (82.9)
Continuously	73 (17.1)
Medication	
No	301 (70.49)
Yes	126 (29.51)

TABLE 6 Questionnaire data of the COVID-19 infected patients with aural fullness.

Survey question	Value(s) *N* (%) 386
Side	
Unilateral	189 (48.96)
Bilateral	197 (51.04)
Severity	
Occasionally	155 (40.16)
Intermittently, tolerable	147 (38.08)
Continuously, tolerable	75 (19.43)
Intolerable	9 (2.33)
Aural fullness history	
No	298 (77.20)
Yes	88 (22.80)
Medication	
No	343 (88.86)
Yes	43 (11.14)
Prognosis	
Complete resolution	179 (46.37)
Partial recovery	155 (40.16)
Persistent	52 (13.47)

In general, there was not much difference in incidence between the unilateral and bilateral otologic symptoms except otorrhea and facial paralysis (Figure 2A). It was noteworthy that more than half suffered from new onset of otologic symptoms except those with facial paralysis (Figure 2B). In addition, the proportion of individuals taking no medications was overwhelmingly higher (Figure 2C). Furthermore, these symptoms mostly recovered partially or completely (Figure 2D).

TABLE 7 Questionnaire data of the COVID-19 infected patients with hearing loss.

Survey question	Value(s) *N* (%) 261
Side	
Unilateral	109 (41.76)
Bilateral	152 (58.24)
Severity	
Mild	204 (78.16)
Medium	46 (17.62)
Severe	11 (4.21)
Hearing loss history	
No	195 (74.71)
Yes	66 (25.29)
Medication	
No	217 (83.14)
Yes	44 (16.86)
Prognosis	
Complete resolution	70 (26.82)
Partial recovery	144 (55.17)
Persistent	47 (18.01)

3.3. General symptoms of COVID-19

Overall, 0.63% of the participants with otologic symptoms had no general symptoms. Similar to previous studies, the most frequently reported general symptoms were cough (90.44%), asthenia (84.40%), fever (78.63%), nasal congestion (78.45%), sore throat (73.85%), and runny nose (71.15%) (Figure 3).

4. Discussion

COVID-19 primarily infects the respiratory system (12). However, recent studies have demonstrated the involvement of not only major organs and systems like respiratory systems and cardiovascular system, but also cochleovestibular system and facial nerve (1, 13).

In this cross-sectional descriptive study, we found that nearly half the participants with positive nucleic acid test or antigen test of COVID-19 had one or more otologic symptoms through an online questionnaire survey during the COVID-19 pandemic period in China. Furthermore, occurrences of otologic symptoms were associated with gender, since female participants were

TABLE 8 Questionnaire data of the COVID-19 infected patients with otorrhea.

Survey question	Value(s)
	N (%)
	28
Side	
Unilateral	20 (71.43)
Bilateral	8 (28.57)
Severity	
Occasionally	13 (46.43)
Intermittently	7 (25.00)
Continuously	8 (28.57)
Otitis media history	
No	15 (53.57)
Yes	13 (46.43)
Medication	
No	24 (85.71)
Yes	4 (14.29)
Prognosis	
Complete resolution	5 (17.86)
Partial recovery	14 (50.00)
Persistent	9 (32.14)

TABLE 9 Questionnaire data of the COVID-19 infected patients with facial paralysis.

Survey question	Value(s)
	N (%)
	6
Side	
Unilateral	4 (66.67%)
Bilateral	2 (33.33%)
Facial paralysis history	
No	1 (16.67%)
Yes	5 (83.33%)
Medication	
No	5 (83.33%)
Yes	1 (16.67%)
Prognosis	
Complete resolution	1 (16.67%)
Partial recovery	5 (83.33%)
Persistent	0 (0.00%)

significantly more affected than males. This finding is consistent with previous studies reporting that post-COVID-19 symptoms are more prevalent in women than in men (14, 15). Various theories have been proposed to explain the gender-related differences. For example, the higher expression level of the angiotensin-converting-enzyme-2 (ACE2) and lower level of pro-inflammatory cytokines (i.e., interleukin-6) in women after viral infections could explain their higher susceptibility to developing post-COVID otologic symptoms (16, 17). Additionally, unfavorable psychological factors, such as stress, sleep, anxiety, and depressive disorders, were observed to a greater extent in women and might also have an impact on perception of sensory symptoms such as dizziness (18).

Surprisingly, we found that the older adults were less likely to have otologic symptoms. Previous reports have shown that infected older individuals have more severe clinical symptoms and outcomes, and the association between age and symptoms/outcomes is often attributed to increased comorbidities in the older adults. Nonetheless, Almishaal et al. (19) did not find a statistically significant increase in audiovestibular symptoms among the old compared to younger participants. Moreover, our result was consistent with the study by Lechien et al. (3) who reported that young people more often manifested ENT symptoms.

In addition, the professions such as healthcare worker and personnel of enterprises or institutions were positive factors for otologic symptoms. This finding has extended our knowledge of the association between occupation and post-COVID-19 otologic symptoms. We speculated that this might be due to richer awareness of disease development from healthcare worker. In addition, the nature of the personnel of enterprises or institutions makes it easier for them to observe subtle changes in the body.

It has been well-known that vertigo may significantly affect the quality of life. Our study identified that vertigo was the most common otologic symptom. In the studies by Korkmaz et al. (20) and Zieba et al. (21) the incidence rates of vertigo were similar to ours (31.8%, 34%, and 25.95% respectively). There might be multiple explanations why individuals with COVID-19 infection experience vertigo (dizziness, disequilibrium). Firstly, the neuroinvasive and neurotropic properties of COVID-19 virus in the central and peripheral nervous systems have been reported (22–25). Recent studies have shown a high affinity of this virus for angiotensin-converting enzyme 2 (ACE2), which is frequently found in the nervous system and nasal mucosa (26, 27). COVID-19 can be transferred through the olfactory nerve and bulb to the central nervous system directly, or through viremia (24, 28). Secondly, novel coronavirus can directly infect the human inner ear and cochleovestibular nerve because human inner ear tissue co-expresses the ACE2 receptor for novel coronavirus (29). Furthermore, an autoimmune-mediated mechanism has also been proposed as a potential mechanism. In severe cases of COVID-19 infection, an autoimmune-mediated process causes an uncontrolled viral replication and an exaggerated systemic response leading to an increase of pro-inflammatory cytokine levels (i.e., cytokine storm) which may constitute a potential source of damage for many body organs including the inner ear (30–32). Vascular pathologies were also proposed as a potential mechanism for COVID-19-mediated vestibular manifestations given the evidence that a significant proportion of COVID-19 patients develop coagulation abnormalities (33, 34). It is worth of note that these are the mechanisms that not only may cause vertigo but also may cause other otologic symptoms such as tinnitus, otalgia, and hearing loss. It is important to highlight that

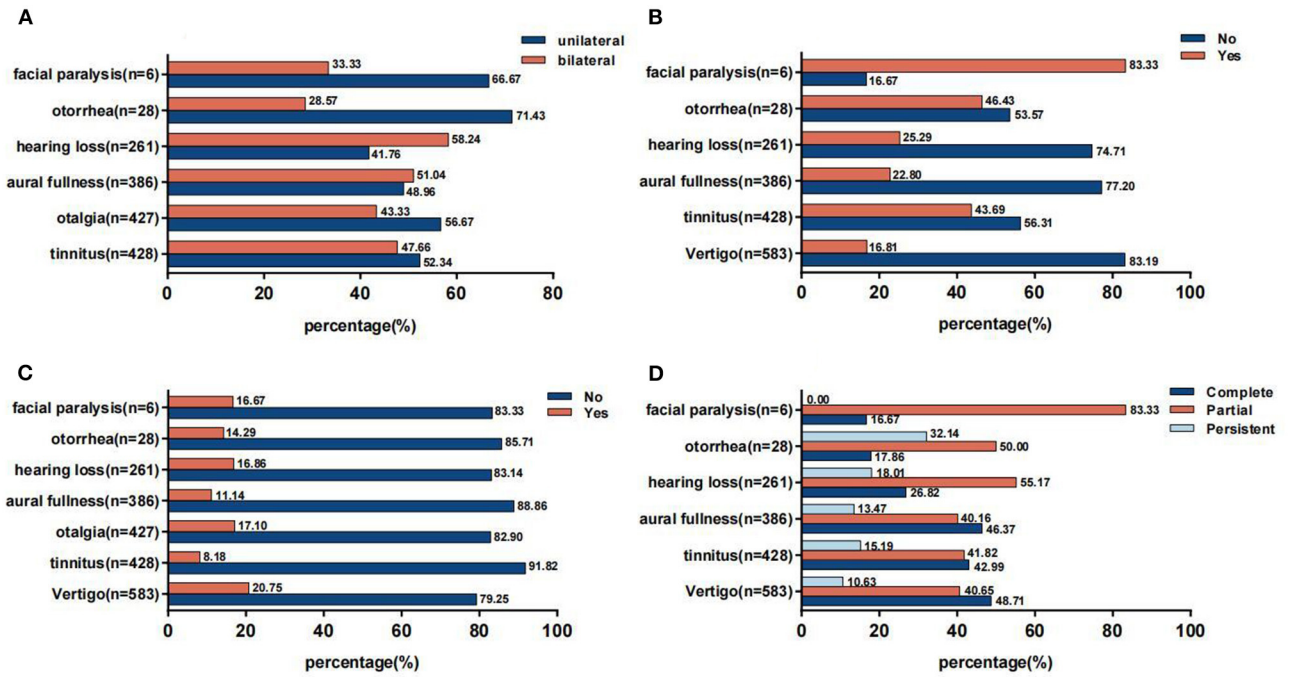

FIGURE 2
(A) Side of otologic symptoms. **(B)** Previous history of otologic symptoms. **(C)** Usage of medication for otologic symptoms. **(D)** Prognosis of otologic symptoms.

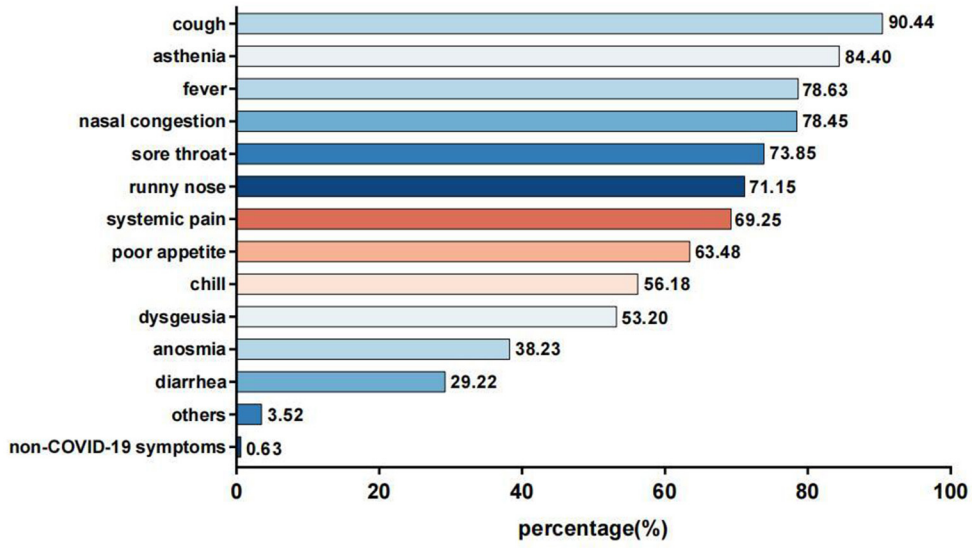

FIGURE 3
General symptoms of the COVID-19 infected with otologic symptoms.

factors other than the primary infection itself can generate vertigo symptoms. Studies have revealed a significant contribution of body weakness and fatigue resulting from metabolic and nutritional dysfunctions to the manifestation of vestibular symptoms during the acute phase of COVID-19 infection (19, 35).

Tinnitus was the second most common otologic symptom shown in the present study. It is unclear whether tinnitus is directly related to COVID-19 infection or not. Additionally, as stated in literatures, the relationship between COVID-19 and the onset of tinnitus or worsening of the preexisting tinnitus may depend on the negative effects of stress and anxiety generated by the pandemic process (36, 37). Our result falls within previously reported estimates ranging from 0.35% to as high as 35% (16). In addition, our study identified that nearly half participants with

tinnitus presented worsening tinnitus, which was consistent with previous study by Xia et al. (38) who reported an increase in tinnitus severity during the pandemic.

We investigated otalgia following COVID-19 infection and found a similar incidence rate with that of tinnitus. The majority of sufferers presented with mild to moderate intermittent otalgia. Most patients with otalgia presented pain around the ear or in the ear canal without local redness, swelling, or heat. We speculate it as neuropathic pain due to direct involvement of trigeminal nerve and greater auricular nerve by virus (39). Indeed, certain aural diseases such as otitis media can also cause otalgia (39, 40).

Aural fullness is a recognized classical symptom of transient Eustachian tube dysfunction which may be triggered by many causes, the most common being upper respiratory viral infections (41). Indeed, as the cellular receptor for the COVID-19, ACE2 was detected in the Eustachian tube of mice and in the autopsy of the middle ear tissues of COVID-19 positive decedents, which indicates that these structures are likely susceptible to COVID-19 infection leading to aural fullness (42, 43).

Hearing loss following COVID-19 infection may be sensorineural, conductive, or mixed. Classification of hearing loss in this study was not available since audiometry is not possible for an online survey. A growing body of evidence suggests that patients with COVID-19 are at risk of developing sudden sensorineural hearing loss (SSNHL) (44, 45). The incidence of hearing loss (11.62%) in our study was consistent with a retrospective observational study, which showed that over 10% of COVID-19 patients with self-reported chemosensory loss complained of hearing loss (46). In contrast, the prevalence was higher than those in some earlier systematic reviews reporting a prevalence of about 5.08%—8.7% (13, 47). The precise reason for SNHL post COVID-19 infection is currently unknown yet, but recent studies have indicated the importance of endothelial dysfunction and micro-thrombosis (48). Also, the virus is associated with the increased activation of immune system (49, 50). It should be noted that the majority of the extant research emphasizes that viral infection of the cochlea nerve or central nervous system is one of the more common presumed etiologies (51, 52). In addition, there could be conductive deafness due to middle ear diseases such as otitis media and Eustachian tube dysfunction resulting from COVID-19 infection.

Few studies investigate otorrhea among COVID-19 infected individuals in detail, and only a few cases with otitis media have been reported. In our study, we found that the prevalence of otorrhea is much lower than the symptoms mentioned earlier. Of these participants, nearly half suffered from otitis media recurrence. A possible explanation for this might be that COVID-19 infects the middle ear directly through the ACE2. Moreover, some studies identified COVID-19 virus in mastoid or middle ear (43, 53). After being infected by COVID-19, on the one hand, the patients may suffer from weakened immunity that tends to result in otitis media. On the other hand, the middle ear may experience secondary bacterial infection, which deteriorates the existed otitis media.

Last but not least, some researches have reported that COVID-19 may cause facial nerve palsy (54, 55). Mehrdad Estakh et al. (11) have shown that there is enough evidence suggesting that patients with COVID-19 infection may present with facial palsy as the initial clinical manifestation. According to recent studies, the COVID-19 virus may damage facial nerve function by direct toxic effects on the nerve or by increasing hypercoagulopathy (23). Increased deterioration of nerve function can occur due to direct viral damage or an autoimmune event that can trigger a boost in inflammation of the nerve (56). For instance, facial palsy was regarded as neuronal damage secondary to severe complications like Guillain-Barré Syndrome (GBS) (57). Previous studies have shown combined facial and trigeminal nerve palsy after COVID-19 infection (58). Nevertheless, the incidence rate of facial palsy is very low. We found that only 6/2247 (0.27%) patients had facial paralysis, of which 5 out of 6 had previous history. Therefore, further studies are needed to investigate the potential mechanism of facial palsy following COVID-19 infection. Physicians, however, should keep undoubtedly in mind the likelihood of facial palsy post COVID-19 infection and treat it accordingly.

A key superiority of our study is that we recruited participants during the acute phase of COVID-19 infection with a large sample size. Furthermore, our questionnaire covers common otologic symptoms and thus enables a comprehensive analysis of the correlation between COVID-19 infection and otologic symptoms. Nevertheless, there are a few limitations that need to be considered in this study. Firstly, the data collected were self-reported by patients via the social media application WeChat without objective diagnostic and audiological tests. Secondly, the use of a single social media platform may result in sampling bias since some older individuals are less likely to complete the online questionnaire. Thirdly, the vast majority of questionnaires were filled out in a few days after COVID-19 infection. Further studies are needed to investigate the long-term impact of COVID-19 infection on ear.

5. Conclusion

Our study shows that otologic symptoms are common among the COVID-19 infected individuals during the acute phase of the pandemic period, and that these symptoms mostly appear to recover spontaneously. However, the true prevalence of involvement of the cochleovestibular system and facial nerve in COVID-19 patients around the world is unknown. Given that most of the studies are from a single institution with a small sample size, the published data must be interpreted with caution. In addition, more comprehensive otologic tests are required to further elucidate the pathogenesis of the underlying dysfunctions of the cochleovestibular system and facial nerve after COVID-19 infection.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (K2023-059).

Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

SZ and LW contributed to the design, analysis, and writing of the manuscript. HP and YH participated in conducting the study and supervised data acquisition. LP contributed to gathering data and drafting of tables and figures. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Effectiveness of SARS-CoV-2 vaccines against Omicron infection and severe events: a systematic review and meta-analysis of test-negative design studies

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Background: A rapidly growing body was observed of literature evaluating the vaccine effectiveness (VE) against Omicron in test-negative design studies.

Methods: We systematically searched papers that evaluated VE of SARS-CoV-2 vaccines on PubMed, Web of Science, Cochrane Library, Google Scholar, Embase, Scopus, bioRxiv, and medRxiv published from November 26th, 2021, to June 27th, 2022 (full doses and the first booster), and to January 8th, 2023 (the second booster). The pooled VE against Omicron-associated infection and severe events were estimated.

Results: From 2,552 citations identified, 42 articles were included. The first booster provided stronger protection against Omicron than full doses alone, shown by VE estimates of 53.1% (95% CI: 48.0–57.8) vs. 28.6% (95% CI: 18.5–37.4) against infection and 82.5% (95% CI: 77.8–86.2) vs. 57.3% (95% CI: 48.5–64.7) against severe events. The second booster offered strong protection among adults within 60 days of vaccination against infection (VE=53.1%, 95% CI: 48.0–57.8) and severe events (VE=87.3% (95% CI: 75.5–93.4), comparable to the first booster with corresponding VE estimates of 59.9% against infection and 84.8% against severe events. The VE estimates of booster doses against severe events among adults sustained beyond 60 days, 77.6% (95% CI: 69.4–83.6) for first and 85.9% (95% CI: 80.3–89.9) for the second booster. The VE estimates against infection were less sustainable regardless of dose type. Pure mRNA vaccines provided comparable protection to partial mRNA vaccines, but both provided higher protection than non-mRNA vaccines.

Conclusions: One or two SARS-CoV-2 booster doses provide considerable protection against Omicron infection and substantial and sustainable protection against Omicron-induced severe clinical outcomes.

KEYWORDS

Omicron, vaccine effectiveness, meta-analysis, test negative, booster dose

Introduction

The Omicron variant (B.1.1.529) was first detected in early November 2021 in South Africa and was designated the fifth variant of concern by the World Health Organization (1). In contrast to the original wild-type variant, Omicron accumulated over 50 mutations in the whole genome, including 26–32 in the spike protein. This altered protein receptor-binding efficiency and immunogenicity, increasing infectivity, ability to evade neutralizing antibodies, and risk of reinfection (2). Additional mutations led to multiple Omicron subvariants with increased transmissibility including BA.2, BA.2.12.1, BA.4, BA.4.6, BA.5, BF.7, BQ.1, BQ.1.1, and XBB.1.5, the latter three of which accounted for most infections in the United States as of February 2023 (3). The effective reproduction number (R_t) and basic reproduction number (R_0) were estimated to be 3.8 and 2.5 times higher for Omicron than for Delta (4). Compared with the wild-type and Delta variants, Omicron replicates less efficiently in the lung parenchymal tissues and more efficiently in the bronchial tissues, which may contribute to increased transmissibility but decreased disease severity (5–7).

There is a rapidly growing body of literature of real-world vaccine effectiveness (VE) against Omicron. Studies reported that individuals vaccinated with two mRNA doses were less susceptible to Omicron infection, though the level of protection conferred was lower than that of earlier variants, and protection waned over time (8, 9). The emergence of new variants coupled with waning vaccine-induced immunity prompted recommendations for booster doses and second booster doses based on the original Wuhan-Hu-1 strain, which were shown to confer greater protection against Omicron than two mRNA doses (10, 11). Omicron-specific bivalent mRNA booster doses were recently authorized for use in the U.S. by the Food and Drug Administration, and early data demonstrated stronger neutralizing antibody responses against Omicron than the original monovalent mRNA vaccines (12). The BNT162b2 bivalent BA.4/5 COVID-19 vaccine was recently shown to elicit greater neutralizing antibody titers against newer Omicron sublineages (BA.4.6, BA.2.75.2, BQ.1.1 and XBB.1) in adults older than 55 than a fourth dose of the original monovalent BNT162b2 (13). Uptake of the bivalent boosters, however, is low with only 15% of the U.S. adult population vaccinated as of February 2023 (14). Therefore, it is important to quantify the effectiveness of the original vaccines against Omicron.

Two early meta-analyses evaluated VE of a primary vaccine series or single booster dose and demonstrated greater protection for the third dose against symptomatic infection and severe events compared to a two-dose regimen (15, 16). However, they focused on hybrid immunity (immunity developed from SARS-CoV-2 infection and vaccination) (15) and relative vaccine effectiveness of the third dose compared to two doses (16) rather than non-vaccination. Nor did they evaluate VE for a second booster, long-term (>60 days) VE for the first booster, or adult- and child-specific VEs. Herein, we aggregate estimates in the literature to evaluate VE for the initial full doses, first booster dose, and second booster dose against Omicron-related infection and severe events for pure mRNA, partial (mixed) mRNA, and non-mRNA vaccines. We focus our review on test-negative design studies, an increasingly popular epidemiological study design for evaluating VE on infectious pathogens including influenza, rotavirus, pneumococcus, and others (17). In this design, the same clinical definition is used to enroll cases and controls and laboratory testing distinguishes "test positive" cases from "test negative" controls, thereby reducing bias from differential healthcare-seeking behavior between cases and controls (18).

Methods

This analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guidelines.

Data sources and searches

A systematic literature search was conducted of PubMed, Web of Science, Cochrane Library, Google Scholar, Embase, Scopus, and preprint servers (bioRxiv and medRxiv) for papers published from November 26th, 2021, when Omicron was classified as a World Health Organization Variant of Concern (1), to June 27th, 2022 (for full doses and booster), and to January 8th, 2023 (for the second booster). We applied Boolean combinations of the following keywords to identify relevant publications: "SARS-CoV-2", "COVID-19", "2019nCoV", "vaccine", "booster", "second booster", "effectiveness", "efficacy", "test-negative case-control", "test-negative design", "Omicron", "infection", "hospitalization"; the detailed search procedures were presented in the Supplementary material. Publication language was not restricted, and reference lists of selected papers were also screened for additional studies.

Study selection

The selection of studies followed Participant (P), Intervention (I), Comparator (C), Outcome (O), and Study Type (S), PICOS criteria (19) (Supplementary Table 1). Published studies were eligible for inclusion if they were original analyses with the test-negative design (TND) and reported VE or corresponding odds ratios (OR) of full doses, booster, or second booster against Omicron infection or severe events. We excluded studies that focused on special populations (e.g., patients with kidney disease); did not include circulation period of Omicron variant; combined VE estimates for Omicron with other viral variants such as Delta; reported relative VE between different vaccines, vaccination doses, or variants among vaccinated individuals; did not evaluate VE (e.g., instead, evaluated neutralizing antibodies); or evaluated outcomes other than infection or severe events. All available ages were included. We did not contact authors for additional data.

After removing duplicated results, we first screened studies by titles and abstracts to identify potentially eligible articles. Two pairs of researchers then independently evaluated full texts and selected those meeting the inclusion criteria. Any disagreements were discussed until a consensus was reached. Preprints were checked and updated with their most recent published version if available as of January 10th, 2023. Zotero was used for literature management.

Due to the scarcity of published TND studies involving Omicron-specific bivalent booster doses by the time this meta-analysis was conducted, we solely focused on monovalent vaccines and booster doses based on the original Wuhan-Hu-1 strain.

Data extraction and quality assessment

Two pairs of researchers independently extracted the following from the included studies: author names, publication year, study region, study design, dose, vaccine type, test time in reference to vaccination time, adjusted VE point estimate and 95% confidence intervals, and adjustment confounders; if available, the number of vaccinated and unvaccinated individuals in the cases and controls were also recorded.

Study quality and risk of bias were independently assessed by two researchers using the Newcastle-Ottawa Scale (NOS). Studies could earn up to 9 points composed of participant selection (4 points), study comparability (1 point), and outcome of interest (4 points). A score >7 was considered as high quality, 5–6 as medium, and <5 as low, and studies classified as low were excluded from the meta-analysis. Publication bias was also evaluated by Egger's test, Begg and Mazumdar rank correlation, and funnel plots when at least ten studies were available, with significance set at $p < 0.1$. If we detected publication bias, we used the Duval and Tweedie trim-and-fill method (20) for adjustment, which consists of imputing missing effect sizes to achieve symmetry.

Data synthesis and analysis

We categorized full doses and booster VE into short-term, long-term, and overall to evaluate potential waning of VE over time. In the collected studies, there is no uniform definition for short-term vs. long-term VE, but most adopted cut-off points of 60–120 days from last vaccination to lab-testing. Considering the lower and upper bounds of the post vaccination test dates, we used the following guidelines. For initial full doses, a lower bound ≤30 days and an upper bound ≤180 days constitute short term, and a lower bound ≥90 (except one study used ≥70 days) days and an upper bound that is either ≥200 days or unspecified are considered long term. For booster doses, a lower bound ≤30 days and an upper bound ≤120 days are considered short term, and a lower bound ≥60 days and an upper bound >120 days or unspecified are considered as long-term. To simplify description, we occasionally use "<90 days" and "≥90 days" to represent short-term vs. long-term VEs for the full doses, and use "<60 days" and "≥60 days" to represent short-term vs. long-term VEs for the booster doses. If a study reported VEs for finer time intervals than we needed, we used an inverse variance weighted (IVW) averaging approach to combine them.

For each time interval, we further categorized VE by the type of vaccine: pure mRNA vaccines, partial mRNA vaccines, and non-mRNA vaccines. Pure mRNA vaccines comprise of homogenous or heterogeneous BNT162b2 and mRNA-1273, or a population-level mixture of the two if a study does not discriminate between them. Partial mRNA vaccines include either a multi-dose course containing at least one mRNA vaccine dose, or the study indiscriminately reported VEs of a population-level mixture of vaccines including at least one mRNA vaccine. Non-mRNA vaccines refer to the regimens that do not involve mRNA vaccines at all (e.g., Ad26.COV2.S, ChAdOx1).

We evaluated VE against Omicron infection and severe events. Analyses of VE against infection or symptomatic infection combined studies that reported either VE against symptomatic infection or VE against any infection (symptomatic or asymptomatic). Severe events included hospitalizations, noncritical hospitalizations, deaths, emergency department (ED) or urgent care (UC) encounters, ED admissions, intensive care unit (ICU) admissions, and invasive ventilation.

We evaluated VE for the overall vaccine-eligible population as well as for age groups defined as adults (≥18 years) and children/adolescents (5–17 years). If VE was not reported but odds ratios (OR) were provided, we calculated VE as (1 – OR) ×100%. The pooled VE and 95% confidence intervals were calculated via a random effects meta-analysis with restricted maximum likelihood estimation. I^2 was used to evaluate between-study heterogeneity with thresholds of 25, 50, and 75% indicating low, moderate, and high heterogeneity, respectively. The *metafor* package in the R statistical software (version 4.0.5) was used for estimation and visualization in this meta-analysis (21).

Results

Study selection and characteristics

For full doses and booster doses, we obtained 1,139 articles from all searched databases (82 from PubMed, 23 from Web of Science, 89 from Embase, 721 from Scopus, 3 from Cochrane Library, 115 from medRxiv, 6 from bioRxiv, and 100 from Google Scholar). After removing duplicates, 952 articles remained, of which 136 were retained for full review following inspection of the title, abstract, and keywords. After full text review of these 136 articles, 33 articles (9, 10, 22–52) with 271 VE estimates were formally included in this meta-analysis (Figure 1A). For the second booster, we obtained 1,413 articles from all databases (56 from PubMed, 22 from Web of Science, 55 from Embase, 1,015 from Scopus, nine from Cochrane Library, 149 from medRxiv, seven from bioRxiv, and 100 from Google Scholar). After removing duplicates, 1,236 articles remained, of which 116 were considered relevant after inspection of the title, abstract, and keywords. These 116 relevant articles were then reviewed in full text for eligibility, and 11 articles (23, 37, 53–61) with 46 VE estimates were finally included in this meta-analysis (Figure 1B).

Among the 33 papers relevant to full doses and booster doses, 14 studies were conducted in the U.S., five in the U.K., four in Canada, three in South Africa, two in Qatar, two in Brazil, and one in each of Belgium, Netherlands and Scotland, respectively. A study could report multiple VEs for different vaccination types and outcomes. In total, there were 271 VE estimates including 124 for full doses and 147 for the first booster doses; 133 for pure mRNA vaccines, 100 for partial mRNA vaccines, and 38 the non-mRNA vaccines; 138 for symptomatic infection, 14 for any infection, and

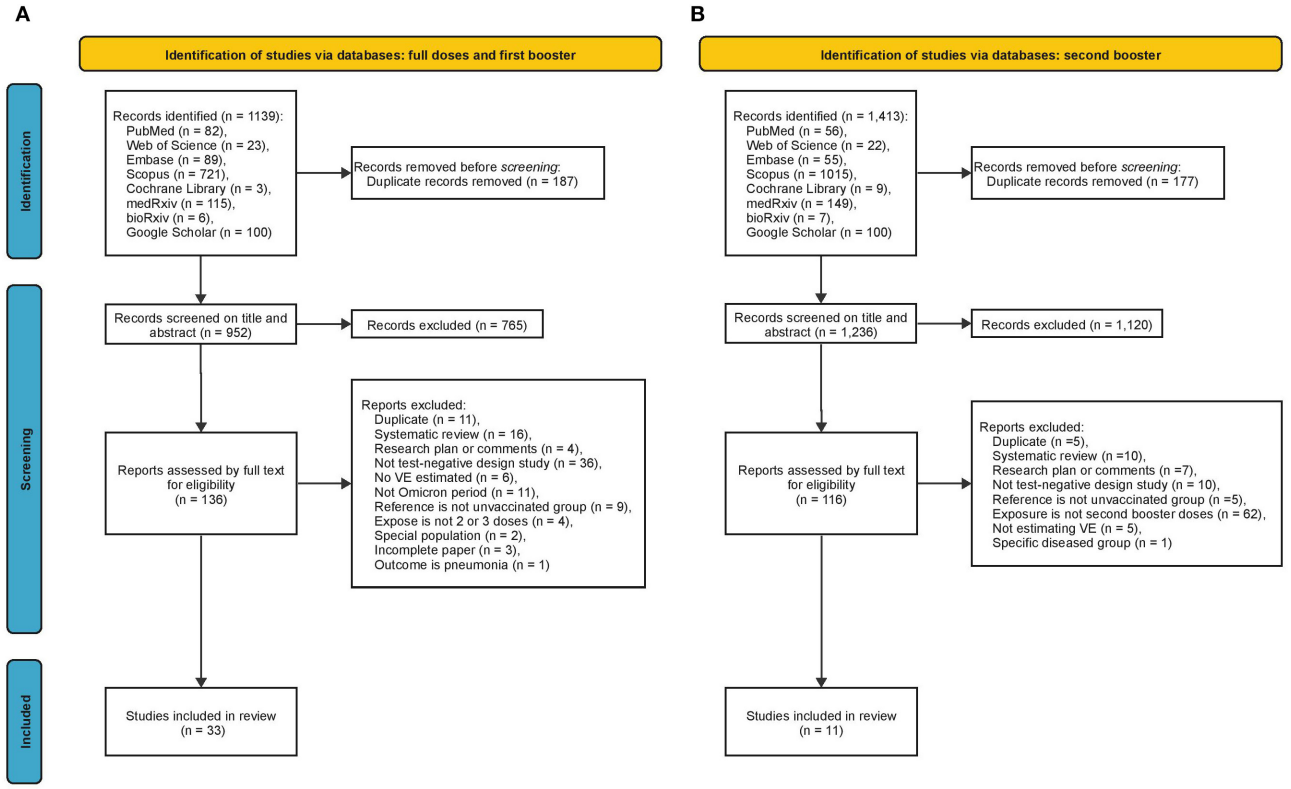


FIGURE 1
Summary of evidence search and selection. **(A)** Full doses and first booster **(B)** second booster.

119 for severe events. For the second booster, out of 11 papers, five studies conducted in the U.S., three in Canada, and three in Thailand. In total, there were 46 VE estimates including 32 for pure mRNA vaccines, 13 for partial mRNA vaccines, and one for non-mRNA vaccines; three for symptomatic infection, 24 for any infections, and 19 for severe events.

Vaccine effectiveness against Omicron symptomatic infection or any infection

The VE estimates for the initial full doses against Omicron symptomatic infection or any infection were summarized in Figure 2A. Pooling all vaccine types and time intervals, the overall VE was estimated to be 28.6% (95% CI: 18.5–37.4%, 25 studies) for all ages and 24.4% (95% CI: 16.2–31.8%, 15 studies) for adults. The overall VE of the pure mRNA vaccines was estimated to be 30.6% (95% CI: 17.1–41.8%, 18 studies) for all ages, 25.4% (95% CI: 11.5–37.1%, 8 studies) for adults, and 54.2% (95% CI: 35.2–67.7%, 5 studies) for children and adolescents. Overall VE estimates for partial mRNA vaccines and non-mRNA vaccines were only available for adults, 28.1% (95% CI: 19.8–35.6%, 5 studies) and 1.5% (95% CI: 0.4–2.7%, 2 studies) respectively. This is also why we do not have a separate overall VE estimate for children and adolescents pooling all vaccine types.

Short-term full-dose VE estimates pooling all vaccine types were 40.7% (95% CI: 34.3–46.5%, 19 studies) for all ages and 37.5% (95% CI: 31.4–43.1%, 10 studies) for adults (Supplementary Figure 1). Short-term VE of pure mRNA vaccines was estimated to be 43.5% (95% CI: 35.4–50.6%, 13 studies) for all ages, 41.3% (95% CI: 40.2–42.4%, 4 studies) for adults, and 45.3% (95% CI: 28.7–58.1%, 6 studies) for children and adolescents. Short-term VE estimate of partial mRNA vaccines was 34.7% (95% CI: 25.4–42.9%, 6 studies) for adults, slightly lower than that of the pure mRNA vaccines.

Long-term full-dose VE estimates against symptomatic or any infection were in general much lower than their short-term counterparts. Pooling all vaccine types, long-term full-dose VE was estimated to be 17.6% (95% CI: 13.2–21.8%, 22 studies) for all ages and 16.6% (95% CI: 10.5–22.3%, 15 studies) for adults (Supplementary Figure 2). Long-term full-dose VE of pure mRNA vaccines was estimated to be 16.4% (95% CI: 13.6–19.1%, 11 studies) for all ages, 13.1% (95% CI: 11.7–14.6%, 4 studies) for adults, and 22.3% (95% CI: 13.6–30.1%, 4 studies) for children and adolescents. Long-term full-dose VE among adults was estimated to be 22.6% (95% CI: 10.8–32.7%, 5 studies) for partial mRNA vaccines and 13.2% (95% CI: 2.6–22.6%, 6 studies) for non-mRNA vaccines.

Compared to unvaccinated controls, the overall VE of the first booster dose against Omicron symptomatic infection or any infection was 53.1% (95% CI: 48.0–57.8%, 31 studies) for

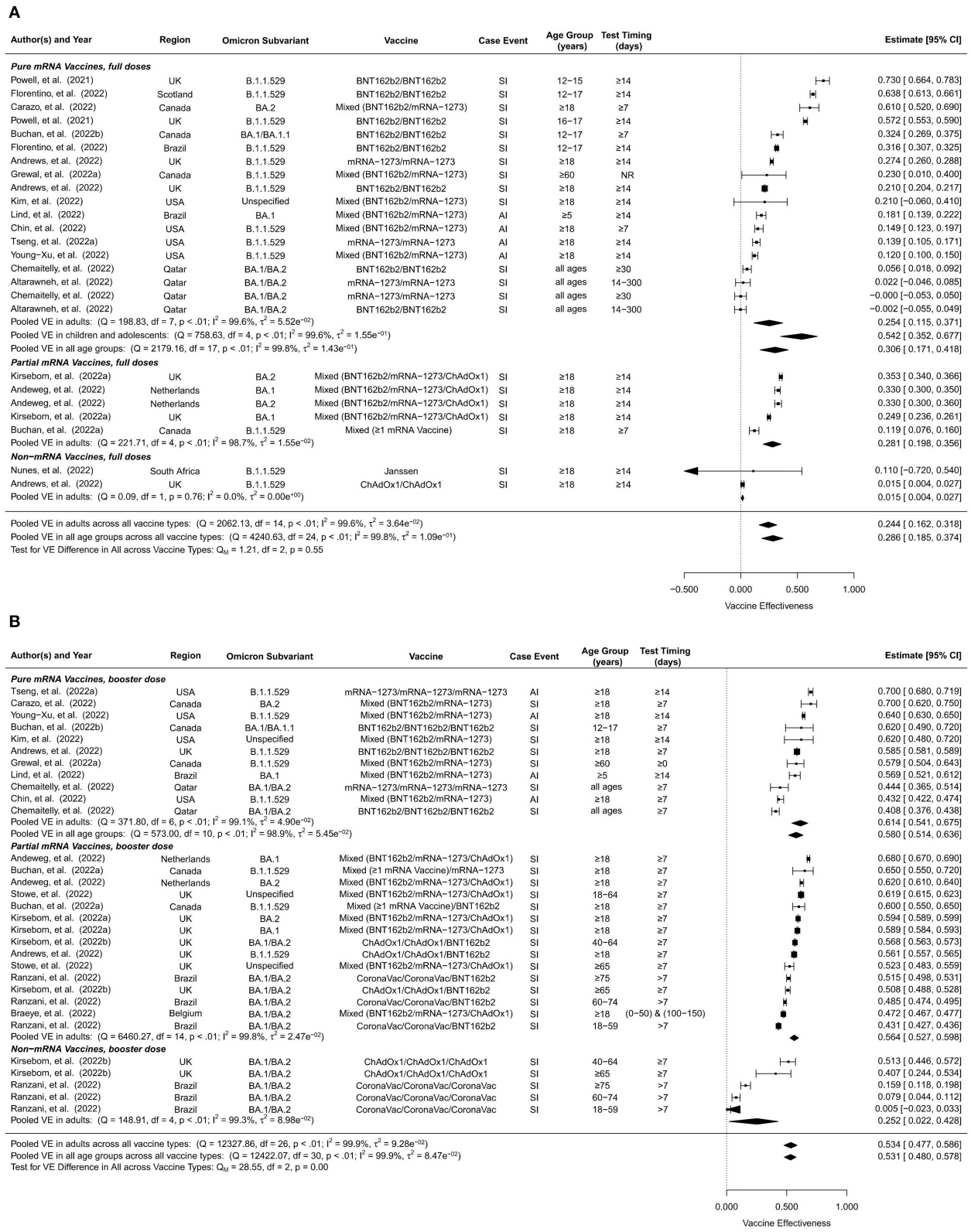


FIGURE 2
Overall vaccine effectiveness of full doses and first booster against infection or symptomatic infection. **(A)** Pooled VE of full doses estimated from all 25 studies combined as well as for each vaccine type. **(B)** Pooled VE of first booster estimated from all 31 studies combined as well as for each vaccine type. Statistics Cochran's Q, I^2 and τ^2 measure the heterogeneity between studies. End points of the studies are either symptomatic infection (SI) or any infection (AI). Mixed vaccine type indicates the study reported VEs of these vaccines combined without distinguishing between them.

all ages and 53.4% (95% CI: 47.7–58.6%, 27 studies) for adults (Figure 2B). No studies included in this analysis reported VE of booster doses for children. When stratified by vaccine type, the overall first-booster VE estimates were 58.0% (95% CI: 51.4–63.6%, 11 studies) for all ages and 61.4% (95% CI: 54.1–67.5%, 7 studies) in adults for pure mRNA vaccination, 56.4% (95% CI: 52.7–59.8%, 15 studies) for adults for partial mRNA vaccines, and 25.2% (95% CI: 2.2–42.8%, 5 studies) for adults for non-mRNA vaccines.

In comparison to its overall VE, the short-term VE estimates of the first booster dose were slightly higher, 59.4% (95% CI: 55.1–63.3%, 33 studies) for all ages and 59.9% (95% CI: 55.1–64.1%, 28 studies) for adults (Supplementary Figure 3). When stratified by vaccine type, the short-term first-booster VE estimates were 63.7% (95% CI: 59.2–67.7%, 15 studies) for all ages and 67.3 % (95% CI: 64.5–69.9%, 10 studies) for adults for pure mRNA vaccination, 62.3% (95% CI: 59.2–65.1%, 12 studies) for adults for partial mRNA vaccines, and 37.2% (95% CI: 19.5–51.0%, 6 studies) for adults for non-mRNA vaccines.

Long-term VE estimates of the first booster dose were moderately lower than their overall counterparts, 34.9% (95% CI: 27.6–41.5%, 22 studies) for all ages and 31.5% (95% CI: 22.7–39.4%, 20 studies) for adults (Supplementary Figure 4). Long-term first-booster VE estimates stratified by vaccine type were 46.6% (95% CI: 36.8–54.8%, 7 studies) for all ages and 50.9% (95% CI: 45.0–56.2%, 5 studies) for adults for pure mRNA vaccination, 34.6% (95% CI: 28.6–40.2%, 11 studies) for adults for partial mRNA vaccines, and 4.6% (95% CI:−9.5–16.9%, 4 studies) for adults for non-mRNA vaccines.

Due to lack of data, we were only able to estimate short-term and long-term VE but not overall VE of the second booster (Figure 3). Furthermore, we were unable to distinguish between vaccine types for the second booster, but the majority of these studies were based on four doses of mRNA vaccines. The short-term second-booster VE against symptomatic infection or any infection for Omicron was 59.6% (95% CI: 52.0–66.1%, 17 studies) in adults, similar to the overall and the short-term first-booster VE estimates in adults. The long-term second-booster VE was 32.7% (95% CI: 15.4–46.4%, 10 studies) in adults, comparable to that of the first booster.

Vaccine effectiveness against omicron-associated severe events

The overall VE of the full doses against Omicron-associated severe events was estimated to be 57.3% (95% CI: 48.5–64.7%, 24 studies) for all ages and 57.9% (95% CI: 51.5%-63.4%, 16 studies) for adults (Figure 4A). The overall VE estimates of pure mRNA vaccines were 60.9% (95% CI: 50.7–68.9%, 18 studies) for all ages, 60.1% (95% CI: 53.1–66.0%, 10 studies) for adults, and 59.9% (95% CI: 24.7–78.6%, 6 studies) for children and adolescents. The overall VE of partial mRNA vaccines for adults was slightly lower than that of pure mRNA vaccines, 54.5% (95% CI: 41.1–64.8%, 6 studies). We did not find studies estimating the overall VE of non-mRNA vaccines against Omicron-related severe events.

The short-term VE of the full doses against Omicron-associated severe events was estimated to be 66.9% (95% CI: 58.3–73.8%, 16 studies) for all ages and 69.9% (95% CI: 62.8–75.6%, 10 studies) for adults (Supplementary Figure 5). Stratified by vaccine type, the short-term VE estimates were 64.0% (95% CI: 50.2–74.0%, 9 studies) for all ages, 70.5% (95% CI: 64.9–75.2%, 3 studies) for adults, 60.7% (95% CI: 36.6–75.6%, 6 studies) for children and adolescents for pure mRNA vaccines and 70.7% (95% CI: 59.2%-78.9%, 7 studies) for adults for partial mRNA vaccines.

Long-term VE estimates of the full doses against Omicron-associated severe events were comparable to the overall VE estimates, 58.3% (95% CI: 45.5–68.1%, 18 studies) for all ages and 59.0% (95% CI: 49.0–67.1%, 13 studies) for adults (Supplementary Figure 6). Stratified by vaccine type, the long-term VE estimates were 62.4% (95% CI: 38.9–76.8%, 9 studies) for all ages, 67.7% (95% CI: 56.3–76.1%, 4 studies) for adults, and 56.4% (95% CI:−3.6–81.7%, 5 studies) for children and adolescents for pure mRNA vaccines, 50.7% (95% CI: 29.9–65.2%, 6 studies) for adults for partial mRNA vaccines, and 60.1% (95% CI: 39.7–73.6%, 3 studies) for adults for non-mRNA vaccines.

First booster doses generally showed higher VEs against Omicron-associated severe disease than full doses. The pooled overall VE of the first booster dose was estimated to be 82.5% (95% CI: 77.8%-86.2%, 28 studies) for all ages and 82.0% (95% CI: 77.0%-86.0%, 25 studies) for adults (Figure 4B). Pure mRNA vaccines and partial mRNA vaccines showed similar overall VEs against severe events, 83.6% (95% CI: 77.0–88.2%, 11 studies) for all ages, 82.5% (95% CI: 74.7–88.0%, 8 studies) for adults for the former, and 84.6% (95% CI: 77.6–89.5%, 12 studies) for adults for the latter. The overall VE was moderately lower for non-mRNA vaccines, 71.4% (95% CI: 52.1–82.9%, 5 studies) for adults.

Short-term and long-term VEs of the booster dose against Omicron-associated severe events were only available for adults (Supplementary Figure 7). We estimated the short-term VE to be 84.8% (95% CI: 80.4–88.1%, 17 studies) and the long-term VE to be 77.6% (95% CI: 69.4–83.6%, 16 studies) for all vaccine types combined. Short-term vs. long-term booster VE estimates were 85.3% (95% CI: 79.8–89.3%, 6 studies) vs. 80.1% (95% CI: 64.6–88.8%, 5 studies) for pure mRNA vaccines, 88.1% (95% CI: 83.4–91.4%, 7 studies) vs. 78.0% (95% CI: 64.3–86.4%, 8 studies) for partial mRNA vaccines, and 73.0% (95% CI: 53.7–84.3%, 4 studies) vs. 70.5% (95% CI: 47.3–83.5%, 3 studies) for non-mRNA vaccines.

Pooled short-term and long-term VE estimates for the second booster against Omicron-associated severe events among adults were 87.3% (95% CI: 75.5–93.4%, 14 studies), and 85.9% (95% CI: 80.3–89.9%, 5 studies) respectively (Figure 3), both of which are comparable to those of the first booster, though the long-term VE of the second booster appears to decay at a slower rate.

Assessment of publication bias

Publication bias was detected in the pooled estimates of overall VE of the full doses against severe events (Egger's test $p = 0.073$, Begg's test $p = 0.208$), long-term VE of the full doses against severe

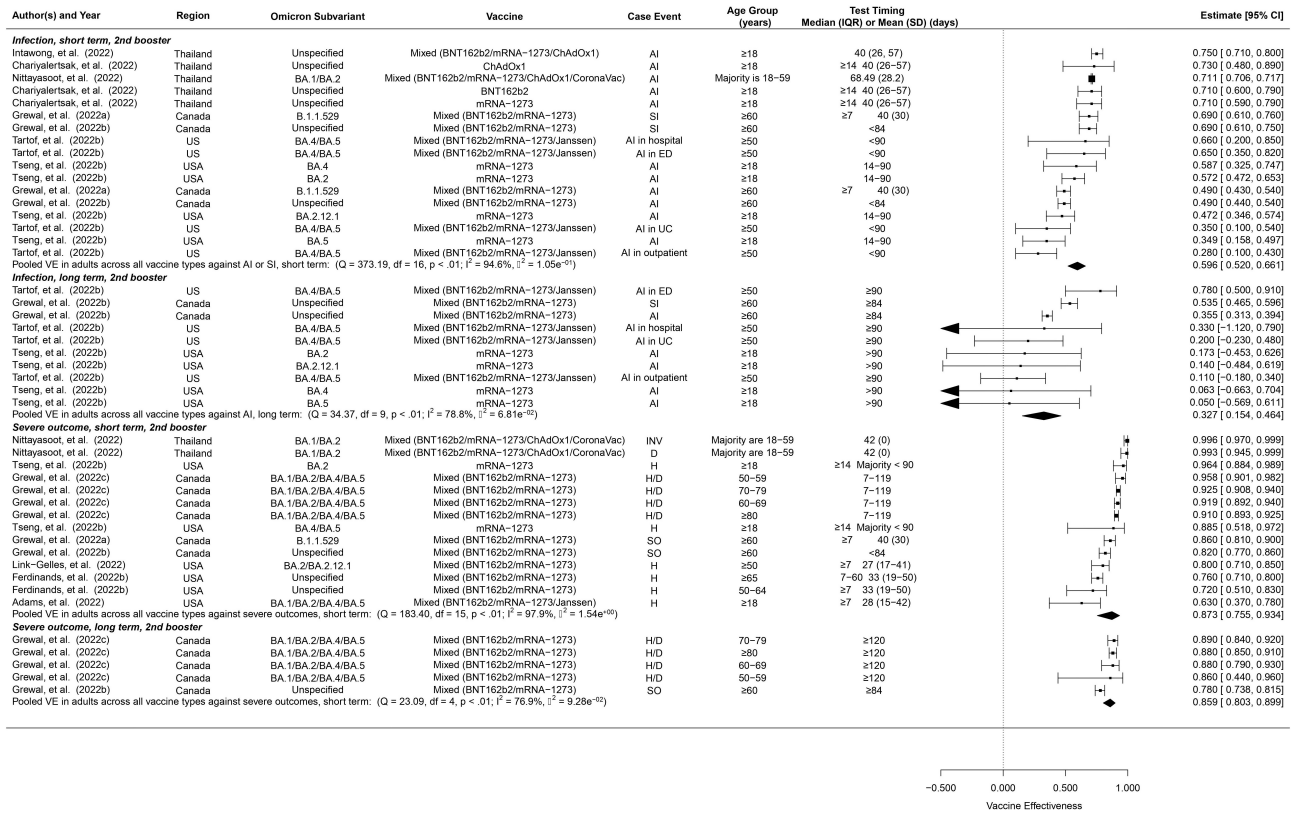


FIGURE 3
Overall vaccine effectiveness of second booster dose against infection or symptomatic infection and against severe events. Pooled VE estimates are stratified by short-term (<60 days) vs. long-term (≥60 days). Statistics Cochran's Q, I^2 and τ^2 measure the heterogeneity between studies. For infection, possible end points of the studies are symptomatic infection (SI) or any infection (AI). For severe events, possible end points are hospitalization (H), death (D), severe outcomes (SO) or invasive procedures (INV). Mixed vaccine type indicates the study reported VEs of these vaccines combined without distinguishing between them.

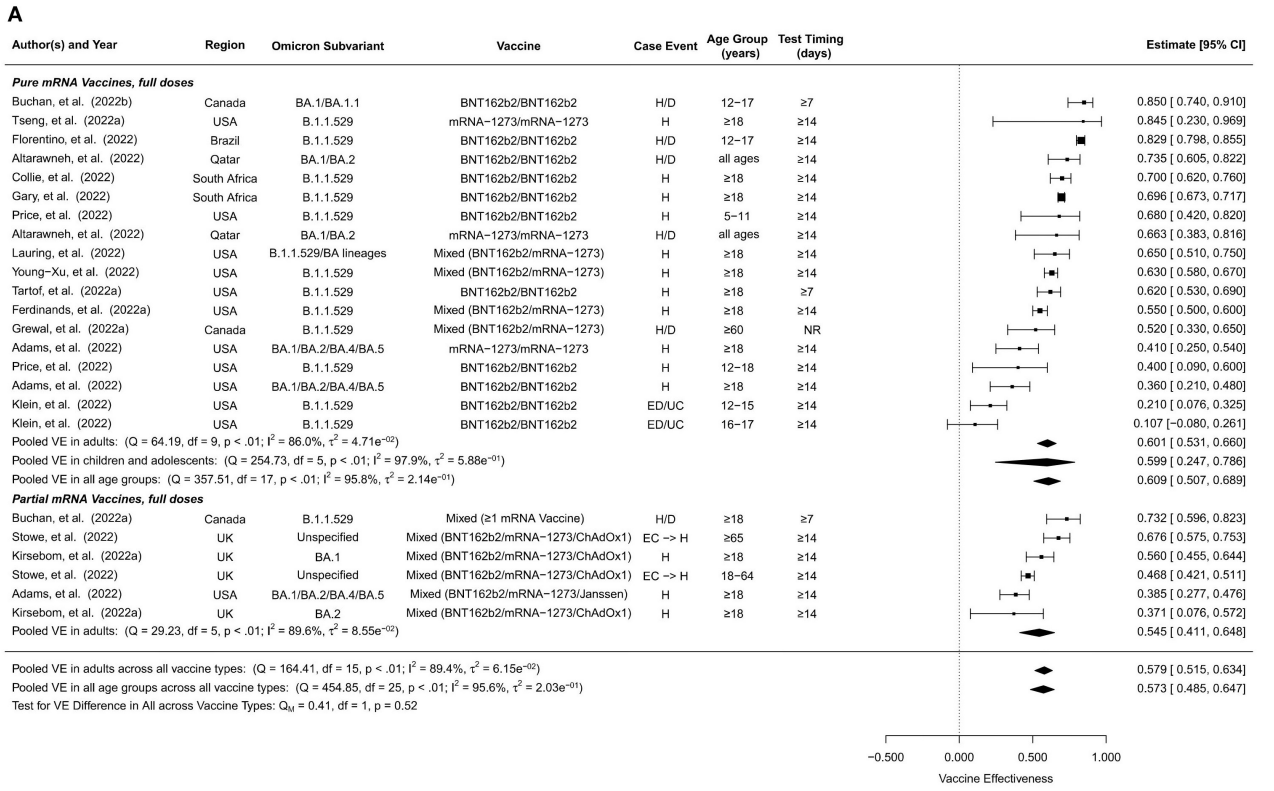
events (Egger's test $p = 0.027$, Begg's test $p = 0.369$), short-term VE of the first booster dose against severe events (Egger's test $p = 0.098$, Begg's test $p = 0.49$), and short-term VE of the second booster dose against severe events (Egger's test $p = 0.001$, Begg's test $p = 0.747$), as shown in Supplementary Figures 8–11. Additionally, publication bias was found in four subgroups defined by age group and vaccine type (Supplementary Figure 12). Results were corrected for these biases using the trim-and-fill method.

Discussion

In this systematic review and meta-analysis of 42 studies, we found that one or two booster doses in addition to the initial full COVID-19 vaccine series provided substantial protection against Omicron infection with VE ≥ 50% and severe events with VE ≥ 80%, compared to no vaccination. In general, pure and partial mRNA vaccines provided comparable protection levels against infection or severe disease, and both were more effective than non-mRNA vaccines, though the difference was less dramatic in terms of protection against severe disease. The VEs of the full doses and the booster doses against severe disease only wane slightly after 3 months, but the VEs against infection wane more quickly.

Both the first and second booster doses provided considerably higher VE against infection and severe events compared to completion of the initial full series only. Studies have reported higher anti-receptor binding domain specific memory B cells and anti-spike antibodies after booster doses compared to full series only (23, 62). Similarly, T cell immunity against Omicron is provided by booster doses though at a reduced level compared to ancestral variants (63). While the initial full doses provided inadequate protection against infection (Figure 2A), they did render practically meaningful (≥50%) VE against severe disease (Figure 4A).

Pure and partial mRNA vaccines offered comparable protection levels against infection, 25.4% vs. 28.1% for the full doses and 61.4% vs. 56.4% for the first booster among adults, and both were much more effective than the non-mRNA vaccines (1.5% for the full doses and 25.2% for the first booster). Studies included in this analysis reported lower binding activities between anti-spike and anti-receptor among Ad26.COV2 recipients compared to mRNA recipients (23). Similar trends were observed against severe events, though the gap between mRNA and non-mRNA vaccines was much narrower. In particular, full-dose non-mRNA vaccines provided a similar level of sustained protection against severe disease (VE = 60%) compared to full-dose mRNA vaccines (Supplementary Figure 6), suggesting that the initial full doses of



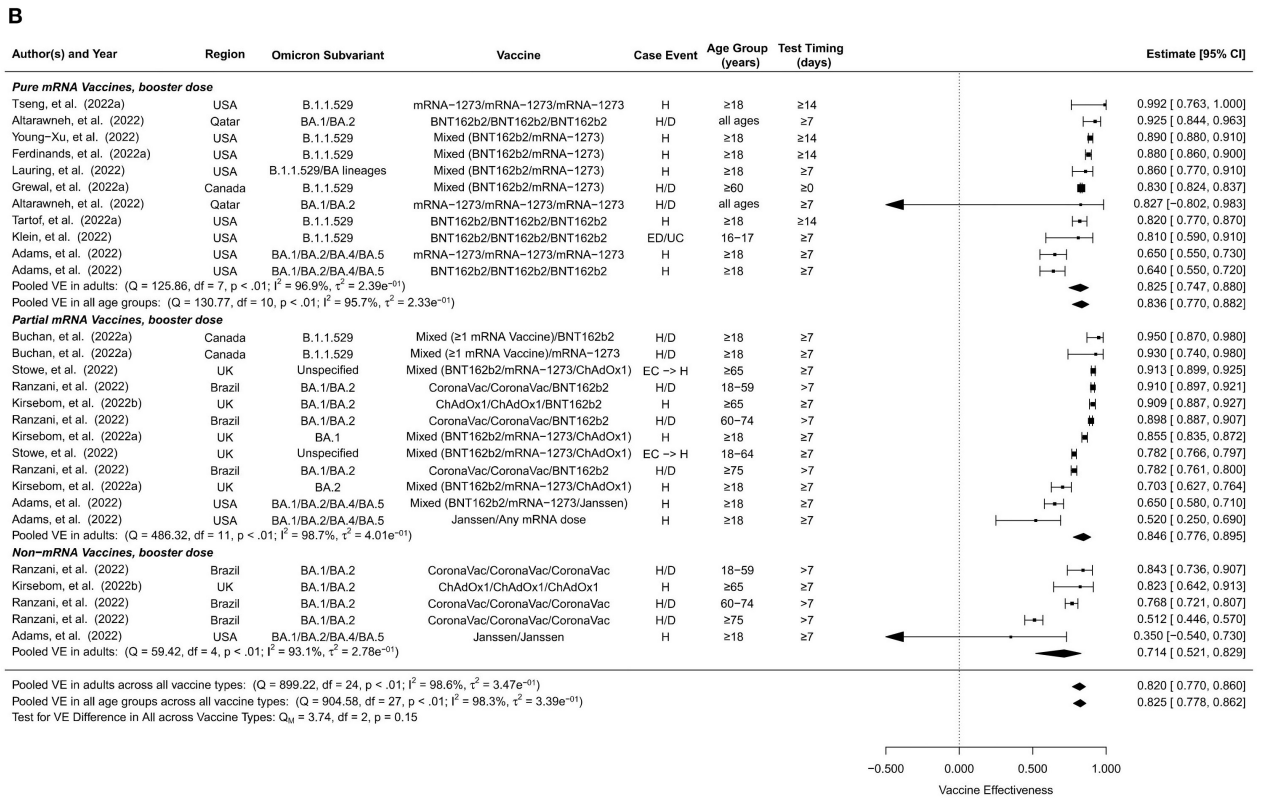


FIGURE 4
Overall vaccine effectiveness of full doses and first booster against severe events. **(A)** Pooled VE of full doses estimated from all 24 studies combined as well as for each vaccine type. **(B)** Pooled VE of first booster estimated from all 28 studies combined as well as for each vaccine type. Statistics Cochran's Q, I^2 and τ^2 measure the heterogeneity between studies. Possible end points of the studies are hospitalization (H), hospitalization or death (H/D), emergency department or urgent care encounter (ED/UC), or hospital admissions from emergency care (EC→ H). Mixed vaccine type indicates the study reported VEs of these vaccines combined without distinguishing between them.

non-mRNA vaccines should be encouraged among unvaccinated individuals in regions where mRNA vaccine supply is insufficient.

The VEs of the initial full doses and the first booster dose against Omicron infection waned substantially over time, from 40.7% within 3 months of boosting to 17.6% for full doses and 59.4 to 34.9% for the first booster. The VEs against Omicron-associated severe disease waned at a slower pace, from 66.9% to 58.3% for the full doses and from 84.8% to 77.6% (in adults) for the first booster dose. Our findings are consistent with other studies reporting waning immunity of COVID-19 vaccines for earlier variants (19, 58) as well as for Omicron regardless of age, immunocompromised status, and vaccine product (55). One study reported that VE against symptomatic infection waned more rapidly among older adults (64), which was also reflected in this meta-analysis, e.g., the full-dose VE of pure mRNA vaccines against infection declined from 45.3 to 22.3% among children and from 41.3 to 13.1% among adults (Supplementary Figures 1, 2). These age differences in decay rates were not observed for the VEs against severe disease (Supplementary Figures 5, 6).

The second booster of pure or partial mRNA vaccines protected adults from Omicron infection with a VE of 59.6% which is slightly lower than the short-term VE of the first booster for pure mRNA (67.3%) or partial mRNA vaccines (62.3%) among adults. A similar gap was seen for the long-term VE among adults as well, 32.7% for the second booster vs. 50.9% for pure mRNA and 34.6% for partial mRNA first boosters. This seemingly unexpected gap (not statistically significant) may result from the fact that the dominant Omicron subvariants were mostly BA.1 and BA.2 for the first booster studies but BA.4 and BA.5 were taking over for the second booster studies. BA.4 and BA.5 are known to be associated with high immune escape and transmissibility compared to BA.1 and BA.2, e.g., the effective reproductive number was estimated to be 5.11 and 5.22 for BA.4 and BA.5 compared to 3.22 and 5.04 for BA.1 and BA.2 (65).

In terms of protection against severe disease among adults, we observed comparable VE estimates between the second booster and the first booster doses for both short term (87.3% for the second booster vs. 85.3% and 88.1% for pure and partial mRNA first boosters) and long term (85.9% for second booster vs. 80.1% and 78.0 for pure and partial mRNA first boosters). The second booster appears to wane to a lesser extent over time. However, a caveat is that nearly all data used to estimate the long-term VE of the second booster against severe disease came from the same study among elderly residents of long-term care facilities in Ontario, Canada (60). In addition, this long-term VE is against BA.1 and BA.2, the dominant subvariants during the study period of 31 December 2021 to 27 April 2022, according to the Ontario Ministry of Health.

Our study had several limitations. First, in several test-negative studies, we included, the same control group for multiple vaccine groups, which introduces dependence among the VE estimates. However, such dependence was not accounted for in our analysis due to lack of covariance estimates. Second, there was significant heterogeneity in VE estimates, which may be attributable to differences between studies in terms of a whole host of characteristics, including study design, follow-up duration, definitions of VE, time since vaccination, dosing intervals, confounders adjusted for, and others. Finally, as most studies did not provide subvariant-specific VE estimates and there is ambiguity in which Omicron subvariants were dominant for many studies, we were not able to stratify the meta-analysis by subvariant.

Our findings demonstrate that completion of a full COVID-19 vaccine series plus one or two booster doses provides considerable VE against Omicron infection and strong VE against severe events compared to non-vaccination. Although VEs generally wane after 2–3 months, the second booster clearly generates more sustainable protection. As the Omicron family continues to evolve with more genetic and antigenic variation, e.g., the XBB* and BQ.1* sublineages, lower VEs and faster waning of protection of the Wuhan-Hu-1-based boosters should be expected. Meanwhile, the level and longevity of efficacies of Omicron-specific bivalent vaccines should be closely monitored using meta-analytic approaches. To facilitate comparison and synthesis of VE estimates across studies, we recommend the following improvements to future vaccine studies: (i) longer follow-up to better understand long-term VE; (ii) stratification of VE by age group, vaccine type and variant whenever possible; and (iii) when multiple VE estimates are reported, providing covariance or correlation among the estimates via, e.g., resampling the data.

Data availability statement

Publicly available datasets were analyzed in this study. The raw summary-level data were derived from publicly available papers cited in the reference. Extracted data and programming codes will be made available upon request by email to the corresponding authors.

Author contributions

YY and IL conceived the study. SS, ZM, and ML collected the data. SS, ZM, ML, and YY reviewed the data. SS analyzed data under the supervision of YY, ZM, and IL. SS, ZM, and YY drafted the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1195908/full#supplementary-material

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Transmission dynamics of *SARS-CoV-2* variants in the Brazilian state of Pará

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Introduction: After three years since the beginning of the pandemic, the new coronavirus continues to raise several questions regarding its infectious process and host response. Several mutations occurred in different regions of the *SARS-CoV-2* genome, such as in the spike gene, causing the emergence of variants of concern and interest (VOCs and VOIs), of which some present higher transmissibility and virulence, especially among patients with previous comorbidities. It is essential to understand its spread dynamics to prevent and control new biological threats that may occur in the future. In this population_based retrospective observational study, we generated data and used public databases to understand *SARS-CoV-2* dynamics.

Methods: We sequenced 1,003 *SARS-CoV-2* genomes from naso-oropharyngeal swabs and saliva samples from Pará from May 2020 to October 2022. To gather epidemiological data from Brazil and the world, we used FIOCRUZ and GISAID databases.

Results: Regarding our samples, 496 (49.45%) were derived from female participants and 507 (50.55%) from male participants, and the average age was 43 years old. The Gamma variant presented the highest number of cases, with 290 (28.91%) cases, followed by delta with 53 (5.28%). Moreover, we found seven (0.69%) Omicron cases and 651 (64.9%) non-VOC cases. A significant association was observed between sex and the clinical condition (female, p=8.65e-08; male, p=0.008961) and age (p=3.6e-10).

Discussion: Although gamma had been officially identified only in December 2020/January 2021, we identified a gamma case from Belém (capital of Pará State) dated May 2020 and three other cases in October 2020. This indicates that this variant was circulating in the North region of Brazil several months before its formal identification and that Gamma demonstrated its actual transmission capacity only at the end of 2020. Furthermore, the public data analysis showed that *SARS-CoV-2* dispersion dynamics differed in Brazil as Gamma played an important role here, while most other countries reported a new infection caused by the Delta variant. The genetic and epidemiological information of this study reinforces the relevance of having a robust genomic surveillance service that allows better management of the pandemic and that provides efficient solutions to possible new disease-causing agents.

KEYWORDS

genomic surveillance, Brazil, *SARS-CoV-2*, COVID-19, Amazon, gamma

1. Introduction

In December 2019, an outbreak of pneumonia was reported in Wuhan, the capital of the Hubei province in China. The associated symptoms are referred to as coronavirus disease 2019 (COVID-19), and its rapid spread caused a global pandemic officially declared by the World Health Organization (WHO) on March 11, 2020. The COVID-19 pandemic, whose etiologic agent is the severe acute respiratory syndrome coronavirus 2 (*SARS-CoV-2*), has caused almost seven million worldwide deaths in the three years since its discovery in Wuhan (1, 2). To this date (may/2023), the Americas present more than 192.4 million cases, while Brazil has around 37.4 million cases (2).

SARS-CoV-2 is a Betacoronavirus that belongs to the *Coronaviridae* family, which is constituted mainly by agents that cause the common cold and severe acute respiratory syndrome (SARS), such as *SARS-CoV-1* (3, 4). *SARS-CoV-2* has a 30-kb genome that encodes four major structural proteins: nucleocapsid (N), membrane (M), envelope proteins (E), and spike (S) (5). The spike protein mediates the virus entry into host cells, and once inside the cell, the virus replicates and may cause high levels of inflammation, which favors opportunistic infections (6, 7).

Clinical manifestations of COVID-19 are highly heterogeneous, even among patients with the same gender, age, and risk group. Some are asymptomatic, while others may present dry cough, fever, headache, fatigue, odynophagia, and diarrhea. Some patients present the most severe symptoms, such as pneumonia and thromboembolic events, which may lead them to death (8, 9).

The cause of the differential patterns of symptoms in COVID-19 remains unclear. Still, it seems to be explained by several factors, including the patient's genetic background, previous comorbidities, and the *SARS-CoV-2* variants (8–10). These variants are classified according to the set of mutations in the viral genome. Since March 2020, several lineages of *SARS-CoV-2* have emerged, and those with higher virulence and ability to propagate were classified as variants of concern (VOCs), followed by the variants of interest (VOIs) and variants under monitoring (VUMs), in descending order of virulence. Five VOCs have been recognized: B.1.1.7 (U.K, Alpha variant), B.1.351 (South Africa, Beta variant), P.1 (Brazil, Gamma variant), B.1.617.2 (India, Delta variant), and B.1.1.529 (multiple countries, Omicron variant) (11).

Most mutations present in these variants are in the spike gene and enable a more effective entry of the virus into the host cells, resulting in both high viral load and levels of inflammation, which leads to an increased risk of developing the severe form of COVID-19 (12). Even though Omicron has a higher number of mutations, the symptoms caused by it were less severe when compared with the other lineages, probably due to the higher vaccine coverage of the world population at the time of its emergence (13–15).

VOCs have presented different patterns of prevalence across the globe. For instance, Gamma, which is a descendant of B.1.1.28, showed a high prevalence in Brazil and South America. Since the first Gamma case, in December 2020, FIOCRUZ database has reported 56.877 infections caused by Gamma. It is important to mention that these numbers are probably lower than reality, due to the notification missing data (16).

Gamma originated in Brazil's Amazon region and displays 12 mutations in the *S* gene, three of them (K417T, E484K, and N501Y) in the receptor binding domain (RBD). These mutations potentially increase transmissibility and prevent virus immune recognition by the host (7, 11). This VOC was first identified in Brazil in December 2020, in the city of Manaus, capital of the Amazonas state, and since then until the end of 2021 it caused several cases in Brazil, especially in the North region (17).

According to Our World in Data,[1] more than 765 million cases and more than 6.9 million deaths worldwide have been reported. In Brazil, there are estimated to be more than 36 million cases and about 695,000 deaths. Pará (PA) was the most affected by the pandemic in the North region of Brazil, with 861,000 cases and more than 18,000 deaths (18).

In this study, we provide data collected by a regional network for COVID-19 genomic surveillance in the state of Pará in the North of Brazil and map up the status of transmission dynamics of *SARS-CoV-2* during these three years of the pandemic of COVID-19 worldwide. Besides that, this study's main aim is to help the scientific community, specially the Brazilian one, to understand the spreading of coronavirus variants in the North region of Brazil and also to help this community to prevent and control new biological threats that may occur in the future. Furthermore, this study may have significant contributions to

1 https://ourworldindata.org/coronavirus

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TABLE 1 Clinical characteristics of COVID-19 patients (asymptomatic, mild symptoms, and severe disease) versus variants (Alpha, Beta, Gamma, Delta, Omicron, and non-VOCs).

Clinical characteristics	Asymptomatic (*n*=122)	Mild (*n*=219)	Severe (*n*=116)	*p*-value*
Sex, *n* (%)				
Female	50 (40.98%)	116 (52.96%)	62 (53.45%)	**8.65e-08**
Male	72 (59.02%)	103 (47.04%)	54 (46.55%)	**0.008961**
Age, mean (SD)				
	43.9 (16)	37.3 (17.8)	51.3 (18.2)	**3.6e-10**
Variants				
Alpha	0	0	0	1
Beta	2 (1.63%)	0	0	0.1353
Gamma	57 (46.72%)	88 (40.18%)	58 (50%)	0.08798
Delta	12 (9.83%)	27 (12.33%)	6 (6.17%)	**0.01019**
Omicron	0	7 (3.20%)	0	**0.0009119**
Non-VOCs	51 (41.80%)	97 (44.30%)	52 (44.83%)	**3.183e-05**

SD, standard deviation. **p*-values were calculated using Chi-squared test (for sex and variants) and Kruskal-Wallis test with correction for multiple comparisons (for age). Bold values are statistical significant.

the understanding of possible correlations between coronavirus variants and the host's clinical conditions and comorbidities.

2. Materials and methods

2.1. Study design and sample collection

This population-based retrospective observational study uses genomic and COVID-19 surveillance data collected from the state of Pará in North Brazil. The data presented in this study result from a regional network headed by Instituto Tecnológico Vale (Belém/PA, Brazil) and Fundação Oswaldo Cruz (Fiocruz - Rio de Janeiro/RJ, Brazil), In which several institutions gathered efforts to accomplish a COVID-19 genomic surveillance in Brazil.[2]

From May 2020 to October 2022, we sequenced 1,003 naso-oropharyngeal swabs and saliva samples (136 saliva samples and 867 swab samples) from Pará. The mean age was 43 years old. We included samples from 67 municipalities of Pará, but most are from Belém, its capital (Supplementary Table S1; Supplementary Figure S1). Swab samples were collected and transferred to viral transport media, and saliva samples were collected in sterile plastic collection tubes. After collection, both sample types were stored at −80°C until further analysis.

The study, including all experimental protocols, was approved by the Ethics Committee of the Center of the University Hospital João de Barros Barreto of the Federal University of Pará (No. 50865721.1.0000.0017). All study participants or their legal guardians provided informed written consent in accordance with the Helsinki Declaration. A summary of clinical data is presented in Table 1, and the detailed metadata is in Supplementary Table S1.

2.2. RNA isolation, qRT-PCR, and sequencing

Viral RNA was isolated from naso-oropharyngeal swabs and saliva samples using MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher Scientific) at KingFisher System (Thermo Fisher Scientific). These kits are designed to work with robots that can isolate genetic material from various sample types, pathogens, and hosts. To ensure a sterilized extraction environment, it is crucial to sanitize both kits before use and expose them to ultraviolet light (UV), if possible.

The Maxwell protocol is a straightforward process that involves adding around 200 µL of the sample (saliva or swab) to a microtube containing a solution of 200 µL and 20 µL of Proteinase K. The microtube is then vortexed briefly and incubated at 56°C for 10 min. While the sample is incubating, extraction cartridges, tips, and microtubes (0.6 mL) containing nuclease-free water are prepared and added to the robot. The robot then performs the extraction, which takes 42 min and yields 50 µL of sample. In contrast, the automated extraction performed by KingFisher can be more time-consuming and complex. It requires four 96-well plates, the first containing the samples, bead solution (binding solution and beads), and proteinase K, the second with alcohol 80%, the third with Wash Solution from the kit, and the fourth with the elution buffer. After preparing the plates with their respective amounts of samples and reagents, the plates are loaded onto the robot for automated extraction, which takes approximately 25 min and yields approximately 30 µL of sample.

Viral RNA was detected using TaqPath 1-step RT-qPCR Master Mix (Thermo Fisher Scientific) according to the CDC 2019-nCoV Real-Time RT-PCR diagnostic panel instructions for use (19). This protocol uses three probes: N1, N2, and RP. The samples were considered as positive when all three probes crossed the threshold line within 40 cycles, and were considered as negatives when only RP crossed the threshold line within 40 cycles. The Non Template Controls (NTC) were nuclease-free water and the positive controls were samples with confirmed coronavirus infection.

Samples with Ct value ≤35 were selected for sequencing and viral genomic libraries were constructed using Illumina COVIDSeq Test

2 https://www.itv.org/imprensa/projeto-genoma-covid-19/

(Illumina) and checked for quality using 2,200 TapeStation (Agilent Technologies). Libraries were sequenced on NextSeq 500 Sequencing System (Illumina) using NextSeq 500/550 Mid Output Kit v2.5 (300 cycles - Illumina).

2.3. Bioinformatic analysis and statistical methods

The quality treatment, mapping, assembly, and variants identifications were performed using the PipeCoV pipeline (20). First, FASTQ files were trimmed using the Phred score (−q 20) as parameters with small reads discarded (−l 20). Later, high-quality reads were mapped to the reference sequence EPI_ISL_402124 (hCoV-19/Wuhan/WIV04/2019) available in the EpiCoV database in GISAID[3] and the assembly was performed with a kmer size (−k 31). Pangolin v2.3.8 performed lineage identification with the default parameters. Clinical characteristics were analyzed using the Chi-squared test for categorical variables and the Kruskal-Wallis test with correction for multiple comparisons for continuous variables (age). The normality of the dataset was assessed using the Shapiro–Wilk test. All graphs and statistical analyzes were made using R (v.4.2.1). p-values <0.05 were considered statistically significant.

2.4. Data sources

In addition to our data, we obtained *SARS-CoV-2* genomic, epidemiological, and population information from Brazil and from all continents. The databases used were FIOCRUZ[4] for all Brazilian regions information and GISAID[3] for information about all continents. These data from FIOCRUZ and GISAID were collected on November 2nd, 2022 and December 3rd, 2022, respectively, concerning data from November 2020 to September 2022 for FIOCRUZ and from October 2020 to November 2022 for GISAID. No ethical board approval was requested, given that these databases are public and anonymous.

3. Results

We analyzed 1,003 *SARS-CoV-2* genomes obtained from positive COVID-19 patients from Pará (Brazil), divided into three groups according to their clinical symptoms: asymptomatic, mild, and severe (Table 1). Among our samples, 496 (49.45%) were derived from female participants and 507 (50.55%) from male participants, and the average age was 43 years old.

Of the 1,003 samples sequenced, none were infected with Alpha, and two (0.19%) were infected with the Beta variant. The Gamma variant presented the most significant number of cases with 290 (28.91%) cases, followed by Delta with 53 (5.28%). Plus, we found seven (0.69%) Omicron samples in our internal data and 651 (64.9%) non-VOC cases.

Concerning the clinical conditions, 122 (26.70%) of the participants were asymptomatic, 219 (47.92%) had mild symptoms, and 116 (25.38%) had severe disease. A significant association was observed between gender and the clinical condition (female value of $p = 8.65e\text{-}08$; male value of $p = 0.008961$) and age (value of $p = 3.6e\text{-}10$; Supplementary Figure S2). Regarding variants, Delta (value of $p < 0.01$), Omicron (value of $p < 0.01$), and non-VOCs (value of $p < 0.01$) were significantly associated with the clinical groups.

We analyzed the distribution of *SARS-CoV-2* variants within a specific time interval in all continents, Brazil and the state of Pará. On a global scale, according to the data deposited in GISAID, until October 2021 the proportion of different variants alters between the continents along the time scale. For instance, until May 2021, Alpha and Beta variants presented a major epidemiological prevalence in Africa, Asia, Europe, North America, and Oceania but not in South America (Figure 1). This dynamic is also observed between June and August 2021, whereas Delta is the dominant variant in all continents except South America. From September, however, the lineages' pattern is the same across continents, with Delta being the most prevalent between September and December 2021 and Omicron between January 2022 and now (January 2023).

In South America, Gamma was the variant responsible for most cases during the first semester of 2021. As mentioned above, the prevalence of Delta cases was only observed months after its emergence and dominance in other continents. However, from August 2021, the Delta variant modified the epidemiological scenario. It became the most prevalent variant just when the Gamma variant started to disappear until the emergence of Omicron in December 2021 (Figure 1).

Brazil's epidemiological scenario can vastly modify the scenario displayed in South America, given its large population size, as shown in Figure 2. Other South American countries also suffered from an increase in the number of cases after Gamma, such as Argentina, Colombia, Venezuela and Bolivia (21).

In Figure 3, on the other hand, we showed that the period between November 2020 and January 2021 in Brazil was marked by the prevalence of non-VOC in all five Brazilian regions (North, Northeast, Midwest, South and Southeast) (Supplementary Figure S3). However, as 2021 started, Gamma was responsible for most COVID-19 countrywide cases between January 2021 and July 2021, followed by Delta in August 2021 and Omicron in January 2022.

It is worth mentioning that there are huge differences between the Brazilian regions. The North region has several differences compared to the other regions of Brazil, like socioeconomic, cultural and health dissemblances. Usually, the Southeast region has more financing, especially due to its large concentration of people. These differences between the regions were also demonstrated in the pandemic given that some regions were financially poorly assisted to combat COVID-19, while others had enough fundings. Another example of these variations is the fact that quite a few Alpha cases were observed in the North, where Gamma prevailed for the longest time (April–September 2021).

Figure 3 shows the distribution of *SARS-CoV-2* variants. Observing the Brazilian regions, we can see that Gamma (in green) was the most prevalent variant for an extended period rather than Alpha and Beta (which were the most prevalent in other countries), especially in northern Brazil. Furthermore, we observed that in Belém, until May 2021, non-VOC were the most dominant variants. This

3 https://www.gisaid.org/

4 http://www.genomahcov.fiocruz.br/dashboard/

FIGURE 1
Prevalence of the VOCs and their sub-variants in all continents between October 2020 and November 2022, according to the GISAID database.

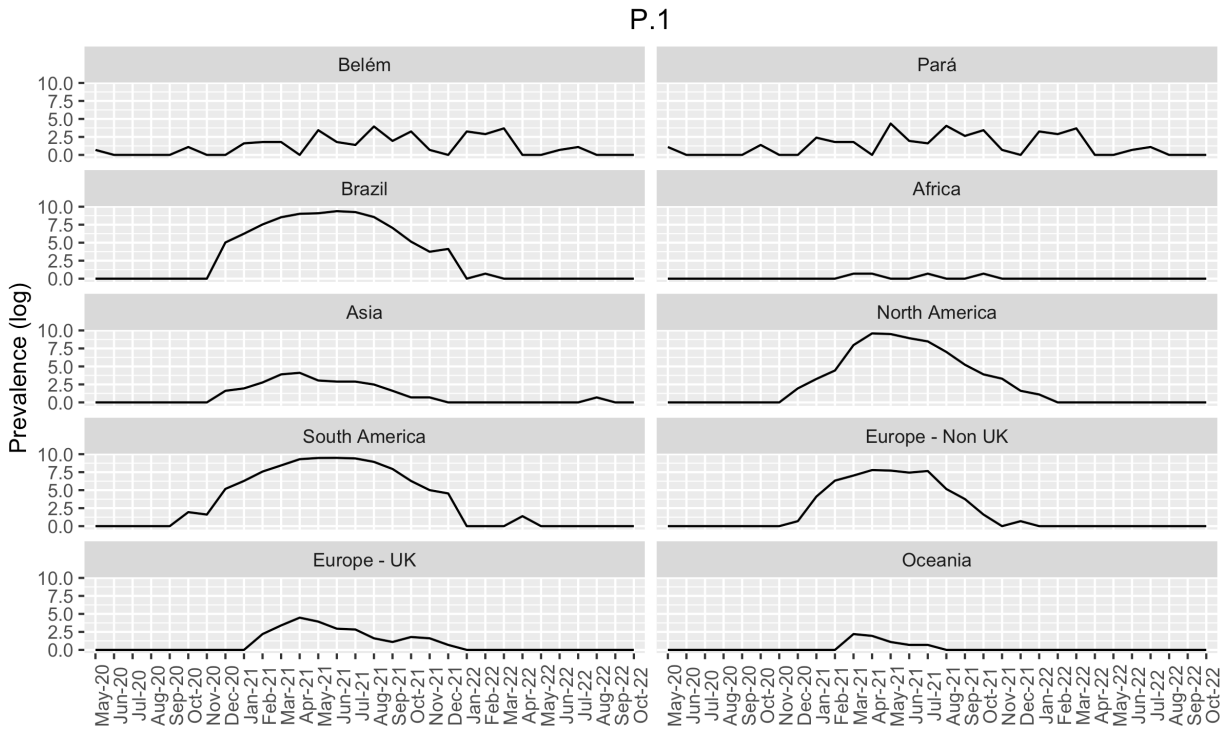

FIGURE 2
Prevalence of Gamma-like variants in all continents, in Brazil (both from GISAID), and in the state of Pará and in its capital, Belém (own data), in the period of May 2020 to October 2022. Data is presented as the log of the absolute prevalence count.

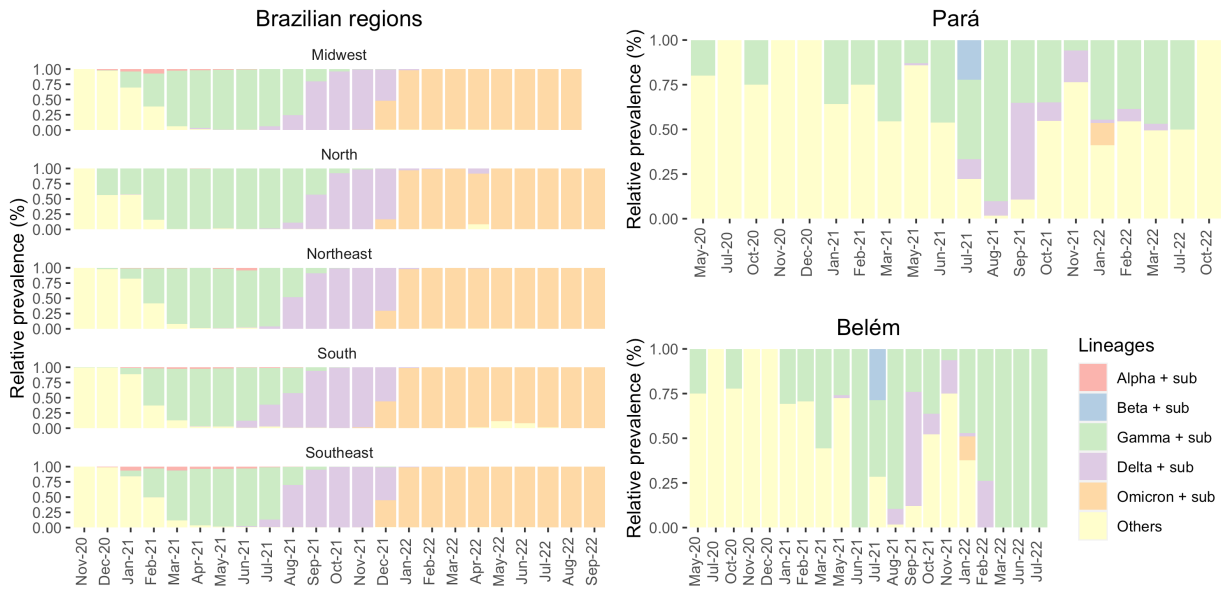

FIGURE 3
Prevalence of the VOCs and its sub-variants in the Brazilian regions (from GISAID) and in the state of Pará and in its capital, Belém (own data), in the period of May 2020 to October 2022.

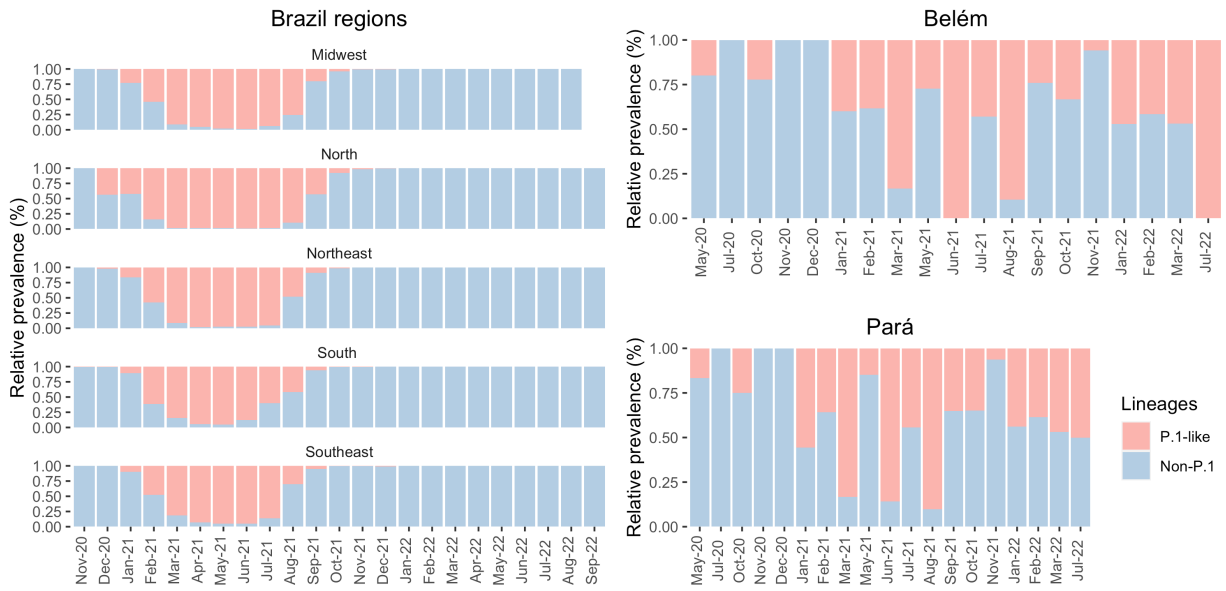

FIGURE 4
Prevalence of P1-like and non-Gamma variants in the Brazilian regions (from GISAID) and in the state of Pará and in its capital, Belém (own data), in the period of May 2020 to September 2022.

demonstrates a greater diversity of circulating variants that diminished with the increase of Gamma cases from June 2021. In addition, according to FIOCRUZ data, P.2 variant had its greater transmission between December 2020 and February 2021, while Gamma (P.1) presented higher prevalence in Pará state between April 2021 and September 2021.

Our data showed a few cases of Gamma infections in May 2020 and October 2020 in Belém (Figure 4). Therefore, we decided to compare the distribution of the Gamma variantin Brazil. As shown in Figure 2, Gamma caused a new wave of infections between January and August 2021 in Brazil, with cases until September in Midwest and North regions. However, according to our own dataset, in both Pará and Belém, the first case of Gamma infection was reported in May 2020, and it persisted with high prevalence until 2022. In Pará, Gamma may have prevented a wave of infections by the Delta variant in the state, by dominating the area before Delta arrived.

When comparing Brazil, Pará, and Belém with the continents between May 2020 and October 2022, it becomes clear that Brazil's epidemiological status has molded the South Americas' given that their Gamma prevalence patterns are very similar (Figure 2). In parallel, the Brazilian North region had an important role in the maintenance of the Gamma variant in Brazil. In addition, there was a lower prevalence of Gamma in Asia, Africa, Europe, and Oceania, indicating that this variant did not present dissemination success in these continents. On the other hand, the Delta variant has emerged in Asia and has fastly disseminated across those continents (Figure 2).

4. Discussion

The State of Pará is located in the Brazilian Amazon and is the second largest state in Brazil, with a population of more than 8.7 million inhabitants. Belém is the capital and has about 1.5 million inhabitants (22). Pará comprises 144 municipalities, and we obtained 1,003 samples from 67 of them (Supplementary Figure S1).

Among these samples, we found a significant association between sex and clinical condition (female value of $p = 8.65e\text{-}08$; male value of $p = 0.008961$) and age and clinical condition (value of $p = 3.6e\text{-}10$). Delta, Omicron, and the non-VOC presented significant associations with clinical conditions. In contrast, despite being the most incident VOC, Gamma did not show any significant association with the clinical condition in our analysis. The P.1-like variants demonstrated massive participation in the Pará and Belém infections (Figure 2), appearing even when Delta and Omicron dominated the national epidemiological scenario, despite not having influenced the proportion of deaths caused by COVID-19 in the first (7.7%) and second wave (2.3%) in Pará However, according to Freitas et al. (23), Gamma may be related to the increase in deaths between young (20 to 59 years) and female patients in the State of Amazonas. They showed that the proportion of deaths in female patients increased from 34% in the first wave to 47% in the second wave of COVID-19 and that the number of hospitalizations between young patients was approximately 2.7 times higher in the second wave in comparison to the first one. Gamma also presented an association with the increase in deaths and severe cases among patients without previous comorbidities - it increased from 31 to 50% in the first to the second wave, respectively, after the emergence of the Gamma variant (23). These differences in the outcome between these two states may be explained by the higher incidence of Gamma in Amazonas since it emerged there and by the health system collapse.

According to FIOCRUZ data, in Brazil the first wave of COVID-19 started in April 2020, and the second wave in December 2020, this last one strongly influenced by the increasing Gamma variant cases (16). It is important to highlight that during the first wave, we observed a greater diversity of circulating variants worldwide and in Brazil. However, after the second wave, the epidemiological scenario was dominated by Delta, and Omicron in December 2020, July 2021, and December 2021, respectively (24). Data from FIOCRUZ showed that the Gamma variant was the first VOC to vastly dominate the epidemiological scenario in all Brazilian regions from 2020 until July 2021 (16). In the second semester of 2021, Delta caused a new wave of infections and hospitalizations that was followed by Omicron and its sub-variants (such as BA.1, BA.2, XBB.1., BQ., and FE) in December 2021, which quickly became the most detected strains in the country, reaching almost 100% of COVID-19 cases until May 2023 (25).

In contrast, our data has shown another pattern: non-VOC were responsible for most of the cases at the beginning of the pandemic. From December 2020/January 2021 onwards, Gamma became the most prevalent variant, followed by Delta and Omicron. Gamma (P.1) caused severe public health problems in the North region of Brazil as it was decisive in increasing the number of cases and death of COVID-19. This variant emerged in Amazonas (AM), a neighboring state of Pará (Figure 3), and presents a high transmission capacity and virulence (17, 26).

Gamma reached its highest level of transmission between January and June 2021, a period that coincides with the second wave of COVID-19 and with the highest number of hospitalizations and deaths caused by the coronavirus in Amazonas, Pará, in the rest of Brazil and in South America (27–29). According to De Souza et al., there was an increase to almost 3,000 daily deaths and hospitalizations during the second wave in Amazonas caused mostly by the P.1 variant (30). At this time, the Brazilian National Public Health System (SUS) almost collapsed - the occupancy rate of ICU (Intensive Care Unit) was 90%, leading to the most severe health crisis ever experienced in Brazil (31). Therefore, Gamma had a central role in the vast proportion of COVID-19 during its second wave in Brazil and South America since Brazil represented more than 55% of total cases and deaths from this continent (32). However, it is essential to highlight that Gamma infection itself may not have been the sole cause of this high mortality rate - other factors may have influenced it, such as the shortage of respiratory equipment and intensive care units.

In the global scenario, the emergence of the Gamma variant coincided with a transient increase in the number of cases and deaths, especially in January and February 2021. As shown in Figure 2, in 2021 this VOC did not have significant incidence in countries outside the Americas as it had in Brazil and in other countries such as Mexico and Bolivia with Gamma sub variants, like P.1.7.1 and P.1.10.2 (33). In this context, (33) reported that, between June and August 2021, Gamma variant stood out in Argentina. However, according to Bastos and colleagues, the Brazilian second wave significantly increased severe COVID-19 cases in Africa and the UK (34). Soon after, the number of reported cases rapidly decreased and increased in late March 2021, when Delta began to dominate COVID-19 cases worldwide (24).

The first cases of Gamma were officially detected in January 2021 (35, 36), but there is evidence of this variant in the state of Amazonas before the mentioned date. A few days later, the Instituto Leônidas & Maria Deane (ILMD/FIOCRUZ Amazônia), in collaboration with Fundação de Vigilância em Saúde do Amazonas (FVS-AM), released a technical note stating that Gamma would be derived from B.1.1.28. This note also declared that in December 2020, about 51% of the SARS-CoV-2 genomes in Amazonas were Gamma — this number increased to 91% in January 2021 (27). Although Gamma was officially identified in December 2020/January 2021 (35, 36), there were four sequenced samples from Belém, one in May 2020 and three in October 2020, that were infected with Gamma-like variants, according to

Pangolin, UShER and Scorpion databases. To the best of our knowledge, there are no studies before 2021 that discuss the transmission of Gamma-like variants in Brazil. Therefore, this fact may indicate that this variant emerged in northern Brazil several months before its identification and that Gamma and its sub variants (like P.1.4, P.1.7, P.4, and others) demonstrated their actual transmission capacity only at the end of 2020 The significant prevalence of Gamma in the North of Brazil may have delayed the entry of the Delta variant in the region. According to Figure 4, Gamma represented most cases in the Southeast region between March and July 2021 and in the North region between February and August 2021. Meanwhile, Delta was detected in the Southeast region in July 2021 and prevailed over the other VOCs from August to December 2021. In the North region, Delta appeared in August 2021 and presented the highest number of cases only from September 2021 until December 2021. It demonstrates that Gamma dominated the prevalence scores for a longer period, which may explain the late detection of Delta in the North region of Brazil (37).

In these pandemic times, next-generation sequencing has proven to be extremely useful in the fight against SARS-CoV-2 - it allows real-time identification of the virus variants. It provided strong evidence about its transmission dynamics. This genetic and epidemiological information is precious to direct sequenced-based public health surveillance and can be applied against other infectious diseases, such as chikungunya and malaria (38). Brazil's zoonotic pathogens sequencing and genomic surveillance capacity are conducted by only a few research institutions and universities capable of supporting Brazilian regions, using different types of sequencing technologies. So, a significant investment in genomic capacity is critical to empower surveillance in Brazil and would vastly improve global efforts to combat this pandemic and any new emergent pathogen.

5. Conclusion

This study shows the importance of investigating the SARS-CoV-2 transmission dynamics to understand the different prevalence patterns of its variants across global regions. Here, we focused especially on the state of Pará, in the North of Brazil. Our results show that Brazil represented a unique epidemiological status since it was where Gamma (P.1) emerged This variant had a higher transmission ability and presented an increased prevalence in South America compared to the rest of the world, especially during 2021 as seen in Figures 1, 2. From our sequencing, we also suggest an early case of Gamma-like infection in the state of Pará, indicating that this VOC may have appeared a few months before its formal identification. It reinforces the relevance of having a robust genomic surveillance service as it allows better management of the pandemic and efficient solutions to possible new disease-causing agents, like viruses, bacteria, and fungi.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

The studies involving human participants were reviewed and approved by 50865721.1.0000.0017. The patients/participants provided their written informed consent to participate in this study.

Author contributions

CP and AV: writing—original draft preparation. TN, RO, JA-P, GS, and GN: bioinformatic analysis. CP, AV, MB, LC, JP, CB-d-S, CS, LM, and PP: sample processing and sequencing. AV: statistical analysis. JV, RB, MSS, MBS, PFC, CGS, and ÂR-d-S: sample acquisition. WD and GO: funding. GO: study conception and supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1186463/full#supplementary-material

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An ecological study on reinfection rates using a large dataset of RT-qPCR tests for SARS-CoV-2 in Santiago of Chile

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Introduction: As the SARS-CoV-2 continues to evolve, new variants pose a significant threat by potentially overriding the immunity conferred by vaccination and natural infection. This scenario can lead to an upswing in reinfections, amplified baseline epidemic activity, and localized outbreaks. In various global regions, estimates of breakthrough cases associated with the currently circulating viral variants, such as Omicron, have been reported. Nonetheless, specific data on the reinfection rate in Chile still needs to be included.

Methods: Our study has focused on estimating COVID-19 reinfections per wave based on a sample of 578,670 RT-qPCR tests conducted at the University of Santiago of Chile (USACH) from April 2020 to July 2022, encompassing 345,997 individuals.

Results: The analysis reveals that the highest rate of reinfections transpired during the fourth and fifth COVID-19 waves, primarily driven by the Omicron variant. These findings hold despite 80% of the Chilean population receiving complete vaccination under the primary scheme and 60% receiving at least one booster dose. On average, the interval between initial infection and reinfection was found to be 372 days. Interestingly, reinfection incidence was higher in women aged between 30 and 55. Additionally, the viral load during the second infection episode was lower, likely attributed to Chile's high vaccination rate.

Discussion: This study demonstrates that the Omicron variant is behind Chile's highest number of reinfection cases, underscoring its potential for immune evasion. This vital epidemiological information contributes to developing and implementing effective public health policies.

KEYWORDS

COVID-19 pandemic, SARS-CoV-2, vaccines, variants of concern, reinfection

1. Introduction

The SARS-CoV-2 is responsible for the current global COVID-19 pandemic, which has resulted in more than 690 million infections and almost 6.8 million deaths worldwide (1). Even though governments and health authorities have implemented numerous measures to prevent or mitigate contagion, the most effective way to control the

pandemic is by reducing the number of susceptible individuals in the population. While natural infection by SARS-CoV-2 leads to robust humoral and cellular responses (2), most vaccines can also induce high titers of neutralizing antibodies (3). Various studies on the duration of humoral immunity after a natural infection have reported anti-spike IgG antibodies lasting up to 90 days (4), 6 months (5), and even 11 months (6) after the clinical recovery of the patient.

On the other hand, although immunity from vaccines is still being actively studied, it has been found to last at least 6 months (7). Additionally, new variants of SARS-CoV-2—compared to the ancestral virus—have emerged that can overcome patient immunity due to immune-evading mutations (4, 8). In effect, the Omicron variant, which was declared a variant of concern on November 26, 2022 by the World Health Organization (WHO) (9), has led to a surge of cases in different parts of the world (10, 11), albeit associated with reduced case fatality rates compared to other previous waves of infections, such as the Delta variant waves (12, 13). Omicron subvariants (including BA.1, BA.2, and BA.4/5) have higher immune evasion ability because of additional individual mutations in the S protein (14). Therefore, newer subvariants of Omicron, BQ.1 and BQ.1.1, characterized by increased resistance to neutralizing antibodies, are becoming predominant (15).

The decrease in immunity over time after infection or vaccination and the appearance of new, more elusive variants of SARS-CoV-2 are closely related to reinfections and can result in COVID-19 outbreaks. However, probably, many patients have not been correctly diagnosed, underestimating the official global COVID-19 statistics [revised in (16)]. While numerous isolated reinfections with new variants of SARS-CoV-2 have been reported (17–19), the frequency of reinfection continues to be the subject of the study.

The risk of reinfection has been reported in some studies. The first reports considered reinfections in individuals who previously had COVID-19, which were infrequent (20, 21). The extension of vaccination in different populations and the emergence of new variants during the pandemic became critical factors in determining the incidence of reinfection cases. For example, a study in Malaysia in 2022 reported that the reinfection rate was 6.6 times higher during Omicron circulation than other variants, regardless of the age group, while booster doses decreased the frequency of reinfection compared to sub-optimally vaccinated individuals (22). A study in Iceland in 2022 found that the reinfection rate was 15% among people aged 18–29 during the Omicron wave (23). Multiple reinfections have been reported in South Africa (24), although the incidence of reinfection is higher due to the low percentage of vaccination. However, there are no reports on the rate of reinfection in Chile during the Omicron surge. This country has one of the highest vaccination rates in Latin America [revised in (1)]. This study involves the RT-qPCR tests carried out during the pandemic in the Diagnostic Laboratory of the University of Santiago de Chile (USACH), which reached 578,670 samples of nasopharyngeal swabs (NPSs) from different communes of Santiago de Chile up to July 2022, to analyze the incidence of reinfections. Our results reaffirm the high capacity to evade the immune response presented by the Omicron variant compared to other surges of infections generated by different variants due to the higher prevalence of reinfections under the domain of this variant, even in a population with a complete vaccination schedule ~80%. This report suggests special attention to the increase in reinfection events since they could be related to possible risk groups or the appearance of new, more evasive SARS-CoV-2 viral variants in the population.

2. Methods

2.1. Study design, sample collection, and COVID-19 diagnosis

The Virology Laboratory of the Universidad de Santiago de Chile (USACH) performed 578,670 RT-qPCR diagnostic tests on nasopharyngeal swab samples (NPSs) from the Central Metropolitan Health Service (CMHS). The CMHS has a catchment population of approximately one and a half million and overseas two hospitals and 17 primary care centers in the western area of the Santiago Metropolitan Region (M.R.). Of these 578,670 RT-QPCR tests, 345,908 corresponded to patients (including positive and negative diagnoses), with 44,181 positive tests from 43,638 patients infected. Diagnostic testing was done from 1 April 2020 to 31 July 2022. The RT-qPCR tests of the M.R. began in the first few days of March 2020. In addition, total RNA from 250 µl of NPSs was extracted as previously described using the Total RNA purification Kit (Norgen Biotek Corp) (25). The detection of SARS-CoV-2 was carried out using the ORF1ab gene probe from TaqMan™ 2019nCoV Assay Kit v1 (Thermo Fisher Scientific, Reference code. A47532) as previously reported by our group (26). We included samples with matching identification numbers to accurately identify RT-qPCR tests from the same patient across different periods. These tests were then grouped based on the Chilean ID number and the date of sample collection. Any discrepancies in dates were individually resolved by cross-referencing the sample ID number with the sample collection date. In case of sex discrepancies, these were manually rectified. Considering the duration of this study extended beyond 2 years, the age reported corresponds to the patient's age at the time of the first test. This methodology provided unambiguous identification of all tests conducted per patient and facilitated the tracking of respective reinfections.

2.2. Analysis of infections and reinfections

Five surges were registered in Chile during the study period, which was defined using the data from the M.R. The onset of each wave was determined using the moving average of daily cases, with a window of 7 days to decrease day-to-day variability. The start of each surge was defined when the number of new cases exceeded three standard deviations compared to the previous 3 days. This change in standard deviation coincided with the rate change (first derivative) of the number of cases. The difference in standard deviation could not always be used to determine the end of each surge because it did not always coincide with the change in the slope of the number of points. Moreover, in some cases, the number of points at the end of the surge reached a different level than at the beginning. For those reasons, the end of the wave was defined as the date when the rate of change in the number of cases was closer to zero for at least five consecutive days. Thus, five waves were defined and are shown in Table 1. Reinfections

TABLE 1 Waves of infections in Chile.

	First wave	Second wave	Third wave	Fourth wave	Fifth wave
Start	22 April 2020	1 December 2020	16 September 2021	25 December 2021	2 May 2022
Final	1 August 2020	1 August 2021	5 December 2021	10 April 2022	31 July 2022
Duration	101 days, 3.4 months	243 days, 8.1 months	80 days, 2.7 months	106 days, 3.5 months	90 days, 3.0 months

were identified by analyzing wave pairs, with the first infection occurring during the first wave of the couple and reinfection in the second wave. In addition, the second positive test must be at least >90 days after the first positive diagnosis, as previously reported in reinfection studies (27) and according to the Pan American Health Organization (PAHO) criteria (28) and Centers for Disease Control and Prevention (CDC) in 2023 (29). The percentage of reinfection was calculated as the number of reinfections over the number of positive cases in the first surge of the pair. The incidence of reinfection was computed as the number of reinfected patients divided by the cumulative number of persons-day at risk. This follow-up time was calculated as the sum of days from the first positive test to the second positive test or the end of the last surge of the pair. Confidence intervals were computed at 95%.

To study reinfections independently of the surges (overall reinfection in the study period), a dataset was built with the only criterion being the interval between positive tests >90 days. The percentage of reinfection was calculated as described above. For the incidence rate, the follow-up time for patients with only one positive test was calculated up to the end of the study.

2.3. Public data sources

National PCR data from M.R. were obtained from Ministerio de Ciencias Tecnologia Conocimiento e Innovacion (30). SARS-CoV-2 variants were obtained from the GISAID platform (https://gisaid.org/) and Genomic Surveillance Program from Instituto de Salud Publica de Chile (31). Data were analyzed with custom software written in Python.

2.4. Ethics

This study was authorized by the Ethics Committee of the University of Santiago of Chile (No. 226/2021) and the Scientific Ethical Committee of the Central Metropolitan Health Service, Ministry of Health, Government of Chile (No. 370/2021), following Chilean legislation.

3. Results

3.1. Dynamics of epidemiological surges

From 1 April 2020 to 31 July 2022, the COVID-19 diagnostic laboratory at the Universidad de Santiago de Chile (USACH) conducted a total of 578,670 RT-qPCR tests. These tests, performed on nasopharyngeal swab samples, were referred by the Central Metropolitan Health Service. The number of tests correspond to 345,908 distinct individuals. Of these individuals, 43,658 were diagnosed positive for COVID-19, accounting for 44,181 of the positive RT-qPCR tests conducted.

During the period under review, the quantity of RT-qPCR tests conducted by USACH, represented in red, exhibited significant variability when compared to the entire Metropolitan Region of Santiago, Chile (M.R.), represented in black, as shown in Figure 1A. While the data from the M.R. also demonstrated some degree of weekly variability, it was markedly less pronounced.

By February 2022—the fourth wave—USACH testing capacity peaked at 2,000 tests per day, and by the end of the fifth wave had dropped to 500 tests per day. In the M.R., there was a significant shift in the volume of RT-qPCR tests performed daily between the fourth and fifth waves of the pandemic. During the fourth wave, the M.R. reached a peak of 40,000 tests per day, but this number nearly halved to ∼20,000 tests per day during the fifth wave. This represented a considerable reduction of nearly 50% in regional testing at the national level (Figure 1A). The volume of positive cases began notably high at the onset of the pandemic, during the first wave. However, this number subsequently experienced a substantial surge during the fourth wave. This pattern of case incidence was observed similarly in the USACH and the M.R. (Figure 1B). The increased number of infected patients is directly related to increased positivity (defined as positive cases over the total tests analyzed) during the study period. In the first wave, the positivity reached 0.7 for both the overall M.R. testing and the USACH tests. This positivity varied during the pandemic, reaching its lowest value in the third surge, with a relative value of 0.05 for M.R. and USACH. For the fourth surge, the positivity came to 0.4, while, by the end of the fifth surge, it reached 0.25 in USACH data, while for the rest of the wave, the M.R. was close to 0.3 (Figure 1C). The behavior of the results obtained by the USACH was similar to those reported by the total M.R. during the pandemic. This data can closely represent the reinfection incidence in the greater Santiago area.

In April 2020, corresponding to the first wave, the viral Ct values associated with the RT-qPCR diagnosis and viral load were between 22 and 25 (Figure 1D). As the pandemic unfolded, these Ct values increased and reached a maximum close to 27 for the fifth wave of infections ($R^2 = 0.833$, Supplementary Figure 1), indicating a decrease in the SARS-CoV-2 viral load during the pandemic (32). During the study and within the total number of tests analyzed (578,670), 247,542 patients underwent one RT-qPCR test, 56,007 patients underwent two tests, 42,217 patients underwent more than three tests, and 28 patients underwent up to 45 RT-qPCR tests during the study period (Supplementary Figure 2A). In addition, patients with multiple tests were outnumbered by patients with only one test; hence, the number of tests per patient had a mean of 1.67, a median of 1.0, and a mode of 1.0 test, respectively.

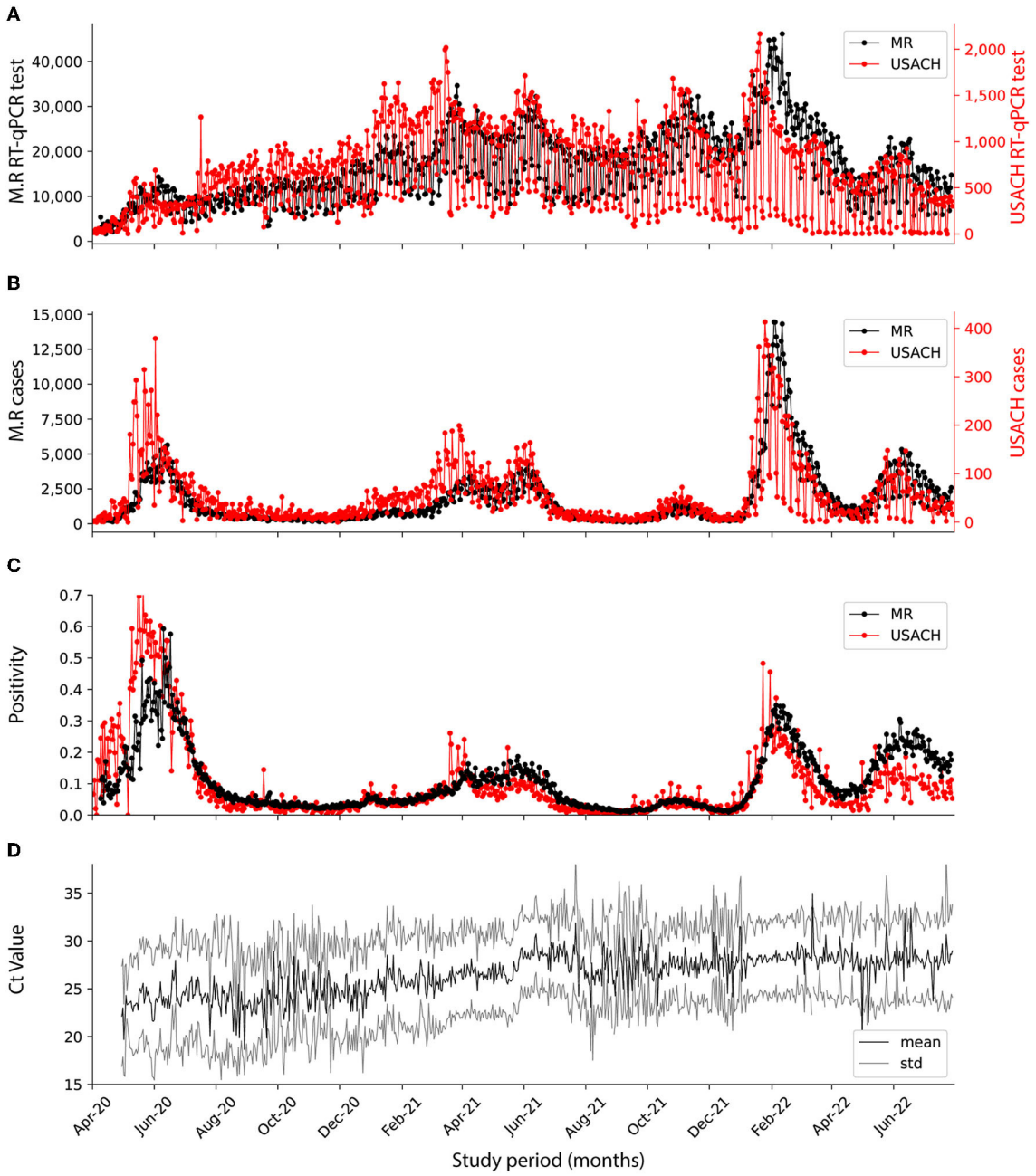

FIGURE 1
Dynamics of SARS-CoV-2 pandemics in USACH and M.R. at the analyzed period. Comparison of USACH (red) and M.R. (black). **(A)** Number of daily RT-qPCR tests. **(B)** Number of daily cases. **(C)** Daily positivity. **(D)** Daily mean Ct for positive cases (black) with standard deviation (gray).

Of the total patients examined, 47.7% were women and 45.3% were men, with respective positivity rates of 40.6 and 37.8% (refer to Supplementary Figure 2B). An anomalously high count was observed in patients whose gender could not be ascertained. Throughout the analysis period, the average and median ages of the male patient cohort were 39.4 and 37 years, respectively (Supplementary Figure 2C). In contrast, the female patient cohort had an average age of 41.2 years and a median of 39 years (Supplementary Figure 2D). Among the men who tested positive for COVID-19, the mean age was 39.2 years with a median of 36 years (Supplementary Figure 2E), while for women who tested positive, the average and median ages were 40.3 and 38 years, respectively (Supplementary Figure 2F).

3.2. Reinfections

To detect potential SARS-CoV-2 reinfections, we focused our analysis on positive cases within paired surge periods. Cases that surfaced during the interim period between surges were not

included in this analysis. Reinfections were identified if a patient tested positive during the initial wave of the pair, followed by a second positive test during the subsequent surge. Furthermore, these two test results needed to be spaced apart by more than 90 days, a criterion established based on precedents set in several similar studies (33, 34). With these criteria, 261 reinfections were detected. The period between the two positive tests ranged from 94 to 788 days, with an average duration of 371.6 days and a median of 335 days (Figure 2A). The histogram demonstrating the course of these intervals presents a multimodal distribution due to encompassing intervals from all surge periods. Upon dissecting the data, individual histograms depicting reinfections during the second, third, fourth, and fifth surges reveal diverse populations with varying reinfection intervals (Figures 2B–E, Supplementary Table 1). The shortest reinfection interval occurred in patients who were infected in the first and then in the second wave, with an average time of 294 days before reinfection (Figure 2B). The most extended period between initial infection and reinfection was observed during the fifth wave, with certain patients experiencing over 700 days before a subsequent infection occurred (predominantly with the Omicron variant in the fifth wave). Figure 2 displays histograms of reinfection intervals for each pair of surges, while Supplementary Table 2 provides additional descriptive statistics regarding these durations. Interestingly, the duration between infections tended to be shorter in men (mean = 341.9 days, median = 317.5 days) compared to women (mean = 381.7 days, median = 347 days). The reinfection time intervals for patients in all wave pairs are shown in Supplementary Figure 3.

Figure 3 shows the five waves highlighted in gray stripes (Figure 3A) and the reinfection intervals for the 261 patients identified, sorted by date of infection (Figure 3B). For patients infected in the first wave, the highest reinfection rates occurred when the reinfection occurred in the second and fourth waves [0.41 positive tests per 100,000 people (95% CI: 0.213–0.615) and 0.63 (95% CI: 0.43–0.821), respectively, Table 2]. During the second wave, Gamma and Lambda were the most prevalent variants, and during the fourth wave, Omicron was the most prevalent variant (Figure 3D). By the end of the second wave, more than 60% of the Chilean population presented a complete vaccination scheme with two doses (Figure 3C). For patients infected in the second wave, the highest reinfection rate occurred during the fourth wave [2.08 positive tests per 100,000 people (95% CI: 1.687–2.473), Table 2], where the most abundant variant was Omicron (98%, Figure 3D). At the end of the fourth wave, more than 60% of the population had taken two doses and a booster dose (Figure 3C). For patients infected in the third and fourth waves, the highest reinfection rates occurred during the fifth wave [waves 3–4: 0.97 test per 100,000 people (95% CI: 0–2.1), waves 3–5: 1.34 (95% CI 0.327–2.354), and waves 4–5: 1.36 (95% CI: 0.823–1.886), Table 2]. During this wave, the Omicron variant was 100% predominant, and close to 50% of the population had a fourth vaccination, corresponding to the second booster (Figures 3C, D). Overall, the highest incidence rates of reinfection occurred between waves 2–4, 3–4, 3–5, and 4–5, where Omicron was the most abundant variant.

On the other hand, with 100% predominance of the Omicron variant for the fifth surge, close to 50% of the population had a fourth vaccination, corresponding to the second booster. In addition, two patients had a triple infection (Figure 3B). For one,

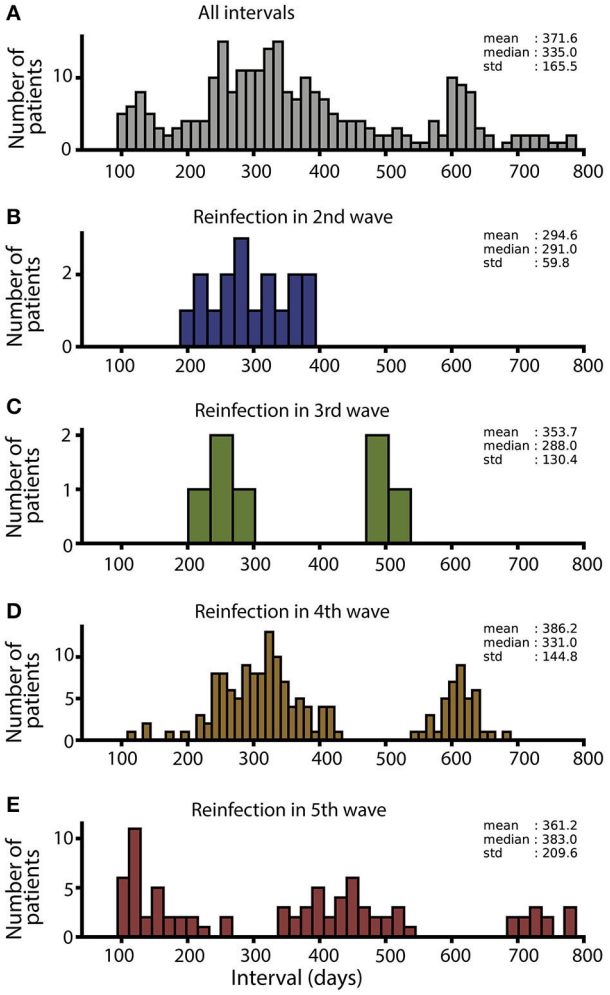

FIGURE 2
Duration of interval (days) between infections. **(A)** Interval histogram for all reinfections. **(B)** Interval histogram for the 17 reinfections that occurred during the second surge. **(C)** Interval histogram for the seven reinfections that occurred in the third surge. **(D)** Interval histogram for the 159 reinfections that occurred during the fourth surge. **(E)** Interval histogram for the 82 reinfections that occurred in the fifth surge.

the last infection occurred during the fourth surge and another on the fifth surge, with a predominance of the Omicron variant. However, triple infection only represented 0.8% of all reinfections analyzed in this study.

Regarding sex differences in the incidence of reinfection, we observed notable differences. Women presented a higher rate of reinfection than men, with 62.8 and 36.8%, respectively (Figure 4A). There is a bimodal age distribution for both sexes; the mean age of reinfected men was 26.6 and 56 years (Figure 4B). While for women, the means were of 30.0 and 56.3 years, respectively. However, women have more reinfections at lower ages than men (Figure 4C). The average period of reinfection in men was 347.35 days, while in women, 385.49, indicating that women in Chile last longer before becoming infected again (Supplementary Figure 4). To quantify these differences, the proportion of patients with lower and higher ages for

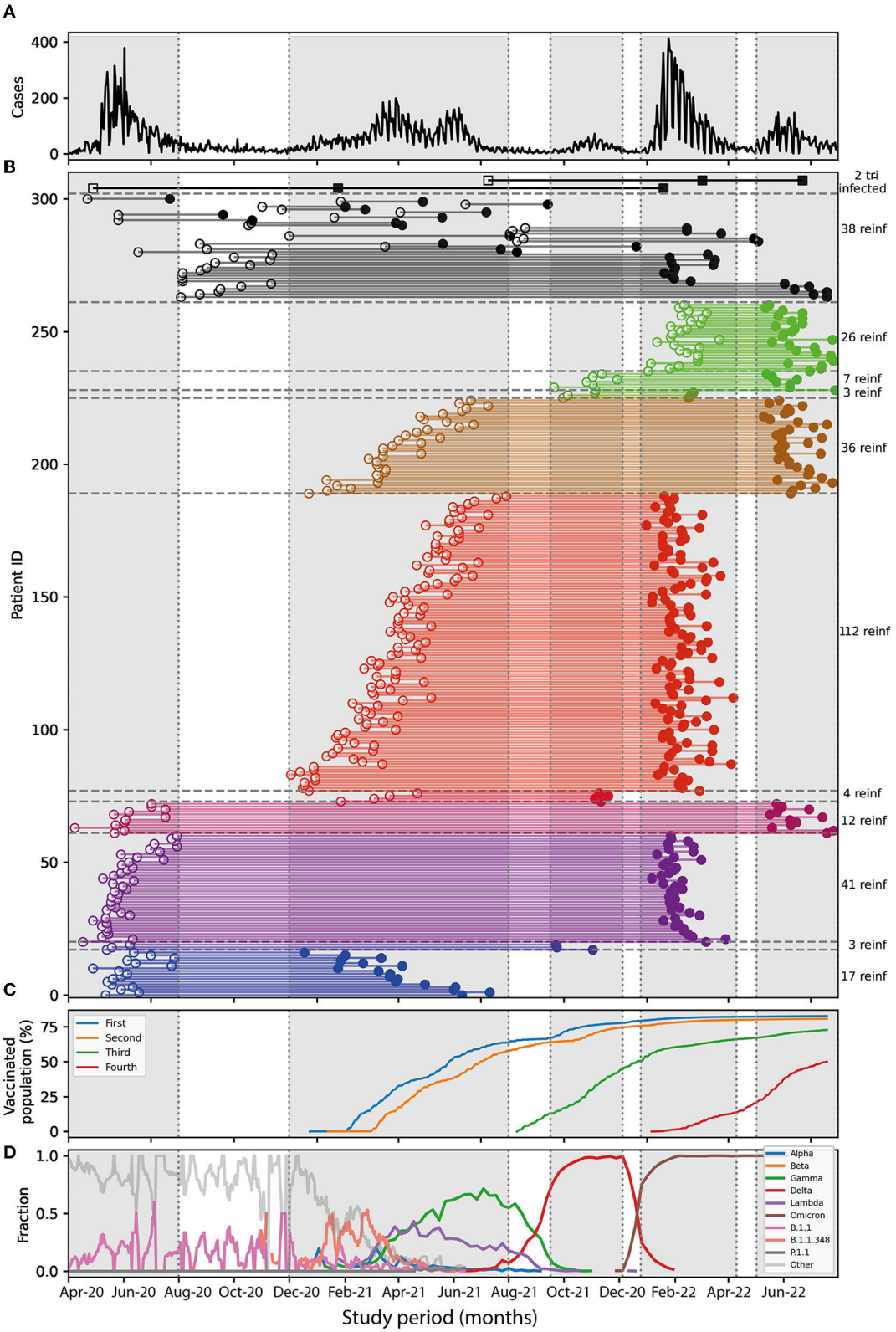

FIGURE 3
Reinfections between surges. **(A)** Daily cases over time, each surge is enclosed in gray rectangles. **(B)** Interval duration for each reinfected patient, showing first (open circle) and second positive test (filled circle) connected by a colored line representing surges 1–2 (blue), 1–3 (dark blue), 1–4 (purple), 1–5 (magenta), 2–3 (orange), 2–4 (red), 2–5 (golden), 3–4 (pistachio), 3–5 (green), and 4–5 (light green). Reinfections not falling into the surges are represented by black circles. The two cases of tri-infections are shown in squares. To the right, is the number of reinfections for each group. **(C)** Percentage of Chilean population vaccinated with fist dose (blue), second dose (orange), booster dose (green), and second booster (red). **(D)** Time course of the different SARS-CoV-2 variants detected by genome sequencing in Chile. The percentages of the predominance of the variants are the following: First wave: Lambda 48%, others 52%. Second wave: Gamma 62%, Lambda 25, and Alpha 2%. Third wave: Delta 98%, Lambda 0.5%, Gamma 0.25%, and Alpha 0.1%. Fourth wave: Omicron 97% and Delta 3%. The fifth wave, Omicron 100%.

TABLE 2 Reinfections and incidence rate for all pairs if surges.

Pairs of surges	Number of reinfections	Number cases	% Reinfection	CI % reinfection	Days follow-up	Inc. Day/ 100,000 hab	CI Inc. day
1–2	17	9,723	0.18	(0.092, 0.258)	4,102,245	0.41	(0.213, 0.615)
1–3	3	9,723	0.03	(0.0, 0.066)	5,329,306	0.06	(0.0, 0.121)
1–4	41	9,723	0.42	(0.293, 0.55)	6,551,920	0.63	(0.43, 0.821)
1–5	12	9,723	0.12	(0.054, 0.193)	7,642,998	0.16	(0.066, 0.248)
2–3	4	14,643	0.03	(0.001, 0.054)	3,545,956	0.11	(0.0, 0.226)
2–4	112	14,643	0.77	(0.624, 0.906)	5,383,844	2.08	(1.687, 2.473)
2–5	36	14,643	0.25	(0.166, 0.326)	7,029,365	0.51	(0.341, 0.683)
3–4	3	1,914	0.16	(0.0, 0.334)	307,875	0.97	(0.0, 2.1)
3–5	7	1,914	0.37	(0.095, 0.636)	522,114	1.34	(0.327, 2.354)
4–5	26	11,119	0.23	(0.144, 0.324)	1,919,446	1.36	(0.823, 1.886)

each group and the amplitudes from each component of the Gaussians were used to obtain the ratio of low/high patients ages. For male patients with positive reinfections, the ratio of low/high ages was ∼1.5. However, women reinfected have a ratio of 3.0, meaning a greater number of reinfected young women patients. The most significant differences in the number of men and women occurred in reinfections in the fourth and fifth surges (Supplementary Figure 5A). Higher numbers of women of low age were reinfected between surges 2–4, 2–5, and 4–5, while for surges 1–4 and 1–5, the increase was at all ages (Supplementary Figure 5B). Men were also reinfected at young ages but with fewer events in these last two surges. These data, therefore, indicate that the highest prevalence of reinfection occurred during the predominance of the Omicron variant in women, even in a scenario where the Chilean population had high vaccination rates.

3.3. PCR cycle threshold (Ct) value in a reinfection event

The cycle threshold (Ct) value during the diagnosis of COVID-19 is closely related to the viral load of the infected patient (35), the risk of mortality during infection (36), and a greater capacity for the transmission and generation of contagion outbreaks (37). We evaluated the relationship between the Ct values of the second diagnosis (Ct2) against the initial infection (Ct1) to determine whether this reinfection was associated with a lower or higher viral load. Ratios of Ct2/Ct1 values from all intervals show a multimodal distribution with a mean of 1.18 and a median of 1.14 (Figure 5A). Ratios from patients reinfected in the second surge showed a higher mean and median (1.25 and 1.32, respectively, Figure 5B). In contrast, patients reinfected in the third and fourth surges have means closer to the value of overall reinfection patients Ct2/Ct1 ratio (Figures 5C, D). Patients reinfected in the fifth surge show a mean and median closer to 1, indicating similar Ct values in the second infection (Figure 5E). Since the histograms of the distribution of ratios show multimodal components, we analyzed each pair of surges. Average ratios higher than one were found in all pairs of waves, except surges 2–3, which are lower to one, and surges 4–5 are closer to one (Supplementary Figures 6, 7, Supplementary Table 4). In addition, all the pairs of waves showed a high percentage of values >1, including surges 4–5 (Supplementary Figure 7B). These results show that the behavior of ratios in surges 4–5 is unique and not shared with the other reinfections events that occurred in the fifth surge. Overall, these data indicate that the Ct2 values were higher in the second contagion in a population of patients, so the viral load in a reinfection event was mainly lower.

3.4. Overall reinfection analysis

In addition, we use an alternative analysis of reinfections independent of the date of occurrence without restricting surge dates. With this criterion, we found 283 patients with intervals between infections longer than 90 days, which occurred within surges and inter-surge periods. The cases that do not fall within waves are plotted in Figure 4B below the intervals between waves. The mean duration of all 283 intervals was 372.5; the median was 336, and the standard deviation was 171.4. The results of this cohort show a reinfection rate of 1.52 (95% CI: 1.34–1.70) per 100,000 inhabitants. These values are within the range obtained for the analysis using only reinfections between surges.

4. Discussion

Previous studies have documented the rate of reinfection processes in different countries and localities concerning the appearance of new variants of SARS-CoV-2 and various vaccination schemes. Gazit et al. (38) reported that in Israel, people who were naturally infected and then had a dose of vaccine significantly decreased the risk of reinfection by the Delta variant compared to people infected without any dose. A similar effect was determined by Malhotra et al. (39), in New Delhi, India, who indicated that unvaccinated patients had a 12.7% chance of reinfection compared to 1.6% of patients with two doses of vaccine in a cohort of 4,978 health workers. While Medić et al.

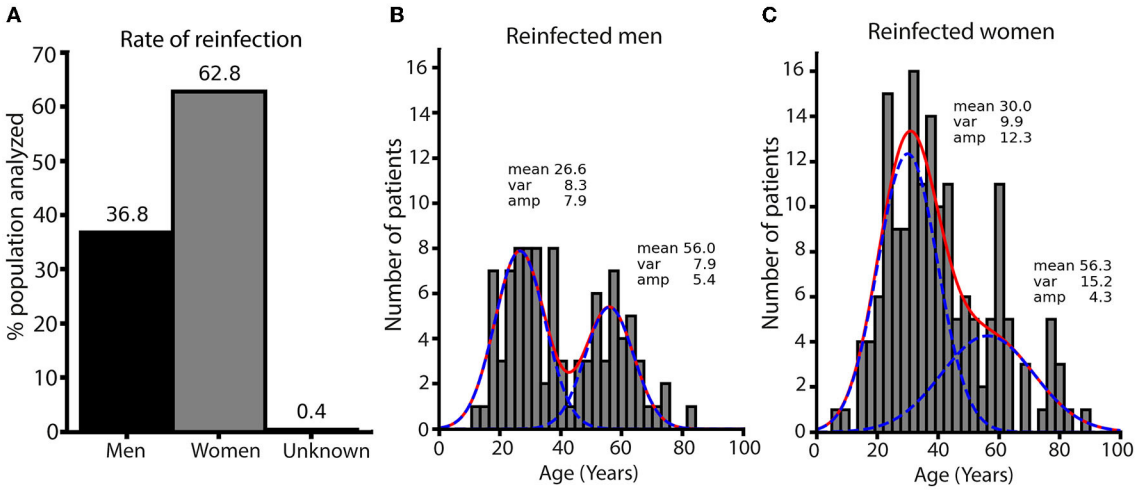

FIGURE 4
Distribution of age and sex in reinfected patients. **(A)** Percentages of reinfected men and women patients. The numbers above each bar are percentages. **(B)** Histogram of the age distribution for reinfected men. The red line is the fit to two Gaussians, and the blue lines are each Gaussian. **(C)** Histogram of the age distribution for reinfected women. Lines are the same as for men. Both histograms were built with the same range and number of bins to allow the comparison of the count. Fitted parameters are on the top of each Gaussian distribution. The ratio of low/high age calculated from fitted amplitudes is 1.56 for men and 2.82 for women.

(40), in a study carried out in Vojvodina, Serbia, highlighted that reinfections occurred mainly in women between the age of 30 and 39 years and 95% of the cases in patients without vaccination, while an event of reinfection occurred in 0.16% of people with complete vaccination (two doses) plus a booster dose. Reinfections increased significantly with the Omicron variant but with less severity than the Delta variant (40) when a similar situation was observed in South Africa (24). However, there are no reports on the analysis of the rate of reinfection concerning the variants, vaccination, and epidemiology in Chile. This study corresponds to a retrospective and descriptive analysis of reinfection events in a western zone of Santiago de Chile during the five surges of infections that have affected the country. A total of 578,670 tests were analyzed, corresponding to a total of 345,908 patients, of which 43,658 were diagnosed positive for COVID-19, at the laboratory of the University of Santiago de Chile (USACH), between 1 April 2020 and 31 July 2022. Reinfection events were considered >90 days after the first positive diagnosis, although viral persistence events have been reported even after 380 days (41), which are unusual and isolated cases. At the same time, even some reinfection criteria of ≥40 days have been reported (42). It was found that most of the reinfection events occurred during the Omicron propagation wave, where up to 0.772% of the total number of infections in the period was recorded. In comparison, the lowest prevalence of reinfection was recorded in the third surge, with the majority of the Delta variant. Although the Omicron variant is highly evasive of the immune system (43), our data on the reinfection rate were lower than that reported by the other studies. In this sense, e.g., the study by Nguyen et al. (44), in the city of Marseille, reported up to 6.8% reinfection, with an inclusion criterion of 90 days from the first positive diagnosis, where the Omicron variant was responsible. This difference can probably be explained due to the high vaccination rate in Chile (close to 80% with a full two-dose vaccination schedule and 70% with the first booster dose) when facing a surge of contagion from Omicron. Chile is one of the countries with the highest vaccination rate per 100 inhabitants in Latin America (45) and one of the countries that implemented vaccination the fastest worldwide (46). The policies implemented by the Government of Chile with the Ministry of Health generated a low incidence of reinfection. Even though, our data show a higher rate of reinfection in younger women than men, similar to finding reported by other studies (47, 48), even with an inclusion criterion of reinfection of >90 days after the first positive diagnosis. This could be reflected in less disease severity in contagion outbreaks since women are less likely to develop the severe disease than men (47). This higher rate of reinfections in women can be explained by women's more significant number of interpersonal contacts (49).

Regarding the number of intervals of days for reinfection to occur, an average of 358 days was found. This is similar to previously reported studies; for example, by Özüdogru et al. (50), who indicated an average of 361 days for reinfection, and Wilson (51), with an average of 343 days. The data obtained by the University of Santiago (USACH) were closely related to what was reported by the rest of the M.R., even up to the fifth wave of infections in July 2022. Therefore, since M.R. involves 40% of the total population of Chile, we could suggest that our data could closely represent the behavior of the rate of reinfection in the whole of Chile. In this sense, since patients' clinical history is not public, we speculate that reinfections would tend to represent a less severe disease (52–54) and lower transmissibility (55). In addition, the low viral load observed in patients reinfected by each contagion may be related to the efficacy of mass vaccination in Chile or simply to the immunity conferred after a natural infection by Omicron, as previously seen (24).

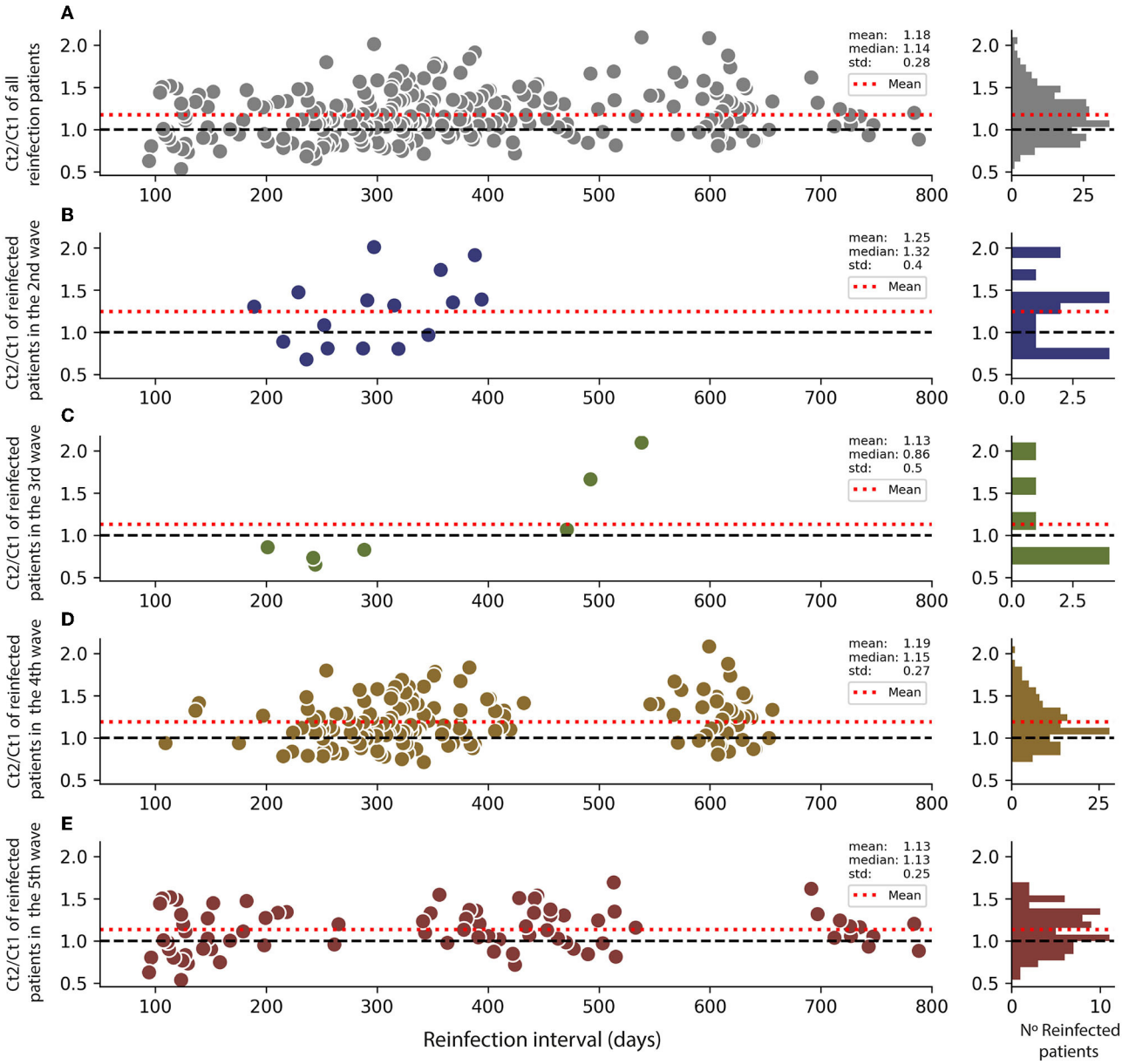

FIGURE 5
Variations of viral load in reinfected patients in different surges. Ct2 indicates the reinfection event, and Ct1 is the first infection. Ct values are the cycle threshold in RT-qPCR assay. **(A)** Ratio Ct2/Ct1 for all reinfection in the function of the reinfection interval. The red line shows the ratio average. A histogram of interval distribution is shown to the right. **(B)** Ratio Ct2/Ct1 for all reinfection during the second surge. **(C)** Ratio Ct2/Ct1 for all reinfection during the third surge. **(D)** Ratio Ct2/Ct1 for all reinfection during the fourth surge. **(E)** Ratio Ct2/Ct1 for all reinfection during the fifth surge.

Our study has some limitations that are important to highlight. First, it did not differentiate the involvement of reinfection after natural, hybrid, or vaccination-only immunization between the RT-qPCR tests of the patients analyzed. These different ways of generating immunity in a patient could affect a reinfection event since not all yield the same protection capacity (56), resulting in more or fewer days between one infection or another. Our data do not consider cases identified by rapid antigen tests. These tests were used massively during the waves of Omicron. This reduced the proportion of reinfection cases determined by RT-qPCR in our dataset. Rapid antigen tests in Chile represent 18% of the total tests carried out during the pandemic (57); therefore, our data may be underrepresented. However, our results show that the highest reinfection rate occurred with Omicron's arrival, which is consistent with other reports (40, 50).

On the other hand, the relationship between the vaccination rate and the number of reinfection events occurring in a period was only descriptive, because there is no information on the vaccination status of the study patients. At the same time, there was no discrimination between the different types of vaccines administered (58), which generated different degrees of protection efficacy against a new SARS-CoV-2 infection. Finally, no difference

was made between the Omicron subvariants, which can cause reinfections with different time intervals (59). However, despite these limitations, our study indicates a rate of reinfection in Chile, similar to research from other countries, supported by extensive RT-qPCR test data.

This is the first retrospective report on the prevalence of reinfection in Chile, with the largest dataset of patients analyzed to date, giving our analyses greater robustness. These data could be helpful and of particular interest to government authorities for continuously implementing public health policies to control the pandemic and for epidemiological groups with a greater predisposition to reinfection. Although reinfection seems to be a rare process, there is a probability that it can occur, even in populations with a high vaccination rate. Our study demonstrates the need for epidemiological monitoring of SARS-CoV-2 since an increase in reinfection rates in a locality could account for the appearance of new, more transmissible, and evasive variants.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the University of Santiago of Chile (No. 226/2021) and the Scientific Ethical Committee of the Central Metropolitan Health Service, Ministry of Health, Government of Chile (No. 370/2021), following Chilean legislation. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

CA-C and FER-L: conceptualization. PR and CA-C: methodology and data curation. AMS, FER-L, and MI: validation. PR: formal analysis. VB, AI-M, MV, RL, EV-V, and AM-T: investigation. CA-C, CB-A, MI, FER-L, and AMS: resources. CA-C and CB-A: writing—original draft preparation. CA-C, LM, FER-L, PR, CB-A, and VB: writing—review and editing. DV and MI: visualization. CA-C, FER-L, and AMS: supervision. FER-L and AMS: project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1191377/full#supplementary-material

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Willingness and hesitancy of parents to vaccinate against COVID-19 their children ages 6 months to 4 years with frail conditions in Italy

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Background: In Italy, on December 2022, COVID-19 vaccination was recommended for children aged 6 months-4 years with frail conditions and for those healthy. The purposes of the survey were to understand parental willingness and hesitancy toward COVID-19 vaccination of children with frail conditions in Italy and related influencing factors.

Methods: A cross-sectional survey was performed among 445 parents with a child aged 6 months-4 years with frail conditions who attended a teaching hospital and a public hospital randomly selected in the city of Naples, Italy.

Results: Almost one third (29.9%) were willing to vaccinate their frail children against COVID-19, whereas 21.3% were uncertain, and 48.8% did not intend to vaccinate. Parents with a higher level of perception that the vaccine is useful and safe and those who had received information by pediatrician were more likely to be willing to vaccinate their child. The mean Parent Attitudes About Childhood Vaccines (PACV-5) score was 3.4, with 13.5% of parents high-hesitant for the COVID-19 vaccination for their child. Parents with a higher COVID-19 vaccine-related safety concerns, those who have delayed at least one shot of a recommended vaccine for their child, and those who did not have received at least three doses of the vaccine against SARS-CoV-2 were more likely to be high-hesitant.

Conclusion: The survey findings have important implications for designing interventions to increase willingness and to reduce hesitancy for COVID-19 vaccine among parents of frail children aged 6 months-4 years in Italy.

KEYWORDS

COVID-19, frail children, hesitancy, Italy, vaccination

1. Introduction

The novel coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), determined over 767 million confirmed cases and 6 million deaths worldwide, whereas in Italy the total number of cases was more than 25.7 million and over 190 thousand people died (1). It is interesting to observe that the previous

SARS-CoV-1 epidemic caused more than 8 thousand cases and 774 deaths worldwide (2), while in Italy only 4 non-fatal cases were reported (3).

It is well-known that COVID-19 vaccination has been the most important measure to mitigate the spread of the disease. The use of the mRNA COVID-19 vaccines in children ages 6 months to 4 years has been recommended by the European Medicines Agency on October 19, 2022. In children from 6 months to 4 years of age, Comirnaty can be given as primary vaccination with three doses (of 3 micrograms each) and the first two doses are given 3 weeks apart, followed by a third dose at least after 8 weeks, whereas Spikevax with two doses (of 25 micrograms each), 4 weeks apart (4). In Italy, on December 9, 2022, the Ministry of Health recommended the vaccination with Comirnaty for children with high frail conditions and for those healthy (5). However, to date, no vaccination campaign among this age group has been implemented. This is a public health concern as of May 3, 2023, among the 26 million confirmed individuals infected by SARS-CoV-2 in Italy, 785 thousand cases have been reported among children ages 6 months to 4 years and there have been over 14 thousand hospitalizations (6).

These children, mainly those with frail conditions, are at increased risk of SARS-CoV-2 infection and this is an important cause of morbidity and mortality (7–9). So, is even more important in them compared to the healthy population, to achieve an adequate coverage of the COVID-19 vaccination in order to reduce the burden of the disease and to control the transmission. There is overwhelming evidence supporting that parents are the decision-makers regarding the vaccination for their children and the attitudes are key determinants in the vaccine acceptance and uptake. Cross-sectional studies have investigated the willingness of parents of children ages 6 months to 4 years in many places (10–20) but limited information was available regarding children with frail conditions (21–23). Such data are paramount to COVID-19 vaccine uptake rates. Therefore, to address these gaps in the literature, the two purposes of the present survey were to understand the willingness and hesitancy of parents toward COVID-19 vaccination of their children ages 6 months to 4 years with frail conditions in Italy and to identify the influencing factors.

2. Materials and methods

2.1. Setting and participants

This cross-sectional survey was conducted between February and April, 2023, as part of a larger project, which aimed to investigate COVID-19 vaccination perceptions and behaviors of different groups in Southern Italy (24–33). The source population was all parents who had a child aged between 6 months to 4 years with frail conditions who attended a teaching hospital and a public hospital randomly selected in the city of Naples, Southern part of Italy, from January 1, 2023 to April 5, 2023.

The sample size was determined by using a single proportion formula by assuming the prevalence of parents' willingness of COVID-19 vaccination for their children of 50%, with a 95% confidence interval, a 5% margin of error, and then adding a 10% non-response rate (34). Thus, the minimum total number of participants was 427.

2.2. Procedures

The parent of each child was contacted by trained investigators via telephone from Monday to Friday in morning, afternoon, and evening, to ensure that either working or not working parents were reached, or was approached while waiting for their child's clinical appointment. At the beginning of the interview, the research team illustrated at the respondent parent the survey objectives and their rights as research participants, that the time commitment for completion of the survey was 10 min, that the participation was voluntary, that no subject identifiers were recorded, that the data would be maintained anonymously, and that they may freely stop answering the questionnaire at any point. If the parents had more than one frail child within this age range, they were requested to respond for the child whose age was closest to 6 months. All parents gave verbal informed consent prior to the interview. Participants received no incentives whatsoever for completing the survey. A maximum of 3 attempts were made to contact the parents via telephone. If the individual selected was unreachable he was not replaced.

The study protocol and procedures were approved by the Ethics Committee of the Teaching Hospital of the University of Campania "Luigi Vanvitelli" (protocol number 0001816/i).

2.3. Survey instrument

Data were collected using a structured questionnaire which was modified and adapted from those used by some of us enrolling different populations (24–33). A pilot study was conducted with 10 participants to assess the survey's comprehensibility and feasibility of the questionnaire. Since, no modifications were made, the parents were included in the final sample.

The questionnaire, uploaded as Supplementary material, entailed the following three major sections: (1) socio-demographic and general characteristics of the respondent (i.e., gender, age, partnership status, education background, employment status, history of chronic medical condition, personal and family history of having been infected by SARS-CoV-2, and personal and family history of COVID-19 vaccination) and of the child (i.e., gender, age, birth order, and chronic medical condition); (2) attitudes toward the COVID-19 infection (perceived severity of COVID-19, perceived risk for the child of being infected by SARS-CoV-2) and the COVID-19 vaccine (perceived utility and safety). For these four items the responses were with a 10-point Likert scale with a score ranging from "1: Not at all" to "10: At all", and for two items, whether they delayed or refused at least one shot of vaccinations for their children, with "yes/no/do not know" responses. Participants were also asked their willingness to vaccinate their child and the response options were "yes", "no", and "uncertain" and they were then asked to select from a provided list with 12 options of response one or more reasons for their willingness or unwillingness or uncertain to vaccinate their child. Participants' vaccine hesitancy was measured based on the 5-item version of the 15-item Parent Attitudes About Childhood Vaccines (PACV) Survey Tool, with 5-point Likert categorical responses (strongly disagree, disagree, uncertain, agree, or strongly agree) (30, 35). Each item received a score of 0 for "non-hesitant" responses, a score of 1 for responses of

"not sure" and "I do not know," and a score of 2 for "hesitant" responses. The scores were summed to a total score ranging from 0 to 10 and each parent was considered low hesitant with a score of 0–4, moderate hesitant with a score of 5–6, and high hesitant with a score of 7–10; and (3) the last section is about the relevant information source(s) about this vaccination for their child and the need to receive additional information. In the question regarding the source(s) of information, the respondents were asked to select, from a list of 7 possible options, all sources that have been used. Finally, the response regarding the need of additional information was in the yes/no format.

2.4. Statistical analysis

First, descriptive statistics were expressed as frequencies, proportions, means, and standard deviations to assess the characteristics of the sample and of the different variables. Second, bivariate analysis with chi-square test or Student's t-test were used to examine the association between categorical or continuous variables. Third, two multivariate logistic regression models were estimated to examine the extent to which independent variables, having in the bivariate analysis a p-value equal to or less than 0.25, were associated with the outcomes of interest. The models were built using a stepwise variable selection procedure, with a $p = 0.2$ for a variable to stay and a $p = 0.4$ to exclude it. The two outcomes of interest were the following: parental willingness to vaccinate against COVID-19 their child (Model 1); and parental COVID-19 vaccine high hesitancy for their child (Model 2). For Model 1, the response options were combined into "not willing/uncertain" and "willing", to form a dichotomous outcome; for Model 2, the response options were combined into a dichotomous outcome "low hesitant with a score of 0–4 and moderate hesitant with a score of 5–6" and "high hesitant with a score of 7–10". The independent variables included in the models were the following: gender (male = 0; female = 1), age, in years (continuous), partnership status (unmarried = 0; married/living with a partner = 1), at least one parent having baccalaureate/graduate degree (no = 0; yes = 1), at least one parent being a healthcare worker (no = 0; yes = 1), at least one parent/family member with one chronic medical condition (no = 0; yes = 1), having had a personal or family member history of SARS-CoV-2 infection (no = 0; yes = 1), having received at least three doses of the vaccine against SARS-CoV-2 (no = 0; yes = 1), at least one child who had received at least two doses of the vaccine against SARS-CoV-2 (no = 0; yes = 1), and having more than one child (no = 0; yes = 1). The following were the independent variables regarding the frail child: believing that COVID-19 is a severe illness (continuous), risk perception of getting SARS-CoV-2 infection (continuous), perceived utility of the COVID-19 vaccine (continuous), perceived safety of the COVID-19 vaccine (continuous), having delayed at least one shot of vaccine (no = 0; yes = 1), source of information about the COVID-19 vaccine (none = 1; pediatrician = 2; other = 3), age, in years (<1 = 1; 1 = 2; 2 = 3; 3 = 4; 4 = 5), gender (male = 0; female = 1), and having been infected by SARS-CoV-2 (no = 0; yes = 1).

The results of the logistic regression models were presented as odds ratios (OR) with 95% confidence intervals (CI). All hypothesis testing used two-tailed p-value equal to or less than 0.05 to be considered statistically significant. STATA software version 17 was used to conduct all statistical analyses.

3. Results

Of the 476 parents contacted, a total of 445 completed the interview with an effective response rate of 93.4%. The principal characteristics of the parent and of the child are provided in Table 1. The mean age of respondents was 35.4 years, the majority were female (86.1%) and married or living with a partner (93.7%), less than one-third had a university education (30.6%), half were employed (52.6%), less than one-fifth of the parents and of the other family members had at least one chronic medical condition, 82.7% have had a personal or family member history of SARS-CoV-2 infection, and 42.7% had received at least three doses of the vaccine against SARS-CoV-2 some time prior to the survey. Most of the frail children were female, the most frequent causes of frailty were prematurity (29.2%), kidney (26.5%), and cardiovascular diseases (22.9%), and almost half had been infected by SARS-CoV-2.

The results of the attitudes toward COVID-19 and its vaccination, measured on a 10-point Likert type scale, among parents who responded to the questionnaire are showed in Table 2. Only 19.5% of the sample perceived that their children were at risk of being infected by SARS-CoV-2 with an overall mean value of 6.6. Similar attitude has been observed regarding their perception that COVID-19 was a serious illness with only 16.4% indicated the value of 10 and the overall mean value was 6.5. Regarding the vaccination, respectively 13.9% and 12.4% of the respondents indicated that was useful to vaccinate their children and that the vaccine was safe with overall mean values of 4.8 and 5.6.

Overall, almost one third (29.9%) of the participants responded that they were willing to vaccinate their frail children against COVID-19, whereas 21.3% were uncertain, and 48.8% did not intend to vaccinate. Table 3 presented the multivariate logistic regression analysis of the factors affecting each of the different outcomes of interest. Eight variables were incorporated into the final model regarding the parental willingness to vaccinate their child against COVID-19 and four of them were found to be significantly associated with the outcome. These included parents' perceived utility and safety of the vaccination and source of information. Respondents who had a higher level of perception that the vaccine is useful (OR = 2.58; 95% CI = 1.99–3.34) and safe (OR = 1.66; 95% CI = 1.27–2.17) and those who had received information by pediatrician, with the odds of being willing 83% higher as compared to those who had received information from other sources (OR = 0.17; 95% CI = 0.04–0.75) and 68% higher compared to those who did not receive any (OR = 0.32; 95% CI = 0.11–0.99), were more likely to be willing to vaccinate their child (Model 1). The most prevalent reasons among the parents who said that they would vaccinate against COVID-19 their frail children were to protect them (55.6%), the vaccines' efficacy (48.1%), and the child is at risk of getting a COVID-19 infection (46.6%). The main reasons why parents were uncertain to vaccinate their children were the concern over side effects of the COVID-19 vaccine (63.1%) and not having received a pediatrician's recommendation (26.3%), whereas the main reasons for their unwillingness were the concern for the side effects (75.6%) and that the child is not at risk of getting a COVID-19 infection (31.8%).

The mean PACV-5 score was 3.4 with 13.5% of participating parents classified as high-hesitant for the COVID-19 vaccination for their child with a score ≥ 7, 14.2% as moderate-hesitant scoring between 5 and 6, and 72.3% as low-hesitant scoring ≤ 4. Responses to the individual items of the PACV-5 are shown in Table 4. Only 11.3% of parents thought

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TABLE 1 Socio-demographic and general characteristics of the study population.

Characteristics	N	%
Parent		
Age, years	35.4 ± 5.6 (20–57)*	
Gender		
Female	383	86.1
Male	62	13.9
Partnership status		
Married/living with a partner	417	93.7
Unmarried	28	6.3
Educational level		
High school degree or less	305	69.4
Baccalaureate/graduate degree	136	30.6
Employment status		
Unemployed	211	47.4
Employed	234	52.6
At least one parent being a healthcare worker		
No	417	93.7
Yes	28	6.3
At least one parent/family member with one chronic medical condition		
No	361	81.1
Yes	84	18.9
Having had a personal/family member history of SARS-CoV-2 infection		
No	77	17.3
Yes	368	82.7
Having received at least three doses of the vaccine against SARS-CoV-2		
No	255	57.3
Yes	190	42.7
Having more than one child		
No	246	55.3
Yes	199	44.7
At least one child who had received at least two doses of the vaccine against SARS-CoV-2		
No	171	85.9
Yes	28	14.1
Child		
Age, years		
<1	44	10.1
1	121	27.8
2	96	22
3	76	17.4
4	99	22.7
Gender		
Female	239	54.6
Male	199	45.4
Frailty condition**		
Prematurity (<2 years of age)	130	29.2
Kidney	118	26.5
Cardiovascular	102	22.9
Metabolic	29	6.5
Onco-hematologic	25	5.6
Rheumatic	23	5.2
Genetic syndromes	25	2.9
Others (obesity, neurological)	12	2.7
Having been infected by SARS-CoV-2		
No	229	51.5
Yes	216	48.5

Number for each item may not add up to total number of study population due to missing value.
*Mean ± Standard deviation (range).
**More than one chronic medical condition was allowed to be indicated.

their child received more vaccines than are good for them, more than one-fourth (28.6%) wanted children to receive fewer vaccines at the same time and more than three-quarters (80.8%) strongly disagreed or disagreed that it is better for children to develop immunity by getting sick than to get a shot. Approximately two-thirds (63.5%) of respondents considered themselves to be vaccine hesitant. Less than half (41.6%) said they trusted the information they received about childhood COVID-19 vaccine. The results of the multivariable logistic regression analysis examining the factors associated with the hesitancy showed that parents with a higher COVID-19 vaccine-related safety concerns (OR = 0.63; 95% CI = 0.56–0.72), those who have delayed at least one shot of a recommended vaccine for their child (OR = 2.33; 95% CI = 1.01–5.36), and parents who did not have received at least three doses of the vaccine against SARS-CoV-2 (OR = 0.49; 95% CI = 0.25–0.99) were more likely to be high-hesitant (Model 2 in Table 3).

Only one-fourth of parents (26%) reported having previously received information on the COVID-19 vaccine for their child. Of those who had acquired information, the pediatrician was indicated as the principal source by 57.7%. The second most reported source was Internet (31.9%), followed by friends and family members (28.5%) and mass media (22.4%). Finally, 44.3% participants indicated the desire to get additional information about the COVID-19 vaccine.

4. Discussion

This survey, to the best of our knowledge the first conducted in Italy, provided important and useful insights into the parents' willingness and hesitancy to have their children ages 6 months to 4 years with frail conditions vaccinated against COVID-19 as well as the influencing factors.

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TABLE 2 Respondents' attitudes toward COVID-19 and its vaccination measured on a 10-point Likert type scale.

Item	Mean ± SD*	Score of 1 *N* (%)	Score of 10 *N* (%)
How serious do you consider COVID-19 for your child?	6.5 ± 2.5	16 (3.6)	73 (16.4)
How much do you perceive your child at risk of getting COVID-19?	6.6 ± 2.6	20 (4.5)	87 (19.5)
How useful do you consider COVID-19 vaccination for your child?	4.8 ± 3.3	129 (29.1)	62 (13.9)
How safe do you consider COVID-19 vaccination for your child?	5.6 ± 2.9	78 (17.5)	55 (12.4)

*Standard deviation.

TABLE 3 Multivariate logistic regression analysis results examining the determinants of the different outcomes of interest.

Variable	OR	SE	95% CI	*p*
Model 1. Parental willingness to vaccinate their child against COVID-19 Log likelihood = −86.17, χ^2 = 56.53 (8 df), $p < 0.0001$				
Higher perceived utility of the vaccine against SARS-CoV-2	2.58	0.34	1.99–3.34	<0.001
Higher perceived safety of the vaccine against SARS-CoV-2	1.66	1.23	1.27–2.17	<0.001
Source of information about the vaccine against SARS-CoV-2 for their child				
Pediatrician	1.00°			
Other sources	0.17	0.13	0.04–0.75	0.02
None	0.32	0.18	0.11–0.99	0.049
Child's age, years				
<1	1.00°			
1	0.59	0.31	0.21–1.67	0.326
3	0.36	0.24	0.11–1.31	0.123
4	0.59	0.31	0.21–1.64	0.312
Not having had a personal or family member history of SARS-CoV-2 infection	0.59	0.31	0.21–1.65	0.322
Model 2. Parental COVID-19 vaccine high hesitancy for their child Log likelihood = −126.59, χ^2 = 86.12 (4 df), $p < 0.0001$				
Lower perceived safety of the vaccine against SARS-CoV-2	0.63	0.04	0.56–0.72	<0.001
Having delayed at least one shot of a recommended vaccine for their child	2.33	0.99	1.01–5.36	0.047
Not having received at least three doses of the vaccine against SARS-CoV-2	0.49	0.17	0.25–0.99	0.05
Male child	0.69	0.23	0.36–1.32	0.267

°Reference category.

The first key finding is that of the parents surveyed, only 29.9% said that were willing to vaccinate their children against COVID-19 and this is of great concern although no data are available on the effectiveness and safety of vaccines in this age group. This result was like that of 31.3% in the United States among parents of healthy children 2–4 years (15). This value was higher than those observed in other countries, in which the prevalence was 19.8 and 25.2% among parents of healthy children, respectively, aged <5 years (20) and 0–4 years (16) in the United States, but it was lower than the 71% in Brazil (14) and 50.6% in Ireland for children aged 0–4 years (23), 50% for 4 years in Australia (19), 45.1% for <5 years with few had an underlying disease in Malaysia (13), 42% for <2 years in the United States (12), and 41.9 and 45.4% for 2–4 years and 6–23 months in Canada (17). Furthermore, the value was also likewise lower as compared to the 36% found among parents of children 0 to 5 years with developmental disabilities in the United States (22), and to the 42.1% of children 0 to 4 years with neurodevelopmental disorders in Bangladesh (21). However, it is necessary to underline that the variations in reported prevalence across countries may in part be attributed to the differences in, for example, the frequency of the disease, study setting and period, characteristics of the sample, and data collection methodology. Moreover, among the parents who participated in this survey, 13.5% reported being high-hesitant assessed using the PACV-5 questionnaire against the COVID-19 vaccine for their frail children, whereas the moderate-hesitant and low-hesitant accounted, respectively, for 14.2% and 72.3% of the total participants. In a study among parents of healthy children aged 0–60 months in Turkey it has been observed that 9.38% of participants were vaccine hesitant measured using the PACV-15 (36). It is important to underline that the sample of this survey was constituted by children with chronic medical conditions and this may partially explain the parents' hesitancy, possibly due to the presence of individuals for whom vaccines are contraindicated (37, 38).

The second key finding is that identifying the primary reasons for parents' willingness or unwillingness of the COVID-19 vaccination for their child are needed by the healthcare workers, mainly pediatricians with whom they have a closer and deeper interaction, to tailor information. More than half of the surveyed parents answered that the prevention of the onset of the disease was the main reason taken into consideration for their willingness in favor of the COVID-19 vaccination for their child, consistent with the existing literature among parents of children of different age (30, 39, 40). The

TABLE 4 Descriptive characteristics of PACV-5 about COVID-19 vaccine.

Item	Parent response	*N* (%)
Children get more shots than are good for them	Strongly agree/agree	50 (11.3)
	Strongly disagree/disagree	369 (83.1)
	Not sure	25 (5.6)
It is better for my child to develop immunity by getting sick than to get a shot	Strongly agree/agree	43 (9.7)
	Strongly disagree/disagree	359 (80.8)
	Not sure	42 (9.5)
It is better for children to get fewer shots at the same time	Strongly agree/agree	127 (28.6)
	Strongly disagree/disagree	281 (63.3)
	Not sure	36 (8.1)
Overall, how hesitant about the COVID-19-vaccine for your child would you consider yourself to be?	Very hesitant/ somewhat hesitant	282 (63.5)
	Not hesitant at all/not too hesitant	12 (2.7)
	Not sure	150 (33.8)
I trust the information I receive about the COVID-19-vaccine	Strongly agree/agree	185 (41.6)
	Strongly disagree/disagree	118 (26.6)
	Not sure	141 (31.8)

Number for each item may not add up to total number of study population due to missing value.

fact that the parents have indicated this reason underlined the stone that the disease severity is a leverage point to get their children vaccinated. Among those parents who were uncertain or did not intend to vaccinate their child, the most common reason was the parental concerns about the safety of the vaccination. This result is in accordance with those that have been observed in other recent studies worldwide examining immunization confidence among different groups of individuals (14, 22, 41–44). Therefore, it is of great importance targeting the interventions to address these concerns raised by the parents by a participatory approach and it is also necessary to stress the dangers of this vaccine-preventable disease.

The third key finding was, unexpectedly and unfortunately, a widespread lack of information among the sample of this study with only slightly more than one-third of the parents had acquired information about the COVID-19 vaccination for their children through multiple sources. The most frequently chosen sources of information were pediatricians and Internet. It is worth mentioning that the information from pediatricians is crucial and it was significantly associated with the parents' willingness to vaccinate against COVID-19 their children. Among parents who have sought information about this vaccination for their child from this source the odds of being willing to vaccinate were 83% higher as compared to those who had received information from other sources and 68% higher compared to those who did not receive any. This result support previous publications in the literature which underline the significant and positive influence of pediatricians and other healthcare professionals on the individuals' attitudes and behaviors with those who had received information from this source that were more likely to accept or to receive a vaccination than those who did not use this source (11, 13, 30, 45, 46). However, since slightly more than half had received information by their pediatricians about the COVID-19 vaccine this may represents a limitation to receive the vaccine in the future. This indicates the need for specific strategies and actions to ensure healthcare professionals communication and education programs to inform and to persuade parents of frail children to get the vaccine against COVID-19 and also to impart knowledge about its benefits in order to change their behavioral intentions. Moreover, although the amount of health-related information seeking online is continuing increasing and Internet is a very common used platform, the fact that 31.9% used this source is of concern because previous studies have reported the availability of widespread misinformation with the vaccines on SARS-CoV-2 that are considered unsafe and harmful and this is likely to negatively influence parents' knowledge and to reduce the intentions to vaccinate their child (46–48). Therefore, public health campaigns regarding the benefits of this vaccination on this source should be more frequent so that the misinformation is less likely to be seen and, as such, will have a less negative impact.

The fourth key finding was that in the final multivariate logistic regression models, several factors significantly predicted the parents' willingness and hesitancy toward the vaccination against SARS-CoV-2 for their child. It is important to note that, in addition with the association already reported with the source of information, respondent's attitudes have been identified as important determinants. Indeed, this study revealed that willingness to vaccinate their child against COVID-19 is heavily influenced by parental positive attitude toward the utility and safety of this vaccination. Parents who had these positive attitudes were 2.58 and 1.66 times more willing to vaccinate their child when compared with parents who had unfavorable attitudes. This finding is consistent with those of several recently conducted studies showing that parents' opinions about the efficacy of the vaccines in general have a significant impact in vaccine acceptance (13, 15, 18, 23, 49, 50). Therefore, public health education programs for parents with a negative attitude toward the utility of the COVID-19 vaccine would result in a high vaccine acceptance with an increase in the uptake. The survey revealed that parents with a lower COVID-19 vaccine-related safety concerns had 37% lower odds of being high-hesitant than those with higher concern and those who delayed at least one shot of a recommended vaccine for their child were 2.33 times more likely to be high-hesitant. This finding is consistent with those observed in previously conducted surveys (49, 51). Moreover, delayed of at least one shot of a recommended vaccine for their child underlined the need of targeted activities through a better parent-physician communication that may be crucial toward enhancing vaccination coverage.

5. Limitations

This survey is subject to at least five potential methodological limitations that merit consideration when interpreting the present findings. First, the survey adopted a cross-sectional design and this limits to make final determinations about the causal relationships between the independent variables and the outcomes of interest. Second, the data collection source was a single-site and therefore generalization of the study findings to other geographic locations of Italy and globally should be done with caution. Third, no record verification was performed regarding the vaccination status. However, the parental interview is the most used method and recall bias is likely

limited as the survey was conducted when no active vaccination campaign among this age group has been implemented. Fourth, responses are prone to social desirability bias in which parents tend to give answers that are thought to be more socially acceptable and may not correlate with future behaviors, such as the intention to vaccinate their children. However, this bias has been minimized by assuring all participants that their responses were anonymous and confidential. Fifth, it is possible that non-respondents were different than respondents. Non-response bias was minimized by attempting to reach parents at least 3 times over the telephone and it can be ruled out since a participation rate of 93.4% has been obtained. Despite the described limitations, the findings of this survey give important information of the parents' willingness and hesitancy toward the COVID-19 vaccination of their frail children ages 6 months to 4 years in Italy.

6. Conclusion

In conclusion, the survey findings have important implications for designing interventions to increase willingness and to reduce hesitancy for COVID-19 vaccine among parents of frail children ages 6 months to 4 years in Italy. Healthcare providers, mainly pediatricians, have a significant role and closer and regular contacts with the parents should be encouraged for increasing awareness about the importance of vaccinating their child and acceptance of COVID-19 vaccination. Furthermore, the finding that less than 60% of the parents have received the recommendation by a pediatrician emphasizes that improving their training with also informative campaigns is essential to assist the parents by communicating with them helpfully and to support them in order to have the correct information regarding the COVID-19 vaccine's safety and utility for reaching a higher coverage.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study protocol and procedures were approved by the Ethics Committee of the Teaching Hospital of the University of Campania "Luigi Vanvitelli" (protocol number 0001816/i). All parents gave verbal informed consent prior to the interview.

Author contributions

GMdG, GDP, and MP participated in the conception and design of the study, collected the data, and contributed to data analysis and interpretation. IFA, the principal investigator, designed the study, was responsible for the statistical analysis and interpretation, and wrote the article. All authors have read and approved the final version of the article and agreed to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1212652/full#supplementary-material

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Impact of the first wave of COVID-19 on Crohn's disease after the end of "zero-COVID" policy in China

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Background: The incidence and severity of coronavirus disease 2019 (COVID-19) among Crohn's disease (CD) patients are unknown in China. This study aimed to clarify the clinical courses and outcomes of CD patients in the first COVID-19 wave after the end of "zero-COVID" policy in China.

Methods: Clinical characteristics, including vaccination doses and medications of 880 CD patients from a prospective cohort were collected for analysis.

Results: Of the enrolled patients (n=880) who underwent nucleic acid or antigen testing for COVID-19 from Dec 7, 2022, to Jan 7, 2023, 779 (88.5%) were infected with COVID-19. Among the infected patients, 755 (96.9%) were mild, 14 (1.8%) were moderate, one patient with leukemia died of cerebral hemorrhage (mortality, 0.1%) and only 9 (1.2%) were asymptomatic. Fever, cough, headache and appetite loss were the most frequently observed symptoms in general, respiratory, neurological and gastrointestinal manifestations, respectively. The age and disease duration were significantly higher (40/32, 5.6/3.6, all p <0.05) in moderate patients than those in mild patients. All other clinical characteristics, including CD activity and medication exposure, showed no significant differences between the above two groups. Furthermore, no significant difference in vaccination or comorbidities was observed between the two groups.

Conclusion: Most CD patients contracted the Omicron infection and experienced mild disease courses in the first COVID-19 wave attack after China ended the "zero-COVID" policy irrespective of vaccination dose or comorbidities.

KEYWORDS

COVID-19, Crohn's disease, cohort study, China, "zero-COVID" policy

1. Introduction

Crohn's disease (CD), one main type of inflammatory bowel disease (IBD), is a chronic inflammatory condition of the gastrointestinal tract with a relapsing–remitting and progressively disabling pattern (1). The management of CD during the coronavirus disease 2019 (COVID-19) pandemic has been a research priority for the IBD community worldwide over the last 3 years (2). Patients with CD, especially in the presence of immunosuppressive medications, are supposed to be at high risk of serious viral and bacterial infections (3). Evidence from studies in the phases of earlier variants (alpha, beta, gamma, and delta) revealed no differences in COVID-19 hospitalization or mortality between patients with IBD or without IBD (4), while advanced age and the presence of comorbid conditions were found to be key risk factors for severe infection (5). Very few data concerning the impact of the new Omicron strain with high transmissibility on CD patients have been reported (6–8). COVID-19 vaccines are believed to play a protective role against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); however, concerns about vaccination efficacy are one reason for hesitancy (9). Along with the adjustment of the "zero-COVID" strategy in China on 7 December 2022, the first nationwide Omicron-based outbreak started shortly after the relaxation of nonpharmaceutical public health intervention measures (including social distancing, mass testing, quarantine and travel restrictions), and passed the peak rapidly within 1 month (Dec 8, 2022 to Jan 7, 2023) with more than 50,000 deaths.[1] The impact of this on CD patients who were naïve to COVID with different vaccination backgrounds should be clarified to promote our understanding of COVID-19 and CD management. This study aimed to clarify the clinical courses and outcomes of CD patients in the first COVID-19 wave after the end of "zero-COVID" policy in China.

1 https://www.chinacdc.cn

2. Patients and methods

2.1. Study design

CD patients from our prospective open cohort (established from July 1st, 2019) who had nucleic acid or rapid antigen tests during the first wave (Dec 8, 2022, to Jan 7, 2023) were enrolled in this study (Figure 1). Clinical data, including comorbidities, medications and vaccinations, were collected from the cohort database and follow-up information. The incidence and severity of COVID-19 among CD patients were analyzed. This study was approved by the Institutional Review Board of the Ethics Committee of the Second Affiliated Hospital, School of Medicine, Zhejiang University in China (approved No. 2023-0134). In all cases, informed written consent was obtained from participants or their legal surrogates before enrollment. The study followed the STROBE reporting guideline.

2.2. Patients

CD was diagnosed based on a combination of clinical, laboratory, endoscopic, cross-sectional imaging, and histological assessments. The exclusion criteria were as follows: (1) patients who were living abroad during the first wave; or (2) patients who did not undergo any nucleic acid or rapid antigen tests for COVID-19. All patients in the cohort were followed up in a short time to reduce recall bias and underreported bias.

2.3. Criteria for COVID-19 diagnosis and severity

COVID-19 diagnosis was based on viral tests (by nucleic acid or rapid antigen tests, irrespective of symptoms) and disease severity was classified according to the ninth edition of the COVID-19 diagnosis and treatment protocol (10). Symptomatic COVID-19 infections were

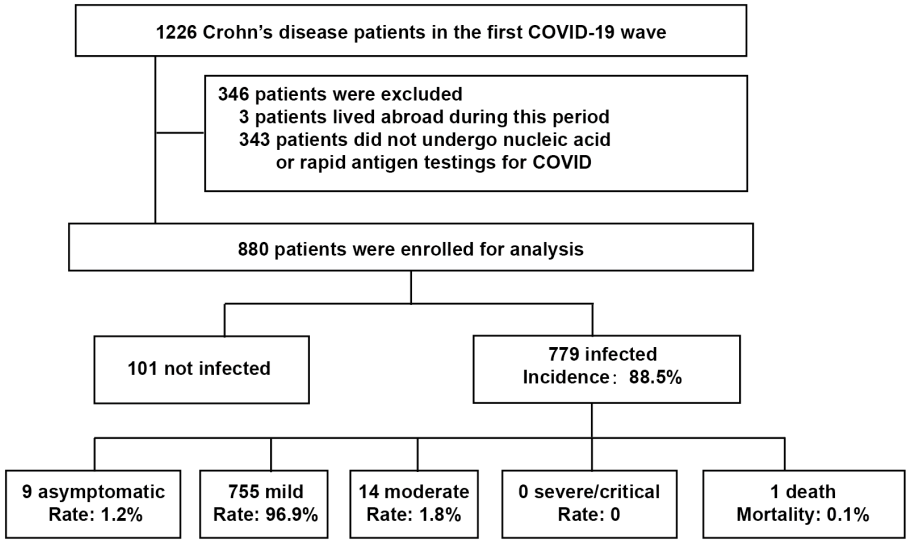

FIGURE 1
Study population flowchart. COVID-19, coronavirus disease 2019.

defined as those that had a positive nucleic acid or rapid antigen test result with at least one of 22 symptoms (11). Asymptomatic infections were defined as a positive nucleic acid or rapid antigen test without any symptoms at the time of testing. Mild infection was defined as slight clinical symptoms without pneumonia on chest imaging. Moderate infection was defined as clinical symptoms plus pneumonia on chest imaging without evidence of hypoxia. Severe infection was diagnosed according to dyspnea (respiratory rate ≥ 30 times/min), resting finger oxygen saturation ≤ 93%, or artery PaO2/FiO2 ≤ 300 mm Hg (1 mm Hg = 0.133 kPa). Critical infection was defined as respiratory failure with shock and multiorgan failure requiring mechanical ventilation and intensive care unit admission.

2.4. Data collection and procedure

Demographic (including sex and age) and diagnostic profiles (diagnosis, disease duration and chronic illness history) were extracted from the cohort database. The following information was collected during follow-ups: COVID-19 diagnosis status, COVID-19 symptoms, body mass index (BMI), COVID-19 vaccination doses, disease activity prior to COVID-19 infection [as defined by the Harvey-Bradshaw Index, HBI (12)], medication exposure at time of COVID-19 diagnosis and whether medications were discontinued, chest imaging and COVID-19 treatments. Patients with COVID-19 diagnosed less than 7 days prior were followed up again to confirm any progression on Jan 20th, 2023.

2.5. Statistical analysis

For continuous variables, the means (standard deviations, SDs) and medians (interquartile ranges, IQRs) were used for normally and nonnormally distributed data, followed by unpaired t tests and Mann–Whitney *U* tests when appropriate. Categorical variables were expressed as numbers (%) and compared using Fisher's exact test. $p < 0.05$ was considered statistically significant and SPSS (V.26.0) was used for all analyses.

3. Results

A total of 1,226 CD patients were extracted from the cohort database and 880 patients were enrolled for the final analysis. Of the enrolled patients, 779 (88.5%) were diagnosed with COVID-19 infection, of whom, 9 (1.2%) were asymptomatic, 755 (96.9%) were mild, 14 (1.8%) were moderate, and one patient with leukemia died of cerebral hemorrhage after COVID-19 infection (Figure 1 and Table 1).

The clinical characteristics of all enrolled patients are summarized in Table 1. Among 880 CD patients, 620 (70.5%) were male. The age and disease duration distributions were left-skewed, with a median age of 32 (26–41) years and a CD duration of 3.6 (1.6–6.6) years. The median BMI was 21.0 (19.0–23.4). A total of 43 (4.9%), 230 (26.1%), 394 (44.8%) and 19 (2.2%) patients had been vaccinated with one dose, two doses, three doses and four doses, respectively, while 194 (22.0%) patients were not vaccinated. Sixty patients (6.8%) were current smokers and 5 (0.6%) were pregnant during the study period. Fifteen percent of patients had at least one comorbidity in addition to CD, the most common being chronic hepatitis B virus infection (5.6%) and hypertension (3.1%). Comparative analysis between the infection and non-infection groups showed no difference in sex, age, disease duration, vaccination doses or comorbidities.

All 22 clinical symptoms were summarized and ranked by their prevalence among symptomatic patients under categories of general, respiratory, neurological, and gastrointestinal (GI) manifestations in Figure 2. General symptoms were mostly reported, under which fever (86.5%) was the leading complaint followed by fatigue (66.9%), muscle aches (65.2%) and chills (42.1%). Manifestations of the respiratory system had the widest spectrum of symptoms, such as cough (83.4%), stuffy nose (53.6%), runny nose (46.8%), sore throat (38.3%), sneezing (31.9%), hoarse voice (27.1%), chest tightness (16.1%) and chest pain (6.6%). Neurological manifestations were the third most common symptoms, including headache (57.3%), loss of or change in sense of taste (38.8%), dizziness (34.8%), difficulty sleeping (26.8%) and loss of or change in sense of smell (24.8%). GI complaints were ranked as the fourth most common discomfort and included appetite loss (43.5%), diarrhea (30.0%), nausea (14.3%), abdominal pain (10.9%) and vomiting (7.3%). Collectively, fever, cough, headache, and appetite loss were the most observed symptoms in terms of general, respiratory, neurological and GI manifestations, respectively.

The characteristics of CD patients with symptomatic COVID-19 infections are summarized in Table 2 ($n = 769$, one death was excluded) and grouped by disease severity. Seventy percent patients were male, with a median age of 32 (26–40) years. The median CD duration was 3.6 (1.6–6.5) years, and the median BMI was 21.0 (19.0–23.5). Only 42 (5.5%) patients were in an active state (HBI > 3) prior to COVID-19 infection. Apart from the common gastrointestinal symptoms including diarrhea and abdominal pain, some patients presented other fluctuations in CD-related symptoms, including abdominal mass (0.5%), constipation (0.3%), hematochezia (0.8%), bloating (0.5%), perianal symptoms (0.7%) and extraintestinal manifestations (1.3%). Medication exposure was summarized in commonly used categories. Tumor necrosis factor (TNF) antagonists (55.4%) was mostly used, followed by ustekinumab (20.5%) and vedolizumab (2.3%) in the biologics class. Three kinds of immunomodulators were used, including thiopurine (17.2%), methotrexate (2.7%) and thalidomide (1.8%). Only 7 (0.9%) and 2 (0.3%) patients were using corticosteroids and tofacitinib, respectively, before infection. Thirty-nine (5.1%) patients were taking sulfasalazine/mesalamine. Concerning medications for COVID-19, acetaminophen (50.8%) was the most frequently used drug, followed by Chinese patent medicine (23.9%) and anti-cough drugs (22.4%). Between-group comparisons showed that the moderate group was older (40 vs. 32, $p < 0.05$) and had a longer CD disease duration (5.6 vs. 3.6, $p < 0.05$) than the mild group. All other clinical characteristics, including CD activity and medication exposure, showed no significant differences between the above two groups.

The vaccination doses and comorbidities of symptomatic patients are summarized in Table 3. Five (35.7%) and 7 (50.0%) patients in the moderate group had been vaccinated with two doses and three doses, while 38 (5.0%), 203 (26.9%), 335 (44.4%) and 18 (2.4%) patients in the mild group had been vaccinated with one dose, two doses, three doses and four doses, respectively. The two leading comorbidities were chronic hepatitis B virus infection (5.4% in mild and 7.1% in

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TABLE 1 Clinical characteristics of Crohn's disease patients enrolled for COVID-19 analysis.

Characteristic	Total (*n* =880)	Infection (*n* =779)	Non-infection (*n* =101)	*p* value
Sex, *n* (%)				0.729
Male	620 (70.5)	547 (70.2)	73 (72.3)	
Female	260 (29.5)	232 (29.8)	28 (27.7)	
Age in years	32 [26–41]	32 [26–41]	32 [25–41]	0.698
14–19	40 (4.5)	35 (4.5)	5 (5.0)	0.799
20–29	305 (34.7)	267 (34.3)	38 (37.6)	0.507
30–39	291 (33.1)	262 (33.6)	29 (28.7)	0.369
40–49	131 (14.9)	115 (14.8)	16 (15.8)	0.767
50–59	76 (8.6)	67 (8.6)	9 (8.9)	0.852
60–69	28 (3.2)	24 (3.1)	4 (4.0)	0.552
>=70	9 (1.0)	9 (1.2)	0	>0.999
CD duration (years)	3.6 [1.6–6.6]	3.6 [1.6–6.5]	3.1 [1.5–7.5]	0.803
<1	119 (13.5)	105 (13.5)	14 (13.9)	0.878
1–5	432 (49.1)	384 (49.3)	48 (47.5)	0.752
5–10	225 (25.6)	199 (25.5)	26 (25.7)	>0.999
>10	104 (11.8)	91 (11.7)	13 (12.9)	0.743
BMI, kg/m^2	21.0 [19.0–23.4]	21.0 [19.0–23.5]	21.1 [19.1–22.4]	0.275
≤18.4	160 (18.2)	141 (18.1)	19 (18.8)	0.891
18.5–23.9	547 (62.2)	477 (61.2)	70 (69.3)	0.127
24–27.9	146 (16.6)	136 (17.5)	10 (9.9)	0.064
≥28	27 (3.1)	25 (3.2)	2 (2.0)	0.759
Vaccination, *n* (%)				
No vaccination	194 (22.0)	165 (21.2)	29 (28.7)	0.097
One dose	43 (4.9)	39 (5.0)	4 (4.0)	0.809
Two doses	230 (26.1)	210 (27.0)	20 (19.8)	0.148
Three doses	394 (44.8)	347 (44.5)	47 (46.5)	0.75
Four doses	19 (2.2)	18 (2.3)	1 (1.0)	0.714
Current smoker, *n* (%)	60 (6.8)	50 (6.4)	10 (9.9)	0.206
Current pregnancy, *n* (%)	5 (0.6)	3 (0.4)	2 (2.0)	0.104
Comorbid conditions, *n* (%)				
Any comorbidity	135 (15.3)	119 (15.3)	16 (15.8)	0.999
Cancer	8 (0.9)	8 (1.0)	0	0.607
Hypertension	27 (3.1)	25 (3.2)	2 (2.0)	0.759
Diabetes	4 (0.5)	3 (0.4)	1 (1.0)	0.386
Cardiovascular disease	9 (1.0)	7 (0.9)	2 (2.0)	0.277
Lung disease	14 (1.6)	11 (1.4)	3 (3.0)	0.211
Chronic renal disease	3 (0.3)	3 (0.4)	0	>0.999
Chronic HBV infection	49 (5.6)	45 (5.8)	4 (4.0)	0.644
History of stroke	4 (0.5)	3 (0.4)	1 (1.0)	0.386

Categorical variables are expressed as *n* (%); continuous variables are expressed as the medians [Q1-Q3]. Comparisons were applied between the infection and non-infection group (Mann–Whitney *U* test or Fisher's exact test). COVID-19: coronavirus disease 2019; CD: Crohn's disease; HBV: hepatitis B virus.

moderate group) and hypertension (1.3% in mild and 7.1% in moderate group). There was no significant difference in either vaccination doses or comorbidities between patients in the mild and moderate groups.

4. Discussion

We used cohort-based clinical data and follow-up COVID-19 information to clarify the impact of Omicron on the infection-naïve

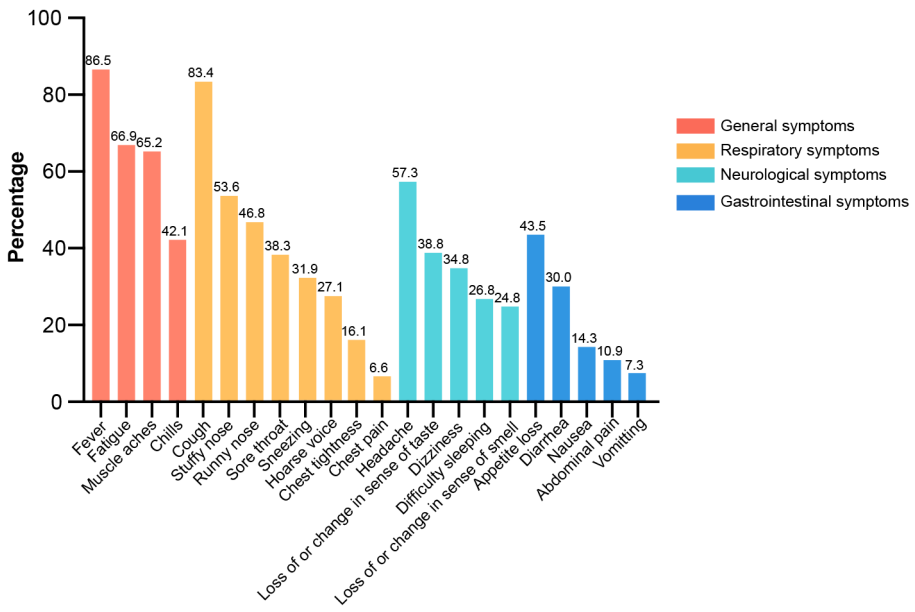

FIGURE 2
Categorization and ranking of symptom profiles.

CD population during the China's first wave after the end of "zero-COVID" policy. The results showed that most CD patients experienced symptomatic infections and mild clinical courses.

The first nationwide COVID-19 wave in China started shortly after the implementation of measures, peaked in late December, then declined continuously and ended in late January[2]. From Dec 8th, 2022, to Jan 7th, 2023, the first wave of COVID-19 in China claimed more than 50,000 lives (see footnote 1). Although the dominant strains that drove the wave were Omicron BF.7 and BA.5.2, which were regarded as highly transmissible but low-virulence subvariants, COVID-19 still triggered great anxiety and stress among CD patients who had never been exposed to COVID. During the observation time, the incidence rate of COVID-19 among CD patients was 88.5%, which was approximately equal to that in the general population reported in Henan province in early January (urban 89.1%, rural 88.9%) (13). Among the infected patients, the majority of them experienced mild (96.9%) or moderate (1.8%) courses. Lu et al. recently reported that 96.97% of the general population infected with COVID-19 experienced mild or moderate symptoms during the same time of our study (14). This similar outcome may be associated with the younger age structure and use of biologics and immunosuppressants in our CD population (15). The lack of biosamples for further genetic analysis is our limitation, as CD patients were encouraged to follow home treatment during the pandemic. No severe/critical cases were observed in our study, and the only death case was caused by complications of leukemia, exacerbated by COVID-19. Given that hematological malignancies have a high mortality rate (29.3–40%) during COVID-19 (16, 17), these groups of patients should be a priority for protection in future outbreaks.

Age and comorbidities are the most important prognostic factors for more severe COVID-19 among IBD patients according to previous studies of earlier variants (18, 19). In the analysis of IBD medications, systemic corticosteroids, the combination of TNF antagonists with azathioprine and active IBD were associated with poor outcomes of COVID-19 (20). Older age and longer CD disease duration were found to be associated with moderate COVID-19, indicating that age and accumulated damage may influence the viral-induced immune response. All other clinical characteristics, including CD activity, medication exposure and comorbidities, showed no significant differences between the above two groups. Given the low rates of moderate/severe cases in our study, our findings need validation in large external cohorts.

The symptom profile reflects the potential of COVID-19 to damage multiple systems through immune responses (21), and changes with the evolution of variants (11). Consistent with the findings that influenza-like symptoms were more frequently reported in Omicron (11), this study demonstrated the highest prevalence of general symptoms among symptomatic patients. Fluctuation of CD-related symptoms was another concern for most patients and physicians during the infection. Over 40% of patients in our study reported fluctuations in CD-related symptoms, including common GI symptoms and CD-specific manifestations. In contrast to the data from Surveillance Epidemiology of Coronavirus Under Research Exclusion (22), patients in our cohort presented a higher rate of common GI symptoms (diarrhea 20.9% vs. 30.0%, abdominal pain 8.9% vs.10.9%); this needs further validation in external cohorts. It is necessary to prolong the observation time for activity patterns and outcomes among these patients.

Although vaccination against SARS-CoV-2 has been recommended to IBD patients since the beginning of the pandemic (23), the rate of uptake among our CD patients was approximately 75% which was lower than that in the general population, reflecting the phenomenon of vaccine hesitancy in this immunosuppressed population (24).

2 https://weekly.chinacdc.cn/

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TABLE 2 Clinical characteristics of Crohn's disease patients with symptomatic COVID-19 infection.

Characteristic	Total (*n* =769)[a]	Mild (*n* =755)	Moderate (*n* =14)	*p* value
Male, *n* (%)	539 (70.1)	530 (70.2)	9 (64.3)	0.769
Age (years)	32 [26–40]	32 [26–40]	40 [31–43]	**0.041**
CD duration (years)	3.6 [1.6–6.5]	3.6 [1.6–6.5]	5.6 [3.4–13.1]	**0.042**
BMI, kg/m^2	21.0 [19.0–23.5]	21.0 [19.0–23.5]	22.4 [19.1–25.8]	0.244
CD activity (HBI) prior to infection, *n* (%)				0.476
0	505 (65.7)	494 (65.4)	11 (78.6)	
1–3	222 (28.9)	220 (29.1)	2 (14.3)	
>3	42 (5.5)	41 (5.4)	1 (7.1)	
Fluctuation of activity, *n* (%)				
Any new symptoms	330 (42.9)	322 (42.6)	8 (57.1)	0.416
Abdominal pain	84 (10.9)	81 (10.7)	3 (21.4)	0.190
Diarrhea	231 (30.0)	226 (29.9)	5 (35.7)	0.769
Nausea	110 (14.3)	110 (14.6)	0	0.240
Vomiting	56 (7.3)	56 (7.4)	0	0.615
Abdominal mass	4 (0.5)	4 (0.5)	0	>0.999
Constipation	2 (0.3)	2 (0.3)	0	>0.999
Hematochezia	6 (0.8)	6 (0.8)	0	>0.999
Distension/bloating	4 (0.5)	4 (0.5)	0	>0.999
Perianal symptoms	5 (0.7)	5 (0.7)	0	>0.999
Extraintestinal manifestations	10 (1.3)	9 (1.2)	1 (7.1)	0.169
Medications for CD, *n* (%)				
TNF antagonists	426 (55.4)	418 (55.4)	8 (57.1)	>0.999
Ustekinumab	158 (20.5)	155 (20.5)	3 (21.4)	>0.999
Vedolizumab	18 (2.3)	18 (2.4)	0	>0.999
Thiopurine	132 (17.2)	129 (17.1)	3 (21.4)	0.718
Methotrexate	21 (2.7)	20 (2.6)	1 (7.1)	0.324
Thalidomide	14 (1.8)	14 (1.9)	0	>0.999
Corticosteroids	7 (0.9)	7 (0.9)	0	>0.999
Tofacitinib	2 (0.3)	2 (0.3)	0	>0.999
Sulfasalazine/mesalamine	39 (5.1)	39 (5.2)	0	>0.999
Medications for COVID-19, *n* (%)				
Acetaminophen	391 (50.8)	384 (50.9)	7 (50.0)	>0.999
NSAIDs	151 (19.6)	149 (19.7)	2 (14.3)	>0.999
Chinese patent medicine	184 (23.9)	178 (23.6)	6 (42.9)	0.113
Traditional Chinese medicine	23 (3.0)	22 (2.9)	1 (7.1)	0.349
Anti-cough drugs	172 (22.4)	164 (21.7)	8 (57.1)	**0.005**
Anti-diarrheal drugs	14 (1.8)	14 (1.9)	0	>0.999
Anti-viral drugs	5 (0.7)	4 (0.5)	1 (7.1)	0.088

Categorical variables are expressed as *n* (%); continuous variables are expressed as the medians [Q1-Q3]. Comparisons were applied between the mild and moderate group (Mann–Whitney *U* test or Fisher's exact test).[a]One patient who died of cerebral hemorrhage after COVID-19 infection was removed from the analysis. COVID-19, coronavirus disease 2019; CD, Crohn's disease; HBI, the Harvey-Bradshaw Index; TNF, Tumor necrosis factor; NSAIDs, Nonsteroidal anti-inflammatory drugs.
Significant results are presented in bold.

Vaccine effectiveness against viral acquisition and severe outcomes was assessed in recent population-based studies during Omicron outbreaks, suggesting that a booster dose of COVID-19 vaccine is needed for older patients and high-risk populations against severe or fatal outcomes (25). Bivalent booster vaccines are now encouraged among IBD patients taking TNF antagonists and tofacitinib based on emerging evidence regarding the effectiveness of COVID-19 vaccines (8, 26). No significant difference in vaccination doses was observed between the infection and non-infection group or between the mild and moderate groups in this study despite the same infection-naïve background. It is worth noting that the vaccine effectiveness in IBD patients is influenced by many factors, such as vaccine type, doses, and waning antibodies

TABLE 3 Vaccination and comorbidities of Crohn's disease patients with symptomatic COVID-19 infection.

Characteristic	Total (*n*=769)[a]	Mild (*n*=755)	Moderate (*n*=14)	*p* value
Vaccination, *n* (%)				
No vaccination	162 (21.1)	161 (21.3)	1 (7.1)	0.322
One dose	39 (5.1)	38 (5.0)	1 (7.1)	0.521
Two doses	208 (27.0)	203 (26.9)	5 (35.7)	0.543
Three doses	342 (44.5)	335 (44.4)	7 (50.0)	0.788
Four doses	18 (2.3)	18 (2.4)	0	>0.999
Current smoker, *n* (%)	48 (6.2)	48 (6.4)	0	>0.999
Comorbid conditions, *n* (%)				
Cancer	7 (0.9)	7 (0.9)	0	>0.999
Hypertension	24 (3.1)	23 (3.0)	1 (7.1)	0.361
Diabetes	2 (0.3)	2 (0.3)	0	>0.999
Cardiovascular disease	6 (0.8)	6 (0.8)	0	>0.999
Lung disease	11 (1.4)	10 (1.3)	1 (7.1)	0.184
Chronic renal disease	3 (0.4)	3 (0.4)	0	>0.999
Chronic HBV infection	42 (5.5)	41 (5.4)	1 (7.1)	0.548

Categorical variables are expressed as *n* (%); continuous variables are expressed as the medians [Q1-Q3]. Comparisons were applied between the mild and moderate group (Mann–Whitney *U* test or Fisher's exact test).[a]One patient who died of cerebral hemorrhage after COVID-19 infection was removed from the analysis. COVID-19, coronavirus disease 2019; HBV, hepatitis B virus.

with time. This study was limited by its retrospective design and inadequate sample size; therefore, future prospective studies with large cohorts are needed to evaluate the effectiveness and adjust the vaccination protocol for this special population.

In summary, our study reported the impact of COVID-19 on CD patients from a prospective cohort in the first countrywide wave after the end of "zero-COVID" policy in China. Most CD patients contracted the Omicron infection and experienced mild disease courses irrespective of vaccination dose or comorbidities. Our study presents clinicians with first-hand data on COVID-19 in CD patients during the first wave attack and may help ease the health anxiety of patients in the next wave of the pandemic.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of the Ethics Committee of the Second Affiliated Hospital, School of Medicine, Zhejiang University in China (approved No. 2023-0134). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

JL and YC: conceptualization, writing—review and editing, supervision, and funding acquisition. WH, XL, and ZY: methodology, software, visualization, and project administration. ZY: validation. WH and XL: formal analysis and data curation. XL, ZY, QW, JkL, QY, SuL, SiL, AR, and EG: investigation. YC and SuL: resources. WH, XL, ZY, QW, JkL, QY, and SiL: writing—original draft preparation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

ZY was employed by The China Crohn's & Colitis Foundation.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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SARS-CoV-2 spike-protein targeted serology test results and their association with subsequent COVID-19-related outcomes

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Importance: In the absence of evidence of clinical utility, the United States' Centers for Disease Control and Prevention does not currently recommend the assessment of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spike-protein antibody levels. Clinicians and their patients, especially immunocompromised patients, may benefit from an adjunctive objective clinical laboratory measure of risk, using SARS-CoV-2 serology.

Objective: The aim of this study is to estimate the association between SARS-CoV-2 spike-protein targeted antibody levels and clinically relevant outcomes overall and among clinically relevant subgroups, such as vaccine and immunocompetency statuses.

Design: A retrospective cohort study was conducted using laboratory-based data containing SARS-CoV-2 antibody testing results, as well as medical and pharmacy claim data. SARS-CoV-2 testing was performed by two large United States-based reference clinical laboratories, Labcorp® and Quest Diagnostics, and was linked to medical insurance claims, including vaccination receipt, through the HealthVerity Marketplace. Follow-up for outcomes began after each eligible individual's first SARS-CoV-2 semiquantitative spike-protein targeted antibody test, from 16 November 2020 to 30 December 2021.

Exposures: Exposure is defined as having SARS-CoV-2 spike-protein targeted antibody testing.

Main outcomes and measures: Study outcomes were SARS-CoV-2 infection and a serious composite outcome (hospitalization with an associated SARS-CoV-2 infection or all-cause death). Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). Propensity score matching was used for confounding covariate control.

Results: In total, 143,091 (73.2%) and 52,355 (26.8%) eligible individuals had detectable and non-detectable levels of SARS-CoV-2 spike-protein targeted antibodies, respectively. In the overall population, having detectable vs. non-detectable antibodies was associated with an estimated 44% relative reduction in SARS-CoV-2 subsequent infection risk (HR, 0.56; 95% CI 0.53–0.59) and an 80% relative reduction in the risk of serious composite outcomes (HR 0.20; 95% CI 0.15–0.26). Relative risk reductions were observed across subgroups, including among immunocompromised persons.

Conclusion and relevance: Individuals with detectable SARS-CoV-2 spike-protein targeted antibody levels had fewer associated subsequent SARS-CoV-2 infections

and serious adverse clinical outcomes. Policymakers and clinicians may find SARS-CoV-2 spike-protein targeted serology testing to be a useful adjunct in counseling patients with non-detectable antibody levels about adverse risks and reinforcing appropriate actions to mitigate such risks.

KEYWORDS

SARS-CoV-2 spike antibody, SARS-CoV-2, COVID-19, immunocompromised conditions, immune protection

Introduction

During the coronavirus disease-2019 (COVID-19) pandemic, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spike-protein targeted serology testing has played only a limited role in clinical decision-making, largely based on the advice from the United States Centers for Disease Control and Prevention (CDC) (1). CDC Guidance, updated on 16 December 2022, states, "Antibody testing is not currently recommended to assess for immunity to SARS-CoV-2 following COVID-19 vaccination or to assess the need for vaccination in an unvaccinated person" (2). Furthermore, the United States Food and Drug Administration (FDA) has not designated any Emergency Use Authorization (EUA) SARS-CoV-2 test for assessing individual immunity through antibody testing (The FDA reviews and responds to submissions from *in vitro* diagnostics manufacturers). Currently, the efficacy of detectable antibody levels against subsequent SARS-CoV-2 infection and adverse outcomes is incompletely understood. As a result, only a small number of studies have evaluated the risk of SARS-CoV-2 infection outcomes based on SARS-CoV-2 antibody testing, and none have evaluated this risk among subgroups of the population at the highest risk for severe adverse outcomes of SARS-CoV-2 infection, e.g., immunocompromised individuals (3–5).

The need for clinical guidelines for using SARS-CoV-2 serology testing at the individual level is most acute for immunocompromised persons and those with chronic medical conditions (6). These groups are at increased risk for serious adverse COVID-19-related outcomes, including hospitalization and death (7–10). Immunocompromised patients hospitalized with COVID-19 accounted for 12.2% of hospitalized SARS-CoV-2 infected patients but only 2.7% of the general population (11). Furthermore, immunocompromised persons are more likely to experience adverse COVID-19 outcomes, regardless of vaccination status (11). Having other chronic medical conditions and advanced age are associated with an increased vulnerability to adverse COVID-19 outcomes including age 65 years and older (6), diabetes, cardiovascular disease including hypertension, chronic kidney disease (12), and obesity (13, 14).

The objective of this study was to investigate if having detectable vs. non-detectable SARS-CoV-2 spike-protein targeted antibody levels was associated with a decreased risk of COVID-19-related adverse outcomes overall and among clinically relevant subgroups.

Methods

Data sources

Data were analyzed from HealthVerity-curated real-world sources in the United States, including de-identified closed medical and pharmaceutical claims, clinical laboratory data, COVID-19 vaccination records, and mortality records with services provided between 1 July 2020 and 28 February 2022.

Study design and population

A retrospective cohort study was conducted to investigate the association between having detectable vs. non-detectable SARS-CoV-2 spike-protein targeted antibody levels and the occurrence of subsequent COVID-19-related outcomes. Figure 1 displays the flow diagram of the study cohort selection. Individuals entered the study cohort if their first eligible SARS-CoV-2 spike-protein targeted semiquantitative antibody test was performed between 16 November 2020 and 30 December 2021. Subjects were excluded if they met one or more of the following criteria: (1) lacked continuous insurance enrollment during 181 days prior to and including the antibody test date (allowing for 30-day gaps), (2) had discordant antibody test results, (3) were <18 years of age, (4) had missing age or sex information, or (5) experienced a study outcome or censoring event on the antibody test date.

Exposure, outcomes, and covariates

The study individual's index date was defined as the date of the individual's first eligible SARS-CoV-2 spike-protein targeted semiquantitative antibody test. The exposure of interest was having detectable vs. non-detectable levels of SARS-CoV-2 spike protein antibody levels, as measured by commercially available semiquantitative assays. Four different FDA EUA assays, intended to identify individuals who may have developed an adaptive immune response to SARS-CoV-2, were in use by Labcorp and Quest Diagnostics during the study period: Siemens Healthcare Diagnostics, Inc ADVIA Centaur® SARS-CoV-2 IgG (COV2G) and ADVIA Centaur/Atellica® (sCOVG) assays, Roche Diagnostics Inc. Elecsys® Anti-SARS-CoV-2 S, and DiaSorin Inc. LIAISON® SARS-CoV-2 TrimericS IgG. The threshold for

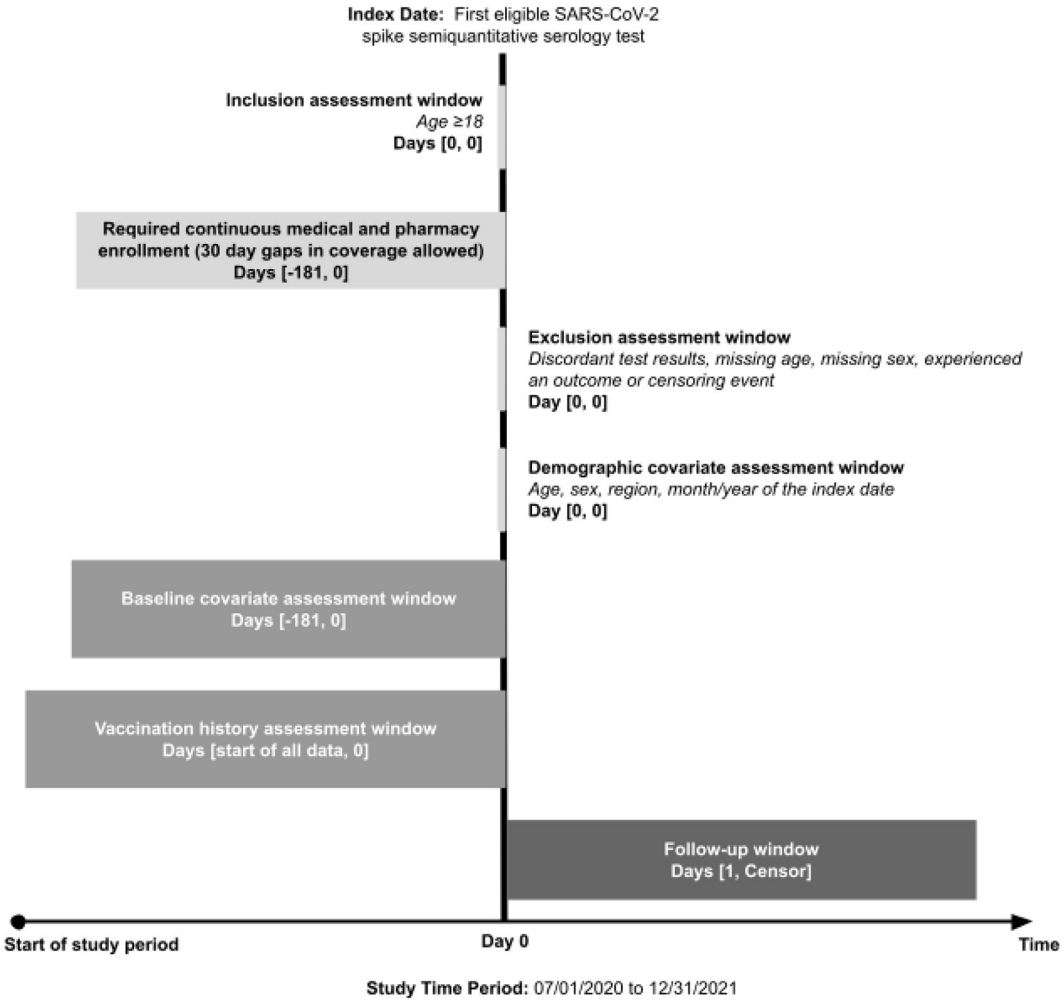

FIGURE 1
Study design. COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

classifying people as having either detectable or non-detectable antibody levels differed across assays, and thus, test-specific levels were defined (Supplementary Table 1).

Outcomes of interest were as follows: (1) subsequent SARS-CoV-2 infection [i.e., positive polymerase chain reaction (PCR) or other nucleic acid amplification tests (NAATs)] and separately (2) a composite of serious events, hospitalization with an associated SARS-CoV-2 infection, or all-cause mortality (Supplementary Table 2). The follow-up period for outcome assessment started on the day after the index date, and outcomes were ascertained using medical claims, laboratory, and mortality data.

Baseline covariates included potential confounders and variables known to be strong risk factors for the study outcomes (15). Covariates of interest were identified prior to or on the index date and included demographic characteristics, skilled nursing facility or nursing home residence, the presence of an immunocompromising condition, and other clinical conditions associated with a heightened risk of severe COVID-19 (i.e., vulnerable medication conditions) and COVID-19 vaccination status (Supplementary Tables 3–4).

Statistical analysis

All statistical analyses were performed using the Aetion Evidence Platform® version 4.63 with R version 3.4.2. Baseline characteristics are described across individuals with detectable and non-detectable levels of antibodies against SARS-CoV-2 spike protein as count (%) for categorical variables and as mean ± standard deviation for continuous variables. Covariate distributions were compared between exposure groups using absolute standardized mean differences (ASDs). An ASD $\leq$0.10 indicates adequate covariate balance between groups (16).

An as-treated analytic approach was used to evaluate the association between having detectable vs. non-detectable levels of SARS-CoV-2 spike-protein targeted antibodies and the occurrence

TABLE 1 Characteristics of individuals with detectable and non-detectable SARS-CoV-2 spike-protein targeted antibody levels.

Characteristic	Unmatched[a]			Matched[a]		
	Detectable antibody level n = 143,091	Non-detectable antibody level n = 52,355	Std diff[b]	Detectable antibody level n = 51,807	Non-detectable antibody level n = 51,807	Std diff[b]
Year/season of index[c]			0.30			0.02
Winter 2020–2021	1,224 (0.9%)	577 (1.1%)		511 (1.0%)	572 (1.1%)	
Spring 2021	22,683 (15.9%)	12,828 (24.5%)		12,323 (23.8%)	12,437 (24.0%)	
Summer 2021	42,750 (29.9%)	17,433 (33.3%)		17,215 (33.2%)	17,285 (33.4%)	
Fall 2021	57,416 (40.1%)	17,600 (33.6%)		17,842 (34.4%)	17,596 (34.0%)	
Winter 2021	19,018 (13.3%)	3,917 (7.5%)		3,916 (7.6%)	3,917 (7.6%)	
Age (years)	51.6 ± 15.6	48.0 ± 14.5	0.24	47.9 ± 14.7	48.1 ± 14.5	0.01
Female	86,764 (60.6%)	30,983 (59.2%)	0.03	30,962 (59.8%)	30,773 (59.4%)	0.01
Region			0.28			0.01
Midwest	11,755 (8.2%)	6,717 (12.8%)		6,371 (12.3%)	6,459 (12.5%)	
South	51,701 (36.1%)	19,411 (37.1%)		19,483 (37.6%)	19,374 (37.4%)	
West	19,448 (13.6%)	10,214 (19.5%)		9,948 (19.2%)	9,961 (19.2%)	
Northeast	60,166 (42.0%)	16,007 (30.6%)		16,004 (30.9%)	16,007 (30.9%)	
Other or unknown	21 (0.0%)	6 (0.0%)		1 (0.0%)	6 (0.0%)	
SNF or nursing home utilization	1,117 (0.8%)	303 (0.6%)	0.03	196 (0.4%)	299 (0.6%)	0.03
Had ≥ 1 immunocompromising condition	10,467 (7.3%)	3,263 (6.2%)	0.04	3,179 (6.1%)	3,192 (6.2%)	0.00
Had ≥ 1 vulnerable condition	69,032 (48.2%)	20,671 (39.5%)	0.18	20,524 (39.6%)	20,572 (39.7%)	0.00
COVID-19 vaccination status			0.77			0.00
Fully vaccinated plus a booster	1,067 (0.7%)	30 (0.1%)		33 (0.1%)	30 (0.1%)	
Fully vaccinated	35,415 (24.7%)	1,726 (3.3%)		1,709 (3.3%)	1,726 (3.3%)	
Partially vaccinated	9,891 (6.9%)	582 (1.1%)		590 (1.1%)	582 (1.1%)	
Unvaccinated	96,718 (67.6%)	50,017 (95.5%)		49,475 (95.5%)	49,469 (95.5%)	

[a] Values presented as number (%) for categorical variables and as mean ± standard deviation for continuous variables.
[b] A standardized difference >0.10 represents a meaningful imbalance between exposure groups.
[c] Winter 2020–2021 was defined as 1 December 2022 to 28 February 2021. Spring 2021 was defined as 1 March 2021 to 31 May 2021. Summer 2021 was defined as 1 June 2021 to 31 August 2021. Fall 2021 was defined as 1 September 2021 to 30 November 2021. Winter 2021 was defined as 1 December 2021 to 31 December 2021.
COVID-19, coronavirus disease 2019; SNF, SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; skilled nursing facility; std diff, standardized different.

of COVID-19-related outcomes. Individuals were followed forward in historical time starting from the day after the index date until the occurrence of an outcome or censoring event. Censoring events included the following: (1) change in exposure status, (2) insurance disenrolment, and (3) study end (31 December 2021).

In primary analyses, 1:1 propensity score matching was used for confounding control. Nearest-neighbor matching without replacement was performed using a caliper of 1.0%. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) in both the unmatched and propensity score matched cohorts.

Given that associations may differ across clinically relevant subgroups, secondary subgroup analyses were conducted using analogous methods. Subgroups of interest included COVID-19 vaccination status subgroups (vaccinated and unvaccinated individuals) and health status subgroups (immunocompromised, vulnerable, and other healthy individuals) (Supplementary Table 5).

Finally, to understand whether higher SARS-CoV-2 spike-protein antibody levels may be associated with subsequent protection against SARS-CoV-2 infection, additional analyses were conducted among individuals with detectable levels of SARS-CoV-2 antibodies. In these analyses, all semiquantitative antibody test result values were converted to a common scale, WHO binding antibody units (BAUs; Supplementary Table 6) (16–18). Higher antibody levels were defined as having test results of ≥250 BAU/ml and lower antibody levels as having detectable test results of <250 BAU/ml, based on studies supporting

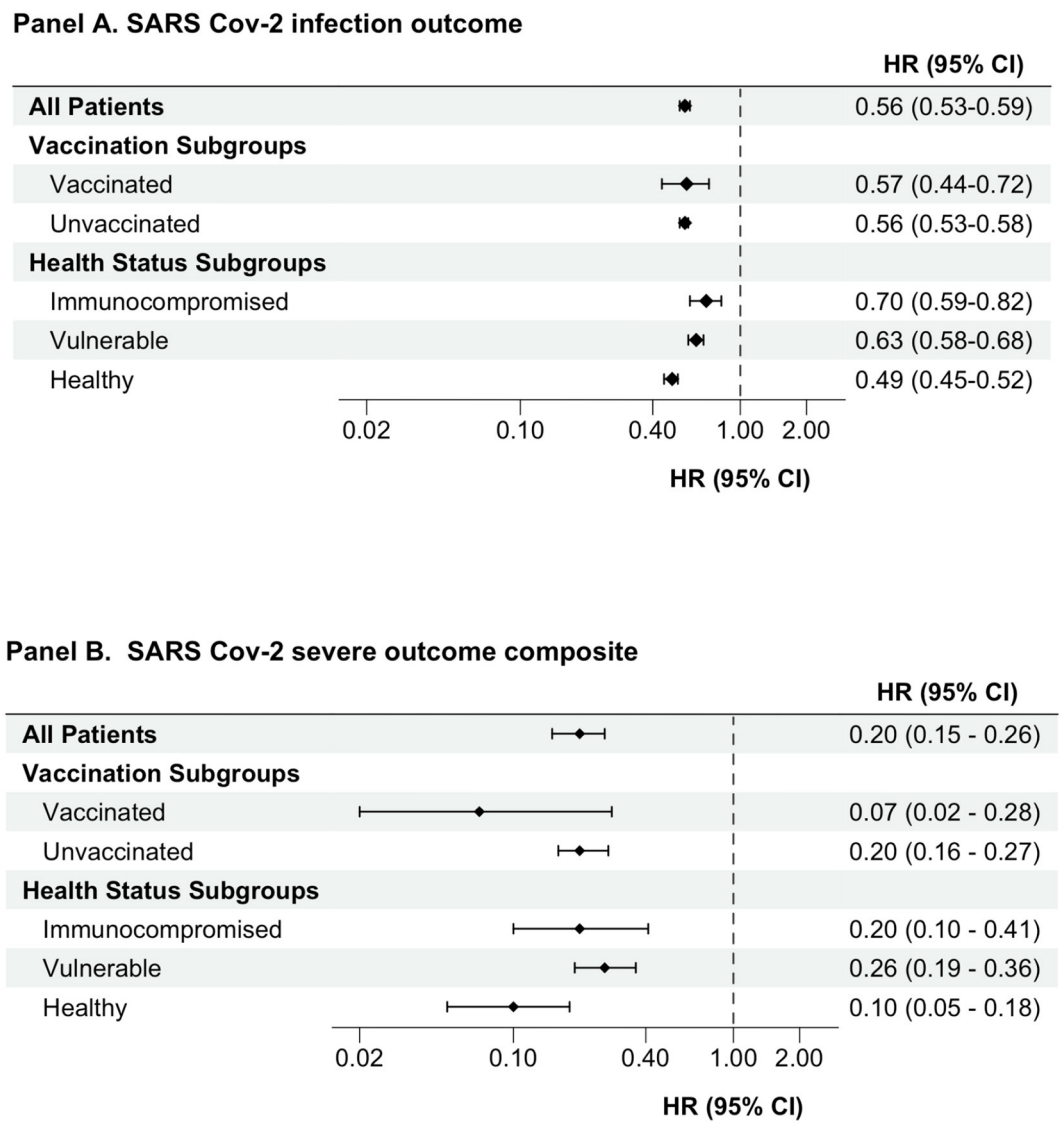

FIGURE 2
Association between having detectable versus non-detectable SARS-CoV-2 spike-protein targeted antibody levels and outcomes. An as-treated analytic approach was used for all analyses. Cox proportional hazards models were used to estimate hazard ratios (CI) comparing individuals with detectable versus non-detectable levels of semi quantitative antibodies against SARS-CoV-2 spike protein. HRs presented is for propensity score-matched cohort. CI, confidence interval; HR, hazard ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

this general threshold (19–21). EQUATOR Reporting Guidelines were followed.

Results

A total of 1,798,606 people had a SARS-CoV-2 spike-protein targeted semiquantitative serology test from 16 November 2020 to 30 December 2021 (Supplementary Figure 1). Lack of baseline insurance enrollment excluded 1,580,452 individuals, and an additional 22,708 persons were excluded for other reasons. Therefore, the study cohort included a total of 195,446 individuals: 143,091 (73.2%) individuals with detectable levels and 52,355 (26.8%) individuals with non-detectable levels of semiquantitative SARS-CoV-2 spike-protein targeted antibodies. Overall, the study cohort had a mean age of 50.6 years, was 60.2% female, and was most commonly from the Northeastern (39.0%) and Southern (36.4%) regions of the United States. Only 24.9% of individuals had a record of receiving at least one dose of a COVID-19 vaccine. Of study individuals with any documented vaccination prior to exposure (SARS-CoV-2 spike-protein antibody testing), 95.5% (46,373 of 48,711) had a detectable antibody. In contrast, only 65.9% (96,718 of 146,735) of study individuals without documented vaccination prior to exposure had detectable antibody levels. Having an immunocompromising medical condition or another vulnerable medical condition associated with a heightened risk of severe COVID-19 was present in 7.0 and 45.9% of the cohort, respectively.

Table 1 shows the baseline characteristics of the study cohort stratified by the exposure groups. Before propensity score matching, baseline covariates were generally well-balanced between exposure groups (ASD $\leq$0.10), with some exceptions (e.g., age,

region of the United States, year/season of cohort entry, and COVID-19 vaccination status). After propensity score matching, all baseline covariates were well-balanced between exposure groups, indicating adequate control of measured confounders.

Primary analyses

During follow-up, a total of 10,735 subsequent SARS-CoV-2 infections occurred in the unmatched cohort: 6,392 events at an incidence rate of 141.1 events per 1,000 person-years in the detectable antibody group compared to 4,343 events at an incidence rate of 229.6 per 1,000 person-years in the non-detectable antibody group. Additionally, 575 serious outcomes (hospitalization with COVID-19 or all-cause mortality) occurred during follow-up: 221 events at an incidence rate of 4.8 per 1,000 person-years in the detectable antibody group compared to 354 events at an incidence rate of 18.1 per 1,000 person-years in the non-detectable antibody group. After propensity score matching, having detectable vs. non-detectable levels of SARS-CoV-2 spike-protein targeted antibodies was associated with a lower risk of subsequent SARS-CoV-2 infection (HR, 0.56; 95% CI 0.53–0.59) and the serious composite outcomes of hospitalization with COVID-19 or all-cause mortality (HR 0.20; 95% CI 0.15–0.26) (Figure 2).

Subgroup analyses

Analyses of clinically relevant subgroups produced results analogous to our primary study findings. In both the unvaccinated and vaccinated subgroups, people with detectable SARS-CoV-2 spike-protein targeted antibody levels had a lower risk of subsequent SARS-CoV-2 infection and the serious outcomes composites compared to people with non-detectable antibody levels (Table 2 and Figure 2). Similarly, in the subgroups of immunocompromised, vulnerable, and otherwise healthy persons, having detectable antibody levels was associated with a lower risk of subsequent SARS-CoV-2 infection and experiencing serious composite outcomes of hospitalization with COVID-19 or all-cause mortality (Table 2 and Figure 2). The HR (95% CI) for subsequent SARS-CoV-2 infection in the groups with detectable SARS-CoV-2 spike-protein antibody levels (referent groups had non-detectable antibody levels) was 0.56 (0.53–0.59), 0.70 (0.59–0.82), 0.63 (0.58–0.68), and 0.49 (0.45–0.52) for the overall study cohort, immunocompromised, vulnerable, and other health groups, respectively. The HR (95% CI) for the serious composite outcomes in the groups with detectable SARS-CoV-2 spike-protein antibody levels (referent groups had non-detectable antibody levels) was 0.20 (0.15–0.26), 0.20 (0.10–0.41), 0.26 (0.19–0.36), and 0.10 (0.05–0.18) for the overall study cohort, immunocompromised, vulnerable, and other health groups, respectively.

Additional analyses

Among the group of individuals who had detectable levels of SARS-CoV-2 spike-protein targeted antibodies (Supplementary Figure 2 and Supplementary Table 5), those with higher (>250 BAU/ml) vs. lower (<250 BAU/ml) antibody levels had a lower risk of serious outcomes (after propensity score matching HR, 0.65, 95% CI 0.45–0.93). Similar associational trends were seen within subgroups, but HR estimates were imprecise (Supplementary Table 7 and Figure 3).

Discussion

Healthcare providers seek guidance on how to evaluate an individual's SARS-CoV-2 risk, especially for those with high-risk conditions, i.e., immunocompromised and vulnerable patients. Kaufman et al. have postulated that SARS-CoV-2 spike-protein targeted serology test results may be clinically useful, notably among these high-risk individuals concerning the subsequent risk of adverse outcomes (22). This position was based on a literature review that revealed (1) individuals at increased risk for severe outcomes following SARS-CoV-2 infection were less likely to develop a robust antibody response following infection and vaccination (23) and (2) studies showing that people with non-detectable or low levels of SARS-CoV-2 spike-protein targeted antibodies were more likely to have subsequent adverse events, i.e., hospitalization with COVID-19 and death, than those with higher levels (8, 24). Furthermore, SARS-CoV-2 spike-targeted antibody titer levels correlate with protection against subsequent SARS-CoV-2 infection or reinfection (25). This study confirms the two prior key findings by demonstrating that people with detectable SARS-CoV-2 spike-targeted antibody levels, as well as those with higher vs. lower detectable antibody levels, have a lower risk of COVID-19 serious outcomes in overall and adds novel findings among subgroups of patients at increased risk of SARS-CoV-2 infection (7–10).

These observations are consistent with other studies that have demonstrated similar associations between specific medical conditions and SARS-CoV-2 spike-protein targeted serology results and between serology results and subsequent outcomes (3–5). A recent study of cancer vs. non-cancer (control) patients in the United Kingdom showed that detectable levels of SARS-CoV-2 spike-protein targeted antibodies were associated with protection against subsequent SARS-CoV-2 infection and serious outcomes (26). Among patients with cancer, a non-detectable vaccine antibody response was associated with more than three times the risk of subsequent SARS-CoV-2 infection and more than six times the risk of a COVID-19-associated hospitalization. The authors suggest that patients with non-detectable antibody levels may benefit from additional vaccine doses, prophylactics, and early treatment.

In the United States, the three COVID-19 vaccines available during the study period were manufactured by BioNtech/Pfizer, Janssen/Johnson & Johnson, and Moderna. SARS-CoV-2 vaccination effectively reduced serious COVID-19 outcome events even though the benefit of vaccination is generally less effective among immunocompromised persons as compared to immunocompetent individuals (27). In this study, 24.9% (48,711 of 195,446) of individuals received at least one vaccine dose. In the matched cohort, the HR for subsequent infection among those with detectable vs. non-detectable SARS-CoV-2 spike-protein

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TABLE 2 Association between having detectable vs. non-detectable SARS-CoV-2 spike-protein targeted antibody levels and outcomes, overall and in COVID-19 vaccination subgroups and in health status subgroups.

SARS-CoV-2 infection, in COVID-19 vaccination subgroups				
Exposure	n	No. events (rate per 1,000 person years)	Unmatched cohort HR (95% CI)	Matched cohort HR (95% CI)
Overall				
Non-detectable antibody levels	52,355	4,343 (229.6)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	143,091	6,392 (141.1)	0.61 (0.59–0.64)	0.56 (0.53–0.59)
Vaccinated				
Non-detectable antibody levels	2,338	173 (236.7)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	46,373	2,109 (159.5)	0.67 (0.57–0.78)	0.57 (0.44–0.72)
Unvaccinated				
Non-detectable antibody levels	50,017	4,170 (229.3)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	96,718	4,283 (133.6)	0.58 (0.55–0.60)	0.56 (0.53–0.58)
Hospitalization with COVID-19 or all-cause mortality, in COVID-19 vaccination subgroups				
Exposure	n	No. events (rate per 1,000 person years)	Unmatched cohort HR (95% CI)	Matched cohort HR (95% CI)
Overall				
Non-detectable antibody levels	52,355	354 (18.1)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	143,091	221 (4.8)	0.26 (0.22–0.31)	0.20 (0.15–0.26)
Vaccinated				
Non-detectable antibody levels	2,338	28 (37.2)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	46,373	79 (5.9)	0.16 (0.10–0.24)	0.07 (0.02–0.28)
Unvaccinated				
Non-detectable antibody levels	50,017	326 (17.3)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	96,718	142 (4.3)	0.25 (0.20–0.30)	0.20 (0.16–0.27)
SARS-CoV-2 Infection, in health status subgroups				
Exposure	n	No. events (rate per 1,000 person years)	Unmatched cohort HR (95% CI)	Matched cohort HR (95% CI)
Overall				
Non-detectable antibody levels	52,355	4,343 (229.6)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	143,091	6,392 (141.1)	0.61 (0.59–0.64)	0.56 (0.53–0.59)
Immunocompromised				
Non-detectable antibody levels	3,263	323 (289.2)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	10,467	642 (193.2)	0.67 (0.59–0.77)	0.70 (0.59–0.82)
Vulnerable				
Non-detectable antibody levels	18,575	1,651 (252.7)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	62,139	3,030 (154.3)	0.61 (0.57–0.65)	0.63 (0.58–0.68)
Other healthy				
Non-detectable antibody levels	30,517	2,369 (210.3)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	70,485	2,720 (121.8)	0.58 (0.55–0.61)	0.49 (0.45–0.52)
Hospitalization with COVID-19 or all-cause mortality, in health status subgroups				
Exposure	n	No. events (rate per 1,000 person years)	Unmatched cohort HR (95% CI)	Matched cohort HR (95% CI)
Overall				
Non-detectable antibody levels	52,355	354 (18.1)	1.00 (ref.)	1.00 (ref.)

(Continued)

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TABLE 2 (Continued)

Hospitalization with COVID-19 or all-cause mortality, in health status subgroups				
Exposure	n	No. events (rate per 1,000 person years)	Unmatched cohort HR (95% CI)	Matched cohort HR (95% CI)
Detectable antibody levels	143,091	221 (4.8)	0.26 (0.22–0.31)	0.20 (0.15–0.26)
Immunocompromised				
Non-detectable antibody levels	3,263	42 (35.9)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	10,467	39 (11.4)	0.31 (0.20–0.49)	0.20 (0.10–0.41)
Vulnerable				
Non-detectable antibody levels	18,575	194 (28.6)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	62,139	154 (7.6)	0.26 (0.21–0.33)	0.26 (0.19–0.36)
Other healthy				
Non-detectable antibody levels	30,517	118 (10.1)	1.00 (ref.)	1.00 (ref.)
Detectable antibody levels	70,485	28 (1.2)	0.12 (0.08–0.18)	0.10 (0.05–0.18)

An as-treated analytic approach was used for all analyses. Cox proportional hazards models were used to estimate hazard ratios (HR) [95% confidence intervals (CI)].
CI, confidence interval; COVID-19, coronavirus disease 2019; HR, hazard ratio; ref., referent; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

targeted antibody levels was 0.56 (95% CI 0.53–0.58) and 0.57 (95% CI 0.44–0.72) for the vaccinated and unvaccinated groups, respectively. In the matched cohort of this study, the HR for serious composite outcomes of hospitalization with COVID-19 or all-cause mortality among those with detectable vs. non-detectable SARS-CoV-2 spike-protein targeted antibody levels was 0.07 (95% CI 0.02–0.28) and 0.20 (95% CI 0.16–0.27) for the vaccinated and unvaccinated groups, respectively. Although the CI are overlapping, this observation supports the clinical value of having detectable antibodies while still recognizing that other immune mechanisms of protection are likely involved in the protection against SARS-CoV-2 infection and COVID-19 outcomes. Furthermore, the direction of the HR supports the concept that vaccination was likely useful in reducing serious outcomes.

Future studies may be useful in delineating the interplay of humoral and cellular immune system components in protection against SARS-CoV-2 infection and its consequences. Tixagevimab/cilgavimab was the only combination therapeutic authorized to date by both the FDA [effective 8 December 2021, revoked 26 January 2023 (28)] and the European Medicine Agency (EMA) (effective 25 March 2022) for pre-exposure prophylaxis of COVID-19. This was especially relevant in the population with immunocompromising conditions who fail to mount a detectable antibody response after multiple vaccine doses. The authors of the primary tixagevimab/cilgavimab study note, "The limitations of our trial include the low number of events in smaller but important subgroups, including immunocompromised persons, so that efficacy in these groups could not be estimated" (29). The FDA subsequently recommended high dosing of tixagevimab/cilgavimab after a significant number of patients in the immunosuppressed group were found to have breakthrough infections (30). Young-Xu et al. at Veteran Affairs Healthcare Systems, found that compared to 251,756 propensity-matched immunocompromised or at-risk historical controls, 1,848 tixagevimab/cilgavimab-treated patients had a lower incidence of SARS-CoV-2 infection, COVID-19 hospitalization, and all-cause mortality (31). Until newer effective prophylactic drugs are approved, respiratory tract masking may be especially valuable within the high-risk population, e.g., with immunocompromising conditions, when visiting healthcare facilities where there are potentially SARS-CoV-2-infected patients (32). The CDC suggests that masks can provide an extra level of protection against SARS-CoV-2 infection and its resulting severe events (6). Early antiviral treatments may be beneficial as well, especially in the immunocompromised population (33). Again, the effectiveness of these multiple infection mitigation measures with current and future SARS-CoV-2 variants is worthy of investigation. Given our findings among immunocompromised persons, the study's findings support the application of these suggested COVID-19 mitigation measures in high-risk populations, particularly those who are SARS-CoV-2 seronegative. Additional studies may indicate the specific potential benefit of additional vaccine dosing and other mitigation efforts to reduce the risk of adverse outcomes in individuals with non-detectable SARS-CoV-2 antibody levels.

The strengths of this study include the use of a large-scale real-world database with information aggregated from diverse sources, inclusive of multiple laboratory antibody test methods, analysis of subsequent SARS-CoV-2 infections, COVID-19 hospitalizations and all-cause mortality, and multivariate modeling with confounding control. Tracking changes in semiquantitative SARS-CoV-2 spike-protein targeted antibody levels over time within an at-risk individual may provide insights into the durability of the antibody response and assist in determining the subsequent risk of infection (5). Alternative assays that measure antibody neutralization of novel spike protein(s) or cellular-based adaptive immunity assays are being studied for their associated clinical utility but they are not yet widely commercially available (34). Of note, this study included SARS-CoV-2 spike-protein targeted antibody tests and did not include rapid antigen tests or nucleocapsid antibody tests. Differences in antibody generation post-infection have been observed with SARS-CoV-2 nucleocapsid antibody tests. SARS-CoV-2 Omicron variants impacted the

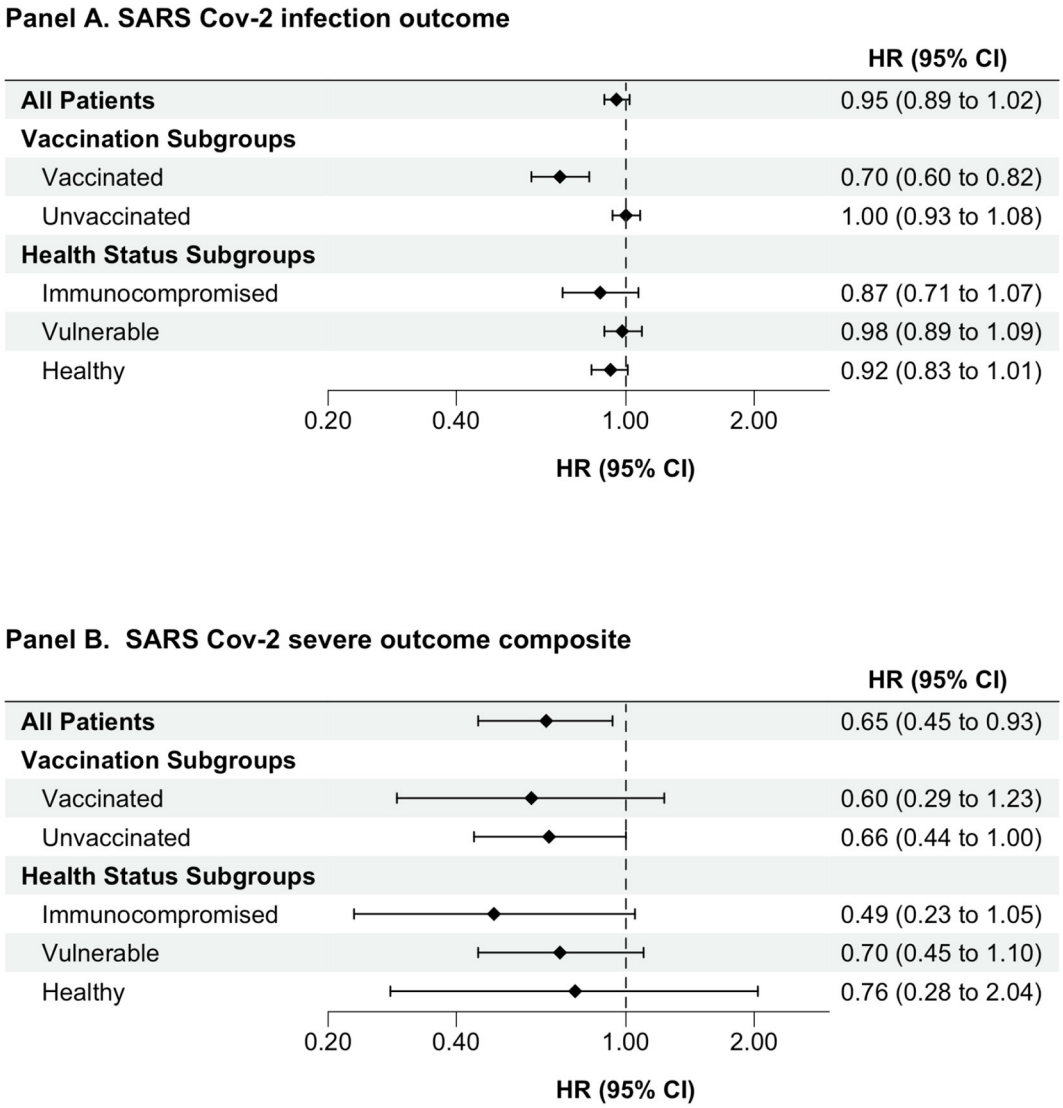

FIGURE 3
Association between having higher (≥250 BAU/mL) versus lower (<250 BAU/mL) SARS-CoV-2 spike-protein antibody level comparison. An as-treated analytic approach was used for all analyses. Cox proportional hazards models were used to estimate hazard ratios (CI) comparing individuals with detectable versus non-detectable levels of semi quantitative antibodies against SARS-CoV-2 spike protein. HRs presented is for propensity score-matched cohort. BAU/ml, binding antibody units per milliliter; CI, confidence interval; HR, hazard ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

performance of some NAAT assay methods. The FDA updates the few assay methods that are adversely affected by viral mutations (35). None of those SARS-CoV-2 NAAT assay methods were used in this study. Therefore, SARS-CoV-2 variants had no reported impact on the performance of the SARS-CoV-2 spike protein targeted antibody or NAAT testing in this study.

The study had some limitations. First, the evaluation time period was early in the pandemic (November 2020 to December 2021) and included a portion of the time when COVID-19 vaccines were not yet widely adopted. Similarly, home laboratory testing that identifies SARS-CoV-2 infections was not captured though also not yet common during the study period. Second, information on the medical reason for the requested SARS-CoV-2 serology testing was unavailable. Third, the COVID-19 infection outcome was largely driven by infections identified in the health insurance claims data. SARS-CoV-2 PCR/NAAT tests performed by LabCorp and Quest Diagnostics, although substantial in aggregate number, represent less than 20% of all total SARS-CoV-2 PCR/NAAT conducted in the United States during the study period (36–38). Finally, deaths were infrequent, precluding studying associations between SARS-CoV-2 antibody levels and mortality alone.

In summary, this large United States-based real-world evidence-based study utilized linked medical claims and clinical laboratory data to examine associations between SARS-CoV-2 spike-protein antibody levels and clinical outcomes. The study demonstrated that people with detectable levels of SARS-CoV-2 spike-protein targeted antibodies had a lower risk of subsequent SARS-CoV-2 infections and serious composite outcomes (hospitalization with an associated SARS-CoV-2 infection or all-cause mortality). This observed effect was seen

in the overall population and also within clinically relevant subgroups, including the immunocompromised population. Analyses of individuals with detectable antibodies >250 vs. <250 BAU/ml generated directionally consistent results, albeit with a less potent magnitude of effect. Thus, federal policymakers and clinicians may find SARS-CoV-2 spike-protein targeted serology testing to be a useful adjunct in counseling immunocompromised persons and other higher at-risk individuals about adverse outcomes and apply appropriate actions to mitigate such risks.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

All authors were involved in study design, analysis, interpretation of data, writing of the manuscript, and the decision to submit the manuscript for publication. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1193246/full#supplementary-material

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Prevalence and factors of COVID-19 vaccine refusal among solid cancer patients in China: an application of the health belief model

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Introduction: It is essential to protect cancer patients from contracting COVID-19 through vaccination. A majority of cancer patients are recommended by international health authorities to take up the vaccines. COVID-19 vaccine refusal among cancer patients during the pandemic period is under-researched. This study investigated factors of vaccine refusal based on the Health Belief Model (HBM).

Methods: A cross-sectional study was conducted among female breast cancer patients, male/female thyroid cancer patients, and gynecological cancer patients in Shantou, China from April to August 2022 (n = 1,115). Multinomial logistic regression analysis adjusted for socio-demographics was conducted to test factors of COVID-19. Adjusted odds ratios of the two models comparing vaccine refusal vs. "vaccine non-refusal" and vaccine refusal vs. ever-vaccination were derived and presented.

Results: Of all the participants, the prevalence of vaccine refusal, "vaccine non-refusal," and ever-vaccination was 25.9, 22.2, and 51.8%, respectively. In both multinomial logistic regression models, significant factors of vaccine refusal included socio-demographics (age, education level, employment status, monthly household income, cancer type, duration since cancer diagnosis, current treatment status) and some vaccine-related HBM (perceived benefits, perceived barriers, cue to action, and self-efficacy). Perceived severity of COVID-19 was significant only in the vaccine refusal vs. ever-vaccination model. In neither model, perceived susceptibility to contract COVID-19 was statistically significant.

Conclusion: About ¼ of the participants expressed vaccine refusal. Interventions are warranted. Future longitudinal studies are needed to verify this study's findings. Pilot interventions should also be launched to test effectiveness of interventions modifying the significant HBM factors found in this study.

KEYWORDS

cancer patients, health belief model, COVID-19, vaccine refusals, vaccine

Introduction

The COVID-19 pandemic has created global severe disease and financial burdens (1). The health consequences of COVID-19 infection are particularly serious in some diseased groups (2, 3). Vaccination is known to be effective in controlling the pandemic (4). It was estimated that COVID-19 vaccines have averted 19.8 million deaths in the first year since their rollout (5). In particular, COVID-19 is a threat to cancer patients who are more vulnerable to severe harms and deaths resulting from COVID-19 than the general population (6–9). One study reported that among COVID-19 patients, those suffering from cancer showed a higher fatality rate and a higher risk of severe complications related to COVID-19 than their counterparts (7). Nationwide data collected in China also showed that cancer patients have higher prevalence of COVID-19 infection than the general population (10). The threat of COVID-19 on cancer patients prevails. Thus, COVID-19 vaccination in this population is highly warranted.

In general, perceived safety and perceived efficacy of COVID-19 vaccination are strong determinants of vaccine hesitancy (11, 12). A study showed that tolerance of COVID-19 vaccination among cancer patients receiving systematic treatments was indistinguishable from that of the general population (13). Another study found a similar incidence of adverse events related to COVID-19 vaccination between cancer patients and non-patients (14). A prospective multicenter study revealed that the predominant adverse events of COVID-19 vaccination among cancer patients were mild and self-resolving reactions of injection site pain and anorexia, suggesting that COVID-19 vaccines among cancer patients are safe in general (15). In addition, a large-scale cohort study showed that COVID-19 vaccines could reduce COVID-19 infection in cancer patients (16). A randomized clinical trial found that cancer patients aged 80 years and older were still able to develop serological responses 1 month after receiving COVID-19 vaccines (17). Thus, there is no evidence that cancer patients should refrain from vaccination.

In contrast, global health authorities, including those specializing in oncology, have recommended that cancer patients should be given a high priority to receive COVID-19 vaccines (18–22). The American Society of Clinical Oncology and the Vaccination Advisory Committee of the National Comprehensive Cancer Network recommend that cancer patients, including those who are active or receiving cancer treatment, should be prioritized for the COVID-19 vaccine, while patients who have recently received hematopoietic stem cell transplantation or chimeric antigen receptor T cell therapy should postpone vaccination for at least 3 months (23, 24). The European Society of Cancer Sciences also recommends vaccination among patients who have finished treatments or who are in stable conditions, while there are reservations for those under active cancer treatments (19). According to the "Chinese Expert Consensus on Issues Related to the Protection, Treatment and Management of Patients with Solid Tumors during COVID-19 (2022)" (25), patients undergoing treatments of surgery, radiotherapy, chemotherapy and immunotherapy and those showing allergy to vaccine components should suspend or refrain from vaccination, while those on endocrine therapy and targeted therapy can receive vaccines immediately after doctor's evaluations. Thus, vaccination is recommended by the majority of cancer patients. High vaccination rates have been observed among cancer patients in countries such as Germany (95%) (26), Japan (75%) (27), and Canada (86.8%) (28). In many countries, the prevalence of COVID-19 vaccination in cancer patients was, however, low or relatively low. For instance, it was 41.8% in Bosnia and Herzegovina (29), 50.5% in Tunisia (30), 66.0% in Mexico (31), and 19.5% in Korea (32). Despite the official recommendations, the prevalence only ranged from 12.6 to 58.8% among cancer patients in China (33–39).

Inclination toward COVID-19 vaccination has been studied in various dimensions, including willingness to take up the vaccines (40), vaccine hesitancy (41), and vaccine refusal (42). Many people had held an initial 'wait-and-see' attitude in response to the uncertainties regarding COVID-19 vaccination had eventually taken up the vaccines (43, 44). Vaccine refusal differs from vaccine hesitancy (45). Instead of considering whether to take up COVID-19 vaccination, people may hold a firm stance on refusing vaccination under any circumstances (46). A better understanding of vaccine refusal has particular importance as its prevalence is critical in determining the eventual vaccination coverage and a high coverage is required to achieve community or herd immunity (47). Notably, a dearth of studies has investigated factors of COVID-19 vaccination behavior and inclinations among cancer patients. Such information may facilitate the design of effective health promotion programs. Similar to other populations, cancer patients' concerns about the safety and efficacy of COVID-19 vaccination were negatively associated with vaccine acceptance (13, 40). Other factors of low vaccine acceptance included female gender, older age, disease status (32), fear of interaction between vaccination and treatment effect (30), and a lack of knowledge about vaccination (33).

Furthermore, it is warranted to understand theory-based factors of COVID-19 vaccination among cancer patients. The Health Belief Model (HBM) (48) was used as the theoretical framework in the present study. It postulates that perceived severity and perceived susceptibility of the disease (COVID-19 in this case) and perceived benefits, perceived barriers, self-efficacy, and cue to action related to the health-related behavior are determinants of the behavior (46, 47, 49–52) [COVID-19 vaccination in this case (53, 54)]. Such HBM factors can be modified through health promotion and interventions (55). The HBM constructs were able to predict vaccination behaviors in the populations (56), such as human papillomavirus vaccines (57), influenza vaccines (58), and COVID-19 vaccines (59, 60) Notably, cancer patients' HBM cognitions related to COVID-19 and COVID-19

Abbreviations: HBM, Health belief model; COVID-19, Coronavirus 2019; BMI, Body mass index; RMB, Renminbi; ORu, Univariate odds ratio; CI, Confidence interval; ORa, adjusted odds ratio.

vaccines may differ from those of the general population (13, 42), and are understudied.

The present study investigated (a) the prevalence of COVID-19 vaccination behavior (i.e., ever-vaccination) and two types of vaccination inclinations ("vaccine non-refusal" and vaccine refusal) among four groups of cancer patients in China who had not taken up COVID-19 vaccination prior to their cancer diagnosis (male and female thyroid cancer patients, female breast cancer patients and gynecological cancer patients), and (b) the levels of related HBM factors. The associations between the HBM factors and vaccine refusal vs. ever-vaccination/vaccine refusal vs. "vaccine non-refusal" were tested. In this study, the "vaccine non-refusal" group referred to those who had neither taken up vaccination nor definitely refusing to take up COVID-19 vaccination in the future. i.e., they planning or thinking about whether to be vaccinated. Our literature search could not locate studies investigating vaccine refusal or applying the HBM to understand COVID-19 vaccination behavior/inclinations among cancer patients.

Methods

Study design and participants

A cross-sectional study was conducted among cancer patients in four major hospitals from April to August 2022 in Shantou city, China, which is located in Guangdong province in southern China and has a population of 5.7 million people. The four conveniently selected hospitals (the Affiliated Cancer Hospital of Shantou University, the First and the Second Affiliated Hospitals of Shantou University, and the Shantou Central Hospital) provided medical care to about 80% of the city's cancer patients. The inclusion criteria included: (1) Chinese residents aged ≥18 years, (2) primary diagnosis of breast cancer (females only) or thyroid cancer (males and females) or gynecological cancer ("gynecological cancer" refers to cancers that specifically originate in the female reproductive organs, including the cervix, ovaries, uterus, fallopian tubes, vulva, and vagina), and (3) provision of written informed consent. The exclusion criteria included: (1) at least one dose of COVID-19 vaccination taken up prior to cancer diagnosis, (2) multiple primary cancer diagnoses, (3) terminal cancer conditions, (4) currently or recently under cancer treatment of palliative care, chemotherapy, radiotherapy, surgery and immunotherapy, (5) physically unfit for vaccination, and (6) cognitive impairment.

Two modes of recruitment were implemented. The first one involved on-site recruitments conducted in the selected hospitals with the assistance of the clinical staff. Cancer patients visiting the hospitals for follow-up consultations were screened according to the inclusion/exclusion criteria. The nurses referred eligible prospective participants who were fit to take up the vaccines to contact the onsite research staff. The trained fieldworkers then explained the objectives, content, and the anonymous nature of the survey to the participants, and guaranteed to them that refusal to participate in the survey or termination at any time point would not cause any negative consequences, nor would affect their rights to use any services. In a private setting and with written informed consent, the participants self-administered an anonymous structured questionnaire which took about 10 min to complete. Upon completion, the investigator collected the questionnaires and conducted onsite quality check and sought clarifications if necessary. Second, a telephone survey was conducted by trained interviewers to further recruit eligible cancer patients who had not visited the hospitals during the study period, using patient records as the sampling frame. With similar inclusion/exclusion criteria, briefing, and consent procedures, the interviewers obtained verbal informed consent and administered the telephone survey using an identical questionnaire. No incentives were given to the participants. This study was approved by the Ethics Committee of the Cancer Hospital of Shantou University Medical College, Shantou, China (Reference Number 2022034).

The initial sample size was 1,303, among which 188 (14.43%) were excluded due to (a) poor quality (e.g., taking less than 1.5 min to fill out the questionnaire; $n = 24$), (b) COVID-19 vaccination prior to cancer diagnosis ($n = 100$), and (c) primary cancer diagnoses other than the breast cancer, thyroid cancer, and gynecological cancers ($n = 64$). The final effective sample size was 1,115, of whom 412 and 703 were recruited on site and via the telephone survey, respectively.

Measures

The expert panel based the development of the questionnaire on a comprehensive literature review of COVID-19 vaccination studies conducted specifically among cancer patients. The literature review encompassed a wide range of research articles, studies, and publications that provided valuable insights into the vaccination experiences, beliefs, and factors influencing vaccination choices in this specific population (61, 62). While established measures and questionnaires exist for assessing the HBM components, the decision to devise new questions was made to ensure the cultural relevance and appropriateness of the items for the population of cancer patients in this study. By developing new questions through the expert panel, we aimed to capture the nuances and context-specific factors that may influence vaccination decision-making among cancer patients in our specific setting. A pilot survey was conducted among 10 cancer patients to assess clarity, readability, and length of the draft questionnaire. With their feedback, the panel finalized the questionnaire.

Background characteristics

(a) Socio-demographic characteristics included age, gender, monthly income, marital status, education level, number of family members, and employment status. (b) Body Mass Index [BMI] (kg/m^2) was calculated by using calibrated machines to measure weight and height (underweight: <18.5 kg/m^2, normal: 18.5–23.9 kg/cm^2, overweight: 24.0–27.9 kg/cm^2, and obese: ≥28.0 kg/cm^2). (c) Cancer-related variables included (i) cancer type (female breast cancer, male thyroid cancer, female thyroid patients, and gynecological cancer patients), (ii) current treatment status [e.g., endocrine therapy, targeted therapy, and treatments that would not affect the suitability of COVID-19 vaccination according to the some official guideline (25)] (yes/no), and (iii) duration since cancer diagnosis.

COVID-19 vaccination behavior/inclination status

Participants were classified into three categories: (a) the ever-vaccination group (those who had taken up COVID-19 vaccines after their cancer diagnosis), (b) the "vaccine non-refusal group," i.e., those

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who were planning or thinking about whether to take up the vaccines instead of definitely refusing any COVID-19 vaccination in the future, and (c) the vaccine refusal group (those who decided that they would definitely not take up the COVID-19 vaccines in the future). In addition, groups (b) and (c) were asked about the reasons for not having taken up the vaccines in a close-ended multi-choice question.

HBM variables

A number of summative scales (see Supplementary Table S1) were constructed in this study, including (a) the 2-item Perceived Susceptibility Scale (Cronbach's alpha = 0.73; range = 0–8; reversed scores), the 3-item Perceived Severity Scale (Cronbach's alpha = 0.89; range = 0–12), the 3-item Perceived Benefits Scale (Cronbach's alpha = 0.81; range = 0–12), the 3-item Perceived Barriers Scale (Cronbach's alpha = 0.81; range = 0–12), the 1-item Self-Efficacy Scale (range = 0–4). Such scales were assessed by 5-point Likert scales (0 = strongly disagree to 4 = strongly agree). High scores represent higher levels of these constructs. "Cue to action" is a concept within the HBM that refers to a trigger or stimulus that prompts an individual to take action toward a specific health behavior. In our research, cue to Action of COVID-19 vaccination was assessed by asking whether the participants had been suggested to take up COVID-19 vaccination by their family members, their friends, doctors/nurses, and members of community or village committees, respectively (yes/no). An indicator variable was formed by counting the number of affirmative responses [0 (the reference group), 1, 2 or above].

Statistical analysis

As the categorical dependent variable of COVID-19 vaccination status had three groups (i.e., ever-vaccination, "vaccine non-refusal" and vaccine refusal), multinomial logistic regression analysis was used to generate two models comparing vaccine refusal vs. ever-vaccination and vaccine refusal vs. "vaccine non-refusal" (63). As the focus was put on vaccine refusal, the results of the third comparison of 'vaccine non-refusal' vs. ever-vaccination was presented in Supplementary Table S2 instead of in the main text. Univariable and adjusted multinomial logistic regression analyses were conducted to test the individual factors (i.e., the HBM variables) of vaccine refusal, both in the absence and presence of adjustment for the significant background factors, respectively. Univariate odds ratios (ORu), adjusted odds ratios (ORa), and their corresponding 95% confidence intervals (CIs) were reported. Data analyses was conducted by using SPSS 25.0. Two-sided $p < 0.05$ was considered statistically significant.

Results

Descriptive statistics

The results are shown in Table 1. The majority (95.2%) of the participants was currently married; 19.9% had received an education level of college or above; 43.5% had had a full-time job; 35.2% had had five or more family members; 56.7% had had a monthly household income of >6,000 RMB (about 880 USD).

TABLE 1 Descriptive statistics (n = 1,115).

Variables	Count	Proportion (%)
Vaccination behavior/inclination status		
Vaccine refusal	289	25.9
"Vaccine non-refusal"	248	22.2
Ever-vaccination	578	51.8
Age group (years)		
>50	535	48.0
≤50	580	52.0
BMI (kg/m²)		
<18.5	52	4.7
<23.9	644	57.8
24 ~ 27.9	359	32.2
≥28	60	5.4
Currently marital status		
Not married	54	4.8
Married	1,061	95.2
Educational level		
Below college level	893	80.1
College or above	222	19.9
Employment status		
Full-time job	485	43.5
Housewife	333	29.9
Retiree	169	15.2
Unemployed	102	9.1
Others	26	2.3
Number of family members		
≥5	393	35.2
3 ~ 4	640	57.4
0 ~ 2	82	7.4
Monthly household income (RMB)		
≤6,000	483	43.3
>6,000	632	56.7
Cancer type		
Thyroid cancer (male)	41	3.7
Thyroid cancer (female)	159	14.3
Breast cancer (female)	553	49.6
Gynecological cancer	362	32.5
Currently under treatment (other than palliative care, surgery, radiation, chemotherapy)		
Yes	580	52.0
No	535	48.0
Duration since cancer diagnosis (year)		
<1	87	7.8
1–3	544	48.8
3–5	244	21.9
>5	240	21.5

(Continued)

TABLE 1 (Continued)

Variables	Count	Proportion (%)
Cue to action indicator (number of types of suggestion)		
0	641	57.5
1	226	20.2
2	132	11.8
3	54	4.8
4	62	5.5

BMI, Body Mass Index; RMB, Renminbi.

About half were aged 50 or below (52%). The BMI data showed that 4.7 and 37.6% were underweight and overweight/obese, respectively. About half (52%) were currently under cancer treatments that should not affect vaccination (e.g., endocrine therapy, oral targeted drugs, Chinese traditional medicine); 21.5% had had disease duration >5 years since cancer diagnosis. Regarding the independent variables, the mean scores of the HBM variables were 2.2 for perceived susceptibility (SD = 1.6, range = 0–8), 6.3 for perceived severity (SD = 2.3, range = 0–12), 8.5 (for perceived benefit SD = 2.1, range = 0–12), 5.5 for perceived barrier (SD = 2.8, range = 0–12), and 2.7 for self-efficacy (SD = 1.2, range = 0–12). Regarding the cue to action indicator, 57.5, 20.2, 11.8, 4.8, and 5.5% of the participants had received suggestions to take up vaccination from 0, 1, 2, 3, and 4 sources (family members: 21.7%; good friends: 11.6%; doctors/nurses: neighborhood community committee members: 21.0%. see Supplementary Table S1).

Prevalence of ever-vaccination, "vaccine non-refusal," and vaccine refusal

The prevalence of ever-vaccination, "vaccine non-refusal," and vaccine refusal was 51.8, 22.2, and 25.9%, respectively. In Table 2, cancer type ($p < 0.001$) but not age ($p = 0.062$) was significantly associated with vaccination behavior/inclination. The prevalence of ever-vaccination was presented in an ascending order of 33.3, 65.5, 76.1, 87.8% for the female breast cancer group, the gynecological cancer group, the female thyroid cancer group, and the male thyroid cancer group, respectively. In reverse, the prevalence of vaccine refusal was 33.6, 22.9, 10.7, 7.3% in the four corresponding groups, respectively ($p < 0.001$).

Reasons for not taking up COVID-19 vaccination after cancer diagnosis

As shown in Table 3, Of the 537 unvaccinated participants, over 10% mentioned the following reasons for not having taken up COVID-19 vaccination: perceived poor health (51.6%), unknown side effects of vaccination in cancer patients (36.5%), fear about potential interactions between COVID-19 vaccines and cancer treatments (35.0%), recommendations against vaccination given by healthcare workers (26.8%), perception that cancer was more serious than COVID-19 (21.2%), perceived stronger side effects in cancer patients than the general population (17.9%), low perceived risk of COVID-19 infection (17.7%), unsupportive attitude among family members or friends (12.9%), and logistics issues (11.4%).

Background factors of COVID-19 vaccine refusal

As shown in Table 4, those aged >50 years (reference group: ≤50), having attained an education level lower than college (reference group: college or above), being currently unemployed (reference group: others), having breast cancer diagnosis (female) having disease duration since cancer diagnosis for <1 year or 1–3 years (reference group ≥5 years), and being currently under treatment were more likely than others to report vaccine refusal than ever-vaccination and only having thyroid cancer diagnosis (both male and female) (reference group: gynecological cancer) was more likely than others to report ever-vaccination than vaccine refusal. Similarly, those having a monthly income ≤6,000 RMB (reference group: >6,000), breast cancer (female) or thyroid cancer diagnosis (female) (reference group: gynecological cancer), duration since cancer diagnosis of <1 year (reference: ≥5 years) were more likely than others to belong to the vaccine refusal group than to the "vaccine non-refusal" group.

Adjusted analysis for the HBM factors of vaccine refusal

As shown in Table 5, the adjusted models showed that those participants with stronger perceived severity of COVID-19 (ORa: 1.31,

TABLE 2 COVID-19 vaccination behavior/inclination by age group and cancer type (n = 1,115).

Variables	Vaccination behavior/inclination status (%)			Chi-square	p value
	Vaccine refusal	"Vaccine non-refusal"	Ever-vaccination		
All	289 (25.9)	248 (22.2)	578 (51.8)		
Age group (years)					
>50	155 (29.0)	119 (22.2)	261 (48.8)	5.54	0.062
≤50	134 (23.1)	129 (22.2)	317 (54.7)		
Cancer type					
Thyroid cancer (male)	3 (7.3)	2 (4.9)	36 (87.8)	170.77	<0.001
Thyroid cancer (female)	17 (10.7)	21 (13.2)	121 (76.1)		
Breast cancer (female)	186 (33.6)	183 (33.1)	184 (33.3)		
Gynecological cancer	83 (22.9)	42 (11.6)	237 (65.5)		

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TABLE 3 Reasons for hesitancy in accepting COVID-19 vaccine (n = 537).

Items	Factors	Count	Proportion (%)
A	I think I'm in poor health to get vaccinated	277	51.6
B	The effect of the vaccine on cancer patients is unknown	196	36.5
C	Fear of interaction of COVID-19 vaccine with the active anticancer treatment	188	35.0
D	Healthcare workers do not recommend	144	26.8
E	COVID-19 is less serious than cancer	114	21.2
F	The side effects of vaccination are higher in cancer patients	96	17.9
G	I think the risk of contracting COVID-19 is very low	95	17.7
H	Family, friends, etc. do not support	69	12.9
I	It is inconvenient and difficult to vaccinate COVID-19 vaccine	61	11.4
J	I don't think the COVID-19 vaccines work very well.	55	10.2
K	The vaccine is unsafe.	48	8.9
L	Other	35	6.5
M	None of the surrounding cancer patients have been vaccinated	22	4.1

95% CI: 1.06–1.61), stronger perceived barrier of COVID-19 vaccination (ORa: 24.84, 95% CI: 16.29–37.88), lower perceived benefit of COVID-19 vaccination (ORa: 0.11, 95% CI: 0.08–0.16), and lower self-efficacy regarding COVID-19 vaccination (ORa: 0.15, 95% CI: 0.12–0.19) were more likely than those ever-vaccinated to show vaccine refusal. Reversely, those exposed to stronger cues to action [reference: no suggestion given; one source (ORa: 0.07, 95% CI: 0.04–0.12), 2–4 sources (ORa: 0.02, 95% CI: 0.01–0.04)] were less likely than others exhibit vaccine refusal than those ever-vaccinated. The same factors were found for the model of refusal vs. 'vaccine non-refusal' except that perceived severity of COVID-19 was non-significant in this but not the former comparison.

Discussion

This study observed that only about half of the sampled cancer patients had taken up at least one dose of the COVID-19 vaccines at the survey time (April to August 2022). Some socio-demographic factors (e.g., cancer type) of vaccine refusal were identified. It is interesting that the HBM factors related to the vaccines (perceived benefits, perceived barriers, cue to action and self-efficacy) were significantly associated with vaccine refusal in both models (vs. ever-vaccination and vs. "vaccine non-refusal"). Yet, perceived susceptibility of COVID-19 was not significant in both models while perceived severity of COVID-19 was significant in the model of vaccine refusal vs. ever-vaccination but not in that of vaccine refusal vs. "vaccine non-refusal."

Notably, the prevalence of COVID-19 vaccination in this study (51.8%) was lower than the concurrent prevalence of vaccination in the general population in Shantou (>90%) where the study was conducted during the concurrent time period (64). It was also much lower than that observed among cancer patients in countries such as Canada, Germany, and Japan (26, 27, 65, 66). The vaccination coverage in the sampled cancer patients was hence sub-optimal and probably inadequate to protect the cancer patients against COVID-19 infection. Completion of two doses of vaccination is required for effective protection against COVID-19; such prevalence must even be lower than that of 1-dose vaccination reported hereby. Health promotion is greatly warranted.

Unvaccinated cancer patients commonly mentioned perceived poor health (51.6%), unknown side effects of vaccination in cancer patients (36.5%), fear about potential interactions between COVID-19 vaccines and cancer treatments (35.0%) as reasons for not taking up the vaccines. Such findings corroborate other COVID-19 vaccination studies targeting cancer patients. A Korean study found a positive correlation between patients' health and acceptance of COVID-19 vaccines (67). A Tunisian study showed that 15.5% of cancer patients refused to take up COVID-19 vaccination as they believed that the vaccines might affect therapeutic effects (67). Among the Italian cancer patients who refused to take up the COVID-19 vaccines, 48.1% worried about adverse reactions and 26.7% were afraid of potential interactions between COVID-19 vaccines and cancer treatments (42, 67). Notably, COVID-19 is still a health threat to cancer patients, presently and in the future. Thus, COVID-19 vaccination is warranted. The aforementioned reasons are specific to cancer patients and are implicative for tailored interventions. Hence, concerns about side effects and interaction effects between vaccines and cancer treatments need to be clarified by health professionals to cancer patients who are suitable for vaccination. In particular, the local and international official expert recommendations for vaccination among cancer patients (25) should be widely disseminated to cancer patients and stakeholders (e.g., family members and health professionals) to facilitate informed vaccination decisions.

Furthermore, about one quarter of the patients did not vaccinate because they had been advised against vaccination by some health professionals, even that these patients seemed eligible according to our inclusion/exclusion criteria and conversations/observations. It is uncertain they were aware of the aforementioned guidelines. Several previous studies have demonstrated that doctors' recommendation was a significant predictor of vaccination behaviors (27, 40, 67). The government should hence ensure both dissemination of those official guidelines about the exact advices about COVID-19 vaccination given to cancer patients to all health professionals that doctors would give such recommendations to oncology patients accordingly. About one fifth of the participants did not vaccinate as they believed that COVID-19 was less severe than

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TABLE 4 Background factors of vaccine refusal (Univariable multinomial logistic regression).

Variables	Vaccine refusal vs. ever-vaccination	Vaccine refusal vs. "vaccine non-refusal"
	ORu (95% CI)	ORu (95% CI)
Age (years)		
>50	1.41 (1.06, 1.87)*	1.25 (0.89, 1.76)
≤50	Ref = 1.0	Ref = 1.0
BMI (kg/m^2)		
<18.5	0.78 (0.32, 1.94)	0.71 (0.24, 2.05)
<23.9	0.89 (0.48, 1.66)	0.86 (0.40, 1.83)
24 ~ 27.9	0.87 (0.46, 1.65)	0.97 (0.44, 2.13)
≥28	Ref = 1.0	Ref = 1.0
Current marital status		
Not married	1.1 (0.53, 2.25)	0.49 (0.24, 1.03)
Married	Ref = 1.0	Ref = 1.0
Educational level		
Below college level	1.52 (1.05, 2.18)*	0.97 (0.61, 1.53)
College or above	Ref = 1.0	Ref = 1.0
Employment status		
Full-time job	0.69 (0.25, 1.86)	1.23 (0.40, 3.80)
Housewife	1.49 (0.55, 4.05)	2.12 (0.68, 6.60)
Retiree	1.06 (0.37, 2.99)	0.84 (0.26, 2.69)
Unemployed	3.25 (1.10, 9.64)*	1.23 (0.38, 4.00)
Other	Ref = 1.0	Ref = 1.0
Number of family members		
≥5	1.69 (0.92, 3.1)	1.38 (0.69, 2.78)
3 ~ 4	1.18 (0.65, 2.12)	1.42 (0.71, 2.82)
0 ~ 2	Ref = 1.0	Ref = 1.0
Monthly household income (RMB)		
≤6,000	0.84 (0.63, 1.12)	0.68 (0.49, 0.97)*
>6,000	Ref = 1.0	Ref = 1.0
Cancer type		
Thyroid cancer (male)	0.24 (0.07, 0.79)*	0.76 (0.12, 4.72)
Thyroid cancer (female)	0.40 (0.23, 0.71)*	0.41 (0.20, 0.86)*
Breast cancer (female)	2.89 (2.09, 3.99)***	0.51 (0.34, 0.79)*
Gynecological cancer	Ref = 1.0	Ref = 1.0
Duration since cancer diagnosis (year)		
<1	4.34 (2.27, 8.28)***	0.49 (0.25, 0.99)*
1 ~ 3	3.27 (2.20, 4.85)***	0.80 (0.47, 1.36)
3 ~ 5	1.25 (0.78, 2.00)	0.91 (0.48, 1.74)
>5	Ref = 1.0	Ref = 1.0
Current treatment status		
Yes	1.78 (1.34, 2.37)***	0.78 (0.55, 1.10)
No	Ref = 1.0	Ref = 1.0

BMI, Body Mass Index; RMB, Renminbi; ORu, Univariate odds ratio; CI, Confidence interval; *$p < 0.05$; ***$p < 0.001$.

TABLE 5 Adjusted associations between the HBM Variables and COVID-19 vaccine refusal.

HBM Variables	Vaccine refusal vs. ever-vaccination	Vaccine refusal vs. "Vaccine non-refusal"
	ORa (95% CI)	ORa (95% CI)
Perceived susceptibility	0.91 (0.74, 1.13)	0.9 (0.71, 1.15)
Perceived severity	1.31 (1.06, 1.61)*	1.06 (0.83, 1.37)
Perceived benefits	0.11 (0.08, 0.16)***	0.37 (0.27, 0.5)***
Perceived barriers	24.84 (16.29, 37.88)***	1.82 (1.36, 2.45)***
Cue to Action Indicator (number of sources of suggestion)		
2–4	0.02 (0.01, 0.04)***	0.34 (0.15, 0.82)*
1	0.07 (0.04, 0.12)***	0.56 (0.32, 0.95)*
0	Ref	Ref
Self-efficacy	0.15 (0.12, 0.19)***	0.47 (0.39, 0.56)***

These models adjusted for age, current marital status, education level, employment status, monthly household income, cancer type, duration since cancer diagnosis, current treatment status. RMB, Renminbi; HBM, Health Belief Model; ORa, adjusted odds ratio; CI, Confidence interval; *$p < 0.05$; ***$p < 0.001$.

COVID-19. Such patients might have under-estimated the severity of COVID-19 for cancer patients and should be informed about the consequences of COVID-19 among cancer patients.

Some significant socio-demographic factors of vaccine refusal have been identified in this study, including older age, lower educational level, and unemployment status, which was consistent with previous surveys (32, 40). It is plausible that those of older age had had stronger concerns over the safety of COVID-19 vaccination (52) as relevant news and social media often mentioned vaccine-related deaths in order people (68). Similarly, those of lower socio-economic status (e.g., lower educational level and unemployment status) might be older in age and/or less informed about the relatively high efficacy and low side effect of COVID-19 vaccination among cancer patients (69), leading to potential vaccine refusal. Health promotion should target such socio-demographic groups.

Three cancer-related background factors of vaccine refusal were identified. First, thyroid cancer patients were less likely and female breast cancer patients were more likely than gynecological cancer patients to indicate vaccine refusal. The primary site of cancer patients may affect cancer patients' vaccination behavior, as it involves different symptoms and treatment plans. However, some previous studies also showed that the primary cancer site did not affect patients' willingness to take up COVID-19 vaccines (32, 70). Such inconsistent results should be examined in future studies. Second, disease duration was inversely associated with vaccine refusal, corroborating a previous multi-center study (71). The sampled cancer patients have relatively good prognosis. The sampled patients might regard a longer duration since diagnosis as a better chance of recovery (72); such patients might hence worry less about the potential side effects of vaccination on their course of the cancer disease. Third, those undergoing treatments other than chemotherapy, radiotherapy and surgery (predominantly endocrinal therapy) were more likely than those not undergoing any treatment to refuse COVID-19 vaccination, possibly due to worries about potential interactions between vaccines and those current treatments. Again, clear, consistent, and transparent information about the suitability of COVID-19 vaccination

should be provided to cancer patients, especially those of specific cancer types and undergoing treatments.

The HBM has been partially supported by the data. It is interesting that all the four constructs related to COVID-19 vaccines (perceived benefit, perceived barriers, cue to action, and self-efficacy) were consistently associated with vaccine refusal and in the expected directions. Although there is a dearth of studies applying the HBM to look at vaccine refusal among cancer patients, this study's findings are consistent with those regarding COVID-19 vaccination behavior (36), acceptance (73), and hesitancy (54) in general populations. Thus, health promotion strategies for reducing vaccine refusal may need to modify such perceptions. A remark for such programs is that the contents should be closely tailored to cancer patients.

The number of sources of cue to action showed a strong negative association with vaccine refusal. As only 21.7, 26.2, 11.6, and 21.0% of the participants had received suggestions for COVID-19 vaccination from family members, health professionals, neighborhood community members, and friends, respectively, there are rooms for improvement. Social influences on COVID-19 vaccination hesitancy are well known (74). As vaccine hesitancy was also common in the general population (75) and family members are influential in determining health-related behaviors of cancer patients (44), family members' objection for COVID-19 vaccination among diseased people is expected to be common and impactful (76–78). It seems that successful vaccine promotion campaigns targeting cancer patients need to involve patients' family members (31, 40). Neighborhood community committee is a special feature in China. It maintains close contacts with the community residents to help dealing with their daily problems and disease prevention (79). It has played an important role in promotion of COVID-19 testing, prevention, and vaccination (33). As the majority of the participants have not received supportive suggestions about COVID-19 vaccination from such committees, improvements could be made. Furthermore, despite significance and potential effectiveness, about 73.8% has not received any suggestions regarding COVID-19 vaccination from health professionals, while 26% had even been advised against vaccination by health professionals. Again, training and improvements are warranted. Health professional need to become facilitators instead of barriers of cancer patients' COVID-19 vaccination.

While the vaccine-related perceptions were significantly associated with vaccine refusal, such was untrue regarding perceptions toward COVID-19. Unlike other studies conducted in some general populations (80), perceive susceptibility was not associated with vaccine refusal. It is plausible that the study was conducted at a time when prevalence of COVID-19 was very low in Shantou. During the study period, indeed, zero cases were detected per day in Shantou (81). In addition, cancer patients were more likely than others to take up preventive measures such as staying at home (82). Such measures might have lowered their perceived susceptibility. Perceived severity was significant when comparing vaccine refusal vs. ever-vaccination but not vs. "vaccine non-refusal," although in general, this construct was a significant factor of COVID-19 vaccination behavior/acceptance (80). It suggests that promotion of perceived severity of COVID-19 might not be effective to shift the cognitions among the unvaccinated cancer patients from refusal to 'non-refusal'. This observation may be particularly true when COVID-19 symptoms become milder in the later phases of the pandemic. A theoretical contribution of the findings is that some HBM constructs might have different applications to COVID-19 vaccination in cancer patients vs. general populations.

This study has some limitations. First, the selection of cancer types focused on female breast cancer, gynecological tumors, and thyroid cancer due to their high prevalence and relatively good prognosis. The sample was hence unrepresentative of all cancer types. Such selection may overrepresented female cancer patients. Consequently, this study did not use sex as an independent background factor of vaccine refusal. Relatedly, this study excluded male breast cancer patients due to the small number in the sample (n = 2). Second, this study was cross-sectional in design, making it unable to determine the causal or temporal relationships between the independent variables and vaccine refusal. Third, this study classified the patients into the three categories of ever-vaccination, "vaccine non-refusal," and vaccine refusal. Notably, "vaccine non-refusal" was a relatively heterogeneous group including those of different stages of change (83) regarding vaccination (e.g., contemplation and preparation stages). As few previous studies have applied the HBM to investigating COVID-19 vaccination among cancer patients, the instruments were created in this study. As COVID-19 vaccination may be seen as a socially desirable behavior (19), reporting bias may have occurred. Finally, some variables affecting COVID-19 vaccination in cancer patients may not have been included in this study, The impact of these factors on vaccination choices in cancer patients and their potential implications for public health interventions should be further investigated.

In conclusion, this study reported relatively high prevalence of vaccine refusal against COVID-19 vaccination and relatively low prevalence of first-dose vaccination behavior among the four groups of cancer patients in a Chinese city. It was based on a relatively large sample size. The associations between the HBM constructs (those related to health beliefs related to the vaccines) and vaccine refusal (vs. ever-vaccination and vs. vaccine refusal) were partially supported by the data. Factors distinguishing vaccine refusal vs. ever-vaccination and vaccine refusal vs. "vaccine non-refusal" were largely similar. Future confirmation of the above findings in longitudinal studies are needed, possibly with an extension to other cancer groups. Pilot randomized control trials are also warranted to modify the significant HBM factors to reduce vaccine refusal in cancer patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved by the Ethics Committee of the Cancer Hospital of Shantou University Medical College, Shantou, China (Reference Number 2022034). Participants provided their written informed consent to participate in this study.

Author contributions

ZX, DZ, HL, and YL contributed to the data acquisition, analysis, interpretation, and drafting the manuscript. JL contributed to conceptualization, questionnaire design, major revision, and finalization of the paper. YY contributed to data analysis, major

revision, and finalization of the paper. ZX, JL, and YY verified the data. QO, JC, SL, KY, and XH contributed to data collection. All authors made substantial contribution to the conception of the work and had full access to all the data in the study, revised the work critically, gave final approval of the manuscript submitted for publication, and agreed to be responsible for all aspects of work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1236376/full#supplementary-material

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Reduced COVID-19 morbidity and mortality in hemodialysis patients across the various Omicron sublineages—A retrospective analysis

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Introduction: Hemodialysis (HD) patients are a COVID-19 high risk population due to comorbidities and impaired immune response. Vaccines, advent of effective treatment and the emergence of novel variants have fundamentally changed the pandemic. We aimed to assess temporal changes of COVID-19 in HD patients of our catchment area, and risk factors for severe and fatal course.

Methods and materials: We retrospectively collected data from 274 patients admitted to the Medical University Graz, Austria for HD between 1st of May 2020 and 31st of August 2022. We analyzed clinical and demographic data between different COVID-19 waves and assessed factors associated with hospitalization, ICU admission and mortality by logistic regression. To further evaluate the dialysis at-risk population, we collected demographic and vaccination data between August 2021 and August 2022.

Results: Time of infection and SARS-CoV-2 sequencing data allowed for distinction of five separate waves of infection with different impact on the dialysis population: While in the initial four waves frequencies of hospitalization, necessity of critical care and mortality were around 60%, 10% and 20%, respectively. These events became rare during the large fifth wave, when Omicron had become the dominant variant. Although only 16.9% had to be hospitalized, this resulted in 29 hospital admissions, due to the high prevalence of COVID-19 during the Omicron era. Furthermore, we observed similar clinical outcomes with BA.4/5 as with BA.1/BA.2 Omicron sublineages. The proportion of previously infected increased simultaneously with the number of vaccination doses in our dialysis population. Vaccination at time of positivity and infection with an Omicron variant conferred protection against hospitalization and mortality in univariate analysis, but only infection with an Omicron variant remained a robust predictor for these outcomes in multivariable analysis.

Discussion: While a fourth of our at-risk population became infected during the Omicron wave, mortality was almost non-existent. Several concomitant factors

have contributed to the decrease of COVID-19 severity in HD patients. This trend appears to be continued with BA.4/5, which was equally mild as BA.1 and BA.2 in our well vaccinated dialysis population.

KEYWORDS

BA.4/5, COVID-19, hemodialysis, Omicron, vaccination

1. Introduction

Individuals on hemodialysis (HD) have been at an increased risk of contracting SARS-CoV-2 (1), and of a severe course of COVID-19 (2, 3). While initial reports suggested a mortality rate of almost 30% (4), the pandemic has fundamentally changed since its emergence in late 2019, especially due to the appearance of variants of concern (VoC).

VoC are a consequence of ongoing mutations and constant selection, and are characterized by increased immune escape, rapid transmission and/or more severe disease.

Each novel VoC dealt a different set of cards, challenging health care systems around the world to rapidly adapt to each VoC's characteristics (5). The most "successful" VoC have been B.1.617.2 (Delta) and B.1.1.529 (Omicron) (6), which differ profoundly in transmissibility and virulence. While Delta posed a major threat to the infected, morbidity and mortality have been low with Omicron (7, 8), which has become the dominant variant due to its ability to rapidly spread (9) and partial escape from antibodies (10). While accumulation of mutations may be slowed by preventive measures, genetic transformation of SARS-CoV-2 cannot be halted completely, and concerns remain high, that a more aggressive variant may arise (11).

Natural immunity after COVID-19 offers a certain degree of protection from future infections in HD patients (12), but it potentially comes at a high cost. Therefore, major efforts have been undertaken to expedite the distribution of vaccines. Although the prospect of sterile immunity has diminished considering novel variants, vaccinations are an effective measure against severe and fatal COVID-19 (13). There is now a large body of evidence displaying that SARS-CoV-2 vaccination elicits a dampened, but still measurable serological response in individuals on HD, who were excluded from initial trials (14). However, frequent booster shots may be necessary in this population to combat waning immunity and non-responders (15). VoC with significant immune escape have further added to the problem (10).

Apart from preventive measures, effective treatments, including antivirals and anti-inflammatory agents have been added to the clinician's armamentarium (16).

In this study, we collected data from SARS-CoV-2 positive HD patients from the first recorded SARS-CoV-2 infection in March 2020 until August 2022. Infected patients were referred to our dialysis center in Graz from our catchment area which consists of around 470 HD patients. We aimed to assess temporal changes between pandemic waves and the influence of potential risk and protective factors on outcomes like hospitalization and mortality. Furthermore, we investigated potential differences between the Omicron sublineages BA.1/BA.2 and BA.4/5.

2. Materials and methods

2.1. Data collection

HD patients were screened for SARS-CoV-2 positivity by antigen testing before each routine dialysis at the Medical University Graz, a tertiary hospital, two secondary care centers and three remote dialysis facilities (Figure 1). All antigen tests adhered to quality criteria provided by WHO, but different kits were used depending on local availability (17). They were additionally tested whenever SARS-CoV-2 infection was clinically suspected. When tested positively, HD patients were transferred to the Medical University of Graz dialysis unit for disease control reasons and confirmation of infection by PCR testing following a nasal swab. Consequently, patients with (1) PCR confirmed SARS-CoV-2 infection, (2) on hemodialysis prior to infection, and (3) on hemodialysis for at least three months in total, were recruited from March 2020 to 31st of August 2022. Duration of SARS-CoV-2 positivity was defined as the interval between the first positive PCR result and the date of the first polymerase chain reaction (PCR) with a CT (cycle threshold) $>$ 30 followed by a consecutive PCR with increasing CT.

Electronical medical records were reviewed for hospitalization, ICU admission, mortality and treatment. "Hospitalization" was defined as hospitalized while SARS-CoV-2 PCR positive, and those who were already hospitalized at the time of viral contraction were excluded. Any admission to the intensive care unit during hospitalization was recorded as "ICU admission". We specified "COVID-19 related mortality" as death within 30 days of SARS-CoV-2 positivity.

The following variables were documented as dichotomous events: usage of antibiotics, antivirals or corticosteroids as COVID-19 treatment; Cardiovascular disease was defined as previous coronary artery disease, peripheral artery disease or cerebrovascular disease; Congestive heart failure (regardless of ejection fraction); Diabetes mellitus (any type); Pulmonary disease was defined as interstitial, obstructive or vascular lung disease; Kidney transplantation prior to HD dependency; Immunosuppression comprised the regular intake of calcineurin inhibitors, antimetabolites, prednisolone (or equivalent) above 10 milligrams daily or treatment with immunomodulatory biologicals at the time of infection.

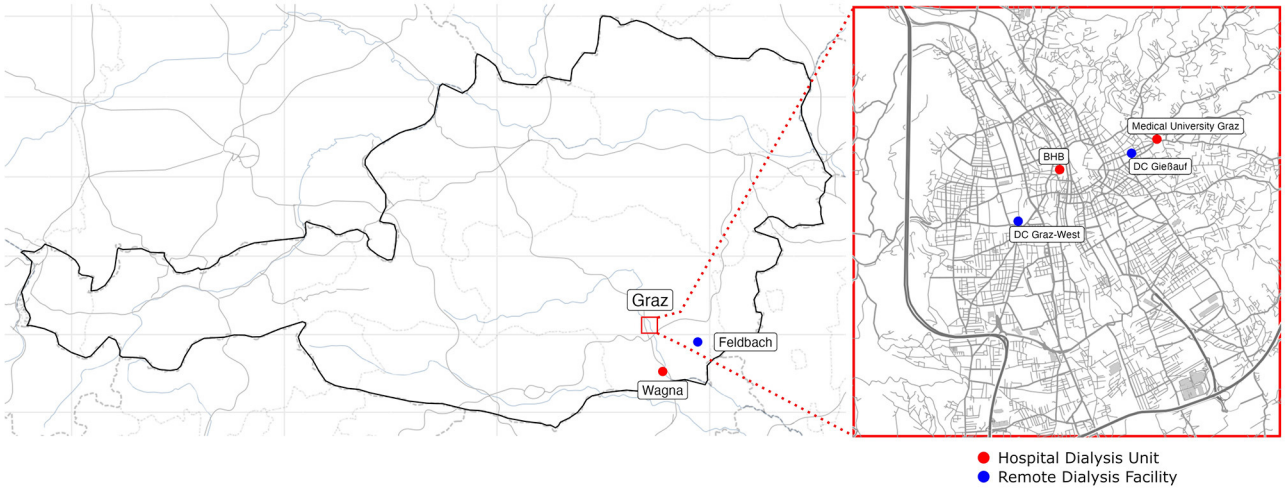

FIGURE 1
Participating dialysis centers are depicted on a geographical map of Austria with an auxiliary map of Graz (right side). Remote dialysis facilities are shown as blue dots and hospital-based dialysis units are represented as red dots.

"Waves" could be distinguished by time and by SARS-CoV-2 sequencing results.

Additionally, we collected clinical and vaccination data of the at-risk HD population between 31st of August 2021 and 31st of August 2022, as provided by the individual dialysis units and/or hospitals (Figure 1). Patients who were on dialysis for at least 3 months were included, and data was censored on the 31st of August 2022. Previous SARS-CoV-2 positivity was defined as any SARS-CoV-2 positive PCR test results within the electronical medical records, regardless of dialysis dependency at the time of positivity.

The study was approved by the ethics committee of the Medical University of Graz (EK 34-372ex21/22).

2.2. Statistical analysis

Descriptive data are given as median with interquartile range for continuous variables, and absolute numbers and percentages for categorical variables. Clinical characteristics of SARS-CoV-2 infected patients were compared between waves by Kruskal-Wallis test or Chi-Square test, depending on the variable. Weekly prevalence and weekly incidence of SARS-CoV-2 were calculated by dividing the number of currently infected and newly infected individuals, respectively, by the total number of prevalent dialysis patients during the same week.

Univariate logistic regression was used to identify risk factors for dichotomous outcome events like hospitalization, ICU admission and mortality. For multivariable analysis of these outcomes, all variables with a significant impact in univariate analysis were included.

Statistical analysis was performed using SPSS 29 (IBM, Endicott, NY, USA) or RStudio (PBC, Boston, MA, USA).

3. Results

3.1. Analysis of SARS-CoV-2 positive patient cases over time reveals five distinguishable waves

By plotting all 274 SARS-CoV-2 positive patients, who met the inclusion criteria, over time, we could identify five separate "waves" of infections from our first recorded case on 20th of March 2020 until the 31st of August 2022 (Figures 2A, B). This segmentation is supported by the available SARS-CoV-2 genome sequencing data, which shows no overlap of variants between these waves (Table 1). Clinical characteristics, disease outcomes and vaccination status at the time of infection are compared between the five waves in Table 1.

The first wave included only five patients at our center, with one fatal case and three patients being hospitalized. The second wave started in October 2020 and ended in March 2021, and 79 SARS-CoV-2 positive HD patients were documented. Hospitalization, ICU admission and mortality were frequent events with 46 (58%), eight (10%) and 17 (21.5%) cases, respectively.

Rollout of vaccines in Austria commenced in early 2021. The first breakthrough infections were recorded during the third wave from 16th of March 2021 to 21st of May 2021. During this period, SARS-CoV-2 sequencing became available at our institution and revealed that the dominant variant infecting our patients was B.1.1.7 (Alpha). All patients infected with the Alpha variant had to be hospitalized. Mortality was particularly high during this wave ($N = 3$, 42.9%). During the following Delta wave, as apparent from sequencing results, 16 patients were infected. Hospitalization and mortality rates remained high at 68.8% and 25%, respectively. In contrast to previous waves, most patients (68.8%) had received two or more doses of vaccination at the time of infection.

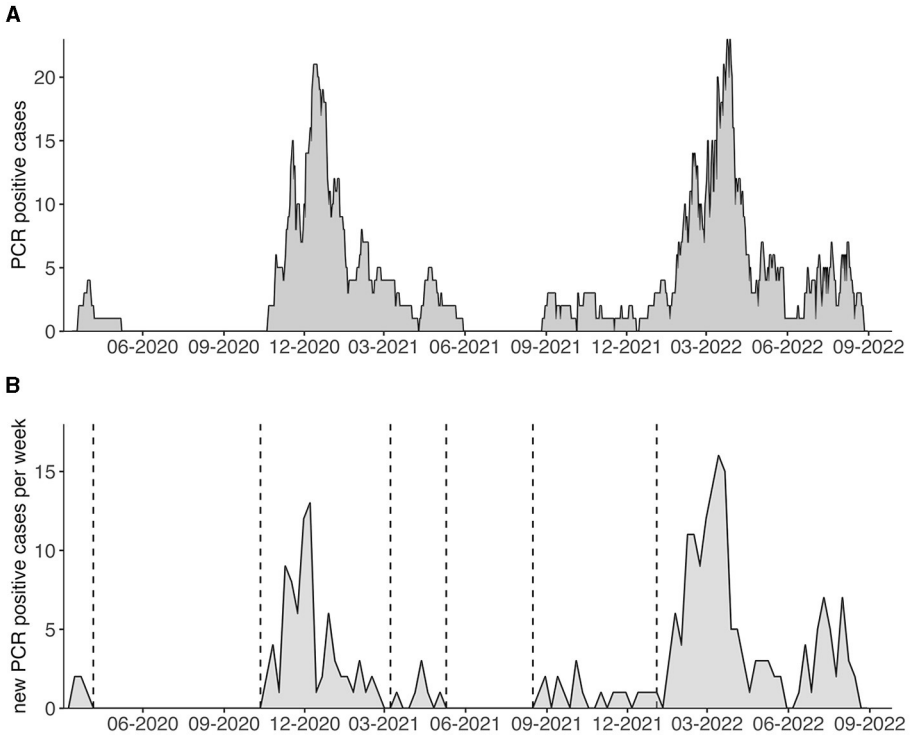

FIGURE 2
(A) PCR confirmed SARS-CoV-2 positive hemodialysis patients in our catchment area are displayed over time and for the duration of PCR positivity. **(B)** Weekly new PCR confirmed SARS-CoV-2 cases are depicted. Vertical dotted lines indicate different waves.

Omicron became dominant in January 2022 and included the largest number of infected individuals with 167 PCR confirmed SARS-CoV-2 cases. A strikingly lower number of hospitalizations ($N = 28$, 16.9%) and mortality ($N = 2$, 1.2%) was seen during this wave. We also observed a decrease in the duration of PCR positivity. From a median of 20 days in the second wave, median positivity diminished to seven days in the fifth wave. Similarly, use of antibiotics in infected individuals was halved in the latter stages of the pandemic compared to earlier waves (77.2–100% compared to 33.5%). Corticosteroids, which are recommended in patients with oxygen dependency during infection and may therefore indicate severe disease, were more frequent in earlier waves compared to the Omicron era (32.9–85.7% vs. 7.8%). Other treatments, like antivirals, convalescent plasma or anti-SARS-CoV-2 antibodies were rarely used in our population. Notably, clinical characteristics and prevalence of pre-existing conditions were similar between waves.

3.2. Infection with the Omicron variant and vaccination are negative predictors of severe COVID-19

Next, we aimed to evaluate risk factors for hospitalization, ICU admission and mortality in all SARS-CoV-2 positive HD patients.

In univariate logistic regression, older age (OR 1.110, 95% CI: 1.011–1.219), diabetes (OR 1.882, 95% CI: 1.138–3.115), and pulmonary disease (OR 2.273, 95% CI: 1.257–4.102) were associated with an increased risk of hospitalization. Contrarily, vaccination (OR 0.580, 95% CI: 0.480–0.701) and infection during the Omicron era (compared to infections during previous waves) (OR 0.120, 95% CI: 0.068–0.211) conferred protection from hospitalization. Although SARS-CoV-2 reinfections were infrequent ($N = 17$), we observed a trend indicating a protective signal for reduced hospitalization rates (OR 0.235, 95% CI: 0.053–1.051) (Table 2). In a multivariable analysis diabetes and pulmonary disease were robust predictors for hospitalization in SARS-CoV-2 infected HD patients, whereas infection with an Omicron variant was associated with improved outcome and an almost 10-fold decrease of risk for hospitalization (Table 3). For ICU admission, which was overall a rare event in our cohort ($N = 13$), vaccination (OR 0.560, 95% CI: 0.353–0.888) and Omicron variant infection (OR 0.177, 95% CI: 0.048–0.661) were protective in univariate analysis (Table 2). However, they did not prevail in a multivariable analysis (Table 3).

Finally, age (OR 1.23, 95% CI 1.029–1.471), heart failure (OR 2.470, 95% CI: 1.106–5.517), diabetes (OR 2.299, 95% CI 1.012–5.224) and pulmonary disease (OR 2.926, 95% CI: 1.275–6.715) were positively associated with mortality in SARS-CoV-2 patients. Again, vaccination prior to infection (OR 0.468, 95% CI 0.325–0.674) and Omicron variant infection (OR 0.040, 95% CI 0.009–0.172) were significant negative predictors. Intriguingly, arterial hypertension lowered the risk for mortality (OR 0.325, 95% CI 0.109–0.966) (Table 2). Pulmonary disease, arterial hypertension, and Omicron variant infection remained significant

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TABLE 1 Clinical characteristics, SARS-CoV-2 sequencing, vaccination status at time of infection, COVID-19 treatment and related outcomes are displayed as absolute and relative frequencies or medians with interquartile range.

Wave	1	2	3	4	5
Time period[a]	20-Mar-2020–31-Mar-2020	21-Oct-2020–22-Feb-2021	16-Mar-2021–05-May-2021	28-Aug-2021–26-Dec-2021	02-Jan-2022–31-Aug-2022
N	5	79	7	16	167
Age (years)	71 (45.5–85)	74 (65–79)	74 (70–77)	67.5 (57.8–80.8)	71 (56–78)
Male gender (%)	3 (60)	35 (44.3)	5 (71.4)	8 (50)	97 (58.1)
BMI (kg/m2)[b]	25.6 (22.4–26.9)	25.6 (22.1–30.1)	23 (21.1–26.9)	26 (21.4–30.4)	26.1 (22.7–30.7)
Kidney disease					
Diabetic nephropathy	2 (40)	20 (25.3)	2 (28.4)	2 (12.5)	59 (35.3)
Hypertensive nephropathy	0	9 (11.4)	1 (14.3)	4 (25)	24 (14.4)
Glomerular disease	1 (20)	14 (17.7)	1 (14.3)	3 (18.8)	27 (16.2)
Polycystic kidney disease	0	2 (2.5)	1 (14.3)	0	6 (3.6)
Other	1 (20)	19 (24.1)	1 (14.3)	3 (18.8)	37 (22.2)
Unknown	1 (20)	15 (19)	1 (14.3)	4 (25)	14 (8.4)
Cardiovascular disease	3 (60)	46 (58.2)	5 (71.4)	8 (50)	101 (60.5)
Congestive heart failure	2 (40)	27 (34.2)	1 (14.3)	8 (50)	60 (35.9)
Diabetes mellitus	3 (60)	31 (39.2)	3 (42.9)	8 (50)	77 (46.1)
Arterial hypertension	5 (100)	70 (88.6)	7 (100)	13 (81.3)	157 (94)
Kidney transplantation	0	9 (11.4)	1 (14.3)	4 (25)	20 (12)
Immunosuppression	2 (40)	10 (12.7)	0	2 (12.5)	14 (8.4)
Pulmonary disease	2 (40)	21 (26.6)	3 (42.9)	2 (12.5)	30 (18)
Dialysis vintage (months)	3 (0.5–87)	31 (8–50)	4 (0–82)	27.5 (10.8–51.3)	34 (12–62)
Previous COVID-19	0	0	0	0	17 (10.2)
SARS-CoV-2 Sequencing	0	0	7 (100)	12 (75)	106 (63.5)
Vaccination					
1 dose	0	3 (3.8)	1 (14.3)	0	0
2 dose	0	0	2 (28.6)	7 (43.8)	19 (11.4)
3 dose	0	0	0	4 (25)	126 (75.5)
4 dose	0	0	0	0	4 (2.4)
Unvaccinated	5 (100)	76 (94.9)	3 (42.8)	4 (25)	15 (9)
Missing information	0	1 (1.3)	0	1 (6.3)	3 (1.8)
Duration PCR positivity (days)	10 (4–27)	12 (7–19)	20 (8–23)	12.5 (7.25–18.75)	7 (3–11)
Hospitalization	3 (60)	46 (58.2)	7 (100)	11 (68.8)	28 (16.9)
Antibiotics	5 (100)	61 (77.2)	7 (100)	13 (81.3)	56 (33.5)
Corticosteroids	0	26 (32.9)	6 (85.7)	8 (50)	13 (7.8)
Convalescent plasma	0	0	1 (14.3)	0	0
Remdesivir	0	2 (2.5)	2 (28.6)	3 (18.8)	3 (1.8)
Anti-SARS-CoV-2 Antibodies	0	0	0	0	3 (1.8)
Duration of hospitalization (days)	7 (6,7)	7.5 (4–19)	14 (5–28)	11 (7–25)	10 (6–17.75)
ICU admission	0	8 (10.1)	1 (14.3)	1 (6.3)	3 (1.8)
Mortality	1 (20)	17 (21.5)	3 (42.9)	4 (25)	2 (1.2)

[a] date of first positive PCR test to last PCR positive test within individual waves.

[b] BMI: One value missing in wave 2 and two values missing in wave 5.

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TABLE 2 Univariate logistic regression for hospitalization.

	Outcome variable								
	Hospitalization			ICU Admission			Mortality		
Variable	Odds ratio	95% confidence interval	*p*-Value	Odds ratio	95% confidence interval	*p*-Value	Odds ratio	95% confidence interval	*p*-Value
Age (per 5 years increase)	1.110	1.011–1.219	0.029	0.963	0.798–1.161	0.689	1.23	1.029–1.471	0.023
Female Gender (compared to male)	1.021	0.620–1.681	0.936	1.392	0.455–4.255	0.562	2.152	0.947–4.889	0.067
BMI (per 1 kg/m^2 increase)[a]	0.970	0.927–1.015	0.181	1.023	0.934–1.122	0.622	0.966	0.894–1.043	0.377
Kidney disease[b]	1.092	0.663–1.800	0.73	0.353	0.095–1.310	0.12	0.483	0.204–1.146	0.099
Cardiovascular disease	1.033	0.622–1.716	0.900	0.568	0.186–1.737	0.321	1.407	0.608–3.257	0.425
Heart failure	1.234	0.737–2.066	0.424	1.129	0.359–3.550	0.836	2.470	1.106–5.517	0.027
Diabetes	1.883	1.138–3.115	0.014	1.481	0.484–4.528	0.491	2.299	1.012–5.224	0.047
Arterial hypertension	1.456	0.550–3.853	0.449	1.050	0.130–8.474	0.963	0.325	0.109–0.966	0.043
Kidney transplantation	1.075	0.518–2.233	0.846	0.538	0.068–4.268	0.558	0.811	0.231–2.843	0.743
Pulmonary disease	2.273	1.257–4.102	0.006	2.453	0.771–7.804	0.129	2.926	1.275–6.715	0.011
Immunosuppression	0.731	0.309–1.729	0.476	0.722	0.090–5.772	0.759	0.680	0.152–3.037	0.613
Previous SARS-CoV-2 positivity	0.235	0.053–1.051	0.058		NA			NA	
Dialysis vintage (per 1 month increase)	0.995	0.990–1.001	0.096	0.991	0.975–1.008	0.319	1.003	0.997–1.008	0.335
Vaccination prior infection (as metric variable)[c]	0.580	0.480–0.701	<0.001	0.560	0.353–0.888	0.014	0.468	0.325–0.674	<0.001
Infection during Omicron Wave (compared to prior Waves)	0.120	0.068–0.211	<0.001	0.177	0.048–0.661	0.01	0.040	0.009–0.172	<0.001

ICU admission and mortality.
[a] three cases missing.
[b] diabetic/hypertensive nephropathy compared to other kidney disease.
[c] five cases missing.
NA, not applicable.

in multivariable analysis, and the latter provides an approximately 20-fold risk reduction (Table 3). While vaccination appeared to provide protection from hospitalization, ICU admission and mortality in univariate analyses, when accounting for other variables, particularly Omicron variant infection, the previously observed beneficial effect of prior vaccination was no longer evident (Table 3).

3.3. At-risk hemodialysis population remained stable between 31st of August 2021 and 31st of August 2022 and displayed a high vaccination coverage

To further evaluate the at-risk population and dynamics of vaccination coverage, we collected data from HD patients in Styria, Austria between 31st of August 2021 and 31st of August 2022 (Table 4). The included dialysis centers are shown in Figure 1. A total of 551 individuals met our inclusion criteria. Over 1 year, the HD population remained stable suggesting no excessive mortality although we lack comparative data from previous years (Figure 3).

Vaccination coverage with at least two doses was around 80% in our population at the beginning of the observational period (Table 4, Figure 4A). By the end of 2021 the majority had received a third booster and by the end of August 2022 the number of four dose vaccinated dialysis patients were climbing ($N = 165$, 34%). Over 90% received mRNA-based vaccination.

At the same time a rapid increase in SARS-CoV-2 infections in our population was observed, which resulted in an approximately three times larger proportion of recovered individuals after 1 year compared to August 2021 (Table 4). Dynamics of the recovered patients, defined as 28 days after the first positive PCR test,

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TABLE 3 Multivariable logistic regression for hospitalization.

Multivariable logistic regression			
Hospitalization			
	Odds ratio	95% confidence interval	*p*-Value
Age (per 5 years increase)	1.048	0.940–1.169	0.401
Diabetes	2.660	1.422–4.977	0.002
Pulmonary disease	2.329	1.171–4.632	0.016
Vaccination prior infection (as metric variable)[a]	0.994	0.684–1.443	0.973
Infection during Omicron Wave (compared to prior Waves)	0.108	0.037–0.316	<0.001
ICU admission			
	Odds ratio	95% confidence interval	*p*-Value
Vaccination prior infection (as metric variable)[a]	0.757	0.369–1.551	0.447
Infection during Omicron Wave (compared to prior Waves)	0.331	0.044–2.504	0.284
Mortality			
	Odds ratio	95% Confidence interval	*p*-Value
Age (per 5 years increase)	1.159	0.937–1.434	0.173
Congestive heart failure	2.381	0.908–6.247	0.078
Diabetes	4.588	1.569–13.414	0.005
Hypertension	0.160	0.037–9.082	0.014
Pulmonary disease	3.250	1.163–9.082	0.025
Vaccination prior infection (as metric variable) [a]	0.935	0.524–1.666	0.819
Infection during Omicron Wave (compared to prior Waves)	0.037	0.005–0.277	0.001

ICU admission and mortality.
[a]five cases missing.

TABLE 4 Clinical and vaccination data of hemodialysis patients on the 31st auf August 2021 and the 31st of August 2022 in Styria, Austria.

	31-Aug-2021	31-Aug-2022
N	478	476
Age (years)	70 (59–77.3)	71 (60–78)
Male gender (%)	291 (60.9)	296 (62.2)
BMI (kg/m^2)[a]	25.9 (22.7–29.8)	26 (22.8–30)
Kidney disease		
Diabetic nephropathy	164 (34.3)	168 (35.3)
Hypertensive nephropathy	72 (15.1)	67 (14.1)
Glomerular disease	72 (15.1)	72 (15.1)
Polycystic kidney disease	28 (5.9)	30 (6.3)
Other	109 (22.8)	111 (23.3)
Unknown	33 (6.9)	28 (5.9)
Cardiovascular disease	319 (66.7)	306 (64.3)
Congestive heart failure	186 (38.9)	188 (39.5)
Diabetes mellitus	222 (46.4)	223 (46.8)
Arterial hypertension	448 (93.7)	448 (94.1)
Kidney transplantation	63 (13.2)	62 (13)
Immunosuppression	45 (9.4)	48 (10.1)
Pulmonary disease	96 (20.1)	87 (18.3)
Dialysis vintage (months)	30.5 (12–66)	35 (18–67)
Previous SARS-CoV-2 positivity	66 (13.8)	216 (45.4)
Vaccination[b]		
1 dose	11 (2.3)	1 (0.2)
2 dose	381 (79.7)	27 (5.7)
3 dose	3 (0.6)	225 (47.3)
4 dose	0	165 (34.7)
Unvaccinated	43 (9)	27 (5.7)
mRNA-1273/BNT162b2[c]	321 (81.3)/70 (17.7)	317 (75.8)/87 (20.8)

[a]Seven and eight cases missing in the 31-Aug-2021 and 31-Aug-2022 group, respectively.
[b]Missing information in 40 and 31 patients, respectively.
[c]Vaccine type referring to first dose. Percentages of vaccinated individuals at specified timepoint are given.

a timepoint at which an antibody response following natural infection should be measurable (18), are shown in Figure 4B.

Our data suggests that Delta and Omicron waves challenged a rapidly changing population with regards to vaccination and infectious history, which resulted in a different vulnerability to severe COVID-19 (Figure 4).

3.4. Incidence and prevalence of SARS-CoV-2 positivity peaked with BA.1/BA.2

Next, weekly prevalence and incidence of SARS-CoV-2 infections were calculated by comparing the at-risk population to the SARS-CoV-2 positive and newly positive patients, respectively. When plotted against time, distinct waves become apparent. The separation of waves is further supported by sequencing data, which allows the differentiation of two distinct Omicron subwaves: the earlier BA.1/BA.2 subwave, which was replaced by end of May 2022 by the BA.4/5 subvariant. Omicron infections were preceded by the Delta wave until January 2022. Sequencing results are summarized in Table 4.

Prevalence and incidence peaked during the BA.1/BA.2 dominated subwave with 47.2 and 33.2 per 1000 dialysis patients, respectively (Figures 5A, B).

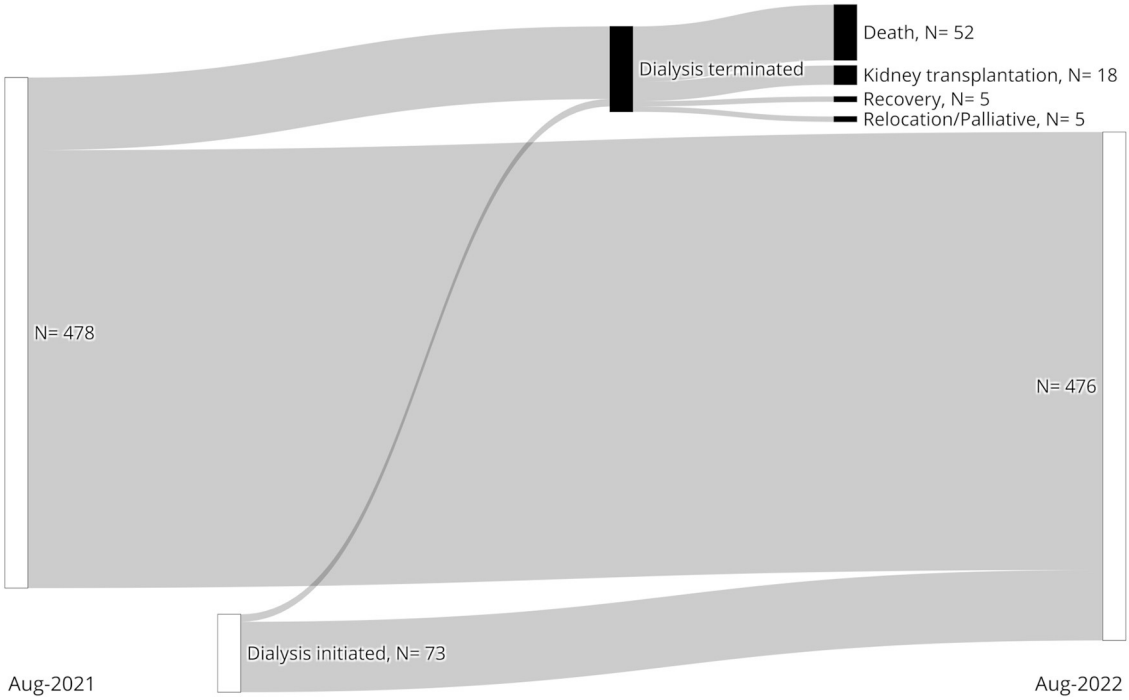

FIGURE 3
Dynamics of the at-risk hemodialysis population between 31st of August 2021 (**Left**) and 31st of August 2022 (**Right**) are shown.

Median prevalence during Delta, BA.1/BA.2 and BA.4/5 were 4.16, 12.4 and 6.21 per 1,000 dialysis patients, respectively ($p \leq 0.001$). Median incidences were also significantly different between these three periods (Delta: 2.07 vs. BA.1/BA.2: 7.29 vs. BA.4/5: 4.20 per 1,000 dialysis patients, $p \leq 0.001$).

3.5. BA.4/5 infections in hemodialysis patients remain equally mild as with BA.1/BA.2

Both Omicron subwaves differ substantially from previous waves, in that infected individuals were vaccinated more frequently (89.2% for BA.1/BA.2 and 89.2% for BA.4/5 with at least two vaccination doses at the time of infection). We also noted repeated COVID-19 in 6.2% and 24.3% for BA.1/BA.2 and BA.4/5, respectively, which underlines the profound immune escape displayed by the Omicron variant. Reinfections were mild with only two hospitalizations and no ICU admissions or deaths.

Severe disease necessitating hospitalization or ICU admission were rare events and similar in both Omicron subwaves (Table 5). However, duration of PCR positivity and hospital stay trended to be shorter in BA.4/5 compared to BA.1/BA.2. Mortality in both Omicron subwaves was almost non-existent and recorded only in one patient each (Table 5).

4. Discussion

COVID-19 has posed a great threat to the lives of HD patients in the early pandemic (2–4), who were often particularly exposed due to regular in-center HD (1). Compared to the general population, diminished antibody response to SARS-CoV-2 has been shown in HD patients (14), leaving them vulnerable after infection and vaccination (12, 19).

Presently, we analyzed COVID-19 cases at our center from the first recorded case in March 2020 until August 2022. In agreement with existing reports, we could show the high morbidity and mortality associated with COVID-19 in the early stages of the pandemic. Furthermore, we could confirm that the Omicron variant has been highly prevalent in dialysis patients, but virulence has been markedly lower than in previous waves. Our at-risk population was extensively vaccinated and exhibited a strong willingness for a third and fourth dose. We also report that infections with Omicron sublineage BA.4/5 do not differ from Omicron sublineages BA.1 and BA.2 with regards to hospitalization, ICU admission, duration of PCR positivity and mortality. We think that our study provides valuable information for nephrologists, who are concerned with these novel sublineages.

High prevalence of comorbidities may render HD patients susceptible to severe and fatal COVID-19. Important risk factors include older age, diabetes, hypertension, and cardiovascular disease (20). In agreement, older age, heart failure and pulmonary disease were predictors of COVID-19 related mortality in our population. However, Ng et al. have shown, that even after adjustment for concomitant disease, end-stage kidney disease

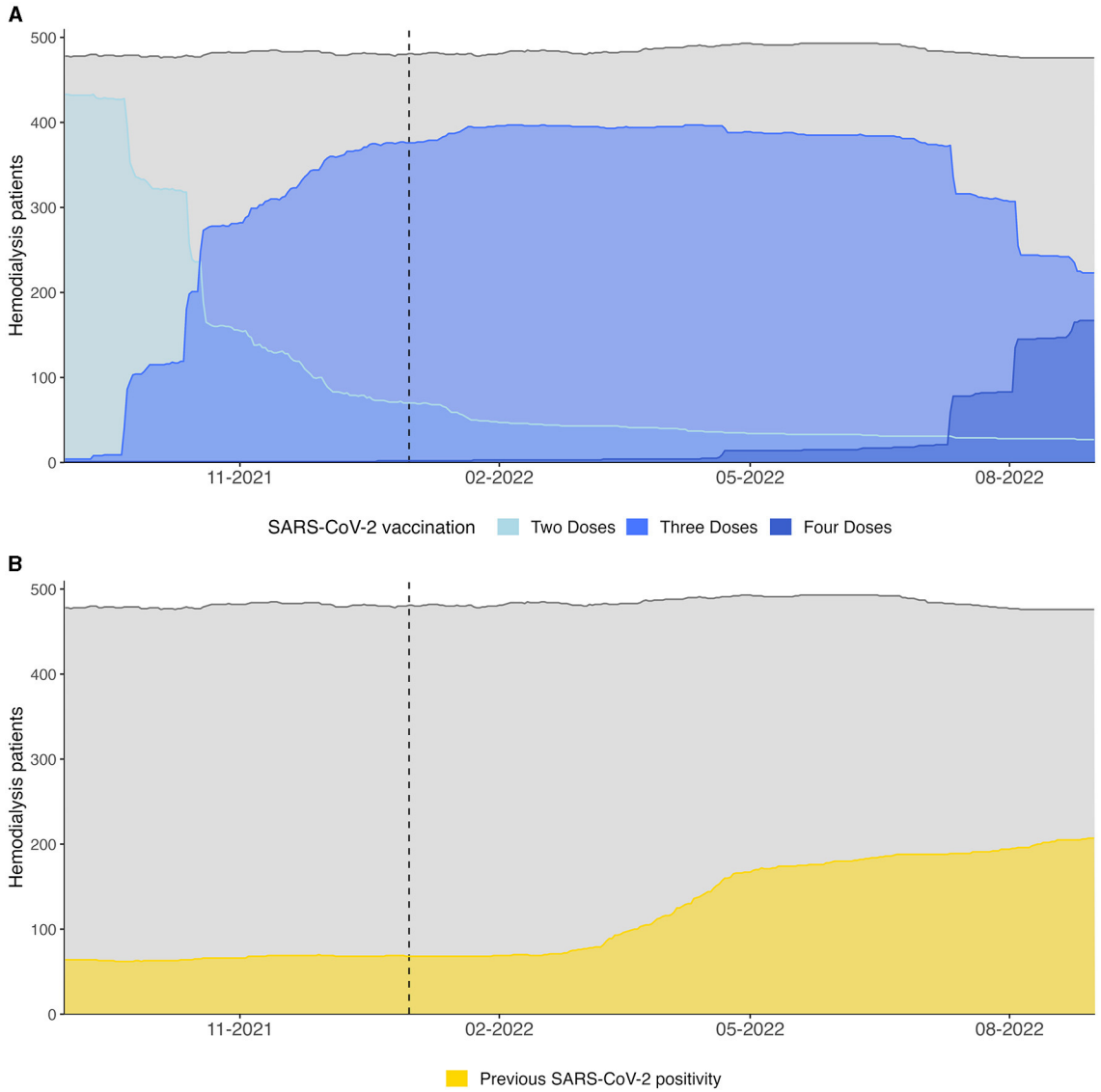

FIGURE 4
(A) Vaccination coverage and **(B)** proportion of recovered HD patients (defined as 28 days after the first PCR positivity) of the at-risk hemodialysis population between 31st of August 2021 and 31st of August 2022 are shown. Gray lines indicate the total number of dialysis patients. The dotted vertical lines indicate the switch from Delta to Omicron wave.

remains a robust predictor of mortality (3). It is speculated, that uremic alterations of the innate and adaptive immune response may predispose to infections (21).

With reduced virulence in latter stages of the pandemic, and with the emergence of Omicron as the dominant variant, mortality has become a rare event (8). Congruently, hospitalization rates declined significantly (22). Nevertheless, due to the high transmissibility of Omicron (9), the total number of hospitalized COVID-19 cases remained high. Even though hospitalization in the Omicron era was necessary in only 16.9% of cases, the absolute number exceeded all previous waves except for the second wave. Although the need for hospitalization is subject of the clinician's assessment and therefore not a strictly objective outcome, it is highly relevant as it poses a substantial cost factor for health care systems. Duration of hospitalization remained similar between waves, and prolonged viral shedding has been described in patients with impaired kidney function further adding to the problem (23). Thus, despite largely losing the threat of a life-threatening disease, COVID-19 still has the potential to overwhelm health care providers.

We report a low number of ICU admissions, which may reflect triage as these patients were often deemed to have no recovery potential. Therefore, caution is warranted when interpreting ICU admission as an outcome parameter of severe disease in the HD population. A recent review found inconsistent evidence regarding ICU admissions in CKD patients, while hospitalization and mortality were robustly increased in CKD with COVID-19 compared to non-CKD (24). Although it is difficult to estimate the "true" need for intensive care, Chan et al. reported that the rate of ICU admissions in HD patients was only about 9% compared

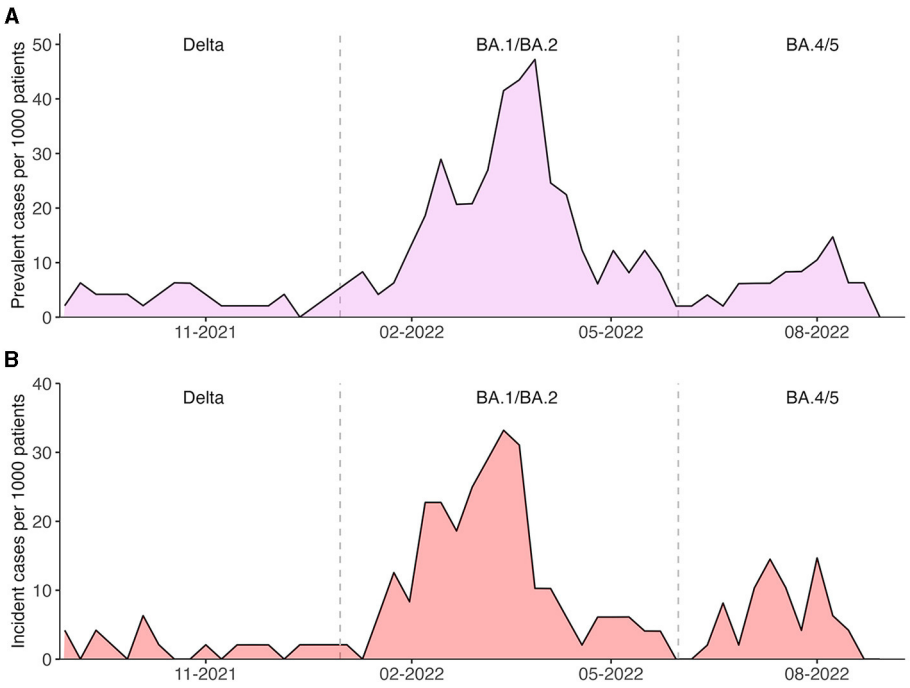

FIGURE 5
(A) Prevalence and **(B)** incidence of SARS-CoV-2 positive cases on hemodialysis between 31st of August 2021 and 31st of August 2022. The dotted lines mark the switch from Delta to BA.1/BA.2 and from BA.1/BA.2 to BA.4/5 infections, respectively.

to 21% in a propensity score matched control group, despite comparable burden of comorbidities and similar symptoms at hospital admission (25).

In agreement with others, we clearly show that the threat for HD patients has progressively diminished over the course of the pandemic (7). Since the emergence of SARS-CoV-2, several important changes ought to be highlighted: first, vaccinations are a safe and effective measure in the prevention of severe disease. Second, treatments have been developed to reduce mortality in those already infected. Third, accumulating infections resulted in a certain degree of natural immunity among survivors, potentially mitigating viral transmission and/or disease severity. Finally, VoCs have profoundly altered the pandemic in terms of transmission dynamics and disease severity. These changes largely coincided with each other, thus making it challenging to quantify the contribution of each individual factor.

Even prior to the emergence of these factors, COVID-19 related morbidity and mortality decreased in the HD population (26), which may simply be a consequence of more widespread testing and the identification of more oligo- and asymptomatic patients (26). Whether the at-risk population was altered with the particularly vulnerable already having succumbed to the initial wave of SARS-CoV-2 remains debated (27, 28). Our at-risk HD population between August 2021 and August 2022 remained stable and prevalence of comorbidities was comparable at both timepoints.

Immune-escape is another hallmark of Omicron (10). While repeated antigenic stimulation by booster vaccination appears to provide some protection from infection (15), neutralizing activity against Omicron BA.1 remains insufficient even after four doses (29). Despite the high vaccination coverage in our dialysis population, we saw a massive surge in infections in 2022. Previous vaccination conferred protection from hospitalization and mortality in our study only in univariate analysis. When controlled for other factors, especially timing of infection (pre-Omicron vs. Omicron era) the protective effect of vaccination disappeared. These findings may be attributable to the profound immune escape displayed by Omicron sublineages. The novel bivalent Omicron BA.4/5-adapated vaccine elicits a robust response in HD patients and may offer improved protection from these sublineages (30). Of note, these adapted vaccines were rolled-out after the end of our observational period in Austria.

Before the advent of vaccines, natural infection was the only way to acquire anti-SARS-CoV-2 antibodies, which have been shown to protect from reinfection in HD patients in the pre-Omicron era (12). A recent meta-analysis concluded that the risk of reinfection in the general population with Omicron sublineages is substantially higher than with previous variants, but natural infection still offers a certain degree of protection especially from severe disease (31). We observed 17 mild reinfections in our cohort, and previous SARS-CoV-2 infection tended to be a protective factor against hospitalization (Table 2). Analysis of the impact on ICU admission and mortality was hindered by the low number of reinfections and events. Reinfections were noted exclusively during the Omicron wave. Since we did not assess for antibody titers, we can only speculate that those individuals either failed to mount a substantial humoral response during the earlier infection or were affected

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TABLE 5 SARS-CoV-2 PCR confirmed infections during the Omicron wave. Cases during the earlier BA.1/BA.2 dominated period are compared to the later BA.4/5 period.

	BA.1/BA.2	BA.4/5	
Time period	01-Jan-2022–31-May-2022	01-Jun-2022–31-Aug-2022	
N	130	37	*p*-Value
Age (years)	71 (55.8–78)	71 (58.5–77.5)	0.901
Male gender (%)	72 (55.4)	25 (67.6)	0.185
BMI (kg/m^2)[a]	26.2 (23–30.8)	25.6 (22.2–29.8)	0.883
Kidney disease			0.729
Diabetic nephropathy	45 (34.6)	14 (37.8)	
Hypertensive nephropathy	17 (13.1)	7 (18.9)	
Glomerular disease	21 (16.2)	6 (16.2)	
Polycystic kidney disease	5 (3.8)	1 (2.7)	
Other	32 (24.6)	5 (13.5)	
Unknown	10 (7.7)	4 (10.8)	
Cardiovascular disease	76 (58.5)	25 (67.6)	0.318
Congestive heart failure	48 (36.9)	12 (32.4)	0.615
Diabetes mellitus	59 (45.4)	18 (48.6)	0.725
Arterial hypertension	121 (93.1)	36 (97.3)	0.340
Kidney transplantation	17 (13.1)	3 (8.1)	0.411
Immunosuppression	11 (8.5)	3 (8.1)	0.945
Pulmonary disease	22 (16.9)	8 (21.6)	0.511
Dialysis vintage (months)	34.5 (10.8–68)	32 (16.5–53.5)	0.967
Previous COVID-19	8 (6.2)	9 (24.3)	0.001
SARS-CoV-2 sequencing	85 (65.4)	21 (56.8)	
BA.1	36 (42.4)	0	<0.001
BA.2	49 (57.6)	0	<0.001
BA.4/5	0	21 (56.8)	<0.001
Vaccination[b]			<0.001
1 dose	0	0	
2 dose	18 (13.8)	1 (2.7)	
3 dose	98 (75.4)	28 (75.7)	
4 dose	0	4 (10.8)	
Unvaccinated	12 (9.2)	3 (8.1)	
Duration PCR positivity (days)	7 (3-12)	5 (3–8)	0.130
Hospitalization	21 (16.2)	7 (18.9)	0.691
Antibiotics	45 (34.6)	11 (29.7)	0.579
Corticosteroids	11 (8.5)	2 (5.4)	0.540
Remdesivir	2 (1.5)	1 (2.7)	0.638
Anti-SARS-CoV-2 Antibodies	3 (2.3)	0	0.351
Duration of hospitalization (days)	13 (8–19)	5 (3–9)	0.077
ICU admission	3 (2.3)	0	0.351
Mortality	1 (0.8)	1 (2.7)	0.340

[a]one value missing in each group.
[b]missing information in 2 and 1 cases, respectively.

by Omicron's heightened immune escape capabilities. Existing data suggests, that the level of protection against BA.4/5 is approximately twice as high when BA.1 was the previous infection compared to pre-Omicron variants (31). Thus, it is tempting to speculate that the increasing number of recovered HD patients during Omicron may have limited further viral spread (Figure 4B). Importantly, the protective efficacy of natural infection compared to vaccination in terms of protection from subsequent Omicron and Omicron sublineage infection in HD patients remains uncertain.

A major strength of this study is the comprehensive and well characterized cohort of HD patients, which was followed over the course of 1 year. Large registry studies have previously reported on COVID-19 in HD patients, but either during a limited observational period (7, 32), or before the emergence of Omicron as dominant variant (33–35). Our study depicts a rapidly changing at-risk population by including extensive information on natural and induced immunity by previous infection and vaccination, respectively. This provides a more complete picture of the real-world impact of the pandemic on the vulnerable HD population. We were also able to characterize and compare infections with BA.1/BA.2 and BA.4/5 in a sizeable number of HD patients.

Apart from the retrospective nature of our study, which comes with inherent bias, our study is limited by its comparatively small population, which may have limited our ability to detect differences especially when comparing smaller waves. While we separated distinct waves based on time and sequencing data, sequencing was not available in all patients. Despite rigorous antigen testing of asymptomatic individuals, we cannot exclude the possibility of undiagnosed SARS-CoV-2 infections, particularly in earlier waves (36). We also acknowledge the reduced sensitivity of antigen testing compared to PCR especially in asymptomatic individuals (37). Yet, diagnostic yield may have been greater in our population due to twice or thrice weekly testing before each dialysis session. We only counted infections if there was evidence within the electronical health records of PCR confirmed SARS-CoV-2 positivity. However, this may have underestimated the number of previous infections in those who became dialysis-dependent later during the pandemic, as PCR results may not have been available, or they may have not been tested as frequently.

While optimization of vaccines and treatments is ongoing, viruses, as well, undergo constant mutations, which may result in the emergence of novel variants and sublineages. Rapid information on new variants or sublineages is paramount to prepare for effective prevention and treatment especially for the vulnerable HD population.

Our findings underline the reduced virulence but increased transmissibility of Omicron in HD patients. Furthermore, we showed that infections with Omicron sublineages BA.4/5 are similarly mild as with BA.1 and BA.2 in HD patients. Although our data is reassuring to clinicians that the situation will remain calm with BA.4/5, we simultaneously acknowledge the importance to remain vigilant for the emergence and spread of novel variants.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the study was approved by the Ethics Committee of the Medical University of Graz (EK 34-372ex21/22). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because retrospective analysis of dialysis cohort. No potentially identifiable images or data are presented in this study.

Author contributions

MS designed the study and interpreted and analyzed the data, drafted the work, finally approved the manuscript, and agreed to be accountable for all aspects of the work. NG, AP, MW, MK, NS, CS, KM, and HH-G acquired the data, revised the manuscript, finally approved the manuscript, and agreed to be accountable for all aspects of the work. AR and PE interpreted the data, revised the manuscript, finally approved the manuscript, and agreed to be accountable for all aspects of the work. KE designed the study and interpreted the data, revised the manuscript, finally approved the manuscript, and agreed to be accountable for all aspects of the work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Pathophysiology and clinical management of coronavirus disease (COVID-19): a mini-review

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An unprecedented global pandemic caused by a novel coronavirus named SARS-CoV-2 has created a severe healthcare threat and become one of the biggest challenges to human health and the global economy. As of July 2023, over 767 million confirmed cases of COVID-19 have been diagnosed, including more than 6.95 million deaths. The S protein of this novel coronavirus binds to the ACE2 receptor to enter the host cells with the help of another transmembrane protease TMPRSS2. Infected subjects that can mount an appropriate host immune response can quickly inhibit the spread of infection into the lower respiratory system and the disease may remain asymptomatic or a mild infection. The inability to mount a strong initial response can allow the virus to replicate unchecked and manifest as severe acute pneumonia or prolonged disease that may manifest as systemic disease manifested as viremia, excessive inflammation, multiple organ failure, and secondary bacterial infection among others, leading to delayed recovery, hospitalization, and even life-threatening consequences. The clinical management should be targeted to specific pathogenic mechanisms present at the specific phase of the disease. Here we summarize distinct phases of COVID-19 pathogenesis and appropriate therapeutic paradigms associated with the specific phase of COVID-19.

KEYWORDS

pathophysiology, clinical management, SARS-CoV-2, COVID-19, prevention

1 Introduction

The ongoing COVID-19 pandemic is entering its fourth year which began with the identification of a group of patients with unknown pneumonia in Wuhan, China in December 2019 (1). This is the third major coronavirus outbreak preceded by severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), however, both of these viruses were contained before causing the global pandemic. Since the emergence of COVID-19, a significant proportion of the human population has been infected with SARS-CoV-2, the causative virus of COVID-19 (2). The rapid spread of the virus allowed the virus

to evolve quickly to become more infectious. Multiple variants of the virus named Alpha, Beta, Gamma, Delta, and Omicron, have emerged during this pandemic and repeated infections have become more common. Many of these variants have much higher infectivity compared to the original strain (3, 4). The continuous evolution of SARS-CoV-2 has caused unprecedented devastating effects on human health and the global economy. As of July 2023, more than 767 million cases of confirmed COVID-19 have been reported and the virus has claimed over 6.95 million lives (5). Beyond the acute disease and death, the COVID-19 pandemic has touched every aspect of life including economic well-being, mental health, and impaired services for other diseases leading to an increase in mortalities due to other diseases (6). Additional impacts include impaired learning in children and stress among adults leading to increased rates of suicide and self-harm (7).

Individuals infected by SARS-CoV-2 present with a wide range of disease severity ranging from asymptomatic to severe disease that leads to death. While in most of the subjects, COVID-19 manifests as a mild flu-like disease, a small but significant number of patients develop severe and often life-threatening diseases. A multitude of factors contributes to the disease severity including viral load, inflammation, and immune response equilibrium. The life-threatening condition occurs due to excessive inflammation and/or impaired viral clearance caused by aging, underlying diseases such as diabetes and hypertension, and many other unknown factors (8, 9). Beyond the acute disease, the chronic consequences of COVID-19 are being documented that include severe life-altering changes such as chronic fatigue, impaired memory, and cognitive functions, among others (10, 11).

Significant progress has been made in our understanding of the transmission mechanisms, preventive measures, vaccinations, and therapeutic approaches to treat COVID-19. Despite these advances, COVID-19 remains one of the foremost healthcare challenges with significant morbidity, mortality, and economic costs each day across the world. Further, the continuous emergence of novel strains threatens our progress regarding both preventative and therapeutic approaches as immune evasion and antiviral resistance are likely consequences of viral evolution.

The chronic sequelae of COVID-19, known as long COVID occurs in at least 10% of the infected individuals and poses a global health challenge (12, 13). More than 200 symptoms have been documented affecting multiple organ systems, with many patients experiencing multiple symptoms simultaneously, affecting their quality of life including the ability to return to work (14, 15). Unfortunately, no validated effective treatments currently exist. This review provides updated insights into the pathophysiology of COVID-19 and available therapeutic avenues to treat this deadly disease, with the aim of reducing COVID-19 incidence, hospitalization, mortality, and long COVID.

2 Pathophysiology

2.1 Interactions and entry of the SARS-CoV-2 into the cell

Confirmed COVID-19 patients with symptoms and asymptomatic carriers are the primary source of new infections (16). In addition to the respiratory droplets and contact with contaminated surfaces, infection by fecal-oral route has been speculated (17). When SARS-CoV-2 initially infects people, the viral spike (S) protein binds to the angiotensin-converting enzyme 2 (ACE2) receptor, which mediates the entry of SARS-CoV-2 into host cells such as nasal, bronchial epithelial cells and pneumocytes (18). The binding affinity of the S protein of SARS-CoV-2 with ACE2 is 10-20 folds higher than that of SARS-CoV, potentially explaining the quick spread of this pandemic (19). S protein undergoes further priming by type 2 transmembrane serine protease (TMPRSS2), a cellular protease particularly present in alveolar epithelial type II cells, which promotes viral uptake and coronavirus entry. Generally, the ACE2 receptor is expressed in multiple tissue cells, including airways, cornea, esophagus, ileum, colon, liver, gallbladder, heart, kidney, and testis. TMPRSS2 expression has an even broader distribution implicating that ACE2, rather than TMPRSS2, may be a limiting and major factor for viral entry at the early stage of the infection (20, 21). Notably, ACE2 and TMPRSS2 can be targeted for drug intervention to prevent the invasion and transmission of SARS-CoV-2 in host cells (22, 23). To further explain the mechanism of viral entry into host cells, the binding of the S protein to ACE2 in the viral entry process involves several stages:

Attachment: The S protein of SARS-CoV-2 binds to the ACE2 receptor on the surface of the host cell.

Priming: The S protein is then cleaved by a host protease enzyme called TMPRSS2. This cleavage allows the S protein to undergo a conformational change, exposing a fusion peptide that facilitates the fusion of the viral membrane with the host cell membrane.

Fusion: The viral membrane fuses with the host cell membrane, allowing the viral genetic material (RNA) to enter the host cell.

Replication: Once inside the host cell, the viral RNA is used as a template to produce more viral proteins and RNA.

Assembly and Release: The newly produced viral proteins and RNA assemble into new viral particles, which are then released from the host cell to infect other cells. Overall, the binding of the SARS-CoV-2 S protein to the ACE2 receptor on host cells plays a crucial role in the viral entry process and the subsequent development of COVID-19. Understanding the molecular mechanisms underlying this process is crucial for developing effective strategies to prevent and treat COVID-19 (15–18). More recent evidence has indicated ACE2 independent entry of SARS-CoV-2 into the cells, especially in the immune cells (24). The pathological consequences of ACE2-independent entry of the SARS-CoV-2 into host cells are not completely understood. Further, it remains unclear if the extra-pulmonary manifestations such as multi-organ failure are direct consequences of viral infection to those tissues or a consequence of host inflammatory response.

2.2 Early stage of infection

Upon entry into cells in the upper respiratory tract, SARS-CoV-2 starts to replicate and propagate in the nose and upper airways. Although infected subjects may remain asymptomatic at this stage,

they are highly infectious with a high viral load, which begins to peak around symptom onset (25, 26). Subsequently, the virus may migrate from the nasal epithelium to the upper respiratory tract via the ciliated cells in the conducting airways (18, 27). The infected individuals can shed viral particles by not only coughing or sneezing but also during their day-to-day activities such as talking, eating, and even exhaling. Pre-symptomatic transmission is considered a significant contributor to viral transmission responsible for 9.1%-62% of positive cases which vary from different literatures among different populations (25, 28, 29).

The viral transmission also occurs during the symptomatic disease and may even continue after symptoms are solved (30). Infected individuals manifest the symptoms of fever, malaise, cough, and sputum production at the early stage. The host mounts an innate response that is mediated by cytokines and antiviral interferons and initiates the adaptive immune response. If the host is able to mount a strong interferon-mediated response at this stage, such as seen in children and adults, they may control the viral replication and limit the disease severity at this stage (26, 31, 32). The precise mediators of early viral clearance are not yet completely understood but given the potent antiviral activity and robust upregulation in those with a mild disease show a critical role of interferons in viral elimination (Figure 1) (32, 33).

2.3 Late stage of infection

Subjects that fail to eradicate the virus in its early stage, may progress to the clinical phase or later stage of the infection, which is manifested by COVID-19 symptoms that may vary in severity and duration (34). It is estimated that 1/5th of the infected patients progresses to the involvement of the lower respiratory tract that

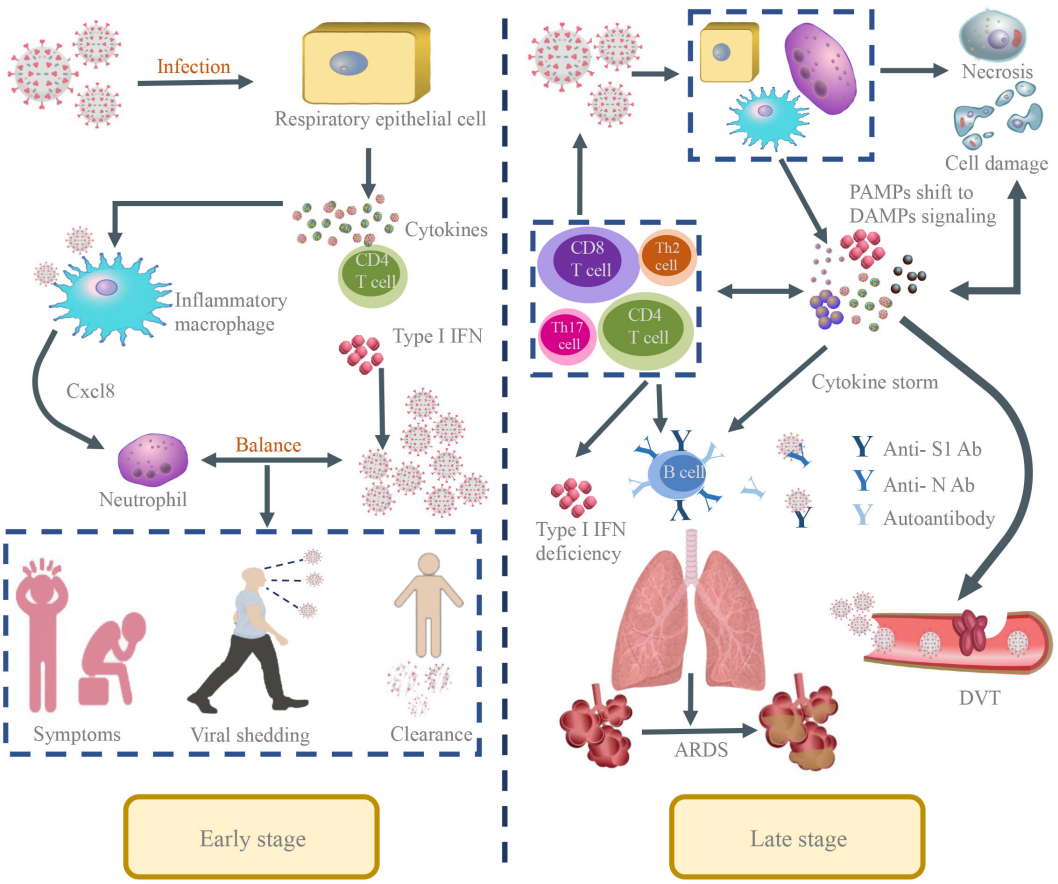

FIGURE 1
The pathological manifestations of SARS-CoV-2 infection in the human body. Early stage: In the early stage of SARS-CoV-2 infection, the respiratory epithelial cells were targets of the virus and the virus begins to replicate and spread in the nose and upper respiratory tract. The host mounts an innate response mediated by cytokines and antiviral interferons that initiates the adaptive immune response. In the early stages, infected people show the symptoms of fever, malaise, cough and sputum production and can transmit viruses to other people. If the host can mount a strong interferon-mediated response at this stage and appropriate regulation of the host immunity relative to the viral burden, viral replication would be arrested and viral clearance initiated to limit disease severity at this stage. Late stage: If the virus can't be eradicated in a timely manner likely due to a delayed PAMP-mediated inflammatory/interferon response, the immune response would shift to a nonspecific inflammatory reaction dominated by damage-associated molecular pattern (DAMP) signaling emanating from damaged or dysfunctional virus-infected host cells. Immune cells including CD4 helper T cells, and CD8 cytotoxic T cells are sequestered in the lung tissue. The host cells undergo persistent apoptosis or necrosis or pyroptosis that may amplify the tissue damage. In addition, the inflammatory environment triggers the expression of activated tissue factor on endothelial cells, macrophages, and neutrophils, thereby enhancing activation of the coagulation cascade in the lungs, causing microcirculatory thrombi and ARDS and increasing disease severity and mortality.

involves infection to the alveolar epithelial type II cells, developing severe symptoms like acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), and pulmonary embolism. The clinical phase of SARS-CoV-2 can be characterized into three distinct phases: acute or pneumonia phase, viremia phase, and lethal/recovery phase.

The acute phase is characterized by pulmonary disease that is manifested by pulmonary symptoms such as dyspnea, cough, and sputum production with imaging evidence of ground-glass opacity or consolidation in the lung. Diffuse alveolar damage, desquamation of pneumocytes, and hyaline membrane formation are observed during the development of ARDS in COVID-19 (35). The increased permeability of the lung vasculature impairs oxygen diffusion and contributes to the fatal disease. Factors contributing to lung permeability during COVID-19 are multifactorial and may include 1. Direct cytopathic effects of coronavirus in infected endothelium resulting in widespread endothelialitis (36). 2. The reduction of ACE2 activity by SARS-CoV-2 and subsequent increase in angiotensin indirectly boosting the kallikrein-bradykinin pathway which promotes vascular permeability (37). 3. Inflammatory cytokines and vasoactive mediators secreted by immune cells such as activated neutrophils induce the contraction of endothelial cells and loosen endothelial tight junctions. 4. Glycocalyx degradation and hyaluronic acid deposition in the extracellular matrix promote fluid retention (38). The increased lung vascular permeability results in impaired lung function that manifests into decreased blood oxygenation, a marker of disease severity.

The viremic phase begins when the virus enters the peripheral blood. The molecular mechanisms of viremia in COVID-19 are still poorly understood, however, ACE2 independent entry of the virus in peripheral monocytes has been shown to promote pyroptotic cell death and disease severity (24). The viremia and subsequent host response contribute to multiple systemic inflammation and multiorgan failure. The inflammatory response during severe COVID-19 is mediated by a simultaneous increase in the multiple inflammatory cytokines such as IL-1α, IL-1β, IL-6, IL-8, IL-12, IL-17), TNF- α, interferons (IFN- β, IFN- λ), MCP-1, and MIP-1 α, making it difficult to pinpoint the specific mediator of inflammatory response (39). Additionally, the early inflammatory response may help the host to limit viral replication, further complicating the role of cytokines in COVID-19. It is not surprising that therapies targeting specific cytokines such as IL-6 or TNF- α led to mixed results (40, 41).

The lethal phase is mediated by a persistent disease that is manifested both locally and systemically. The inflammatory response in the form of cytokine storm and coagulation factors is significantly elevated in severe patients compared to non-severe patients (42–44). In this phase, neutrophils, CD4 helper T cells, and CD8 cytotoxic T cells are sequestered in the lung tissue (45). The host cells undergo persistent apoptosis, necrosis, or pyroptosis, which may amplify the tissue damage. In addition, the inflammatory environment triggers the expression of activated tissue factor on endothelial cells, macrophages, and neutrophils, enhancing activation of the coagulation cascade in the lungs (46). Markers of the coagulation pathway such as upregulation of D-dimer are clearly visible at this stage. In an autopsy study, Wichmann et al. demonstrated that 58% of COVID-19 patients have a concurrency of DVT, and 1/3 of the deaths were directly caused by pulmonary embolism (PE) (47). The potential role of thrombosis in pulmonary veins distal to the alveolar capillary bed, which should act as clot filters, has been pointed out, suggesting that it could be SARS-CoV-2-related vasculitis responsible for ischemic manifestations in various organs (48). For this reason, severe COVID-19 is not restricted to the respiratory system but is a multisystem disease including the development of various cardiovascular manifestations with myocardial injury, arrhythmia, acute coronary syndrome, and venous thromboembolism. These manifestations are closely related to the disease severity and progression to lethal disease (49). Based on all these features, anticoagulant therapy and immunomodulatory agents are probably necessary to attenuate the hyperinflammatory and prothrombotic states (50). These patients may benefit from immune modulators such as steroids while antiviral agents have limited utility at this stage of the disease. Despite the obvious contribution of coagulation pathways in vascular disease, the use of anticoagulant therapy may be filled with the risk of increased bleeding. On the other hand, microcirculatory thrombi in capillaries and large vessels may already cause extensive damage if administered too late.

2.4 The complexity of immune response during COVID-19

Exacerbated immune response manifested as cytokine storm is a common pathological event in many infectious diseases. However, given the wide range of clinical presentations and extensive studies performed on COVID-19, a clearer picture of beneficial and pathological immune responses started to emerge. Host immune response, initiated by sensing the pathogen-associated molecular patterns (PAMP) present on the pathogen strives to eliminate the invading pathogen. A multitude of evidence suggests that the initial host response is dampened markedly in severe COVID-19 patients, especially those mediated by type I interferon (51, 52). Type I IFNs (IFN- α, IFN- β, IFN- ω) are indispensable in viral clearance, and this impairment is associated with high blood viral load and exacerbated inflammatory response (33). Additionally, the nucleocapsid (N) protein, one of the four structural proteins of CoV, serves as an antagonist of IFN, which appears to be beneficial for viral replication (53, 54). It was speculated that a large spectrum of severe clinical presentations could result from a delayed host response towards a specific pathogen-associated molecular pattern (PAMP), which attenuates the antiviral innate immunity required to eliminate the pathogen. In support of this hypothesis, treatment with type I interferon has shown beneficial effects in COVID-19 (55).

Moreover, the postponement of the PAMP-mediated response shifts the immune response to a nonspecific inflammatory reaction dominated by damage-associated molecular pattern (DAMP)

signaling emanating from damaged or dysfunctional virus-infected host cells (56, 57). Although PAMPs and DAMPs can lead to innate and adaptive immune responses, the latter raises additional damage and dysfunction by releasing various pro-inflammatory cytokines by activating dendritic cells and other antigen-presenting cells (APCs). DAMPs initiate innate immune activation and systemic inflammation, which could further upregulate itself by triggering a cytokine storm, providing positive feedback to tissue destruction. It is not surprising that young subjects who remained asymptomatic post-SARS-CoV-2 infection had elevated levels of inflammatory cytokines such as IL-2 while decreasing levels of the anti-inflammatory cytokine IL-10 (57). The current clinical challenge remains to identify and distinguish the DAMP-driven immune response from that driven by PAMPs.

Similar to the innate immune response, the adaptive immune response to SARS-CoV-2 infection has been studied in detail. Overall, it appears that the ability of the host to generate an early humoral response rather than mounting a stronger humoral response is critical in protecting the host against severe disease. Both delayed onset of the humoral response and elevated antibody titers are associated with severe disease in COVID-19 (58, 59). To support this hypothesis, we have shown that the early onset of antibody response was associated with asymptotic disease and patients with severe disease had elevated antibody response (60, 61). Along similar lines, convalescent plasma therapy provided limited benefits in COVID-19, leading the World Health Organization to issue guidelines against plasma therapy for COVID-19 (62).

2.5 Long COVID

Despite the widespread prevalence of long COVID-19, the mechanistic understanding of underlying pathophysiology remains limited. There are several suggested hypotheses for the pathogenesis of long COVID-19 including the persistent exposure of SARS-CoV-2 in tissues, dysregulation of the immune system, reactivation of secondary pathogens (eg. EBV, HHV-6, HCMV, VZV etc.), microbiome dysbiosis in the gastrointestinal system, autoimmunity and immune priming from molecular mimicry, microvascular blood clotting with endothelial abnormalities, and dysfunctional neurological signaling, among others (63–66). Increasing evidence have shown that two third of the population with long COVID is associated with multiple potential risk factors and pre-existing conditions, including female sex, type 2 diabetes, underlying virus reactivation, the presence of specific autoantibodies, and connective tissue disorders (67–69). A higher prevalence of long COVID-19 has been reported in certain ethnicities, including people with Hispanic or Latino heritage (13). Lower income and lack of sufficient rest in the early weeks after SARS-CoV-2 infection are associated with long COVID-19, which is characterized by postexertional malaise, postural orthostatic tachycardia syndrome, pain, fatigue, unrefreshing sleep, brain fog, cognitive dysfunction, gastrointestinal symptoms, neurological symptoms, among others (70, 71).

3 Clinical management

3.1 Prevention and self-protection

The origin of SARS-CoV-2 including the animal host still needs definitive identification (72). Most of the infections occur through human-to-human contact, either directly through droplets emitted from infected objects or indirectly by aerosols suspended in the air (73). Preventive measures such as mask/respirator use are highly effective if used appropriately, although the quality of the mask/respirator is critically important. Although infections in healthcare workers have been reported, but larger outbreaks in the healthcare workers have been rare, even before the availability of vaccines, due to the efficacy of personal protective equipment. A nationwide cross-sectional study in Bangladesh has proved that adequate preventive health measures were associated with a lower risk of infection and death from COVID-19 (74). Among preventive health measures, washing/cleaning hands with soap or hand sanitizer (OR: 0.17, 95% CI: 0.09–0.41), wearing masks properly (OR: 0.02, 95% CI: 0.01–0.43), avoiding crowded places (OR: 0.07, 95% CI: 0.02–0.19), and maintaining social distancing in public places (OR: 0.04, 95% CI: 0.01–0.33), were significantly associated with the reduced number of cases and deaths (74).

3.2 Early isolation and treatment settings

Early isolation and appropriate duration of quarantine are required to stop or slow down the spread of COVID-19 or its newly emerging variants. However, given the ubiquitous spread of COVID-19, the availability of vaccines, effective therapeutic agents, and an overall decrease in the severe disease by new variants, most countries are shifting their focus away from extensive quarantine and isolation of suspected individuals. However, the experience from this pandemic should guide us in future outbreaks to limit both the disease spread and better deal with the social and economic costs associated with the extensive quarantine.

3.3 Vaccines

Vaccines are vital cost-effective tools to prevent the disease and limit the disease severity during the COVID-19 pandemic. Effective vaccine plays a critical role in preventing the viral spread and limiting the disease severity (75). Multiple vaccines are currently available including adenovirus vector vaccines, mRNA, inactivated, and subunit vaccines. The initial data shows that among individuals ≥ 18 years of age, adenoviral vector vaccines were 73% (95% CI = 69- 77) effective and messenger RNA (mRNA) vaccines were 85% (95% CI = 82- 88) (76). Existing adenovirus, mRNAs, and inactivated vaccines can elicit significant immune responses against SARS-CoV-2 RBDs in vaccinated recipients, and individuals develop neutralizing antibodies against the specific area within 30 days of the first and second doses of the vaccine (77). However, the efficacy figures of these vaccines are evaluated within the first 6 months post-vaccination. The immunity gradually decreases over time, and breakthrough infections

became common. In addition, mutations in the SARS-CoV-2 can subvert the immunity to cause infections in vaccinated subjects. Boosting host immunity with additional doses of vaccines is effective in limiting disease severity and death, especially among older patients. The beneficial effects of boosters among the young subjects are difficult to decipher given low hospitalizations and mortality in these subjects and potentially due to strong immune response to initial vaccine doses. However, in patients with carcinomas and other immunosuppressive diseases, currently available vaccines have shown to trigger sufficient immune response (78, 79). Further, variant-specific vaccines have been recently developed and approved, however, the precise supremacy of variant-specific vaccines in real-life settings is yet to be determined.

3.4 Diagnostic evaluation

Reliable clinical or laboratory parameters are of great importance to accurately distinguish between COVID-19 and respiratory infections of other origins. Individuals with typical respiratory symptoms such as fever, cough, and myalgia should be tested by respiratory nucleic acid amplification test (NAAT) through the specimen from bronchoalveolar lavage, sputum, and nasopharyngeal swab using real-time fluorescence polymerase chain reaction with reverse transcription (RT-PCR). Since false-negative results occur in the circumstance of low viral load in the initial screening, repeated testing should be performed if the symptoms continue to persist. Other measures such as computed tomography (CT) examination can be performed for the auxiliary diagnosis of COVID-19 which is manifested as patchy or segmental GGOs (93.3%) and reticular markings distributed by peribronchovascular and subpleural (80). Although limitations exist when some imaging signs of COVID-19 were presented as the same as those of other lung diseases. Chest CT is easy to perform and readily available to quickly detect lung lesions and make imaging diagnoses at an early stage. Particular attention should be paid to the final confirmation of a SARS-CoV-2 infection. In addition, patients with fever in the emergency department (ED) should be monitored for the presence of SARS-CoV-2 (81). While nucleic acid-based tests or antigen detection tests are used for diagnostic purposes, antibody detection tests may be used to assess the overall exposure in the population (82).

3.5 Therapy and clinical management

The clinical management and target of currently used therapeutics against SARS-CoV-2 are demonstrated (Tables 1, 2) (96). The clinical management of COVID-19 involves a wide range of antiviral and immune modulatory drugs that are described below (Figure 2).

3.5.1 Antiviral drugs

In the last four years, aggressive research allowed the development of novel antiviral agents in addition to repurposing other antiviral molecules. These agents vary in efficacy and adverse effect profile. Some of the currently available agents are the following:

3.5.1.1 Remdesivir

Remdesivir was originally developed as an antiviral agent against Ebola, however, the clinical trials failed to show any effectiveness in reducing the mortality (83). The mechanism by which remdesivir acts against viruses is by acting as a nucleoside analog and inhibiting RNA-dependent RNA polymerase, which elicits the delay of the chain termination in the replication of the RNA genome (97). The drug was then tested against SARS-CoV-2, where it was found to have potent antiviral effects during *in vitro* testing (98). These studies led to its approval for emergency use in COVID-19. The initial clinical studies show the clinical benefit of remdesivir in COVID-19 (84). This led to WHO recommending the use of remdesivir for patients without severe or critical illness in April 2022. Subsequent randomized clinical trials failed to show any beneficial effects of remdesivir regardless of the severity of the disease (62, 83, 85). This prompted WHO to recommend against the use of remdesivir in COVID-19 regardless of disease severity (62).

3.5.1.2 Molnupiravir

Molnupiravir is a ribonucleoside prodrug of N-hydroxycytidine (NHC) that effectively inhibits RNA viruses including SARS-CoV-2 (99). Large-scale clinical trials have shown its beneficial effects during COVID-19 including decreasing hospitalizations and mortality (86). Unlike remdesivir, this drug can be used orally, allowing it to be administered early in high-risk subjects. Subsequent studies have confirmed the beneficial findings, but the beneficial effects were limited to a 30% decrease in mortality due to COVID-19 (100, 101). Another major concern is its efficacy against novel variants. Initial data show the clinical benefit of molnupiravir against alpha and beta variants, however, limited clinical data are obtained that molnupiravir exerts activity against delta and omicron variants (86, 102, 103). Recent evidences have indicated the potential benefit of molnupiravir against the omicron variant (104).

3.5.1.3 Paxlovid

Paxlovid is one of the most effective and orally available anticoronaviral medication against SAR-CoV-2. Paxlovid is a combination of nirmatrelvir and ritonavir. Nirmatrelvir targets the main polyprotein protease enzyme of SARS-CoV-2 in the replication cycle, dramatically decreasing the viral loads (105). Ritonavir is a protease inhibitor and a CYP3A4 antagonist, inhibiting nirmatrelvir breakdown and enhancing its pharmacokinetics (106). A double-blind, randomized, controlled trial with 2246 patients who received paxlovid (300mg of nirmatrlvir and 100mg of ritonavir) showed 89% lower incidence of hospitalization and deaths attributed to any cause within 28 days along with decreased viral load (87). Moreover, no obvious safety concerns were observed besides mild generic side effects such as bitter aftertaste, diarrhea, and fatigue. However, one should be careful about drug-drug interactions and should not be co-administrated with other medicines that are metabolized by CYP3A4. Further, this is not indicated in pregnant or breastfeeding patients.

TABLE 1 Main findings of reported literature.

Authors	Journal	Year of publication	Study type	Main findings
Zhou F et al. (1)	Lancet	2020	Retrospective study	Potential risk factors such as older age and markers of disease severity such as high SOFA score, and elevated d-dimer could identify poor prognosis at an early stage.
Zhu N et al. (2)	N Engl J Med	2019	Descriptive study	Isolation of the virus and designated it as a novel beta coronavirus belonging to the sarbecovirus subgenus of the Coronaviridae family. Further, described its specific cytopathic effects and morphology.
Wiersinga WJ et al. (34)	JAMA	2020	Systemic review	Described detailed aspects of transmission, infection, and treatment.
Spinner CD et al. (83)	JAMA	2020	Randomized Clinical Trial	Investigated the effects of Remdesivir on clinical status at 11 days in moderate COVID-19 patients. Compared with standard care, patients in a 5-day course of remdesivir administration had a statistically significant difference in clinical status, while patients treated in a 10-day regimen of remdesivir did not have a statistically significant difference.
Beigel JH et al. (84)	N Engl J Med	2020	Randomized Clinical Trial	Remdesivir was superior in shortening the time to recovery in adults who were hospitalized with COVID-19.
Wang Y et al. (85)	Lancet	2020	Randomized Clinical Trial	Remdesivir was not associated with statistically significant clinical benefits in adult patients admitted to hospital for severe COVID-19.
Jayk Bernal A et al. (86)	New Eng J Med	2021	Randomized Clinical Trial	Early treatment with molnupiravir reduced the risk of hospitalization or death in at-risk, unvaccinated adults with COVID-19.
Hammond J et al. (87)	N Engl J Med	2022	Randomized Clinical Trial	Treatment of symptomatic COVID-19 with nirmatrelvir plus ritonavir resulted in an 89% reduction in the risk of progression to severe COVID-19 than that with a placebo. No major safety concern was reported.
Dougan M et al. (88)	N Engl J Med	2021	Randomized Clinical Trial	In high-risk outpatients, bamlanivimab plus etesevimab resulted in a lower incidence of COVID-19-related hospitalizations and deaths than placebo and accelerated the decline in SARS-CoV-2 viral load.
Weinreich DM et al. (89)	N Engl J Med	2021	Randomized Clinical Trial	The REGN-COV2 antibody cocktail reduced viral loads, with greater effect in patients whose immune response had not yet started or who had high viral loads at baseline. Safety results were similar in the REGN-COV2 combined dose groups and the placebo group.
Chen P et al. (90)	N Engl J Med	2021	Randomized Clinical Trial	Evaluated the quantitative virologic end points and clinical outcomes in patients receiving a single intravenous infusion of neutralizing antibody LY-CoV555 in one of three doses (700 mg, 2800 mg, or 7000 mg) or placebo. This interim analysis of a phase 2 trial showed that one of three doses (2800 mg) of neutralizing antibody LY-CoV555 appeared to accelerate the natural decline in viral load by day 11.
Cohen MS et al. (91)	JAMA	2021	Randomized Clinical Trial	Among residents and staff in skilled nursing and assisted living facilities, treatment during August-November 2020 with bamlanivimab monotherapy reduced the incidence of COVID-19 infection (8.5% vs 15.2%; odds ratio, 0.43 (95% CI, 0.28-0.68); P < .001; absolute risk difference, -6.6 (95% CI, -10.7 to -2.6) percentage points).
Guimaraes PO, et al. (92)	New Eng J Med	2021	Randomized Clinical Trial	Among patients hospitalized with COVID-19 pneumonia, tofacitinib led to a lower risk of death or respiratory failure on day 28 than placebo (18.1% vs 29.0%; risk ratio, 0.63; 95% confidence interval (CI), 0.41 to 0.97; P = 0.04).
Cao Y et al. (93)	J Allergy Clin Immunol	2020	Randomized Clinical Trial	Ruxolitinib recipients had a numerically faster clinical improvement at day 14 (90% from the ruxolitinib group showed computed tomography improvement compared with 61.9% in control group; P =0.0495). Significant chest computed tomography improvement, a faster recovery from lymphopenia, and favorable side-effect profile in the ruxolitinib group.
Shen C et al. (94)	JAMA	2020	Case series	In this preliminary uncontrolled case series of 5 critically ill patients with COVID-19 and ARDS, administration of convalescent plasma containing neutralizing antibody was followed by improvement in their clinical status with decreasing the SOFA score and increasing the Pao2/Fio2 within 12 days. The limited sample size and study design preclude a definitive statement about the potential effectiveness of this treatment, and these observations require evaluation in clinical trials.
Simonovich VA, et al. (95)	N Engl J Med	2021	Randomized Clinical Trial	No significant differences were observed in clinical status (odds ratio, 0.83; 95% confidence interval (CI), 0.52 to 1.35; P = 0.46) or overall mortality (10.96% in the convalescent plasma group and 11.43% in the placebo group, 95% CI, -7.8 to 6.8) between patients treated with convalescent plasma and those who received placebo.

3.5.2 Monoclonal antibodies

Neutralizing antibodies targeting the spike protein of the coronavirus is another important pillar in the fight against SARS-CoV-2 infection, especially in immunocompromised patients (107). Klank et al. reported that monoclonal antibodies (mAbs) such as Bamlanivimab can be used in both preventive and treatment settings (108). In addition, casirivimab and imdevimab also showed a positive protective effects, as SARS-CoV-2 negative individuals who received

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TABLE 2 Indications and contraindications of drugs in the treatment of COVID-19.

Drugs	Indications	Contraindications
Antiviral drugs		
Paxlovid	Paxlovid is a combination therapy for the treatment of mild to moderate COVID-19 in patients who are at high risk of progression to severe COVID-19.	Paxlovid is contraindicated in patients with a known hypersensitivity to any component of the medication.
Molnupiravir	Molnupiravir is an antiviral medication for the treatment of COVID-19 in adults who are at risk of progression to severe COVID-19 and/or hospitalization.	Molnupiravir is contraindicated in patients with a known hypersensitivity to any component of the medication. Also, Molnupiravir is not authorized for use in patients aged <18 years; not authorized for initiation of treatment in patients requiring hospitalization owing to COVID-19; not authorized for use for >5 consecutive days Not authorized for preexposure or postexposure prophylaxis of COVID-19.
Remdesivir	Remdesivir is an antiviral medication for the treatment of COVID-19 in adults and pediatric patients (12 years of age and older and weighing at least 40 kg) requiring hospitalization.	Remdesivir is contraindicated in patients with a known hypersensitivity to any component of the medication.
Monoclonal antibodies		
Bamlanivimab and etesevimab	Bamlanivimab and etesevimab are monoclonal antibodies indicated for the treatment of mild to moderate COVID-19 in patients who are at high risk of progression to severe COVID-19 and/or hospitalization.	There are no known contraindications for Bamlanivimab and etesevimab.
Casirivimab and imdevimab	Casirivimab and imdevimab are monoclonal antibodies indicated for the treatment of mild to moderate COVID-19 in patients who are at high risk of progression to severe COVID-19 and/or hospitalization.	There are no known contraindications for Casirivimab and imdevimab.
IL-6R inhibitor/Immune Modulators		
Tocilizumab	Tocilizumab is an immunosuppressive medication used to treat moderate to severe rheumatoid arthritis, juvenile idiopathic arthritis, giant cell arteritis, and cytokine release syndrome caused by CAR T-cell therapy or COVID-19.	Tocilizumab is contraindicated in patients with a known hypersensitivity to any component of the medication. For all immunosuppressive treatments, secondary bacterial and fungal infections should be closely monitored.
Sarilumab	Sarilumab is an immunosuppressive medication used to treat moderate to severe rheumatoid arthritis and cytokine release syndrome caused by CAR T-cell therapy or COVID-19.	Sarilumab is contraindicated in patients with a known hypersensitivity to any component of the medication.
Baricitinib	Baricitinib is an immunosuppressive medication used to treat moderate to severe rheumatoid arthritis and COVID-19 in combination with remdesivir.	Baricitinib is contraindicated in patients with a known hypersensitivity to any component of the medication.
Ruxolitinib	Ruxolitinib is an immunosuppressive medication used to treat certain types of bone marrow disorders and cytokine release syndrome caused by CAR T-cell therapy or COVID-19.	Ruxolitinib is contraindicated in patients with a known hypersensitivity to any component of the medication.
Tofacitinib	Tofacitinib is an immunosuppressive medication used to treat moderate to severe rheumatoid arthritis and cytokine release syndrome caused by CAR T-cell therapy or COVID-19.	Tofacitinib is contraindicated in patients with a known hypersensitivity to any component of the medication.
Systemic corticosteroids		
Dexamethasone	Dexamethasone is a corticosteroid medication used to treat a variety of inflammatory and autoimmune conditions, as well as to reduce inflammation in certain types of cancer.	Dexamethasone is contraindicated in patients with a known hypersensitivity to any component of the medication. It should also be used with caution in patients with certain infections, such as systemic fungal infections, due to the risk of exacerbating the infection.

the specific mAbs appear to have a lower risk of developing COVID-19 after contact with an infected individual (1.5% versus 7.8%; P<0.001). Further, the symptom duration of patients suffering from COVID-19 was shown to be shorter than that of patients on placebo (109). Given these promising protective effects, a combination of bamlanivimab and etesevimab could be a potential treatment option for immunocompromised patients. In a phase 2/3 study, a combination of bamlanivimab and etesevimab was given to patients with malignancies or to those who were in an immunosuppressive status with COVID-19 (88). Compared to the placebo group, patients receiving the drug showed a lower hospitalization rate (2.1% vs. 7.0%; p<0.001) and a significant reduction in viral load at day 7. Other commercially available mAbs such as casirivimab and imdevimab have also shown a positive effect in protecting against reducing viral loads and hospitalization rates and thereby reducing mortality (89, 90). mAbs probably have the greatest beneficial effects in the early phase of coronavirus infection, where viral replication plays an important role (110, 111). Serious adverse effects, including allergic reactions and cardiac issues such as atrial fibrillation, have occurred and require caution following mAbs infusion (91).

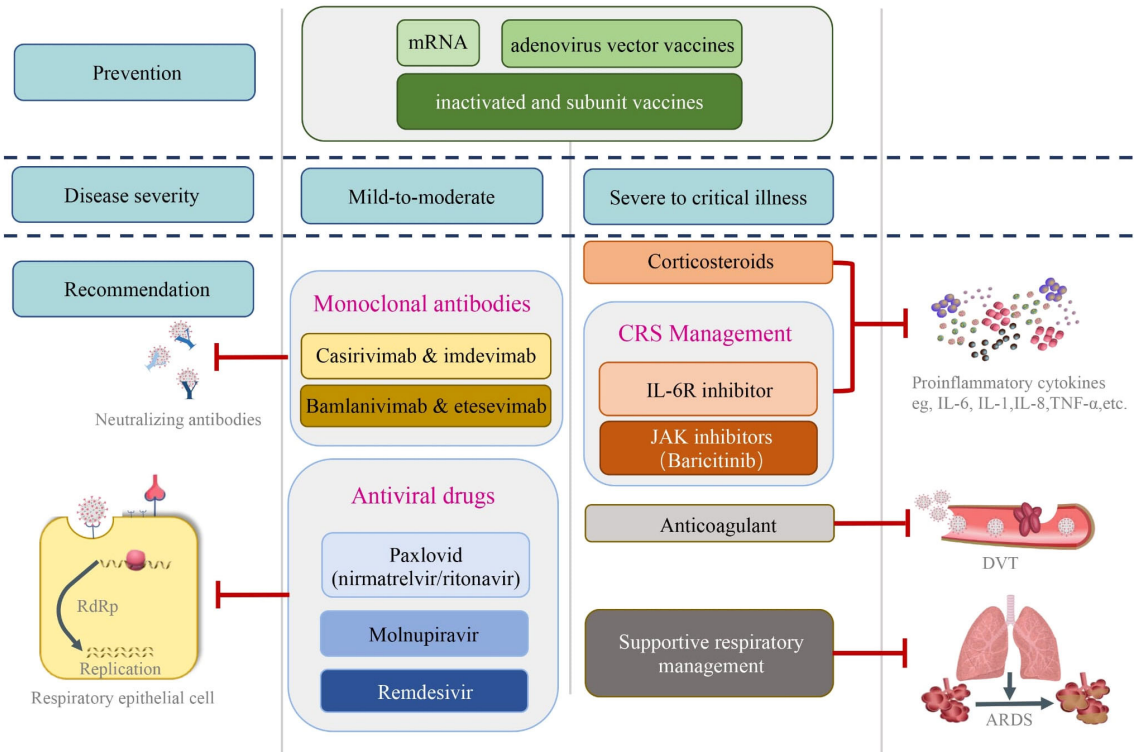

FIGURE 2
The clinical management and target of currently used therapeutics against SARS-CoV-2. Prior to clinical infection with coronavirus, effective vaccines including adenovirus vector vaccines, mRNA, inactivated, and subunit vaccines play critical roles in preventing virus spread and limiting the severity of the infection. The clinical management and target of currently used therapeutics against SARS-CoV-2 are shown based on the disease severity. Monoclonal antibodies including casirivimab & imdevimab and bamlanivimab & etesevemab have been used in non-severe cases. Likewise, antiviral drugs such as Paxlovid (nirmatrelvir & ritonavir), molnupiravir and remdesivir, which target the virus replication in the infected cells have been used in mild-to-moderate patients. For severe to critically illness, corticosteroids and CRS management including IL-6R inhibitor and JAK inhibitors were administrated to control the excessive inflammation. Anticoagulants have been used to prevent and treat DVT. Supportive respiratory managements have been primary measures to treat patients with low blood oxygenation including ARDS. DVT, deep venous thrombosis; ARDS, acute respiratory distress syndrome.

In general, monoclonal antibody therapy is recommended for patients with mild to moderate COVID-19 who are at high risk for disease progression. High-risk factors include older age, obesity, diabetes, chronic kidney disease, or immunosuppression. When it comes to the choice of dose, the specific monoclonal antibody and the patient's weight are taken into consideration. For example, for the monoclonal antibodies bamlanivimab and etesevimab, the recommended dose is 700mg bamlanivimab and 1400mg etesevimab administered together intravenously for patients weighing at least 40 kg (83, 97). The decision to use monoclonal antibodies and the choice of dose should be made by a healthcare professional based on the individual patient's clinical situation.

3.5.3 Management of cytokine release syndrome

A number of therapies targeting a specific or broad range of cytokines have been tested in COVID-19. Here we describe major approaches to reduce the cytokine storm to alleviate the disease severity.

3.5.3.1 IL-6 receptor blocker

Cytokine storm responses pose a significant risk in infectious diseases including COVID-19. IL-6 is a pleiotropic cytokine stimulating and regulating the immune response during infections. Since IL-6 is identified as the key propagator of the cytokine storm reaction, blocking the two forms of the IL-6 receptor, a membrane-bound and a soluble IL-6 receptor was considered. IL-6 receptor blockers, including tocilizumab and sarilumab, are recommended to be used intravenously for severe or critical illness patients of COVID-19 based on evidence of mortality reduction and decreased requirement of mechanical ventilation (112, 113). However, this is only effective in those with severe disease.

3.5.3.2 Janus (JAK) kinase inhibitors

Janus kinases are important mediators of cytokine storm and inflammation and serve as a potential target for limiting cytokine storm during COVID-19. Baricitinib, ruxolitinib and tofacitinib are three major JAK inhibitors tested for severe or critical COVID-19 patients (92, 93, 114). The three JAK blockers are considered nonspecific despite evident differences. Baricitinib is mainly described as a JAK1/2 inhibitor while ruxolitinib also presents the weak suppression of TYK2 except from JAK1/2, while tofacitinib showed more inhibitory potential on JAK1/3 than JAK2/TYK2 (115, 116). Although large randomized clinical trials have been limited, our meta-analysis demonstrated the overall beneficial effects of JAK inhibitors in COVID-19 (55).

3.5.4 Systemic corticosteroids

Systemic corticosteroids are generally recommended in treating severe and critical COVID-19 patients to control the over-activated inflammatory response despite confounding results (117, 118). Patients younger than 70 years with persistent symptoms for more than seven days and who required mechanical ventilation are shown to benefit from dexamethasone therapy (119). In contrast, no clinical improvement was observed in patients with a shorter duration of symptoms and without supplemental oxygen, even in COVID-19-induced mild to moderate ARDS (120). The optimal timing of the therapy from symptom onset is still a matter of debate. Theoretically, it can be speculated that systemic glucocorticoid use should be avoided until viral replication is under control by the immune system or through effective antivirals. The consensus is arising that corticosteroids should be administered in patients with severe or critical COVID-19, even within seven days of symptoms onset, and non-severe cases should not be treated with corticosteroids even though symptoms occur longer than a week (62).

3.5.5 Convalescent plasma therapy

Convalescent plasma therapy is one of the experimental treatments for SARS-CoV-2 infection. The antiviral neutralizing antibodies collected from the plasma of recovered patients are transfused into the COVID-19 patients with an active infection to enhance the immune response (94, 95, 121). Such plasma therapy has shown a promising recovery rate in H5N1 influenza and Ebola viral disease (122–124). However, due to different methodologies, appropriate antibody titers ranging from 1:100 to 1:2560 have been reported in the existing studies from donor to recipient (125, 126). The range is confusing and the uncertainty of the definitive dose lies in the different limit values (127, 128). WHO living guidelines recommend this therapy applied to patients with severe illness, but only in research settings or clinical trial, for the reasons that convalescent plasma therapy has no significant effect on the indicators such as time to symptom improvement, length of hospital stay, duration of mechanical ventilation, or mortality (62, 129).

3.5.6 Supportive respiratory management

Mechanical ventilation and supplemental oxygen are the primary measures for addressing those who present with low blood oxygenation. High flow nasal cannula (HFNC), Non-invasive ventilation (NIV), and lung-protective invasive mechanical ventilation (IMV) are widely used to support respiration (130, 131). Given the rapid decompensation due to early intubation strategies, HFNC was selected early in the pandemic and used in the populations with hypoxemic respiratory failure and patients often respond well (132, 133). However, HFNC and other NIV devices, including non-invasive bi-level positive pressure ventilation (BPAP) and continuous positive airway pressure (CPAP), may pose a potential risk of aerosolization of the virus. For further safety in HFNC use, evidence has supported the use of a surgical mask (134). CPAP via a mask covering the mouth and nose or helmet NIV is recommended to minimize room air contamination and to better contain aerosol leakage, providing superior oxygenation, pressure, and outcomes (135). In addition, it is recommended that negative pressure rooms be used to reduce the risk of the coronavirus spread in the ambient air (45). A large, international systematic review and meta-analysis was conducted to examine global case fatality rate (CFR) reports in adult patients with COVID-19 who received invasive mechanical ventilation (IMV). The results showed that the overall estimate for the initial CFR in IMV was 45%. Reported CFR was higher in elderly patients and in early pandemic epicenters, which may be impacted by limited ICU resources (136). However, IMV is used for crucial supportive care and to provide additional time for patients with severe hypoxemic respiratory failure in the ICU. Further, invasive mechanical ventilation may increase the risk of secondary bacterial infections in COVID-19 patients by either directly breaching the host defense or through coronavirus-impaired host immunity (137).

4 Conclusion

Our understanding of COVID-19 pathology, clinical management, and treatments has improved significantly in the last four years. However, the persistent emergence of variants poses a serious challenge to the effectiveness of both preventive and therapeutic approaches. The clinical management of these infections faces several problems, including failure to administer early antiviral agents, high false-negative diagnosis rates, mixed reports on certain therapeutic drug efficacy, and rapid progression to severe conditions such as ARDS, pulmonary embolism, disseminated intravascular coagulation, sepsis, and cytokine storm. The global fight against COVID-19 is likely to continue for a long time until we develop effective and clinically proven antiviral therapeutics or vaccines that completely prevent transmission of the disease. Further, as we emerge from the pandemic, special emphasis should be given to the chronic consequences such as long COVID-19, which may become a serious healthcare challenge in upcoming years.

Author contributions

YZ wrote the initial draft, prepared the figures, and tables. LS and DC edited the manuscript and provided guidance. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Embracing dynamic public health policy impacts in infectious diseases responses: leveraging implementation science to improve practice

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Rationale: The host-pathogen relationship is inherently dynamic and constantly evolving. Applying an implementation science lens to policy evaluation suggests that policy impacts are variable depending upon key implementation outcomes (feasibility, acceptability, appropriateness costs) and conditions and contexts.

COVID-19 case study: Experiences with non-pharmaceutical interventions (NPIs) including masking, testing, and social distancing/business and school closures during the COVID-19 pandemic response highlight the importance of considering public health policy impacts through an implementation science lens of constantly evolving contexts, conditions, evidence, and public perceptions. As implementation outcomes (feasibility, acceptability) changed, the effectiveness of these interventions changed thereby altering public health policy impact. Sustainment of behavioral change may be a key factor determining the duration of effectiveness and ultimate impact of pandemic policy recommendations, particularly for interventions that require ongoing compliance at the level of the individual.

Practical framework for assessing and evaluating pandemic policy: Updating public health policy recommendations as more data and alternative interventions become available is the evidence-based policy approach and grounded in principles of implementation science and dynamic sustainability. Achieving the ideal of real-time policy updates requires improvements in public health data collection and analysis infrastructure and a shift in public health messaging to incorporate uncertainty and the necessity of ongoing changes. In this review, the Dynamic Infectious Diseases Public Health Response Framework is presented as a model with a practical tool for iteratively incorporating implementation outcomes into public health policy design with the aim of sustaining benefits and identifying when policies are no longer functioning as intended and need to be adapted or de-implemented.

Conclusions and implications: Real-time decision making requires sensitivity to conditions on the ground and adaptation of interventions at all levels. When asking about the public health effectiveness and impact of non-pharmaceutical interventions, the focus should be on *when*, *how,* and *for how long* they can achieve public health impact. In the future, rather than focusing on models of public health intervention effectiveness that assume static impacts, policy impacts

should be considered as dynamic with ongoing re-evaluation as conditions change to meet the ongoing needs of the ultimate end-user of the intervention: the public.

KEYWORDS

pandemic response, public policy, implementation science, non-pharmaceutical interventions, dynamic sustainability framework, infectious diseases, COVID-19

Background

The discovery and subsequent administration of penicillin in 1943 was a major milestone in clinical medicine, saving countless lives (1). However, even before the drug was approved for clinical use, the first reports of antimicrobial resistance were described. Less than 20 years after initial approval, more than 80% of *Staphylococcus aureus* strains were penicillin-resistant (2). In the decades since, a similar story has been described for every antibiotic brought to market. Host-pathogen relationships are inherently dynamic: as hosts develop ways to combat an infectious diseases threat— whether through immunity or treatment—pathogens evolve to evade our advancements.

In addition to an inherently evolving host-pathogen interaction, many factors impacting this interaction are also constantly changing and need to be considered, measured, and integrated into public health policy making. Factors that change over time and therefore determine public policy impact include resource availability, case fatality rate, understanding about modes of transmission, human behaviors, societal expectations, the evidence basis for treatment and prevention and therefore our understanding about the disease, among others.

Maximizing public health policy impact for combating infectious diseases threats necessitates that all of these dynamic factors be measured and evaluated in real-time to continually adapt response plans and achieve maximum public health benefit. Updating policies and recommendations to elevate some interventions and de-escalate others as contextual factors continually evolve is the best evidence-based policy strategy (3, 4). Achieving this ideal requires re-imaging infectious diseases public health policy making as a dynamic and constantly evolving process with the anticipation of change inherently built into health communications and public expectations (5–7). Infrastructure that can support integration of point-of-care data with emerging and evolving evidence and public input to assess ongoing feasibility, acceptability, appropriateness, and costs are needed to achieve safe, efficient and higher quality care and policies (8).

Viewing pandemic responses and the expected impacts of public health policy through the lens of implementation science would enhance emergency preparedness for future pandemics and ultimately improve public health policy impact. The objectives of this review are to: (1) discuss the pipeline from clinical effectiveness to implementation outcomes to ultimate public health impact and introduce the concept of dynamic policy effectiveness (9), (2) to present the COVID-19 pandemic and public health policy as a case study for considering the dynamic host, pathogen, contextual, and evidence changes that evolved over the course of the world-wide emergency responses, and (3) to propose future innovations to support a real-time, learning public health infrastructure that is more adaptable based on changing conditions, context, and evidence. A practical tool for operationalizing the Dynamic Infectious Diseases Public Health Response Framework is presented. The tool is designed to facilitate integration of key implementation outcomes and considerations into infectious diseases response planning (Supplementary material).

Clinical efficacy and effectiveness vs. public health policy effectiveness and impact

Traditional clinical trials evaluate efficacy and effectiveness of an intervention in a controlled setting, whereas implementation trials evaluate how to promote uptake and how to translate potential benefits into tangible improvements (9). Public health policy impact is a downstream consequence of the potential efficacy of the intervention as well as its real-world implementation (Figure 1). The Dynamic Sustainability Framework (DSF) (10) highlights the importance of adapting interventions on *individual* and *systems* levels to maintain and maximize longitudinal impacts in the setting of constantly changing evidence and contexts.

DSF principles also apply to public health systems and public health policy impact. Public health policies are typically composed of a bundle of multiple interventions. To achieve sustained public health impact, public health policies and their component interventions (whether targeted at an individual, organization or society) must adjust and adapt to changing contexts and evidence. In other words, public health policies must be viewed as having *dynamic* public health impacts and *dynamic* effectiveness; a constant level of public health impact while other changes in the system are occurring cannot be assumed.

Implementation outcomes

Efficacy and effectiveness are the traditional measures for evaluating clinical and public health policies and interventions in controlled research studies. These measures focus on the absolute and relative differences in outcomes among exposed and unexposed groups and are generally conceived of as *static* estimates; in other words, the relative risk reduction associated with receipt of a particular medical intervention is assumed to be *constant* over time.

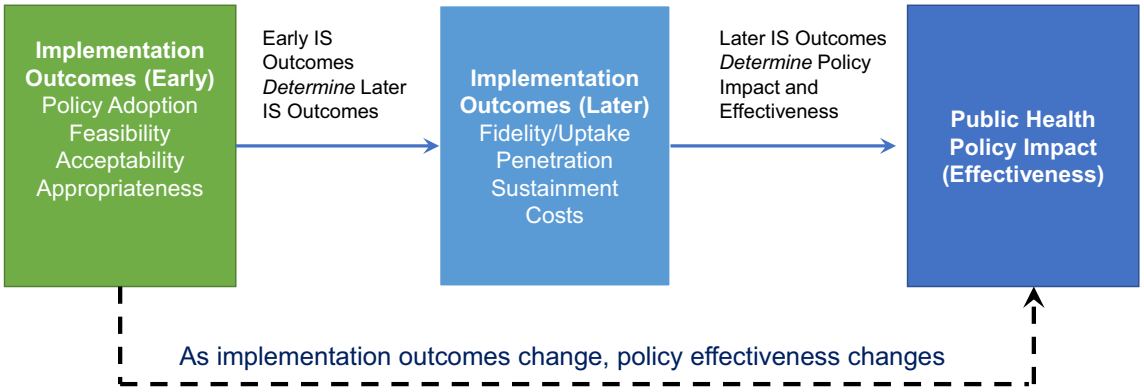

FIGURE 1
Interplay between implementation outcomes and public health policy impact and effectiveness. Early implementation outcomes (adoption, feasibility, acceptability, appropriateness) have direct impacts on later implementation outcomes, which in turn determine the effectiveness of a public health policy. During a pandemic response, implementation outcomes change, and these changes also alter the expected benefits of public health policy interventions.

TABLE 1 Implementation outcomes definitions and impact in the setting of constant change.

Implementation outcome*	Definition	Examples and key longitudinal changes
Acceptability	Perception among interested parties that a policy or practice is agreeable, palatable, or satisfactory	*Business and school closures, social distancing* Attitudes about interventions that limited person-to-person contacts changed substantially and rapidly over time. Very-short term viability, and substantial pressure from the community to limit or refuse these types of mitigation interventions.
Appropriateness	The perceived fit, relevance, or compatibility of the policy or practice for a given context**	*Hospital Admission Surveillance Testing* Initially, given reports of asymptomatic spread, universal hospital admission screening was considered an appropriate means for limiting risk to patients and staff. Over time, as vaccines became available and downsides of the testing program emerged (e.g., identification of false positives, changing views of the role of asymptomatic spread in driving transmission, concerns about impacts of delayed medical care), the perceived appropriateness of the intervention changed.
Adoption	Decision to recommend the intervention or public health policy	*Masking* The decision at a local, state, or national level to officially recommend a specific masking policy, such as a recommendation or a requirement to wear masks in all indoor areas.
Feasibility	The extent to which a policy or practice can be used within a specific context. Closely related to and overlapping with resource *availability*, which has been proposed as an implementation outcome in the context of vaccination. (12)	*Testing Strategies* Early in the pandemic, testing was not feasible due to limited testing resources. Then, as resource limitations decreased, testing of exposed individuals became a more feasible option for limiting time in quarantine. Then, high rates of spread during the omicron wave made contact tracing infeasible. As individual testing became less feasible, alternative surveillance strategies, such as community wastewater testing, became more widely available and thus a feasible alternative.
Fidelity	The degree to which a policy or practice is implemented as planned. For multifaceted interventions, can be considered as the "dose."	*Masking* Masking policy recommendations do not necessarily translate into adherence. Fidelity refers to the rate of adherence, which changed longitudinally with availability of other mitigation measures, and changes to acceptability and appropriateness over time.
Penetration	The reach of the policy or practice (e.g., how many people received the intervention/total number of eligible individuals)	*School-based testing programs* School-based testing programs, such as the test-to-stay modified quarantine program, allowed exposed individuals to continue participation in in-person learning. Penetration, or reach, refers to the number of students and schools who are able to participate in the testing program, and is a function of program availability (access) and participation (e.g, consent)
Cost	The cost or impact of the implementation effort (includes intervention costs, costs of implementation, settings)	*Business and school closures, social distancing* The very short-term (i.e., days to weeks) costs and harms of closures are substantial, and increase as duration and extent of closures increases.

*Sustainability is not listed, as the focus of the framework is on using different contextual factors to predict sustainability. **Context can be defined broadly, and can refer to practice settings, political settings, longitudinal changers, or other factors that impact the perceived fit of a practice or policy.

Implementation science focuses on different outcomes (Table 1) (11). Early implementation outcomes include acceptability, appropriateness, feasibility/availability, and adoption, which is defined as the decision to recommend a specific public health intervention (in contrast with uptake, which is the actual use of the intervention). Later implementation outcomes include fidelity, penetration, and costs. The longest-term implementation outcome is *sustainability*. In implementation science, both *sustainability* and *sustainment* are key terms, with sustainability referring to a property/characteristic of an intervention related to its likely long-term usability whereas sustainment refers to the outcome of whether an intervention was used over a long period of time.

Implementation outcomes: the causal pathway to public health impact

Importantly, while often not considered when evaluating clinical efficacy and effectiveness, implementation outcomes *directly* impact the expected benefit and impact of public health policies and their components (the individual interventions) (Figure 1). Interventions that are promising in laboratory settings or in idealized clinical trial settings have limited or no impact on public and population health if they are infeasible/unavailable, unacceptable, and/or perceived to be inappropriate by end-users. Further complicating longitudinal evaluations of public health impact and ongoing recommendations, these implementation outcomes themselves are not static – feasibility, which is related to availability (12), acceptability, appropriateness, and costs all vary according to contexts, evolving evidence, resource availability, progress, available alternatives and perceived benefits (Figures 2, 3). Sustainability is a perennial challenge in implementation, particularly if day-to-day behavior change is required and if the intervention is perceived to have substantial downsides. Thus, implementation outcomes are key determinants of public health policy and intervention effectiveness and impact. Implementation outcomes are *also* constantly evolving.

The COVID-19 pandemic public health response: a case study in constant change

Consideration of *dynamic* public health policy effectiveness and impact is particularly important for developing and adapting responses to infectious disease threats. As humans make advancements, such as the development of therapeutics or vaccines, the pathogen evolves in response to human progress (Figure 4). For example, delta and omicron variants both emerged in part due to pressure from vaccine and infection-induced immunity. Mutations arose that rendered once highly effective monoclonal antibody therapies for early treatment and prophylaxis obsolete. Antimicrobial resistance, another critical public health threat in infectious diseases, is a direct downstream consequence of pathogen evolution in response to human innovation. Antimicrobial resistance highlights the generalizability of the dynamic nature of the management and containment of infectious diseases beyond the COVID-19 pandemic.

The phases of the COVID-19 pandemic

The COVID-19 pandemic can be viewed as occurring in multiple phases, each characterized by different therapeutic and preventative advancements, resource availability, pathogen infectiousness, evidence and understanding, and varying levels of feasibility, and acceptability of different mitigation policies (Figure 5). Through this lens, the first (early) phase of the pandemic in the United States lasted from approximately February, 2020 through May of 2020, and was characterized by limited understanding about the novel disease, limited access to testing, and no known effective treatments. Case fatality rates were high, as were levels of perceived fear and risk, which translated into high levels of perceived appropriateness of non-pharmaceutical interventions (NPIs). The second (late early) phase lasted from approximately May of 2020 to June of 2020 and was characterized by the identification and availability of effective inpatient therapeutics (remdesivir and dexamethasone). During Phase II, case fatality rates were lower but still relatively high compared to later periods, and access to a variety of different mitigation strategies, including testing, increased substantially. The third (middle) phase occurred from November 2020 to November 2021 and was characterized by advancements in preventative therapies, specifically vaccines with durable protection against severe disease. During Phase III, case fatality rate plummeted, fear and perceived threat fell precipitously, and the acceptability and appropriateness of many NPIs dropped substantially. The fourth (late) phase occurred from December 2021 to December 2022 and was characterized by the expansion of therapeutic options to include outpatient therapies and pre-exposure prophylaxis for those at high risk of disease despite vaccination. These advancements further lowered case fatality rates and perceived fear and therefore appropriateness of various NPIs. A fifth phase may be defined by the loss of pre-exposure prophylaxis due to pathogen evolution (13, 14). Future phases may be defined by the emergence of new variants, the development of next generation vaccines, new pharmaceuticals, or improvements in pre- and post-exposure prophylaxis options, similar to how pre-exposure prophylaxis (PREP) altered the course of the HIV epidemic (15).

Changing resources

Availability of pandemic mitigation tools varied, impacting the feasibility, acceptability, and appropriateness of different pandemic policy responses (Table 2). Initially, NPIs, including masking, testing strategies, ventilation interventions, and distancing were the only available tools to mitigate transmission; during the very early phases, testing and personal protective equipment (PPE) were both limited resources in the United States. Subsequently, advancements in disease management and therapeutics reduced disease severity, and then development and distribution of vaccines further reduced disease severity and increased immunity in

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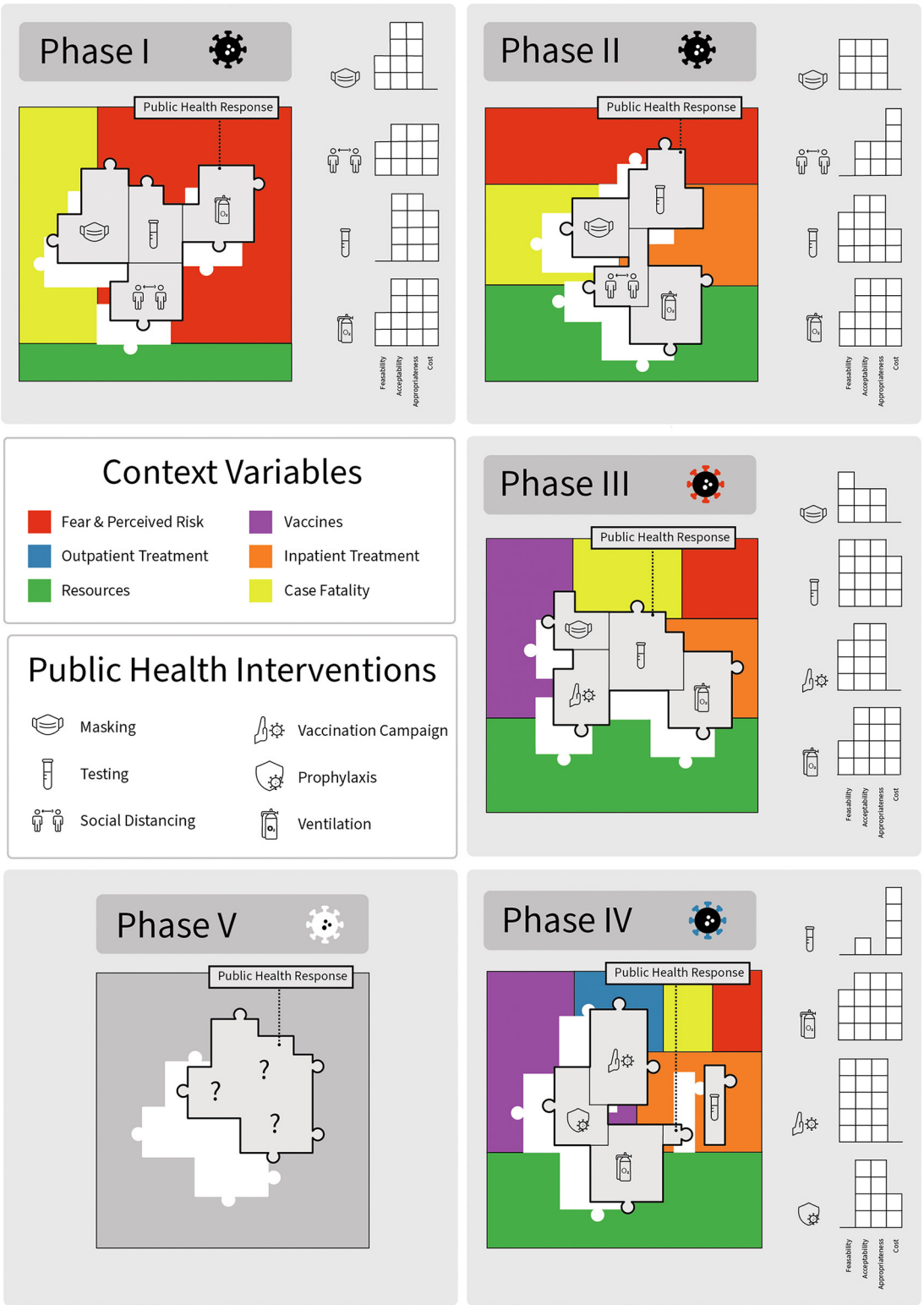

FIGURE 2
Impact of specific public health policy interventions during different phases of the COVID-19 pandemic: changing context and implementation outcomes alter effectiveness. Each box represents a different phase of the pandemic. Contextual factors are indicated by different colors. Public health policy response interventions are puzzle pieces indicated with relevant icons. Graphs represent changing early implementation outcomes as a function of pandemic phase. The viral icon adjacent to the pandemic phase indicates the circulating variant, which also changed over time.

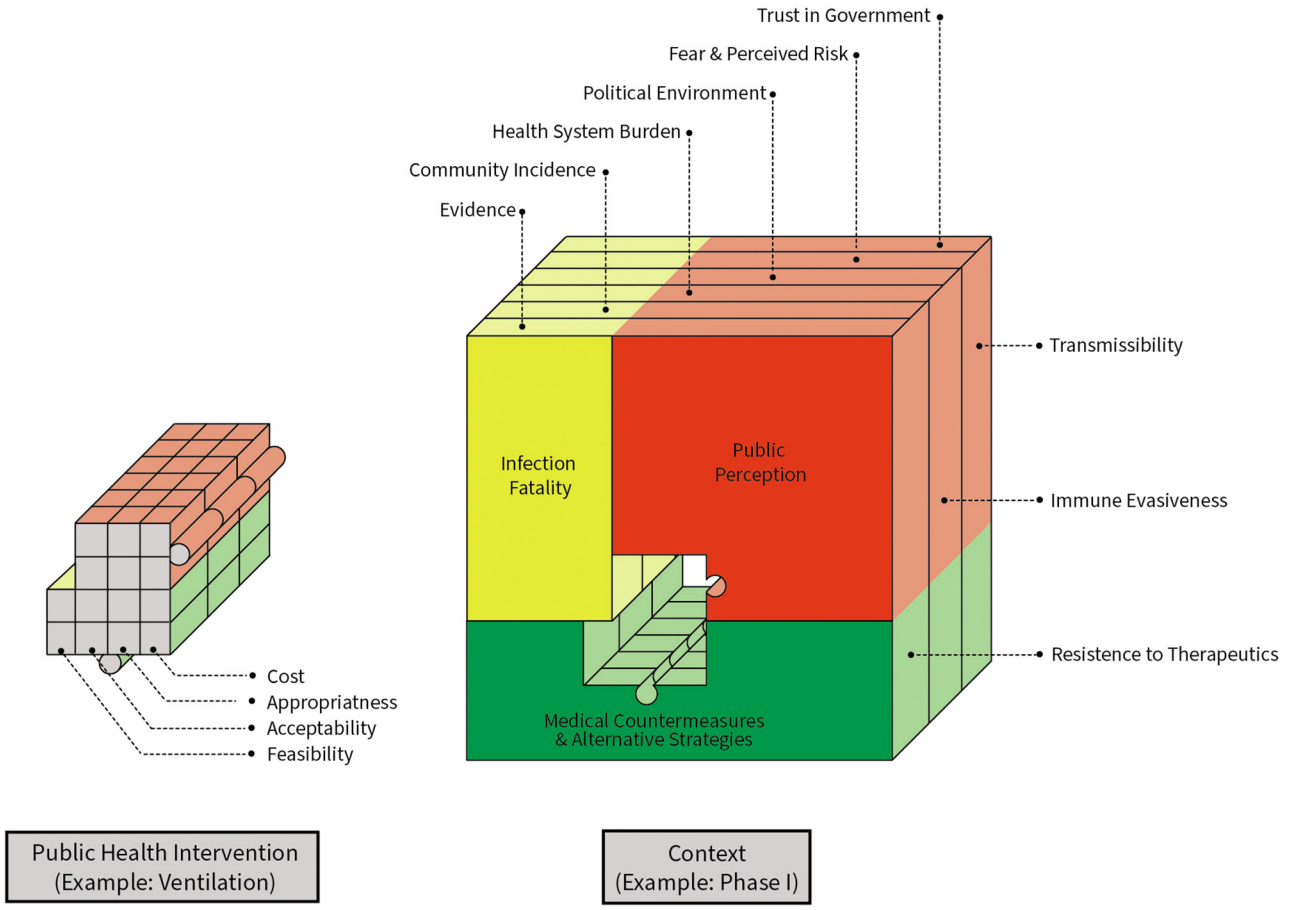

FIGURE 3
Changing contexts and implementation outcomes alter longitudinal impacts of non-pharmaceutical interventions. The puzzle piece represents a non-pharmaceutical intervention and its expected policy effectiveness in the setting of constant change in multiple dimensions. Key considerations for considering ongoing policy effectiveness are highlighted, including contextual factors and pathogen characteristics.

the population. Expanded access to testing and innovations in community-based surveillance methods changed the utility and delivery of this strategy over time (16).

In other countries, resource access was available at different times during the pandemic. For example, in South Korea, an extensive testing program was available very shortly after identification of SARS-CoV-2, and thus the country was able to effectively leverage this strategy for early outbreak control (17–20). Testing in the US was delayed for a variety of reasons, limiting the effectiveness of a test-and-quarantine mitigation strategy. Access to PPE was also highly variable, impacting the feasibility of this approach, and therefore its potential impact.

Implementation outcomes as determinants of NPI impact

With the exception of ventilation upgrades, most NPIs inherently require a high level of ongoing individual effort; if fidelity (or use as-intended) is not maintained, then the effectiveness of the intervention wanes. Masks, for example, only have the potential to work if people wear them (and wear them as-intended); potential policy impact is determined by adherence (21). For these reasons, NPI policy effectiveness at any point in time is necessarily determined by the degree of appropriate use as-intended within the target population or community. Use as-intended, or fidelity, in turn, is determined in part by the feasibility, acceptability, and perceived appropriateness of the intervention for the end-user at any given time. These implementation outcomes all change depending upon a variety of factors and cannot themselves be assumed to be static.

In the longer-term, policy impacts are also determined by the *sustainment* of fidelity to the intervention; because NPIs provide only short-term protection, without sustainment, their impact is to *delay*, rather than to *prevent*, infection. Fidelity, penetration, costs and sustainability are implementation outcomes that provide a means to evaluate the implementation success of interventions, treatments, policies and protocols and are distinct from traditionally measured outcomes, such as clinical or health service outcomes (11). They are also critical for considering NPI policy effectiveness as a dynamic, rather than

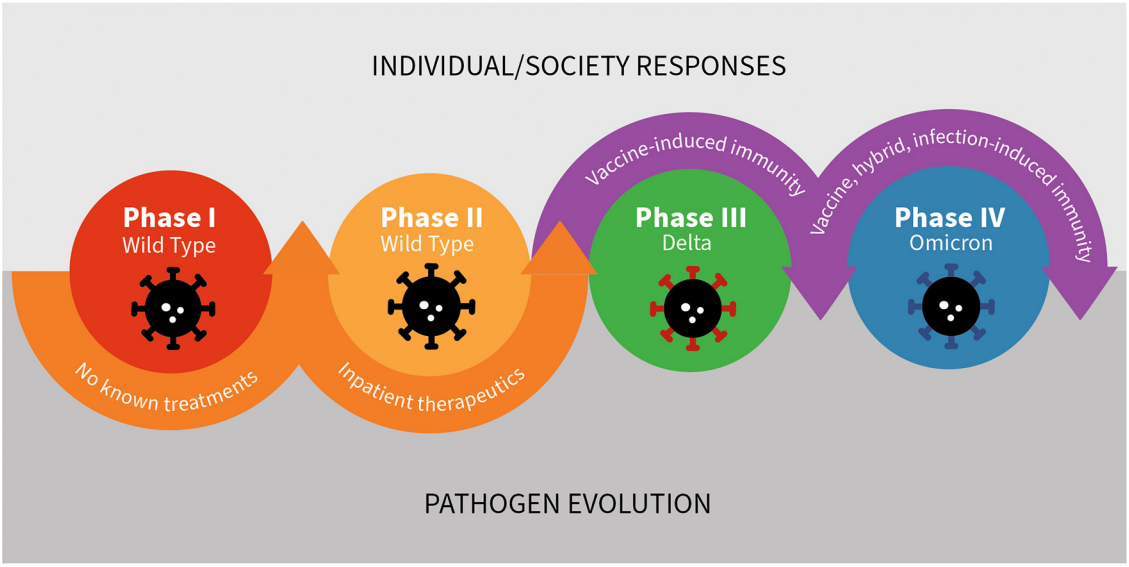

FIGURE 4
The inherently dynamic nature of the host – pathogen interaction: the case of SARS-CoV-2 and human responses.

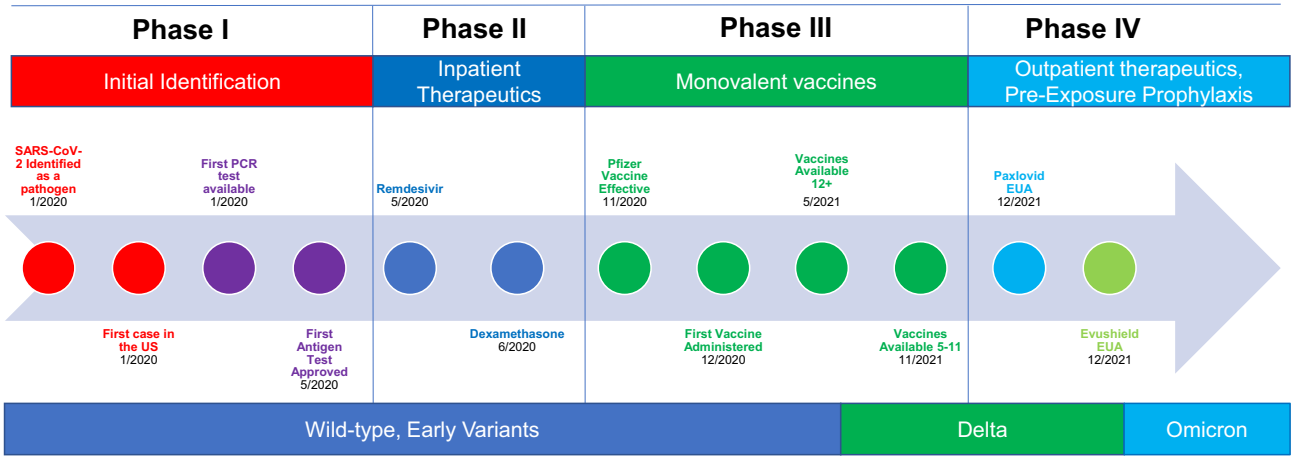

FIGURE 5
Pandemic timeline and phases: interventions, innovations, and the pathogen all experience constant, longitudinal change.

static, entity and point to pathways to improve evidence-based public health policy recommendations during future pandemics through ongoing measurement and re-evaluation of each of these implementation outcomes.

Multiple findings from different contexts and different places in the pandemic highlight the challenges in long-term sustainment of NPI adherence and therefore the potential impact of masking policies as a pandemic control measure. For example, the Bangladesh cluster randomized controlled trial found that a bundle of implementation strategies effectively increased mask use by 29% (to 42%), and that the increase in mask use was associated with a statistically significant reduction of 10% of symptomatic SARS-CoV-2 cases in villages randomized to receive surgical masks and a non-significant reduction of 5% among villages randomized to receive cloth masks (22). An equally important finding of this large, cluster randomized controlled trial was the lack of sustainment of adherence to mask wearing over a relatively short period of time (22). Despite a nationwide masking mandate during the entire study period, a follow-up evaluation of mask use 8-12 weeks after the active intervention found that ongoing fidelity to mask use in villages randomized to receive the bundle of implementation strategies had fallen substantially. The initial 29% increase had fallen to only 10% relative to control villages. Other studies in other settings have similarly found challenges with intervention fidelity (23). Even individuals who undergo training to wear masks properly often are unaware of best practices (24). Thus, the Abaluck study and others highlight two key points about pandemic mitigation policies. First, masking policies have

TABLE 2 Longitudinal changes in person-based testing strategies: how implementation outcomes varied through different pandemic phases.

Pandemic period		Diagnostic testing	Surveillance testing	Test-to-stay
Early	*Key resource considerations*	*Limited availability of all tests; tests limited to those with severe disease and specific exposures*		
	Feasibility	Low	Low	Low
	Acceptability	N/a	N/a	N/a
	Appropriateness	N/a	N/a	N/a
	Cost	N/a	N/a	N/a
Pre-Vaccine	*Key resource considerations*	*Antigen testing remained in limited supply, long delays in PCR testing results*		
	Feasibility	Moderate	Variable, possible in some settings (e.g., healthcare)	N/a
	Acceptability	High	High	N/a
	Appropriateness	High	High	N/a
	Cost	Low	Low	N/a
Post-Vaccine, Pre-Omicron	*Key resource and contextual considerations*	*Both antigen testing and PCR widely available in community and healthcare settings; contact tracing feasible due to specifics of the circulating variant*		
	Feasibility	High	High	High
	Acceptability	High	Moderate	High
	Appropriateness	High	Moderate	High
	Cost	Low	High	Low
Post-Omicron	*Key resource and contextual considerations*	*More transmissible and immune-evasive variant with frequent exposures in a variety of settings. Vaccine availability to all school-aged children reduced risk of severe disease in this population*		
	Feasibility	High	High	Low
	Acceptability	High	Low	Low
	Appropriateness	High	Low	Low
	Cost	Low	High	High

Applying implementation outcomes to testing strategies at different stages in the pandemic helps to determine adoption/uptake and also public health impact. Changes in resources, feasibility, perceived acceptability and appropriateness, costs of the intervention and changes in the host-pathogen relationship determined the longtidunal public policy impacts of the same mitigation measure (testing). Categories do not apply to community-based surveillance methodologies, such as wastewater testing.

the potential to be beneficial, but the long-term sustainment of appropriate use or fidelity to the intervention (and therefore its impact on transmission) means that in the real world, the *public health policy impact* may change substantially as motivations, such as perceived risk and intervention fatigue, change. Recent nation-wide data provides empirical support for the condition-dependent and dynamic impacts of masking policies, highlighting the importance of incorporating implementation science principles as part of ongoing policy re-evaluation (25, 26).

The perennial challenge of sustaining fidelity to interventions that require ongoing behavioral changes (and therefore public health policy impact) is also supported by evidence about adherence to other NPI policies. A cross-sectional survey conducted in the United States about self-reported adoption of social distancing recommendations found a slow but steady decline in a variety of different settings from May to July of 2020; these findings are also corroborated by Google movements data, which demonstrated a slow but steady return to usual activity after the initial disruption (27, 28). Similarly, although school closures temporarily reduced childhood social interactions, over a relatively short time horizon and long before schools re-opened, the number of contacts increased among children, providing at least a partial explanation for the limited real-world effectiveness of this intervention (29–31). Additional data from the spring of 2020 demonstrate that movement increased most substantially among counties that lifted stay-at-home orders, but that increased community activity was also evident among counties that maintained stay-at-home policies (32). Of note, these data were collected during an early pandemic period *before* widespread immunity from natural infection and vaccination when therapeutic options were still minimal. Despite these contextual factors, fidelity to the intervention nonetheless fell, likely driven by changes in risk perception and costs associated with social distancing policies, particularly business and school closures.

Six et al. evaluated factors associated with self-reported adoption of government-recommended NPIs at different snapshots during the first phase of the pandemic in Belgium (33). The first survey occurred during the country-wide lockdown, when cases, hospitalizations and deaths were all close to the initial peak. The second survey was collected after cases, hospitalizations and deaths had all fallen, and relaxation of mitigation measures had been announced. The third survey occurred when cases, hospitalizations, and deaths were all very low, and all mitigation measures were about to be lifted. In these different contexts, authors found that factors and perceptions associated with self-reported compliance varied. At all three data points, fear of

COVID-19 severity, perceived rule appropriateness, and observing others respect the rules were positively associated with self-reported fidelity to recommended interventions. Perceptions about individual risk of exposure to COVID-19 were positively associated with increased support in the second and third surveys. Perceived rule effectiveness was positively associated with fidelity to interventions during the second survey, and measures of altruism were positively associated with self-reported fidelity to interventions during the third survey. Notably, authors also found that self-reported fidelity was *negatively* associated with trust in government. This finding diverged from a body of prior evidence suggesting that increasing trust in government is associated with increases in uptake of policy recommendations. Although the reasons for this finding about trust in the specific context of the pandemic could not be entirely delineated, authors theorized that those who were the most fearful about COVID-19, and who therefore were strongly supportive of ongoing restrictions, were also those who lost the most faith in the government response when mitigation measures were relaxed.

Findings about self-reported factors associated with appropriate use of mitigation measures align with real-world findings about policy impacts and effectiveness. Drivers of *appropriate use*, which is essential for *policy effectiveness*, and therefore *public health impact* changed over time (Table 3). Early in the pandemic, fear of COVID-19 was a major factor that drove adherence and willingness to support NPIs. Changing perceptions about disease severity – as treatments and vaccines became available and as estimates of case fatality changed – likely drove behavioral changes which, in turn, impacted policy effectiveness. Thus, Six et al.'s study also highlights the importance of updating policies to align with current contexts and public perceptions; in a system of constant and dynamic change, public health policy impacts cannot be assumed to be static. Processes for identifying key inflection points in public opinion and contextual changes to trigger policy updates are needed to improve public health policy impact.

Dynamic policy impact: implementation outcomes as determinants of real-world effectiveness

Stemming from changing contexts, evidence, and perceptions, the effectiveness of community public health measures are dynamic. Acknowledging dynamic impact implies that ongoing adaptation of public health policy recommendations as new data emerge and resource availability changes will lead to optimal evidence-based policy making and suggests a path for improving future public policy responses to infectious diseases threats.

Each of the four phases of the COVID-19 pandemic is characterized by different levels of fear, public perceptions about appropriateness, resource availability, feasibility, and differences in estimates of policy impacts (Table 3, Figure 3). The evidence basis to support NPI measures, and our understanding about how, when, and for how long they are effective also changed over time. In addition to NPI fatigue, these constantly evolving factors changed mitigation measure effectiveness via several mechanisms. Feasibility of some interventions, such as testing and contact tracing, was variable. During the early phases of the pandemic in the US, testing was not a viable prevention strategy in community settings due to limited resource availability (34); however, a test-and-quarantine strategy was successfully implemented in other countries (18). As the resource became more widely available in the US, applications of testing interventions as a mitigation measure were expanded. For example, test-to-stay programs for school settings replaced disruptive at-home quarantines in favor of in-person learning opportunities for asymptomatic students (35). However, after the emergence of the more transmissible omicron variant, contact tracing became infeasible due to the frequency of possible exposures both inside and outside of school settings, and some test-to-stay programs had to be retired or adapted (36). As noted above, longitudinal reductions in intervention fidelity were found with social distancing measures as early as the first phase of the pandemic.

Decreasing levels of use as-intended (e.g., fidelity) should be anticipated and factored into public health policy recommendations. Perceived appropriateness of different measures is also variable and dependent upon key milestones (e.g., vaccine development and distribution to high-risk populations). Widespread availability and access to vaccines decreased case fatality rates and decreased fear of COVID-19. Decreased case fatality and fear then altered the perceived appropriateness of ongoing strict mitigation policies. Alterations in perceived appropriateness led to changes in NPI uptake, which then decreased potential NPI policy impact. The lower risk of severe and fatal infections conferred by natural and vaccine-induced immunity also caused smaller absolute risk reduction associated with NPIs policies. Simultaneous with decreasing impact due to fidelity and sustainment challenges, as the duration of some of the interventions increased, most notably school and business closures, their negative impacts became increasingly apparent (37–40). Overtime, these costs altered perceived appropriateness and acceptability of these pandemic mitigation policies. Thus, multiple changes at multiple levels changed the net public health impact of NPI policies, all in the direction of decreasing potential public health impact.

Owing to a confluence of these different mechanisms, the effectiveness of NPIs is likely reduced every time they are recommended or reintroduced. Maximal potential impact occurred during the early phases of the pandemic and subsided with each subsequent wave and medical advancement. Empirical evidence from business closures suggests a potent short-term benefit with rapidly decreasing returns and increasing harms, supporting the theoretical view of longitudinal decreasing expected benefit (41).

Sustainment versus sustainability – defining infectious disease policy goals

A core concept in implementation science is that improving uptake of an evidence-based intervention, and sustaining that increased use longitudinally, leads to improvements in clinical and public health outcomes. Embedded in this view is the concept that

TABLE 3 Availability of different COVID-19 mitigation measures and their acceptability, feasibility, sustainability, and potential policy adjustments as conditions and contexts changed.

	Intervention	Sustainability	Policy adaptations
	Masking mandates	Low	Shift to individual choice, change recommendations about mask type
	Testing programs	Variable	Based on community risk level and resources, consider non-invasive options
	Social distancing	Very Low	Avoid in current context
	Business and Other Closures	Very Low	Avoid in current context
	Ventilation	High to Very High	Focus on infrastructure upgrades, research
	Vaccination	High to Very High	Focus on first doses and tailor boosting messaging
	Pre-exposure Prophylaxis	High	Distribute to immunocompromised, encourage additional research

the effectiveness of the intervention for improving the outcome of interest is *static;* that is the effectiveness of the intervention is a *constant* value that is not inherently variable.

Responses to infectious disease threats, which always involve a dynamic host-pathogen relationship, raise the question of how public health policy goals should be defined. A traditional view of sustainability is the "fidelity approach," which Berta et al. define as "the extent to which an intervention program follows the originally intended implementation plan and faithfully delivers the research-informed components of the intervention (42)." In the traditional view of sustainability, maintaining compliance with the originally intended evidence-based intervention is critical for achieving public health benefit. An alternate view, particularly germane to infectious diseases policy response planning, is the "adaptive approach," which highlights the importance of the "co-evolution of the intervention" and the context. The "adaptive approach" postulates that adapting the intervention (and reducing fidelity to the original program) to better fit the context may in fact *enhance* outcomes.

Thus, rather than focusing sustainability efforts on maintaining compliance with interventions (such as masking policies), an alternate ("adaptive approach") view is that sustainability efforts should focus on prioritizing the shifting use and form of interventions in order to maintain (or improve) public health *outcomes*. In the example of COVID-19, this translates to sustained use of interventions that prevent severe disease, long-term disability, and death, rather than focusing on sustained use of any specific intervention. Early in the pandemic, when medical countermeasures were unavailable, SARS-CoV-2 cases were highly correlated with severe disease and death. Thus, strategies that prevented cases led to reductions in mortality. However, after the availability of medical countermeasures, infections and severe outcomes became uncoupled (43–45). After this uncoupling, preventing cases had a substantially lower public health impact. Focusing on sustaining specific *interventions,* such as masking policies, therefore had a progressively decreasing impact on population health outcomes. Focusing public health policy on sustaining and improving *outcomes* is expected to have ongoing population health benefits. Notably, viewed through this lens and the "adaptive approach," de-implementation is an inherent aspect of dynamic sustainability for infectious diseases threats, as interventions that are no longer effective should no longer be recommended or enforced.

Maximizing public health policy impact: toward a dynamic infectious diseases public health response framework

COVID-19 is presented as a case study for considering public health policy impacts and adaption through an implementation science lens. Principles about dynamic change are inherent to the host-pathogen interaction and generalizable beyond the specifics of the public health response to the COVID-19 pandemic. Key lessons learned include the changing public health policy effectiveness of interventions as a function of conditions, contexts, and political environments and the need to consider the aims of sustainability. Rather than focusing on maintaining compliance with any specific intervention, to improve health, public health policy goals should aim to reduce severe health outcomes.

Achieving the ideal of adapting policy to sustain benefit will require re-focusing public health surveillance and evaluation methods to include consideration of implementation outcomes and changing contextual factors. Ideally, systems will be developed so that key inflection points, or phase transitions (e.g., from the early phases characterized by fear and limited resources to the

later phases characterized by reduced mortality) can be measured and acted upon in real time through ongoing policy updates. Empirical evidence for the importance of novel data sources and integrating implementation outcomes into infectious diseases pandemic planning is illustrated in the modeling data from Chang et al., which demonstrated that integration of cell phone movement data improved outbreak prediction model accuracy (46). Authors also found that integration of data about compliance with social distancing policy (fidelity to the public health intervention), which varied with time, lead to persistent improvements in model prediction accuracy. This study therefore highlights the importance of integrating data about key implementation outcomes, such as fidelity, to improve infectious diseases management and public health policy.

The *Dynamic Infectious Diseases Public Health Response Framework* is a model for evaluating ongoing public health policy impact in the context of a constantly changing and evolving system (Figures 3, 6). Concepts are grounded in implementation science theory and are a direct extension of the Dynamic Sustainability Framework (10). Key considerations are generalizable to many infectious diseases threats and associated mitigation measures and prevention responses.

Focusing on maximizing *public health outcomes* has additional implications. For populations that are high-risk of severe disease despite vaccination (or for other reasons, depending upon the specific infectious disease in question) (47–49), different policy recommendations may be needed to achieve public health goals. For example, mitigation measures designed to prevent any COVID-19 case applied in skilled nursing facilities, dialysis centers, and chemotherapy units are expected to have a more substantial direct public health impact than when those same mitigation measures are applied to lower-risk populations, such as interventions implemented in elementary and secondary school settings. Acknowledgment of differential population risk was one of the reasons masking requirements were maintained in hospital settings longer than in the community (50). Thus, implementation of a Dynamic Infectious Diseases Public Health response requires measurement of population risk and adaptation of policy to match level of risk. In this setting, risk should be defined broadly, and harms and costs of the intervention should be included when crafting public health policy.

Achieving future improvements in real-time infectious disease policy responses will require major infrastructure investments to collect the data necessary to inform ongoing public policy decisions. Traditional surveillance systems focus on measuring cases via reporting from state and local health departments. These traditional systems do not have the infrastructure or linkage to data elements that would facilitate evaluation and integration of implementation outcomes. Novel mechanisms for gathering and interpreting data in near-real time are needed; practically, this will likely include a national data repository, technologic advancements in data cleaning and real-time analysis, and integration of non-traditional sources of information, such as social media, for ongoing assessment of public perceptions about different public health policies. For example, measuring trends about discussion of masking policies and school closures on Twitter and other social media sites likely would have provided valuable insight into changing acceptability of these public health recommendations, and allowed for public health policy makers to integrate perceptions of end-users into ongoing policy updates. Similarly, early in the pandemic, ongoing discussions of lack of access to testing may have served as a signal to invest more heavily in this strategy.

Inherent in the Dynamic Infectious Diseases Public Health Framework is the concept that as implementation outcomes evolve longitudinally, so does the effectiveness of public health policy responses (Figure 1). Thus, as a direct result of constantly evolving conditions, risk of the setting and to the individual, public perceptions, and political contexts, public health policy needs constant revision and evolution to maintain the same societal benefits (7).

Challenges with sustainment of behavior change to control disease spread are not specific to COVID-19. Recommendations for use of barrier methods to prevent transmission of sexually transmitted infections, particularly HIV infection, and to reduce the number of sexual contacts, were eventually abandoned in favor of a more harm-reduction focused approach (51), as early abstinence-based public health recommendations were found to be unacceptable to many and therefore less effective than other approaches, such as treatment and PrEP (52–55). In the summer of 2022, when the Mpox outbreak occurred, public health recommendations that focused on reducing sexual contacts were heavily criticized, due in part to lack of acceptability and perceived appropriateness of the intervention among recipients of the public health campaign (56–59).

The dynamic infectious diseases public health response: an extension of the real-time, learning health system approach

The ideal of the Learning Health System is that data generated in real time can be leveraged to advance our scientific understanding of a problem and that advancement in our understanding can be leveraged to improve care delivery (6). Inherent in this model is the idea that "the evidence" is constantly changing and evolving, and that a variety of data elements are needed to realize potential benefits of this approach (60). Particularly when applied to the context of public health programs, data elements include not only traditional data elements but also other factors that impact real-world effectiveness – cultures, believes, attitudes and contexts. Integration of these other elements into policy evaluation is critical for achieving and sustaining public health benefit.

Adaptation to changing conditions and contexts is the best way to incorporate evidence into policy changes but achieving the ideal synergy of specific policy recommendation to context and public perception requires substantial advancements and investment in data collection, analysis, and implementation. Substantial changes in contexts, evidence and phases need to be identified in near-real time, so that public health policy can be aligned with the current conditions. The more heavily reliant an intervention is on day-to-day, person-level appropriate use for effectiveness,

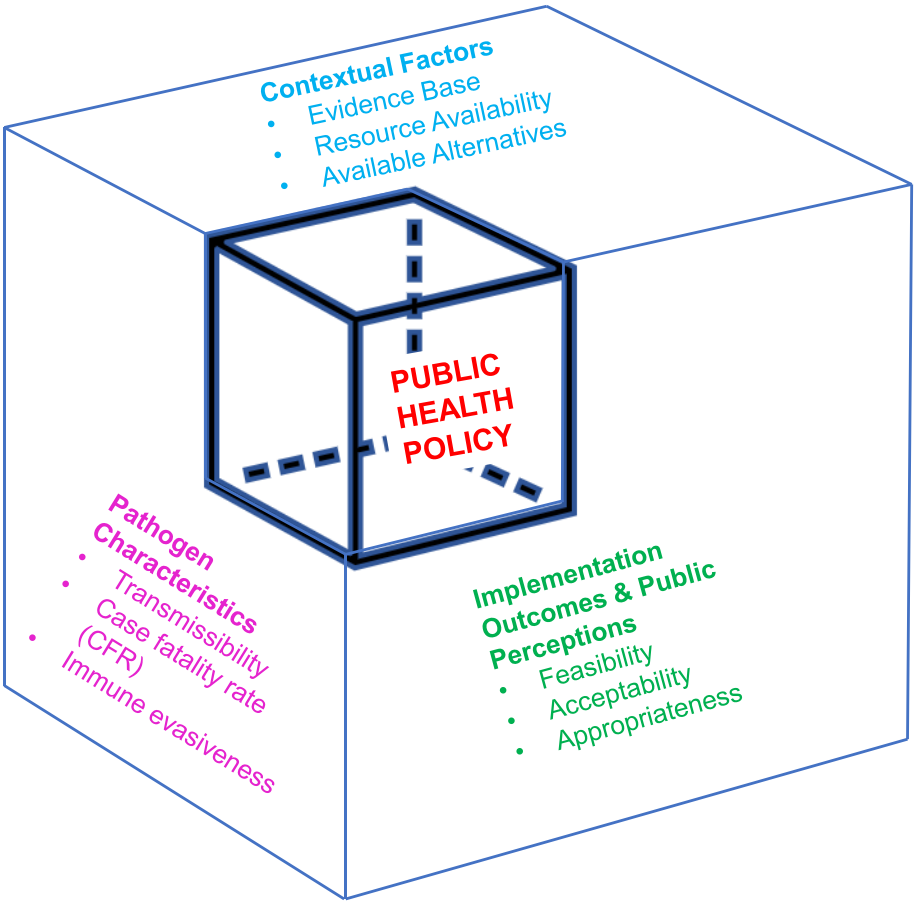

FIGURE 6
Dynamic infectious diseases public health response framework for incorporating implementation outcomes into public health policy adaptation. The dynamic infectious diseases public health response framework presents variables that impact public health policy effectiveness. Optimizing the effectiveness of public health policy necessitates ongoing evaluation of each of these different variables, with updates as they change.

the more strongly the duration of the intervention should be considered in weighing recommendations. Ideally, evaluations of the effectiveness of individual-level interventions should be ongoing, with constant reassessments of whether the intervention is continuing to achieve the desired outcome and immediate policy change once the intervention is no longer effective or found to be harmful. Costs and harms of an intervention should be evaluated in parallel with benefits and on an ongoing basis. Improvements in real-time data analytics are likely to result in earlier policy de-implementation as human behaviors and expectations are inherently integrated into public health policy making. Once the balance tips away from benefit and toward harm, the policy should be de-implemented, with clear and open communication about the emerging evidence and rationale for the change.

Recognizing the inherent limitations of observational data, advancements in data management and rapid data analysis are needed for the next pandemic to enhance our understanding about real-time policy effectiveness. Given the role of perceived appropriateness of policy in driving compliance—and therefore effectiveness—improvements should also include mechanisms to assess and integrate public feedback into public health policy and messaging. Data about the feasibility, acceptability, and costs of different interventions, such as social distancing policies and business and school closures should be collected by public health officials and state and local governments from publicly available sources, such as social media platforms and google movements as well as directly from interested parties and individuals impacted by specific policy interventions. These data elements should then be integrated into planning and policy responses. Data about ongoing use could be used to inform whether policy effectiveness is likely to have changed due to changing conditions and public perceptions. Potential harms of interventions should be prospectively measured and incorporated into assessments of the ongoing acceptability and appropriateness of the public health policy. These data elements could then be mapped to quantitative data and used to tailor public health policy recommendations based on input from those most impacted and ongoing expected benefits and harms. An example application of integrating implementation outcomes into public policy design is presented in Table 3, which ranks NPIs according to feasibility and acceptability. In the future, information about implementation outcomes and public perceptions could be integrated with evidence about relative policy effectiveness to adapt public health policy recommendations to align with current conditions to sustain benefits that are informed by factors that are important to the public.

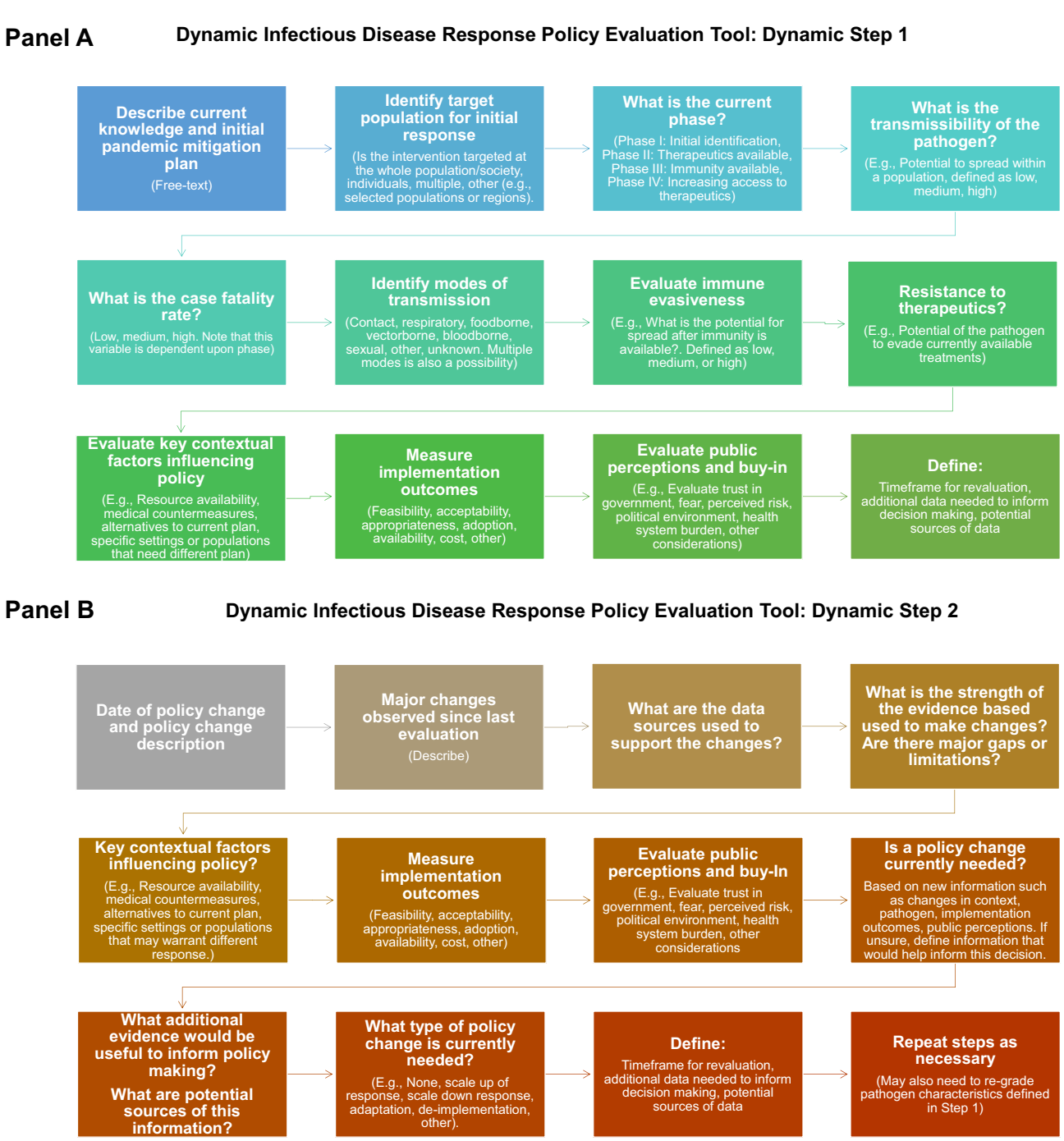

FIGURE 7
Steps in the dynamic infectious diseases public health response framework practical took. **(Panel A)** depicts the questions evaluated during Step 1 of the policy making process, which includes initial plan, evaluation pathogen characteristics, current evidence, implementation outcomes, and public perceptions. At the end of step 1, users define the timeframe for re-evaluation and key information gaps and potential sources of data to inform ongoing policy evaluation and adaptation. **(Panel B)** depicts the questions evaluated during step 2, which should be repeated as often as is necessary to achieve public health policy goals.

Practical considerations

Achieving this ideal – rapid data collection and analysis to inform on-the-ground policy recommendations will require substantial investments in national informatics infrastructure to achieve a public health 'Learning Health System' (6, 7, 61). Leveraging advancements in artificial intelligence to link and analyze novel data sources, such as social media commentary, to assess ongoing public perceptions, feasibility, and acceptability may help in the future to realize these ideals. Setting up systems where data are assembled, analyzed and interpreted, leading to knowledge of the needed interventions and how to manage them, and then tracking data to understand practices changes in real-time are learning health system steps required for the real-world

application of the Dynamic Infectious Diseases Public Health Framework (60).

A practical tool to assist policy makers in applying the framework is presented in Supplementary material 1. The practical tool with pre-populated responses to key determinants of public health policy impacts directs iterative assessments of key implementation outcomes and contextual factors that can be evaluated in real-time to inform and adapt public health policy. It is designed to facilitate measurements and incorporation of implementation outcomes, such as feasibility, appropriateness and costs to inform and adapt decision making, which may include policy adaptation, policy de-implementation, or determination that additional information is needed. If additional information is needed, the tool can help to identify knowledge gaps and direct scientific investigation to close these gaps. Integration of novel information sources, such as public input from social media platforms, is encouraged as part of ongoing assessments of public perceptions about policy appropriateness and harms. Key population-specific factors that may impact decision-making, such as risk of disease, are also included to inform policy development and adaptation. Optimizing the effectiveness of public health policy necessitates pre-planned, ongoing evaluation of each of these different variables, with updates as changes are identified.

A flow chart to direct application and use of the tool is presented in Figures 7A, B. Figure 7A depicts the first step of the dynamic response, which includes consideration of pathogen characteristics, key contextual factors including the current evidence base and potential alternatives to the current strategy, assessment of implementation outcomes (defined in Table 1), and public perceptions. These steps are delineated to assist with policy making and to identify key challenges and evidence gaps to inform subsequent decision-making. At the end of dynamic step 1, users should define the timeframe for re-evaluation. Dynamic step 2 is depicted in Panel B. Step 2 defines key factors that may merit a policy change and directs ongoing planning, including collection of new evidence. Step 2 is designed to be repeated as many times as is necessary for the duration of the response.

Summary and conclusions

A refrain throughout the pandemic has been "the science has changed!" Public health policy making is complex – and the message that impacts of public health policies are dynamic is a difficult one to convey. While knowledge and evidence have evolved and expanded, fundamental scientific principles have not. Diagnostics, therapeutics, preventative interventions, and viral variants did change, as did public perceptions, tolerances, and behaviors. In future policy responses and policy messaging, uncertainty must be acknowledged and embraced. Public health officials should also be upfront that change in policy is an expected outcome of the most evidence-based practice, as we learn more, the context, conditions, and evidence change, and even the goals of the public health response evolve. Both implementation and de-implementation plans should be incorporated into planning.

The Dynamic Infectious Diseases Public Health Response Framework is presented through the lens of the COVID-19 pandemic response but is broadly applicable to public health interventions that include complex and ever-evolving host-pathogen interactions. Consideration of implementation outcomes, in addition to more traditional measures of clinical effectiveness, may help to improve evaluations of public health programs and impact and to facilitate matching policy recommendations with evolving contexts. Public health policy goals, feasibility, costs, and perceived acceptability and appropriateness change as the context, evidence, and resources change, highlighting the importance of viewing the impact and effectiveness of public health policies and impacts as dynamic elements of a larger constantly evolving and changing system. These implementation outcomes are determinants of ultimate public health policy impact.

Public health policy and pandemic responses are not just about the evidence– or just about the evidence at one moment in time. This is particularly true for the management and control of infectious diseases, which always involve a dynamic interplay between the host and the pathogen. Real-time decision making requires sensitivity to conditions on the ground and adaptation of intervention at all levels as implementation outcomes, such as acceptability, appropriateness, and fidelity change and as contexts evolve. When asking about the public health effectiveness and impact of non-pharmaceutical interventions, the focus should be on *when*, *how,* and *for how long* they can achieve public health impact – definitive statements such as "masks work" or "masks don't work" fail to capture how interventions work in real world settings and contexts.

Static effectiveness estimates cannot be assumed in a constantly changing system. Policy impacts are dynamic and need to be recognized and evaluated as such. Just as NPI policy should change, our public health infrastructure needs to adapt to maintain effectiveness in the background of constant change and to maintain relevance and benefit to the end-user of these policy recommendations: the public.

Author contributions

All authors contributed to concept review and analysis, drafting, and editing of the manuscript. In addition, all authors met with the graphic designer and assisted in the design and revision of the figures presented in the manuscript.

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Supplementary material

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Willingness of people living with HIV to receive a second COVID-19 booster dose: a multicenter cross-sectional study in China

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Background: The coronavirus disease 2019 (COVID-19) pandemic has significantly affected the global population, with People Living with HIV (PLWH) being particularly vulnerable due to their compromised immune systems. Although vaccination is a crucial preventative measure against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, little is understood about the willingness of PLWH to receive a second COVID-19 booster dose and the factors that may influence this decision. This study investigates the willingness of PLWH in China to receive a second COVID-19 booster dose and its influencing factors, comparing these with a group of healthy individuals.

Methods: A multicenter cross-sectional study was conducted across five Chinese cities, namely, Beijing, Tianjin, Zhengzhou, Hohhot, and Harbin. Participants were recruited through five community-based organizations. Data were collected *via* participant self-administered questionnaires included demographic information, willingness to receive a second COVID-19 booster dose, and knowledge about HIV and COVID-19 vaccination. Factors influencing vaccination willingness were identified using multivariable logistic regression analyzes.

Results: A total of 156 PLWH and 151 healthy individuals were included in the study. After adjusting for potential confounders, it was found that PLWH demonstrated a lower willingness to receive a second COVID-19 booster dose compared to healthy individuals (77.6% vs. 88.7%, $p = 0.009$). Lower willingness was associated with HIV positive status (Adjusted Odds Ratio [AOR]: 0.39, 95%CI: 0.20, 0.75), perceived barriers (AOR: 0.05, 95%CI: 0.01, 0.26), and perceived severity (AOR: 0.32, 95%CI: 0.12, 0.90).

Conclusion: PLWH in China demonstrated a lower willingness to receive a second COVID-19 booster dose compared to healthy individuals. The findings suggest that perceptions and understanding of the COVID-19 vaccination and its necessity for protection against SARS-CoV-2 could influence this willingness. Efforts should be made to strengthen and disseminate knowledge about HIV and COVID-19 vaccinations among this population. In addition, developing interventions and policies that target specific subgroups and address misconceptions about vaccination could be instrumental in improving vaccination rates among PLWH.

KEYWORDS
COVID-19 vaccines, PLWH, health belief model, willingness, booster dose

1. Introduction

The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has escalated into a global pandemic, with over 750 million infections and 6.8 million deaths recorded as of February 1, 2023 (1). The impacts are particularly severe for specific populations, including people living with HIV (PLWH), who have been shown to have a higher risk of SARS-CoV-2 infection, serious illness, hospitalization, and death than the general population (2–6).

COVID-19 vaccines have been recognized as one of the most effective methods for preventing infection with SARS-CoV-2 and its variants (7, 8). Vaccination has significantly reduced the risk of SARS-CoV-2 infection and severe COVID-19 disease outcomes in PLWH (9, 10). However, the rise of new SARS-CoV-2 variants has necessitated booster doses of COVID-19 vaccines in some countries. Initial studies indicated the effectiveness of the first COVID-19 booster dose among PLWH against new variants such as Delta and Omicron (11–13). However, other research suggests that immunogenicity and the effectiveness of preventing severe outcomes with the first COVID-19 booster dose among PLWH may diminish over time, especially concerning the Omicron variant (14).

Given the potential decrease in immunogenicity and effectiveness over time, PLWH should receive a second COVID-19 booster dose at an appropriate time. Further research indicates enhanced immunogenicity and safety with the second COVID-19 booster dose in PLWH (15). In response to these findings, several countries, including China, now recommend a second booster dose for PLWH, along with other key populations such as individuals over the age of 60, high-risk groups, those with underlying health conditions, and particularly immunocompromised individuals (16–18).

In the past, significant hesitancy was observed among PLWH in China regarding full-dose COVID-19 vaccination. The vaccination coverage among this group was significantly lower than the international average for the PLWH population (6.2% vs. 63.5%) (19, 20). With the current promotion of the second COVID-19 booster dose both in China and globally, understanding the vaccination willingness of PLWH and exploring the relevant influencing factors have profound theoretical and practical implications for developing and promoting vaccination strategies.

According to the World Health Organization (WHO), it is still necessary for individuals who have previously been infected with SARS-CoV-2 to receive a second COVID-19 booster dose (21). However, only Uganda has reported vaccination rates and willingness to receive the first COVID-19 booster doses among PLWH (22). Given the significant differences in COVID-19 vaccination types and perceptions across countries, the results from other contexts cannot directly guide COVID-19 vaccination strategies for PLWH in China.

Previous studies have highlighted the concern about vaccine side effects as a significant factor influencing the hesitation of PLWH to receive the COVID-19 vaccine. Whether side effects after the first COVID-19 booster dose influence the willingness to receive the second booster dose remains unclear. Addressing these knowledge gaps would offer valuable insights to guide the administration of the second COVID-19 booster dose among PLWH.

The HBM is one of the most extensively utilized theories for understanding health and illness behaviors. The model is predicated on the understanding that a person's belief in a personal threat of an illness or disease and belief in the effectiveness of the recommended health behavior or action will predict the likelihood that the person will adopt the behavior. The HBM has been previously employed to analyze COVID-19 complete vaccination willingness and behavior among cancer patients and PLWH, as well as in health education activities related to vaccine promotion (23, 24). Although applying HBM to COVID-19 vaccination could enhance our understanding of this health behavior, there is still a gap in the literature, particularly about COVID-19 booster vaccination among PLWH.

In this study, we developed a questionnaire based on the HBM to conduct an anonymous survey among the PLWH population in mainland China. This study aims to provide a theoretical basis for guiding the effective adjustment and implementation of vaccination strategies in our country and other nations in response to the continuously evolving disease situation.

2. Materials and methods

2.1. Study design and objective

This cross-sectional survey is derived from a registered prospective cohort study (the Chinese Clinical Trial Registry.ChiCTR2200058989). The prospective cohort study aimed to assess changes in immunogenicity and adverse reactions within 6 months following the first COVID-19 booster dose in China among PLWH. The prospective cohort study initially recruited both PLWH and healthy individuals in five Chinese cities (Beijing, Tianjin, Zhengzhou, Hohhot, and Harbin), with participant recruitment and selection criteria described in our previous work (25). Based on the cohort study, we further conducted a cross-sectional survey from December 2021 to March 2022. The present study has been approved by the Ethics Committee of Peking University Shenzhen Hospital (No. 2021-094).

2.2. Participants

In this study, the inclusion criteria for participants included: (1) aged between 18 and 65 years, (2) no history of SARS-CoV-2 infection, (3) having received full immunization (two doses of

Abbreviations: PLWH, people living with HIV; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; AOR, adjusted odds ratio; WHO, world health organization; HBM, health belief model.

COVID-19 inactivated vaccine) and the first COVID-19 inactivated booster dose, (4) the second COVID-19 booster dose has not been vaccinated yet, and (5) willingness to participate in the study activities and having signed written informed consent. The HIV infection status was preliminarily self-reported by participants before attending this site study. We re-identified the HIV serostatus for PLWH using the Abbott ARCHITECT HIV Ag/Ab Combo assay, which has high sensitivity and specificity (S/CO ≥ 1.0, Reactive) at the study site. The exclusion criteria were: (1) interviewees with severe hearing loss, visual impairment, or intellectual disability and (2) major mental illness (schizophrenia or bipolar disorder) or neurocognitive impairment as assessed by the clinician.

2.3. Study procedures

PLWH was recruited from five community-based organizations that collaborated with HIV clinical service providers and offered services to PLWH, one in each city. Recruitment advertisements were disseminated through WeChat public accounts, a widely used social media platform in China. Then, interested PLWH contacted project staff via social media and were briefly informed of the study's purpose and procedure. Potential PLWH participants and the healthy control population received a detailed informed consent form. Upon signing, they were screened using inclusion criteria and a free HIV test through the HIV rapid test kit. Eligible HIV-negative individuals were also invited to participate in the study. Investigators issued an anonymous questionnaire through the online survey platform (Golden Data) at the prevaccination (before 2–4 weeks of receiving the first COVID-19 booster dose) and the fourth-week follow-up of the prospective cohort study to understand their feelings and willingness after the first COVID-19 booster dose. Questionnaires that did not meet the length (less than 100 s) to fill in the questionnaire and had logical errors (For instance: the time of COVID-19 vaccination was before the occurrence of COVID-19) were excluded.

2.4. Questionnaire

The questionnaire used in this survey consisted of five sections: Socio-demographic characteristics and health status; Adverse reactions after vaccination; Willingness to receive the second COVID-19 booster dose; HBM project; HIV-related information and immunization status.

To ensure effectiveness, all questions were constructed and evaluated by an expert team (including two public health experts and an epidemiologist specializing in infectious diseases).

In the HBM section, we set up 16 items across six dimensions, including perceived susceptibility (3 items), perceived severity (3 items), perceived harm (1 item), perceived benefits (2 items), behavioral cues (1 item), and self-efficacy (1 item). The score for each item ranged from 1 to 5, allocated to "strongly disagree, ""disagree, ""neutral, ""agree, "and "strongly agree." The scale's reliability was verified by Cronbach's α coefficient ($\alpha = 0.835$).

HIV-related information and immunization status included current HIV infection status, HIV infection time, ART conditions, the latest testing results of HIV viral load, and CD4 + T cell absolute count.

The questionnaire was anonymous, with a unique 6-digit number for each participant to protect privacy. A master list with identifiable information was saved on the principal investigator's computer with password protection, accessible only to the principal investigator, and the data were encrypted and regularly backed up to prevent data loss or unauthorized access.

2.5. Sample size

This study aimed to evaluate Chinese PLWH's willingness to vaccinate with the second COVID-19 booster dose relative to healthy individuals. Based on the results of published peer-reviewed studies in Greece, Italy, and China, it was estimated that the acceptance rate of the second COVID-19 booster dose among PLWH is 70%. The acceptance rate among the healthy control group is 85% (26–28). The confidence level of $1-\alpha = 95\%$ and the test efficacy $1-\beta = 0.8$ were specified. After considering a 10% dropout rate, 270 participants were required, with the PLWH group and healthy individuals group allocated in a 1:1 ratio. The sample size was calculated using Power Analysis and Sample Size software (version 15.0.5).

2.6. Statistical analysis

For continuous variables, normality was assessed by the Shapiro–Wilk test. Variables conforming to normal distribution were analyzed using the t-test method, and for non-conforming variables, the Mann–Whitney test method was used. For categorical variables, the chi-square/Fisher method was used. A logistic regression analysis was performed to investigate the factors influencing vaccination willingness. First, a binary logistic regression analysis was performed for demographic characteristics information to obtain variables with $p < 0.05$. After that, the variables with $p < 0.05$ were added to the multivariable logistic regression analysis to correct for the bias introduced by background information. Associations between the independent variables of interest (i.e., variables at the individual, HBM project, and HIV-related information and immunization status) and the dependent variables were assessed by adjusted odds ratios (AORs) and 95% confidence intervals. Each AOR was obtained by fitting a logistic regression model involving an independent variable of interest and all significant background characteristics. All statistical analyzes were performed using SPSS software (version 25.0 IBM Corp., Armonk, NY). A two-tailed p value of less than 0.05 was considered statistically significant.

3. Results

3.1. Background characteristics

A total of 339 participants aged between 18 and 65 years old were approached for participation. Thirty-two participants were excluded from the study for four reasons: non-provision of informed consent, failure to complete the online questionnaire, presence of logical errors in the questionnaire responses, and inappropriate completion time. Of the remaining 307 participants, 50.81% were PLWH (156/307), and 49.19% were healthy individuals (151/307) (Figure 1).

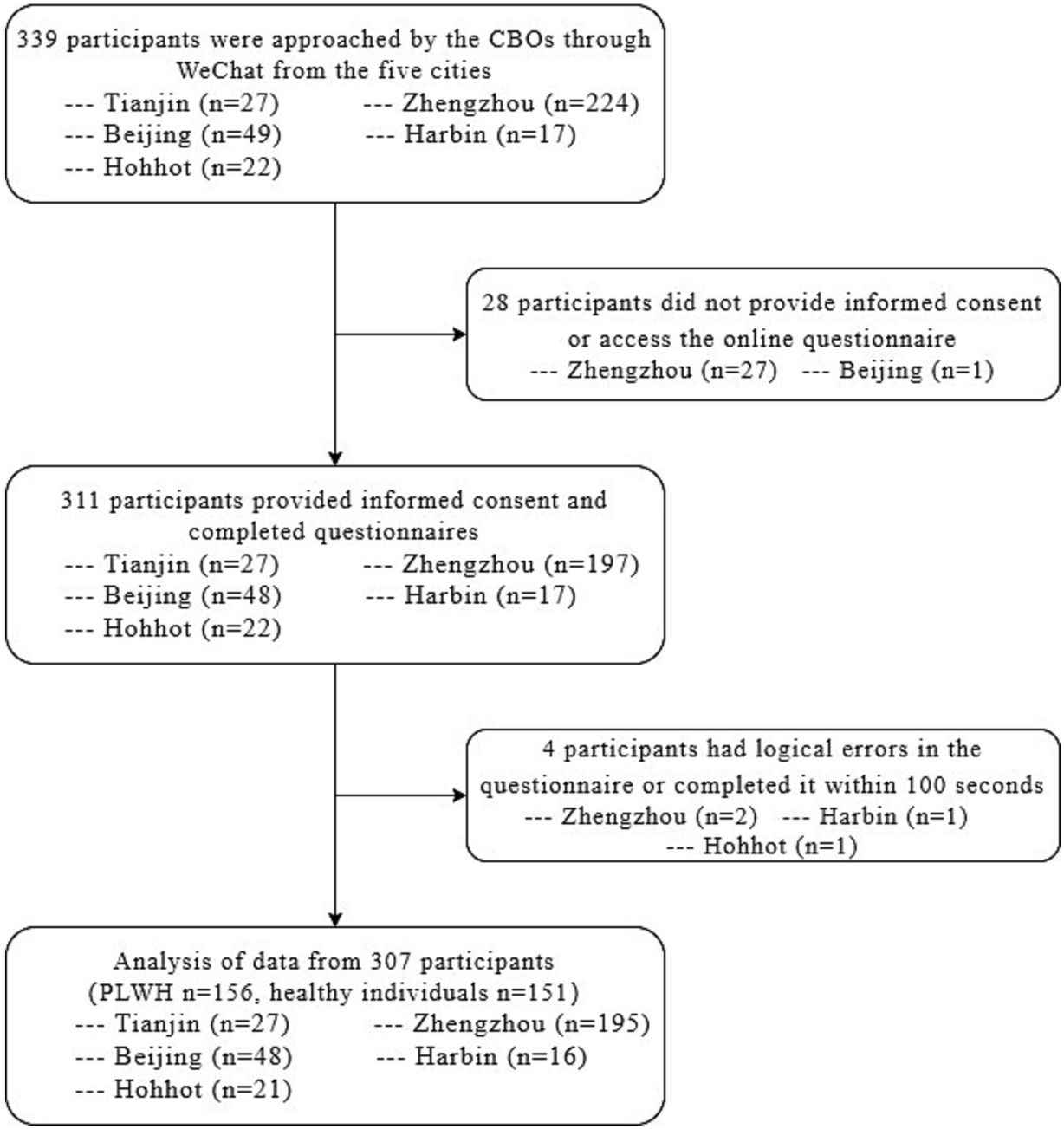

FIGURE 1
Data collection procedures in the study.

All participants had been vaccinated with the preliminary schedule of two doses of inactivated COVID-19 before recruitment for this study. On average, they received the first COVID-19 booster dose 203 days after receiving the initial two doses. The PLWH group had a significantly higher proportion of males (96.2% vs. 86.8%, $p = 0.003$) and individuals aged 30–45 (56.4% vs. 46.4%, $p = 0.045$) than the healthy individual group. Conversely, the healthy individual group had a significantly lower proportion of single/divorced/widowed (56.3% vs. 82.7%, $p < 0.001$) and engaged in full-time work (43.7% vs. 67.3%, $p = 0.047$) than the PLWH group. Regarding HIV-related information and immunization status, 84.6% of PLWH had been infected with HIV for over 2 years, with the majority (92.9%) receiving ART. 51.9% of PLWHs reported that their last HIV viral load test was undetectable, and 78.8% of PLWHs reported that their last CD4+ T cell count was over 200 cells/mm^3. There were no significant differences ($p > 0.05$) between the PLWH and healthy individual group regarding education level, monthly income, or prevalence of chronic underlying diseases. More details of the background characteristics can be found in Table 1.

3.2. Vaccination intention and adverse reactions

A significant difference was observed in the willingness to receive the second COVID-19 booster dose between the PLWH group and the healthy individual group (77.6% vs. 88.7%, $p = 0.009$).

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TABLE 1 Sociodemographic characteristics of 307 PLWH and healthy individuals.

	Total *n* (%) (*N* = 307)	PLWH *n* (%) (*N* = 156)	Non-PLWH *n* (%) (*N* = 151)	*p* value
Sociodemographic characteristics				
Age group (years)				
18–29	71 (23.1)	38 (24.4)	33 (21.9)	0.045
30–45	158 (51.5)	88 (56.4)	70 (46.4)	
46–59	68 (22.1)	28 (17.9)	40 (26.5)	
≥60	10 (3.3)	2 (1.3)	8 (5.3)	
Gender				
Male	281 (91.5)	150 (96.2)	131 (86.8)	0.003
Female	26 (8.5)	6 (3.8)	20 (13.2)	
Education level				
Junior high or below	44 (14.3)	18 (11.5)	26 (17.2)	0.249
Senior high or equivalent	98 (31.9)	48 (30.8)	50 (33.1)	
College and above	165 (53.7)	90 (57.7)	75 (49.7)	
Relationship status				
Single/divorced/widowed	214 (69.7)	129 (82.7)	85 (56.3)	<0.001
Married	93 (30.3)	27 (17.3)	66 (43.7)	
Employment status				
Full-time	190 (61.9)	105 (67.3)	66 (43.7)	0.047
Part-time/self-employed/unemployed/retired/students	117 (38.1)	51 (32.7)	85 (56.3)	
Monthly income (CNY)				
<3,000	71 (23.1)	33 (21.2)	38 (25.2)	0.692
3,000–6,999	181 (59.0)	95 (60.9)	86 (57.0)	
≥7,000	55 (17.9)	28 (17.9)	27 (17.9)	
Presence of chronic disease conditions (not including HIV)				
Yes	24 (7.8)	14 (9.0)	10 (6.6)	0.443
No	283 (92.2)	142 (91.0)	141 (93.4)	
Type of chronic diseases				
Diabetes mellitus	3 (1.0)	2 (1.3)	1 (0.7)	0.581
Hypertension and/or hyperlipidaemia	10 (3.3)	6 (3.8)	4 (2.6)	0.555
Chronic cardiovascular diseases[a]	3 (1.0)	2 (1.3)	1 (0.7)	0.581
Chronic respiratory diseases[b]	4 (1.3)	3 (1.9)	1 (0.7)	0.330
Other chronic diseases[c]	8 (2.6)	5 (3.2)	3 (2.0)	0.503
HIV related characteristics				
Time since HIV diagnosis (years)			N/A	
≤1		13 (8.3)		
2–5		76 (48.7)		
>5		56 (35.9)		
Not sure		11 (7.1)		
On antiretroviral therapy			N/A	
Yes		145 (92.9)		
No		11 (7.1)		
HIV viral load in the most recent episode of testing (copies/mL)			N/A	
Undetectable (<50)		81 (51.9)		
Detectable (≥50)		45 (28.8)		
Not sure		30 (19.2)		
CD4+ T cell count in the most recent episode of testing (cells/mm^3)			N/A	
>500		54 (34.6)		
200–500		69 (44.2)		
<200		6 (3.8)		
Not sure		27 (17.3)		

N/A, not applicable; CNY, Chinese yuan. [a]Chronic cardiovascular disease include chronic heart failure, coronary heart disease, congenital heart disease and valvar heart disease.
[b]Chronic respiratory diseases include chronic obstructive emphysema disease, asthma, chronic cor pulmonale and chronic respiratory failure.
[c]Other chronic diseases include malignant tumors, Immune thrombocytopenia, chronic hepatitis B, gout, etc.

Regarding adverse reactions within 1 month of receiving the first COVID-19 booster dose, 5.1% of the PLWH group and 2.0% of the healthy individual group reported adverse reactions. The primary adverse reactions were local, with 4.5% of the PLWH group and 2.0% of the healthy individual group experiencing them. In the PLWH group, the main complaint was pain at the inoculation site (4.6%), while in the healthy individual group, the main complaint was redness at the inoculation site (2.0%). No significant difference was found between the two groups in the incidence of adverse reactions, local adverse reactions, and systemic adverse reactions ($p > 0.05$). Table 2 presents the specific details of the adverse reactions.

3.3. Health belief model measures

Table 3 presents the attitudes of all participants regarding the six primary dimensions of the HBM and the specific items in each dimension. In five dimensions—perceived benefit, perceived susceptibility, perceived severity, action clues, and self-efficacy—the PLWH group scored significantly lower than the healthy individual group ($p < 0.001$). Conversely, in the dimension of perceived barriers, the PLWH group scored significantly higher than the healthy individual group (7.1% vs. 1.3%, $p = 0.004$). These results suggest that the PLWH group may face more obstacles and be less motivated to receive the COVID-19 booster than the healthy individuals group.

3.4. Factors associated with willingness to receive the second COVID-19 booster dose

Table 4 shows the results from the univariate analysis. Notably, willingness to receive a second COVID-19 booster dose was higher among those aged 18 to 29 years (90.1%) compared to those aged 30 years and older (79.1, 88.2, 60.0%). Similarly, those with a monthly income of 3,000 to 6,999 Yuan were more willing to receive the booster dose (87.8%) compared to those earning less than 3,000 Yuan (77.5%) and more than 7,000 Yuan (74.5%).

After adjusting for statistically significant sociodemographic characteristics, the outcome of lower willingness to receive the second booster dose was independently associated with HIV positivity (AOR: 0.39, 95%CI: 0.20, 0.75), perceived barriers (indicating the expectation of more adverse effects from the COVID-19 vaccine booster) (AOR: 0.05, 95%CI: 0.01, 0.26), and perceived severity (referring to negative attitudes toward the COVID-19 vaccine booster dose) (AOR: 0.32, 95%CI: 0.12, 0.89).

Conversely, a higher inclination towards receiving the second booster dose was associated with perceived benefits (indicating the expectation of more benefits from the COVID-19 vaccine booster) (AOR: 18.57, 95%CI: 4.02, 85.83) and (referring to better physical status after the vaccination) (AOR: 33.37, 95%CI: 4.22, 263.91). Furthermore, consistent with perceived benefits, a stronger inclination towards receiving the second booster dose showed positive correlations (AOR > 1 for all aforementioned variables) with perceived susceptibility, cues to action, self-efficacy, and detectable HIV viral load. Detailed information (e.g., AOR and 95% CI) can be referenced in Table 5.

4. Discussion

To our knowledge, this is the first multicenter cross-sectional study to explore the willingness of PLWH to receive a second COVID-19 booster dose and its influencing factors in China. Our findings suggest that PLWH were more hesitant to receive a second COVID-19 booster dose than the healthy population. The reasons for this hesitation appear to be multifactorial, with HIV infection status, more significant than expected adverse effects after the first COVID-19 booster dose, and negative attitudes toward the

TABLE 2 Adverse reaction after the first COVID-19 booster dose and the willingness regarding the second COVID-19 booster dose ($N = 307$).

	Total *n* (%) ($N = 307$)	PLWH *n* (%) ($N = 156$)	Non-PLWH *n* (%) ($N = 151$)	*p* value
Adverse reaction				
Adverse reactions within one month of the first COVID-19 vaccine booster dose	11 (3.6)	8 (5.1)	3 (2.0)	0.139
Local adverse reactions	10 (3.3)	7 (4.5)	3 (2.0)	0.362
Pain	6 (2.0)	4 (2.6)	2 (1.3)	0.685
Redness	4 (1.3)	1 (0.6)	3 (2.0)	0.365
Pruritus	4 (1.3)	3 (1.9)	1 (0.7)	0.623
Rash	2 (0.7)	2 (1.3)	0 (0.0)	0.498
Induration	1 (0.3)	1 (0.6)	0 (0.0)	>0.999
Systematic adverse reactions	1 (0.3)	1 (0.6)	0 (0.0)	>0.999
Headache	1 (0.3)	1 (0.6)	0 (0.0)	>0.999
Willingness to get the fourth dose of COVID-19 vaccine				
Whether you will receive the second COVID-19 vaccine booster dose				
Very unlikely/unlikely/neutral	52 (16.9)	35 (22.4)	17 (11.3)	0.009
Likely/very likely	255 (83.1)	121 (77.6)	134 (88.7)	

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TABLE 3 HBM items: perceived susceptibility, perceived severity, perceived benefits, perceived barriers, cues of action, and self-efficacy ($N = 307$).

	Total *n* (%) ($N = 307$)	PLWH *n* (%) ($N = 156$)	Non-PLWH *n* (%) ($N = 151$)	*p* value
Perceived benefits				
Feelings after the COVID-19 vaccination booster (the third dose)				
Benefits of COVID-19 vaccination booster compared to expectations				
More (some more/a lot more)	194 (63.2)	88 (56.4)	106 (70.2)	0.033
No change	101 (32.9)	62 (39.7)	39 (25.8)	
Less (less/much less)	12 (3.9)	6 (3.8)	6 (4.0)	
Physical status after COVID-19 vaccination booster compared to expectations				
Good (better/much better)	125 (40.7)	44 (28.2)	81 (53.6)	<0.001
No change	173 (56.4)	105 (67.3)	68 (45.0)	
Poor (worse/much worse)	9 (2.9)	7 (4.5)	2 (1.3)	
Perceived barriers				
Adverse effects (adverse events or side effects) of COVID-19 vaccine booster compared to expected				
More (some more/a lot more)	13 (4.2)	11 (7.1)	2 (1.3)	0.004
No change	127 (41.4)	72 (46.2)	55 (36.4)	
Less (less/much less)	167 (54.4)	73 (46.8)	94 (62.3)	
Perceived susceptibility				
Positive attitudes toward COVID-19 vaccine booster dose (agree/strongly agree)				
Receiving a booster dose can maintain your antibody level and strengthen the protection against COVID-19	226 (73.6)	96 (61.5)	130 (86.1)	<0.001
A booster dose is highly effective in protecting you from COVID-19 variants of concern (e.g., Omicron)	237 (77.2)	110 (70.5)	127 (84.1)	0.006
There is a sufficient supply of COVID-19 vaccine in China to strengthen the vaccination work for many times	251 (81.8)	118 (75.6)	133 (88.1)	0.007
Perceived severity				
Negative attitudes toward COVID-19 vaccine booster dose (agree/strongly agree)				
The side effects of COVID-19 vaccine booster dose are more severe	22 (7.2)	8 (5.1)	14 (9.3)	<0.001
Multiple vaccinations to strengthen the needle will bring unknown long-term health risks	20 (6.5)	6 (3.8)	14 (9.3)	<0.001
The duration of protection of COVID-19 vaccine booster dose is shorter	31 (10.1)	12 (7.7)	19 (12.6)	<0.001
Cues of action				
People who are important to you (e.g., family member, doctors) would support you to receive a booster dose	234 (76.2)	100 (64.1)	134 (88.7)	<0.001
Self-efficacy				
Receiving a COVID-19 vaccine booster dose is easy for you if you want to	240 (78.2)	107 (68.6)	133 (88.1)	<0.001

COVID-19 vaccine booster dose being the main factors contributing to vaccine hesitancy. Our findings provide important insights into the willingness of PLWH in China to receive a second COVID-19 booster dose and the associated factors influencing this decision. Moreover, our results could inform both the theoretical framework and practical measures for institutions aiming to understand and address the vaccination intentions of PLWH and the factors influencing them. This, in turn, may assist in designing more effective public health interventions and educational campaigns for PLWH, aiming to boost vaccination

TABLE 4 Univariate logistic regression of participants' sociodemographic characteristics ($N = 307$).

Variable	Whether you will receive the second COVID-19 booster dose (the fourth dose)		
	Vaccine acceptance *n*/*N* (%)	Odds Ratio (95% CI)	*p* value
Sociodemographic characteristics			
Age group (years)			
18–29	64/71 (90.1)	Reference	
30–45	125/158 (79.1)	0.41 (0.17–0.99)	0.047
46–59	60/68 (88.2)	0.82 (0.28–2.40)	0.718
≥60	6/10 (60.0)	0.16 (0.04–0.73)	0.017
Gender			
Female	21/26 (80.8)	Reference	
Male	234/281 (83.3)	1.19 (0.43–3.30)	0.745
Education level			
Junior high or below	35/44 (79.5)	Reference	
Senior high or equivalent	88/98 (89.8)	2.26 (0.85–6.04)	0.103
College and above	132/165 (80.0)	1.03 (0.45–2.35)	0.947
Relationship status			
Single/divorced/widowed	174/214 (81.3)	Reference	
Married	81/93 (87.1)	1.55 (0.77–3.12)	0.217
Employment status			
Part-time/self-employed/unemployed/ retired/students	94/117 (80.3)	Reference	
Full-time	161/190 (84.7)	1.36 (0.74–2.48)	0.320
Monthly income (Yuan)			
<3,000	55/71 (77.5)	Reference	
3,000–6,999	159/181 (87.8)	2.10 (1.03–4.29)	0.041
≥7,000	41/55 (74.5)	0.85 (0.37–1.94)	0.703
Presence of chronic disease conditions (not including HIV)			
No	236/283 (83.4)	Reference	
Yes	19/24 (79.2)	0.76 (0.27–2.13)	0.597

Statistically significant values are identified in boldface ($\alpha < 0.05$). CI: confidence interval.

coverage and minimize the risk of co-infection and severe clinical outcomes.

Our study observed a lower willingness among PLWH to receive a second COVID-19 booster dose compared to full immunization and the first COVID-19 booster dose reported in the United States, Italy, and Latin America and the Caribbean (78–86.2%) (29–31). One possible explanation for this disparity might be that over time, China's measures to control COVID-19 have not diminished, yet the prolonged duration of such controls has engendered a sense of fatigue among the population. Consequently, this has led to PLWH beginning to underestimate the pathogenic potential of SARS-CoV-2 and its variants. At the same time, our study corroborates previous findings in healthy individuals in China indicating a high willingness to receive a second COVID-19 booster dose (81.1% vs. 88.7%) (32). However, our study, after adjusting for potential confounders, revealed a significant association between HIV status and vaccine hesitancy for the second COVID-19 booster dose. This persistent vaccine hesitancy among PLWH in China warrants further investigation, despite demonstrated safety and preventative efficacy of the fourth COVID-19 dose and ongoing promotion by relevant health departments (15). It suggests the need for targeted interventions and education to address the factors contributing to this hesitancy.

To date, abundant studies investigating COVID-19 vaccine acceptance among PLWH has generated valuable insights into the underlying influencing factors. For instance, a cross-sectional study demonstrated that negative attitudes towards prime vaccines was associated with the diminished likelihood of vaccine acceptance in China (33). Conversely, individuals with positive perceptions of the prime COVID-19 vaccine exhibited higher rates of acceptance. Furthermore, a positive association between the booster vaccine acceptance and beliefs in the safety, benefits, and accessibility of the booster vaccine was also proved in Uganda (22). Our study further demonstrated that negative attitudes towards vaccines and perceived barriers were both associated with reduced acceptance of the second COVID-19 booster Dose. Similar results were found in immunocompromised cancer patients, which strengthened the necessity of vaccination among specific populations (34, 35).

TABLE 5 Univariate and multivariable analysis of factors associated with willing to receive the second COVID-19 booster dose ($N = 307$).

Variable	Willing to receive the second COVID-19 booster dose			
	OR (95% CI)	*p* value	AOR (95% CI)	*p* value
HIV infection status				
Negative	Reference		Reference	
Positive	0.44 (0.23–0.82)	0.010	0.39 (0.20–0.75)	0.005
HIV viral load in the most recent episode of testing (copies/mL)				
Undetectable (<50)	Reference		Reference	
Detectable (≥50)	5.55 (1.56–19.71)	0.008	4.98 (1.35–18.37)	0.016
Not sure	0.93 (0.37–2.32)	0.868	0.77 (0.28–2.14)	0.622
CD4+ T cell count in the most recent episode of testing (cells/mm^3)				
>500	Reference		Reference	
200–500	1.06 (0.11–9.92)	0.963	1.22 (0.13–11.91)	0.865
<200	0.44 (0.05–4.02)	0.463	0.59 (0.06–5.83)	0.651
Not sure	0.70 (0.07–7.20)	0.764	0.82 (0.08–8.81)	0.867
Adverse reaction				
Adverse reactions within one month of the first COVID-19 booster dose				
No	Reference		Reference	
Yes	0.92 (0.19–4.36)	0.911	0.65 (0.13–3.29)	0.603
Perceived benefits				
Feelings about the first COVID-19 booster dose				
Benefits of COVID-19 vaccination booster compared to expectations				
Less (less/much less)	Reference		Reference	
No change	0.70 (0.20–2.49)	0.584	0.81 (0.22–2.98)	0.754
More (some more/a lot more)	15.67 (3.68–66.76)	<0.001	18.57 (4.02–85.83)	<0.001
Physical status after COVID-19 vaccination booster compared to expectations				
Poor (worse/much worse)	Reference		Reference	
No change	1.34 (0.32–5.58)	0.687	1.30 (0.30–5.60)	0.729
Good (better/much better)	30.75 (4.30–220.04)	0.001	33.37 (4.22–263.91)	0.001
Perceived barriers				
Adverse effects (adverse events or side effects) of COVID-19 vaccine booster compared to expectation				
Less (less/much less)	Reference		Reference	
No change	0.05 (0.02–0.13)	<0.001	0.05 (0.02–0.15)	<0.001
More (some more/a lot more)	0.06 (0.01–0.26)	<0.001	0.05 (0.01–0.26)	<0.001
Perceived susceptibility				
Positive attitudes toward COVID-19 vaccine booster dose				
Receiving a booster dose can maintain your antibody level and strengthen the protection against COVID-19				
Disagree/strongly disagree/neutrality	Reference		Reference	
Agree/strongly agree	23.26 (10.78–50.21)	<0.001	28.65 (12.27–66.92)	<0.001
A booster dose is highly effective in protecting you from COVID-19 variants of concern (e.g., Omicron)				
Disagree/strongly disagree/neutrality	Reference		Reference	
Agree/strongly agree	18.92 (9.24–38.71)	<0.001	18.77 (8.81–39.99)	<0.001
There is a sufficient supply of COVID-19 vaccine in China to strengthen the vaccination work for many times				
Disagree/strongly disagree/ neutrality	Reference		Reference	
Agree/strongly agree	26.44 (12.55–55.70)	<0.001	33.14 (13.94–78.83)	<0.001

(Continued)

TABLE 5 (Continued)

Variable	Willing to receive the second COVID-19 booster dose			
	OR (95% CI)	*p* value	AOR (95% CI)	*p* value
Perceived severity				
Negative attitudes toward COVID-19 vaccine booster dose				
The side effects of COVID-19 booster dose are more severe				
Disagree/strongly disagree/neutrality	Reference		Reference	
Agree/strongly agree	0.40 (0.16–1.04)	0.060	0.32 (0.12–0.89)	0.030
Multiple vaccinations to strengthen the needle will bring unknown long-term health risks				
Disagree/strongly disagree/neutrality	Reference		Reference	
Agree/strongly agree	0.35 (0.13–0.91)	0.032	0.32 (0.12–0.90)	0.031
The duration of protection of COVID-19 vaccine booster dose is shorter				
Disagree/strongly disagree/neutrality	Reference		Reference	
Agree/strongly agree	1.42 (0.48–4.25)	0.529	1.47 (0.47–4.54)	0.508
Cues of action				
People who are important to you (e.g., family member, doctors) would support you to receive a booster dose				
Disagree/strongly disagree/neutrality	Reference		Reference	
Agree/strongly agree	30.35 (13.84–66.56)	<0.001	28.89 (12.93–64.57)	<0.001
Self-efficacy				
Receiving a COVID-19 vaccine booster dose is easy for you if you want to				
Disagree/strongly disagree/neutrality	Reference		Reference	
Agree/strongly agree	21.15 (10.25–43.65)	<0.001	19.87 (9.39–42.04)	<0.001

OR, crude odds ratios; AOR, adjusted odds ratios, odds ratios adjusted for significant Sociodemographic characteristics listed in Table 3; CI, confidence interval.

Our study is the first to investigate the relationship between the willingness of PLWH in China to receive the second COVID-19 booster dose and the six main dimensions of the HBM. These dimensions include perceived susceptibility, perceived severity, perceived benefits, perceived barriers, cues to action, and self-efficacy. Our findings indicate that perceived barriers negatively correlate with vaccine willingness, suggesting fears and misconceptions may dissuade PLWH from receiving the booster dose (36). On the contrary, perceived benefits were positively associated with vaccine willingness, highlighting the potential impact of understanding the benefits of vaccination in promoting vaccine acceptance. Interestingly, we found a positive correlation between perceived susceptibility and vaccine willingness, suggesting that individuals at risk of contracting COVID-19 may be more willing to get vaccinated. However, perceived severity was negatively associated with vaccine willingness, which could indicate that those who perceive COVID-19 as a severe disease may have heightened fears about the safety of vaccines (37). We also noted a positive correlation between self-efficacy and preventive behavior, reinforcing that individual belief in their ability to take preventive measures successfully can influence their willingness to vaccinate (38, 39). Finally, our findings showed a positive correlation between cues to action and vaccine willingness. This implies that support and encouragement from family, friends, and doctors could be critical in promoting vaccination among PLWH (33, 40).

In light of these findings, health departments in China should amplify their efforts to communicate the benefits of the second COVID-19 booster dose. This includes providing clear and reassuring information about the vaccine's safety, encouraging social support networks to promote vaccination, and fostering a sense of self-efficacy among PLWH. Addressing these factors can reduce vaccine hesitancy and increase the second COVID-19 booster dose uptake among PLWH.

Our multivariable logistic regression analysis revealed that PLWH with a detectable HIV viral load (≥50 copies/mL) demonstrated a higher willingness to receive the second COVID-19 booster dose than those with an undetectable viral load (<50 copies/mL). This result diverges from a US study, which reported a higher willingness to vaccinate among PLWH with an undetectable HIV viral load (29). The discrepancy could be attributed to differences in study design, participant demographics, cultural attitudes towards vaccination, or the methodology of obtaining HIV viral load data. However, the impact of these factors should be further investigated in future studies.

This study has important practical implications, as it found that the willingness of PLWH to receive the second COVID-19 booster dose in China is notably lower than that of the general adult population. It identifies inhibiting factors such as perception barriers and negative attitudes, suggesting a need for targeted educational campaigns to enhance booster vaccine coverage among PLWH.

However, several limitations in our study should be acknowledged: First, as with all cross-sectional studies, establishing causal relationships between independent variables and different outcomes

of interest is impossible. Longitudinal studies or randomized controlled trials would be needed to examine causal relationships. Second, subjectively self-administered questionnaires may introduce recall bias, which is difficult to avoid considering the need for anonymity in our study. Future research could consider using alternative methods, such as structured interviews or electronic data collection, to minimize this bias. Third, while most of the items and scales used in this study were self-constructed based on those used in the general population, the external validation of these measures was limited. Further research should seek to validate these measures against established scales or through other external validation methods. Finally, there were variations in the distribution of sociodemographic characteristics between the two groups. Although we adjusted for these characteristics in the multivariable logistic regression model, their potential impact on the study results should be considered. Future studies could explore the potential influence of these characteristics on vaccine willingness more comprehensively and consider other statistical techniques, such as propensity score matching, to address these imbalances.

5. Conclusion

In conclusion, our study highlights the lower willingness of Chinese PLWH to receive a second COVID-19 booster dose compared to healthy individuals. Concerns about adverse effects and negative attitudes toward the booster dose primarily drive this reluctance. Strengthening and promoting knowledge about HIV and COVID-19 vaccination, including the importance of vaccine protection against SARS-CoV-2, is crucial. Based on the findings of this study, targeted interventions should be implemented to increase the willingness of PLWH to receive the second COVID-19 booster dose. This may include tailored education and communication strategies, providing comprehensive information and support, and engaging community resources to address the specific concerns and needs of PLWH.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JX was responsible for the conceptualization of the study and funding acquisition. XL was responsible for data curation and analysis. JX, MS, SL, MY, BS, YQ, and LW were responsible for project administration and securing resources. JX and YW were responsible for supervision and reviewing and editing the article. XL and YW were responsible for writing the original draft of the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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High mortality rates among COVID-19 intensive care patients in Iraq: insights from a retrospective cohort study at Médecins Sans Frontières supported hospital in Baghdad

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Background: The Coronavirus Disease 2019 (COVID-19) pandemic has highlighted the challenges of the healthcare system in Iraq, which has limited intensive care unit beds, medical personnel, and equipment, contributing to high infection rates and mortality. The main purpose of the study was to describe the clinical characteristics, the length of Intensive Care Unit (ICU) stay, and the mortality outcomes of COVID-19 patients admitted to the ICU during the first wave and two subsequent surges, spanning from September 2020 to October 2021, in addition to identify potential risk factors for ICU mortality.

Methods: This retrospective cohort study analyzed data from COVID-19 patients admitted to the COVID-19 ICU at Al-Kindi Ministry of Health hospital in Baghdad, Iraq, between September 2020 and October 2021.

Results: The study included 936 COVID-19 patients admitted to the ICU at Al-Kindi Hospital. Results showed a high mortality rate throughout all waves, with 60% of deaths due to respiratory failure. Older age, male gender, pre-existing medical conditions, ICU procedures, and complications were associated with increased odds of ICU mortality. The study also found a decrease in the number of complications and ICU procedures between the first and subsequent waves. There was no significant difference in the length of hospital stay between patients admitted during different waves.

Conclusion: Despite improvements in critical care practices, the mortality rate did not significantly decrease during the second and third waves of the pandemic. The study highlights the challenges of high mortality rates among critical COVID-19 patients in low-resource settings and the importance of effective data collection to monitor clinical presentations and identify opportunities for improvement in ICU care.

KEYWORDS

ICU outcomes, COVID-19, Iraq, Baghdad, ICU mortality, healthcare, limited resource settings, humanitarian

Background

Since the start of the outbreak of the coronavirus disease 2019 (COVID-19), critical patients have required advanced level of intensive medical care (1). The global pandemic has placed a significant strain on the availability of intensive care unit (ICU) beds, leading many countries to implement strategies to improve access and efficiency of intensive care. COVID-19 patients admitted to the ICU need a prolonged hospital stay under a treatment regimen that includes anti-viral or steroid therapy, together with supplemental oxygen often through invasive mechanical ventilation (IMV) (2–4). During the course of this pandemic, low- and middle-income countries (LMICs) were particularly disadvantaged due to their limited resources and difficulty in rapidly expanding ICU bed capacity, providing sufficient oxygen, and maintaining quality of care (5).

The in-hospital mortality rates among critically ill COVID-19 patients remained high throughout the course of the pandemic. A systematic review and meta-analysis of 52 studies revealed an overall ICU mortality rate of 35.5% for COVID-19 patients. The Middle East region had the highest reported mortality rate at 61.9% (1). Older age, male gender, smoking, obesity, co-existing conditions, and complications have been established as major risk factors for increased severity and mortality of COVID-19 globally (6–10). The magnitude of these risk factors is influenced by the context and underlying patient characteristics and clinical conditions.

Iraq was especially vulnerable during the COVID-19 pandemic due to its inadequate healthcare system and limited capacity (11, 12). At the time of the COVID-19 declaration, the country had less than 1,000 ICU beds and continued to struggle with shortages in medical personnel and equipment (13, 14). The pandemic also resulted in a shortage in oxygen supply forcing patients to transfer management to their homes instead of hospital. Additionally, the infection prevention and control measures in Iraq were inadequate, leading to high numbers of cases among healthcare workers and deterring patients from seeking care (15). Over the course of 2020 to 2021, over two million confirmed COVID-19 cases and 24,000 deaths were reported in Iraq (16). Studies from Iraq reported high infection rates among males and predominantly among those aged 30–60 years (14). Furthermore, reports have shown that individuals with chronic conditions, older age, and male gender have a higher risk of mortality, which aligns with the established risk factors globally (17–19).

The Al Kindi Hospital in Baghdad, a tertiary facility under the jurisdiction of the Ministry of Health (MOH), was designated as a center for the isolation and treatment of patients with suspected or confirmed cases of SARS-CoV-2. From July 2020 to October 2021, the Intensive Care Unit was operated with support from Médecins Sans Frontières (MSF), also known as Doctors Without Borders, offering the most optimal medical care and limited to non-invasive mechanical ventilation (Continuous Positive Airway Pressure (CPAP), Flow O_2 25, and ICU ventilators). Invasive mechanical ventilation was not provided, in accordance with the MOH mandate. The focus of the MSF collaboration was to support and improve ICU management, starting with a capacity of 24 beds in September 2020 and gradually increasing to 55 beds by the end of October 2021.

The present study describes the clinical characteristics, the length of Intensive Care Unit (ICU) stay, and the mortality outcomes of COVID-19 patients admitted to the ICU during the first wave and two subsequent surges, spanning from September 2020 to October 2021. The aim of this analysis is to identify potential risk factors for ICU mortality among patients, and to evaluate if there was any improvement in the quality of care, as evidenced by decreased complications, shorter ICU duration, and reduced mortality rate across various stages of the epidemic in Iraq.

Methods

Study design

This study is a retrospective cohort study that analyzed data from confirmed and suspected COVID-19 patients admitted to the COVID-19 ICU at Al-Kindi MOH Hospital in Baghdad, Iraq between September 26, 2020 and October 13, 2021.

Study setting and population

The first confirmed case reported in Iraq was on 24 February 2020 in the Najaf governorate [15]. Consequently, Iraq experienced the first COVID-19 wave between February 2020 and January 2021 reaching a weekly peak of 30,059 confirmed cases and 708 deaths in week 37. The two subsequent waves were recorded in 2021; wave two from 18 January to 17 May 2021 and wave three from 24 May too 27 December 2021 recordeded weekly peaks of 54,301 and 83,098 cases including 281 and 522 deaths, respectively (Figure 1) (20). Accordingly, the study period was divided into three time periods based on the three waves of COVID-19 in Iraq and during which patients were admitted to the MSF supported COVID-19 ICU in Al-Kindi MOH Hospital. The follow-up time was defined from ICU admission to discharge, referral or death.

In this study, we included all patients admitted during the study period and who met the clinical and epidemiological criteria (21). The clinical presentation defined as critical or severe case requiring oxygen support of 7 L/min or more to achieve oxygen saturation levels above 92%. We excluded patients who refused or failed a trial of CPAP in the ER, had a Glasgow Coma Scale (GCS) score lower than 9, were too unstable for transfer, had an untreated medical or surgical problem requiring urgent intervention, or had metastatic cancer. Only patients with complete data on survival outcomes were included in the study.

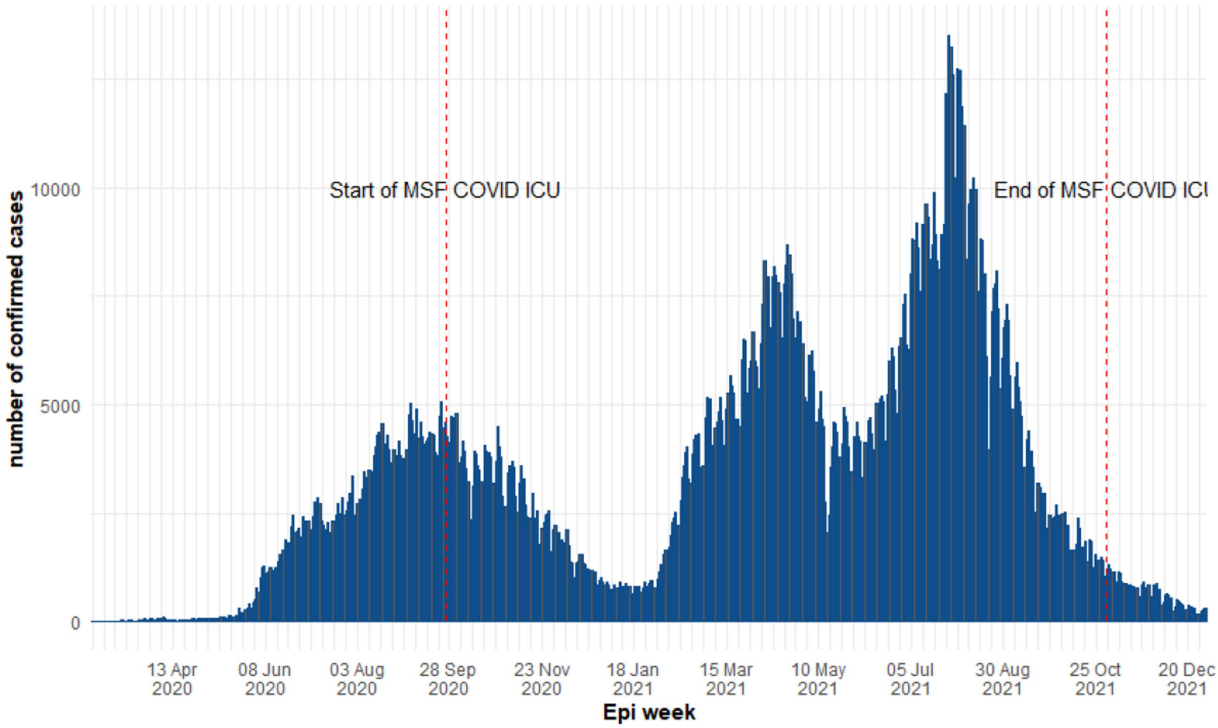

FIGURE 1
Epidemiological curve of COVID-19 daily cases in Iraq between 2020 and 2021 (World Health Organization).

Variables and data sources

Demographic and clinical data were collected and recorded in an Excel-based database by the ICU team as part of routine data collection. However, laboratory findings and treatment regimens provided were not recorded in the database. The severity of the disease was categorized according to the WHO definition. COVID-19 diagnosis was confirmed with a positive reverse transcription-polymerase chain reaction (RT-PCR) test or chest CT scan as determined by the clinical team.

The variables included in the database consisted of the following:

- Demographic characteristics: age and sex of the ICU patients.
- Pre-existing medical conditions: Information regarding any underlying health conditions or comorbidities reported by the study participants for the majority of conditions. As for obesity condition, it was based on BMI results, yet there was no differentiation between obesity and morbid obesity in data collection.
- ICU admission and exit dates: The dates of admission to and discharge from the intensive care unit were recorded to determine the length of ICU stay.
- Chest computerized tomogram (CT) scan findings: Findings from chest CT scans were documented to assess the characteristic features of COVID-19 infection. The primary radiological findings indicative of COVID-19 were bilateral patchy areas of ground glass infiltration/appearance, predominantly observed in lower lobes and periphery of the lungs.
- Non-invasive ventilation: Information on the use of non-invasive ventilation techniques among the COVID-19 patients.
- ICU-related procedures: Any procedures performed on the patients during their stay in the ICU.
- Complications: The occurrence of any complications or adverse events during the ICU stay.
- Outcomes: The final outcomes of the COVID-19 patients.

To ensure data quality control, the ICU team diligently recorded the data during routine patient care under the support of data manager and epidemiologist who implemented data validation techniques after entry.

Study outcomes

The primary outcome of the study was to assess the risk factors associated with ICU mortality and length of stay. The secondary outcome was to evaluate the changes in the clinical and demographic characteristics and outcomes between the different COVID-19 waves during the study period.

Statistical analysis

The sample size was equal to the total number of patients admitted to the ICU during the study period. Descriptive statistics were performed based on the STROBE checklist for observational studies (22). Categorical variables were presented as frequencies and percentages, and continuous variables were reported as medians (interquartile ranges). Differences between groups were assessed using chi-squared or Fisher's exact tests for categorical variables and

Student's t-test or the Kruskal-Wallis rank sum test for continuous variables, as appropriate. Univariate and multiple logistic regression models were used to identify associations between patient characteristics and ICU mortality. A stepwise selection approach based on the Akaike Information Criterion (AIC) to identify the best-fitting regression model was used. The results of the multiple regression analysis were reported as odds ratios (OR) with their 95% confidence intervals (CI). Kaplan–Meier survival curves were plotted and were compared using log-rank test. A level of significance of $p < 0.05$ was considered statistically significant. Missing data were reported in results and were not imputed. Data analysis was performed using R Studio software, version 3.6.3.

Ethical considerations

This study was approved by the Research Committee of the National Centre for Training and Human at the Ministry of Health and Environment, Baghdad, Iraq on 31 May 2022 (protocol number 10/2022) and was exempt from the MSF Ethics Review Board following the approval of the Medical Director.

Results

Baseline characteristics of the study population

The study population consisted of 936 patients admitted to the ICU at Al-Kindi Hospital in Iraq between September 26, 2020 and October 13, 2021. After removing duplicated records and missing data, 924 patients were included in the analysis, with 145 (16%) admitted during the first wave, 425 (46%) during the second wave, and 355 (38%) during the third wave (Figure 2). The majority of patients were men (59%, $n = 545$) with a median age of 60 years (IQR 50–68) and over half of the study cohort had at least one pre-existing medical condition (55%, $n = 511$). The most common pre-existing conditions were cardiovascular disease (38%, n = 354), followed by diabetes (28%, $n = 260$) and hypertension (27%, $n = 246$) (Table 1).

Changes of characteristics and clinical outcomes between waves

Patients admitted in the second and third waves were younger (61[IQR 52,70] and 56[IQR 47,64] vs. 64 [IQR 54,71], $p < 0.001$) and had higher incidence of pre-existing medical conditions compared to the first wave, such as cardiovascular diseases (45, 41% vs. 12%, $p < 0.001$), diabetes (32, 33% vs. 6.2%, $p < 0.001$), hypertension (38, 20% vs. 7.6%, $p < 0.001$), and obesity (14, 10% vs. 3.4%, $p = 0.001$). The distribution of pre-existing conditions shifter across waves. In the first wave, most patients (77%) had no pre-existing conditions, whereas in the second (44%) and third waves (37%), the majority had two or more ($p < 0.001$). The RT-PCR showed a lower rate of negative results in the third wave (13% vs. 29, 26%, $p < 0.001$), while the CT scan results showed no significant difference between waves (44, 38 and 46% respectively, $p = 0.086$). Awake prone positioning was performed in over 80% of patients throughout the waves, reaching 98% in the third wave ($p < 0.001$). However, procedures such as thoracostomy, blood transfusion, and central line were significantly reduced between the first and subsequent waves ($p < 0.001$). The number of complications observed in the hospital decreased from 36% in the first to 24% in the second and 4.5% in the third wave ($p < 0.001$).

Mortality outcomes

Patients were categorized as either survivors (351/924) and non-survivors (573/924) at discharge or death. The weekly death rate was consistently high throughout the three waves (Figure 3) with 60% ($n = 345$) of deaths due to respiratory failure, followed by multi-organ failure (29%, $n = 168$), septic shock (4%, $n = 23$) and cardiogenic shock (2.4%, $n = 14$). Univariate analysis is presented in Table 2.

A multiple logistic regression analysis showed that being 60 years or older, male, and having a pre-existing condition, undergoing aware prone positioning or other ICU procedures, and experiencing ICU complications were all significantly associated with increased odds of ICU mortality (Figure 4).

The median duration between ICU admission and discharge was 11 days (IQR [6, 20]) and that between ICU admission and mortality was 9 days (IQR [5, 15]). Kaplan–Meier curves for different age groups and among those admitted during different waves are shown in Figures 5, 6. The median hospital stay was longer for patients aged 60 or older (10 days, IQR[5–17]) compared to younger patients (9 days, IQR[5–16] log-rank test $p < 0.001$), but there was no statistically significant difference in the length of stay between patients admitted during different waves (log-rank test $p = 0.25$).

Discussion

In this study, the clinical features and outcomes of COVID-19 intensive care unit (ICU) patients who received non-invasive mechanical ventilation at a Ministry of Health (MOH) hospital in Baghdad, Iraq were analyzed. Results showed a considerable ICU mortality rate reaching 63%. We demonstrated that male sex, older age, pre-existing medical conditions, shorter ICU stay, ICU procedures and complications during admission were independent predictors of ICU mortality. After adjusting for baseline and clinical characteristics, no significant change in mortality rates was observed among our study participants between different waves of the COVID-19 epidemic in Iraq.

Globally, poor outcomes and elevated mortality rates of COVID-19 patients in the ICU have been widely reported, with a range of 10–78% (1, 23). An updated meta-analysis of observational studies indicated higher mortality rates during the early months of the pandemic followed by improved outcomes attributed to better therapeutics and clinical management (1). The initial reports from China showed a case-fatality rate of 49% among ICU patients. Similarly, high ICU mortality rates were also reported in high-income settings, such Europe and the United States (24–26).

Despite the limited studies on COVID-19 ICU patients in the Middle East, the high ICU mortality rate observed in our study can be compared to other reported rates in the region of similar contextual challenges. For example, a report from MSF in conflict settings of

TABLE 1 Baseline characteristics and clinical outcomes by COVID-19 waves of ICU patients admitted to Al-kindi MOH Hospital, Baghdad, Iraq. between 26 Sep 2020 and 13 Oct 2021

	Overall *N* = 924	1st wave *n* = 145	2nd wave *n* = 424	3rd wave *n* = 355	*p*-value[a]
Demographics characteristics					
Age in years, (median, IQR)	60 (50, 68)	64 (54, 71)	61 (52, 70)	56 (47, 64)	<0.001
Missing data	1	–	1	–	
Sex, *n* (%)					0.6
Female	379 (41%)	56 (39%)	181 (43%)	142 (40%)	
Male	545 (59%)	89 (61%)	243 (57%)	213 (60%)	
Pre-existing medical condition[b], *n* (%)					
At least one pre-existing condition	511 (55%)	33 (23%)	263 (62%)	215 (61%)	<0.001
Cardiovascular disease	354 (38%)	18 (12%)	192 (45%)	144 (41%)	<0.001
Diabetes	260 (28%)	9 (6.2%)	135 (32%)	116 (33%)	<0.001
Hypertension	246 (27%)	11 (7.6%)	163 (38%)	72 (20%)	<0.001
Obesity	100 (11%)	5 (3.4%)	60 (14%)	35 (9.9%)	0.001
Renal disease	33 (3.6%)	6 (4.1%)	21 (5.0%)	6 (1.7%)	0.047
Lung disease	32 (3.5%)	2 (1.4%)	20 (4.7%)	10 (2.8%)	0.12
Cerebrovascular disease	27 (2.9%)	5 (3.4%)	15 (3.5%)	7 (2.0%)	0.4
Other[b]	38 (4.1%)	5 (3.4%)	20 (4.7%)	13 (3.7%)	0.7
No. of pre-existing medical conditions, *n* (%)					< 0.001
None	413 (45%)	112 (77%)	161 (38%)	140 (39%)	
One	179 (19%)	17 (12%)	77 (18%)	85 (24%)	
Two or more	332 (36%)	16 (11%)	186 (44%)	130 (37%)	
COVID-19 Diagnosis, *n* (%)					
PCR result					<0.001
Negative PCR Result	161 (21%)	35 (29%)	86 (26%)	40 (13%)	
Positive PCR Result	603 (79%)	87 (71%)	242 (74%)	274 (87%)	
Missing data	*160*	*23*	*96*	*41*	
CT scan result					0.086
Non-suggestive of Covid-19	367 (42%)	62 (44%)	158 (38%)	147 (46%)	
Suggestive of Covid-19	507 (58%)	79 (56%)	256 (62%)	172 (54%)	
Missing data	*50*	*4*	*10*	*36*	
ICU procedures, *n* (%)					
At least one ICU procedure	832 (90%)	124 (86%)	361 (85%)	347 (98%)	<0.001
Prone positioning	824 (89%)	119 (82%)	358 (84%)	347 (98%)	<0.001
Thoracostomy tube	18 (1.9%)	8 (5.5%)	9 (2.1%)	1 (0.3%)	<0.001
Blood transfusion	14 (1.5%)	12 (8.3%)	2 (0.5%)	0 (0%)	<0.001
Central line	14 (1.5%)	9 (6.2%)	3 (0.7%)	2 (0.6%)	<0.001
Enteral nutrition	12 (1.3%)	5 (3.4%)	7 (1.7%)	0 (0%)	0.002
Parenteral nutrition	5 (0.5%)	0 (0%)	3 (0.7%)	2 (0.6%)	0.9
Other procedures[c]	17 (1.8%)	3 (2.1%)	13 (3.1%)	1 (0.3%)	0.007
ICU complications, *n* (%)					
Complications	170 (18%)	52 (36%)	102 (24%)	16 (4.5%)	<0.001
Nasal sore	134 (15%)	30 (21%)	88 (21%)	16 (4.5%)	<0.001
Bed sore	55 (6%)	10 (6.9%)	37 (8.7%)	8 (2.3%)	<0.001
Urinal catheter infection	22 (2.4%)	18 (12%)	4 (0.9%)	0 (0%)	<0.001
Ventilator-associated pneumonia (clinical)	16 (1.7%)	7 (4.8%)	9 (2.1%)	0 (0%)	<0.001
Equipment failure	4 (0.4%)	3 (2.1%)	1 (0.2%)	0 (0%)	0.013
Central line infection	2 (0.2%)	2 (1.4%)	0 (0%)	0 (0%)	0.024
Death at ICU, *n* (%)	573(62%)	85 (61%)	268 (63%)	220 (62%)	0.6

[a]Kruskal-Wallis rank sum test; Pearson's Chi-squared test; Fisher's exact test.

[b]Each patient could have more than one pre-existing condition, so the sum of percentages may not add up to 100.

[c]Other procedures include hemodialysis ($n = 4$) and NG tube insertion ($n = 1$), the remaining other procedures were not captured comprehensively in the study database.

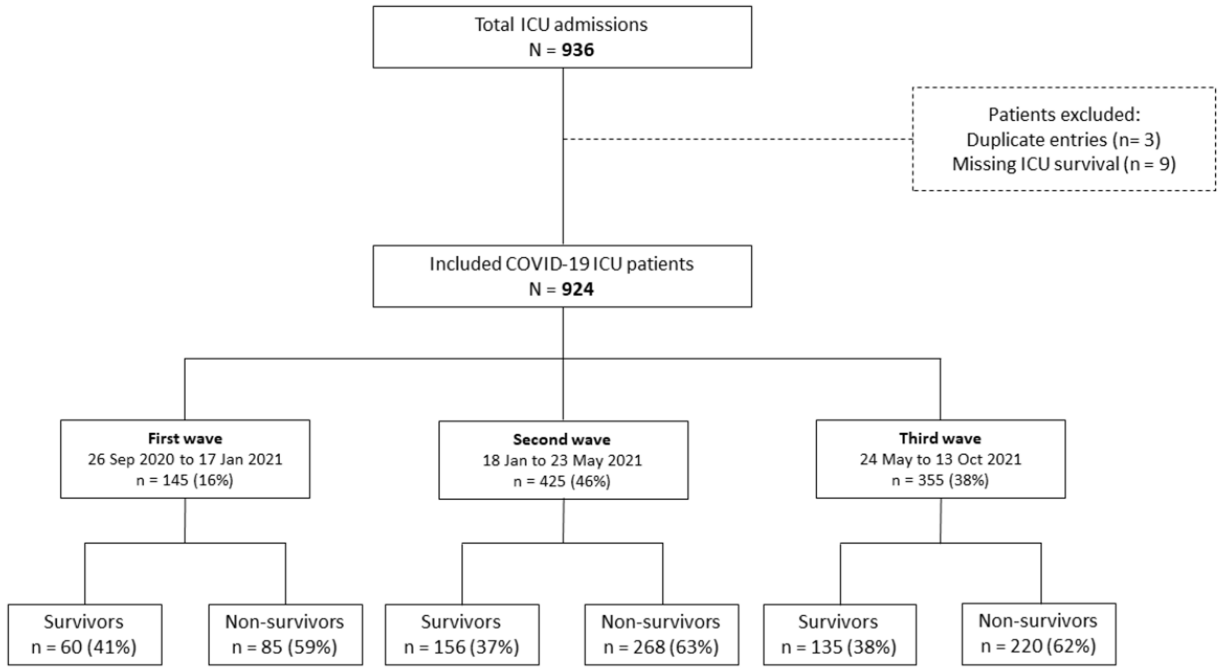

FIGURE 2
Study flowchart of COVID-19 ICU patients admitted at Al-Kindi MOH Hospital, Baghdad.

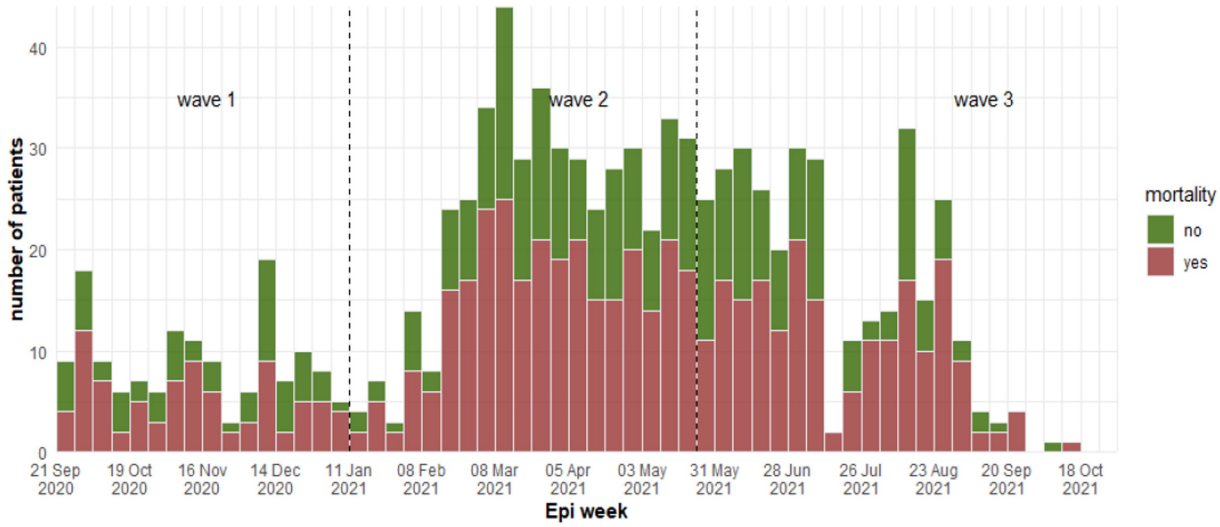

FIGURE 3
Weekly number of fatality cases between waves at Al-Kindi Hospital, Baghdad Iraq 2020–2021.

Yemen revealed an ICU mortality rate of 68%, while a multi-center study in Libya reported an ICU mortality rate of 60.4% (27, 28). In Jordan, a hospital treating ICU COVID-19 patients had a much higher mortality rate of 93.8% among their ICU patients (29). In contrast, an ICU in Egypt had a much lower mortality rate of 24.4% (30) and Lebanon reported 55% among their ICU patients (31). Our results also demonstrate a higher mortality rate compared to other low-resource settings in Africa, which reported an ICU mortality rate of 48.2% (32). Possible reasons behind the variations in ICU mortality rates may include differences in clinical management, access to therapeutics, healthcare infrastructure and staff training in Iraq versus other low-resource settings. Additionally, variations in the prevalence of comorbidities, socioeconomic factors and cultural practices might also contribute to these disparities.

In line with previous studies, several factors were found to be predictors of ICU mortality, including age, male sex, and pre-existing medical conditions (6, 7, 9, 33). Moreover, the presence of complications during hospital admission was also a significant predictor of ICU mortality. The most common complications observed were nose and bed pressure ulcers, which can arise due to

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TABLE 2 Comparison between survivors and non-survivors among the ICU patients admitted to Al-Kindi MOH Hospital, Baghdad, Iraq.

	Survivors *n* = 351	Non-survivors *n* = 573	*p*-value[a]
Demographics			
Age (years), (median, IQR)	55 (46, 65)	61 (53, 70)	<0.001
Missing data			
Sex, *n* (%)			
Male	187 (53%)	358 (62%)	0.006
Pre-existing medical conditions[b], *n* (%)			
At least one pre-existing condition	166 (47%)	345 (60%)	<0.001
Cardiovascular disease	122 (35%)	232 (40%)	0.082
Diabetes	84 (24%)	176 (31%)	0.026
Hypertension	85 (24%)	161 (28%)	0.2
Obesity	38 (11%)	62 (11%)	>0.9
Renal disease	7 (2.0%)	26 (4.5%)	0.043
Lung disease	9 (2.6%)	23 (4.0%)	0.2
Cerebrovascular disease	4 (1.1%)	23 (4.0%)	0.012
Other	11 (3.1%)	27 (4.7%)	0.2
No. of pre-existing medical conditions, *n* (%)			< 0.001
None	185 (53%)	228 (40%)	
One	57 (16%)	122 (21%)	
Two or more	109 (31%)	223 (39%)	
COVID-19 diagnosis, *n*(%)			
PCR result			0.016
Negative PCR Result	75 (26%)	86 (18%)	
Positive PCR Result	218 (74%)	385 (82%)	
Missing data	58	102	
CT Scan result			0.081
Non-suggestive of COVID-19	125 (38%)	242 (44%)	
Suggestive of COVID-19	202 (62%)	305 (56%)	
Missing data	24	26	
ICU procedures, *n* (%)			
At least one ICU procedure	304 (87%)	528 (92%)	0.006
Prone positioning	300 (85%)	524 (91%)	0.005
Thoracostomy tube	6 (1.7%)	12 (2.1%)	0.7
Blood transfusion	4 (1.1%)	10 (1.7%)	0.5
Central line	5 (1.4%)	9 (1.6%)	0.9
Enternal nutrition	3 (0.9%)	9 (1.6%)	0.6
Parenteral nutrition	0 (0%)	5 (0.9%)	0.2
Other procedures[c]	4 (1.1%)	13 (2.3%)	0.2
ICU complications, *n* (%)			
At least one complication	44 (13%)	126 (22%)	<0.001
Nasal sore	31 (8.8%)	103 (18%)	<0.001
Bed sore	15 (4.3%)	40 (7.0%)	0.091
Urinal catheter infection	6 (1.7%)	16 (2.8%)	0.3
Ventilator-associated pneumonia (VAP) (clinical)	2 (0.6%)	14 (2.4%)	0.034
Equipment failure	1 (0.3%)	3 (0.5%)	>0.9
Central line infection	1 (0.3%)	1 (0.2%)	>0.9

[a]Kruskal-Wallis rank sum test; Pearson's Chi-squared test; Fisher's exact test.

[b]Each patient could have more than one pre-existing condition, so the sum of percentages may not add up to 100.

[c]Other procedures include hemodialysis ($n = 4$) and NG tube insertion ($n = 1$), the remaining other procedures were not captured comprehensively in the study database.

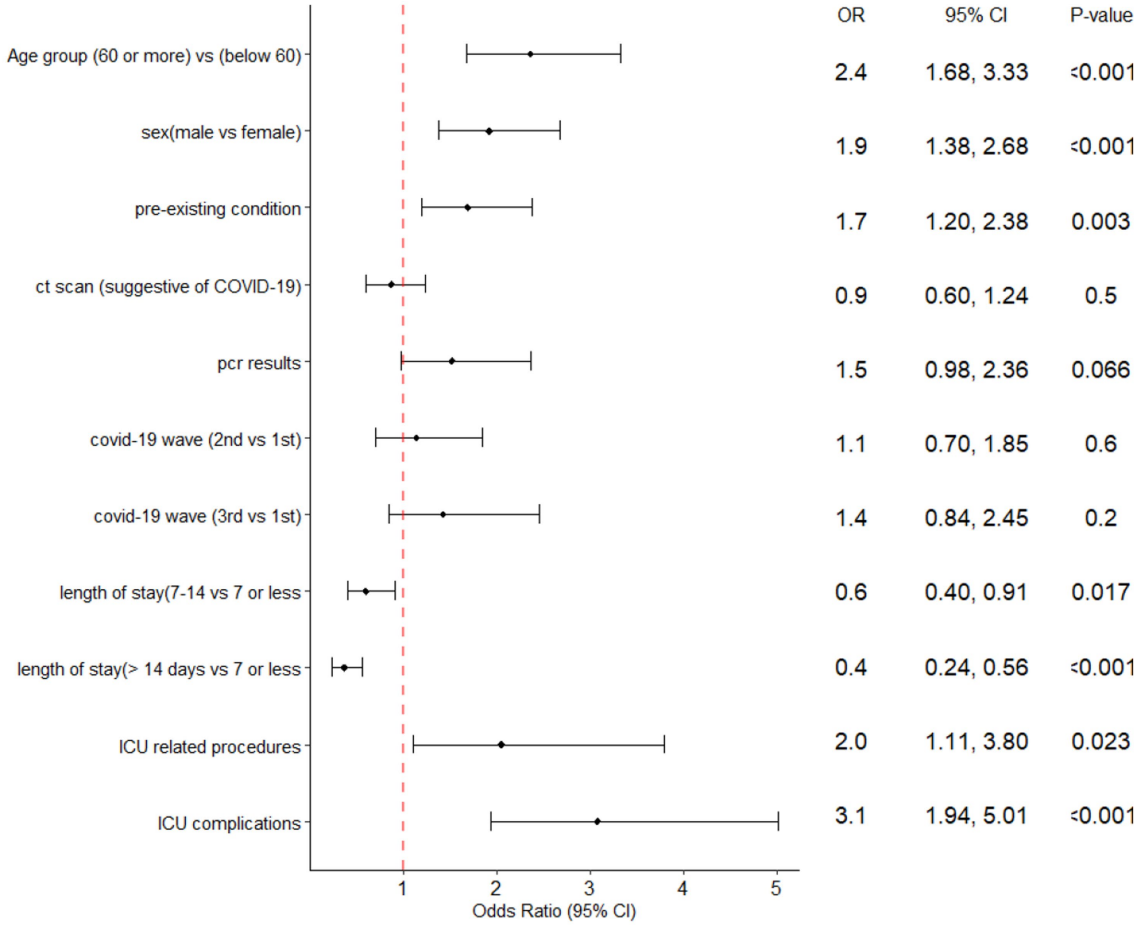

FIGURE 4
Forest plot of the multiple logistic regression analysis with ICU mortality predictors of ICU patients admitted at Al-Kindi Hospital, Baghdad Iraq.

prolonged ICU stay, the use of non-invasive ventilation *via* a mask, and prone positioning (34). Our study found that awake prone positioning, which was performed for a majority of patients, was associated with increased risk of mortality. Nevertheless, other studies have demonstrated that awake prone positioning can be beneficial for patients with Acute Respiratory Distress Syndrome (ARDS) and can reduce the need for intubation among severe cases (35). Due to resource constraints, the ICU in Baghdad did not provide invasive ventilation, hence, we could not measure the impact of awake prone positioning and non-invasive ventilation in reducing the need for intubation in our study cohort. Despite these limitations, the provision of non-invasive ventilation has been widely recognized as a crucial treatment in improving outcomes and reducing the need for intubation (36, 37).

In this study, a high mortality rate was consistently observed among ICU patients with COVID-19 infection despite improvements in critical care practices such as providing ICU training, increasing bed capacity, improving triage, and implementing stringent admission criteria. While such improvements could have played a role in reducing the number of complications and preventing a further increase in the mortality rate despite an increase in bed capacity, we did not observe a significant decrease in mortality during the second and third waves as we had anticipated. The adjusted odds ratio (OR) did not reveal a significant change in the risk of mortality across the three waves of the pandemic. The persistent high mortality rate may be attributed to factors such as resource constraints and a shortage of ICU beds, leading to delayed admission and prolonged wait times for critical care (38–40). Similar scenarios have been observed in other settings and has been shown to be associated with increased mortality due to increased hospital load and strains on critical care capacity (28, 32, 41).

This study had several limitations, including a lack of comprehensive data from medical records and a lack of information on treatment, laboratory findings, and other clinical indicators that could have provided further insight into the results. Additionally, the analysis did not include non-invasive ventilation due to a lack of data on its provision and timing. The impact of high ICU occupancy rates and admission delays was not evaluated as these details were not recorded. Although the MSF team attempted to improve the performance of the ICU, it was not possible to establish a clear connection between changes in clinical management and patient outcomes. Moreover, severity of illness was not included in the analysis since it was not assessed using validated scoring systems and may have resulted in a biased classification. Additionally, vaccination availability and coverage during the study period were limited, with the majority of the population remaining unvaccinated, hence, vaccination status of patients in this study was not consistently recorded and could not be included in this analysis.

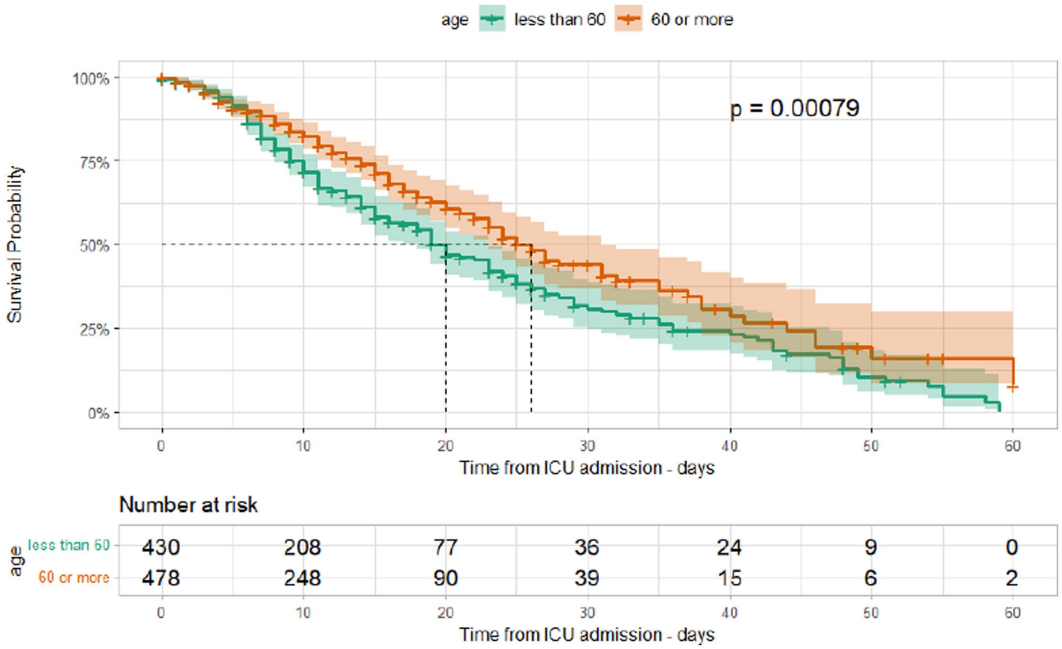

FIGURE 5
Kaplan–Meier survival curves according to age group in ICU patients admitted at Al Kindi Hospital Baghdad Iraq.

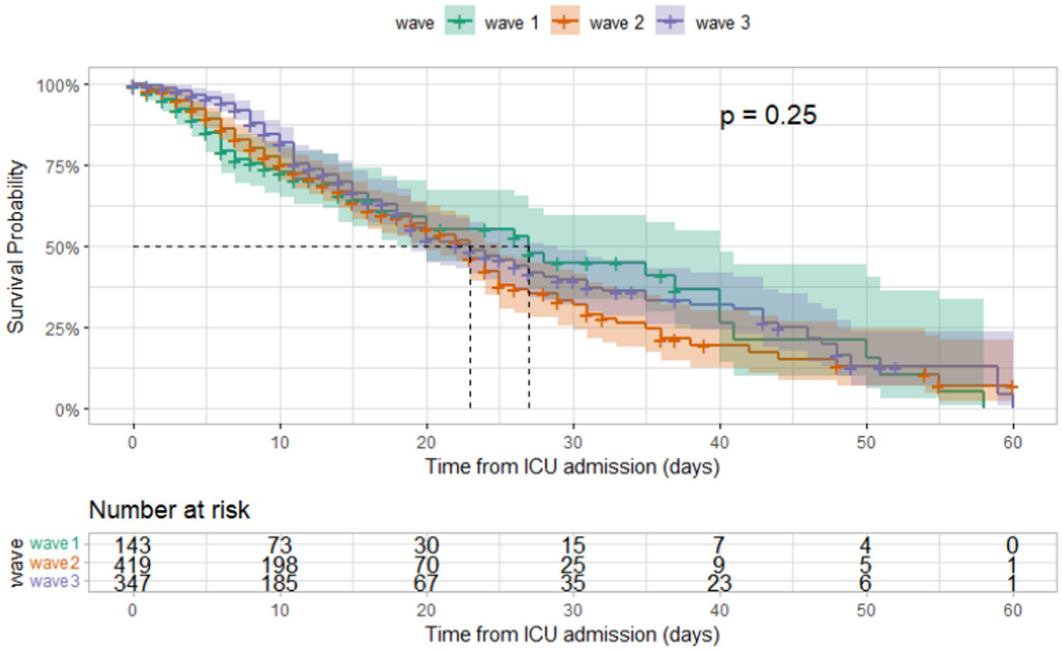

FIGURE 6
Kaplan–Meier survival curves according to covid-19 wave in ICU patients admitted at Al Kindi Hospital Baghdad Iraq.

Conclusion

In low and middle income settings, optimizing ICU care can be challenging due to limited resources and infrastructure. The results of this retrospective observational study demonstrate that despite these challenges, advancements in capacity and clinical practices can significantly mitigate complications in critically ill COVID-19 patients. However, the study also highlights the persistent challenge of high mortality rates in these contexts, with a rate of at least 59% observed throughout the three waves of the pandemic between 2020 and 2021. This necessitates the urgent need for interventions to address this issue and improve patient outcomes.

To address these challenges, it is crucial to prioritize the implementation of effective data collection systems, which can help monitor clinical presentations and identify gaps in clinical management. Although resource limitations may pose barriers to effective data collection, addressing this challenge is essential for improving patient outcomes and identifying opportunities for improvement in ICU care. As the COVID-19 pandemic continues to affect low and middle income settings, it is imperative that we continue to invest in optimizing ICU care and addressing the unique challenges of these contexts.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Research Committee of the National Centre for Training and Human at the Ministry of Health and Environment, Baghdad, Iraq on 31 May 2022 (protocol number 10/2022) and was exempt from the MSF Ethics Review Board following the approval of the Medical Director. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

RM, AH, UD, KP, and AG contributed to the study conception and design. RM, AH, KA, EZ, and AG developed the study protocol. RM and AH managed the data and did the statistical data analysis. UD and IS performed field data collection. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Glossary

ARDS	Acute Respiratory Distress Syndrome
CI	Confidence Interval
COVID-19	Coronavirus disease 2019
CPAP	Continuous positive airway pressure
CT	Computerized Tomogram
ER	Emergency Room
HICs	High-income countries
ICU	Intensive Care Unit
IMV	Invasive mechanical ventilation
IPC	Infection Prevention and Control
IQR	Interquartile range
LMICs	Low- and middle- income countries
LOS	Length of stay
MOH	Ministry of Health
MSF	Médecins Sans Frontières
OR	Odds Ratio
RT-PCR	Reverse transcription-polymerase chain reaction
STROBE	Strengthening the reporting of observational studies in epidemiology
VAP	Ventilator-associated pneumonia
WHO	World Health Organization

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The role of immune suppression in COVID-19 hospitalization: clinical and epidemiological trends over three years of SARS-CoV-2 epidemic

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Specific immune suppression types have been associated with a greater risk of severe COVID-19 disease and death. We analyzed data from patients >17 years that were hospitalized for COVID-19 at the "Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico" in Milan (Lombardy, Northern Italy). The study included 1727 SARS-CoV-2-positive patients (1,131 males, median age of 65 years) hospitalized between February 2020 and November 2022. Of these, 321 (18.6%, CI: 16.8–20.4%) had at least one condition defining immune suppression. Immune suppressed subjects were more likely to have other co-morbidities (80.4% vs. 69.8%, $p < 0.001$) and be vaccinated (37% vs. 12.7%, $p < 0.001$). We evaluated the contribution of immune suppression to hospitalization during the various stages of the epidemic and investigated whether immune suppression contributed to severe outcomes and death, also considering the vaccination status of the patients. The proportion of immune suppressed patients among all hospitalizations (initially stable at <20%) started to increase around December 2021, and remained high (30–50%). This change coincided with an increase in the proportions of older patients and patients with co-morbidities and with a decrease in the proportion of patients with severe outcomes. Vaccinated patients showed a lower proportion of severe outcomes; among non-vaccinated patients, severe outcomes were more common in immune suppressed individuals. Immune suppression was a significant predictor of severe outcomes, after adjusting for age, sex, co-morbidities, period of hospitalization, and vaccination status (OR: 1.64; 95% CI: 1.23–2.19), while vaccination was a protective factor (OR: 0.31; 95% IC: 0.20–0.47). However, after November 2021, differences in disease outcomes between vaccinated and non-vaccinated groups (for both immune suppressed and immune competent subjects) disappeared.

Since December 2021, the spread of the less virulent Omicron variant and an overall higher level of induced and/or natural immunity likely contributed to the observed shift in hospitalized patient characteristics. Nonetheless, vaccination against SARS-CoV-2, likely in combination with naturally acquired immunity, effectively reduced severe outcomes in both immune competent (73.9% vs. 48.2%, $p < 0.001$) and immune suppressed (66.4% vs. 35.2%, $p < 0.001$) patients, confirming previous observations about the value of the vaccine in preventing serious disease.

KEYWORDS

SARS-CoV-2, COVID-19, disease outcome, hospitalization, COVID-19 vaccination, immune suppression

1. Introduction

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infections have highly variable outcomes in different patients, with a clinical spectrum varying from entirely asymptomatic to respiratory failure, septic shock, multiple organ dysfunction, and death (1). Older age and several co-morbidities have been identified to be unequivocally associated with worse COVID-19 (coronavirus disease 2019) outcomes (2). The presence of one or more co-morbidities (multimorbidity) can exacerbate pathological mechanisms occurring during the infection and/or reduce the tolerance of the patient to organ injury (3). For example, chronic kidney, lung, or liver diseases, diabetes, cardiovascular disease, obesity, and cancer have all been associated with an increased risk of progressing to severe COVID-19. Given this variability, the individual immune response to SARS-CoV-2 is likely also affecting the clinical course of the disease (2, 3).

In literature, contradictory opinions about whether immune suppression is a significant risk factor for COVID-19 exist. On one hand, COVID-19 incidence, morbidity, and mortality rates do not seem to differ largely between immune suppressed individuals and the general population, and immune suppressed patients seem to present more favorable outcomes as compared to patients with other types of co-morbidities (3–5) not directly associated with immune suppression. On the other hand, patients with specific types of immune suppression, like those linked to human immunodeficiency virus (HIV) infection, solid organ transplantation, or B-cell depleting therapies, have a greater risk for severe COVID-19 outcomes, such as those requiring ventilation or extracorporeal membrane oxygenation (ECMO), and death (3, 6). Indeed, several factors can influence the immune status of an individual, and immune suppression can have different causes, including genetic disorders, tumors, infections, or pharmacological treatments, and our understanding of COVID-19 clinical outcomes associated with different types of immune suppression is limited. Furthermore, determining the outcome severity in immune suppressed individuals may be complicated as several factors, such as a disease, its treatment, or a disease-related immune suppression, can influence the clinical course of an infection (7).

Another aspect to consider is that a state of immune suppression may reduce the response to vaccine-induced immunizations and subjects with immune dysfunctions may be at higher risk for contracting a breakthrough infection (6). Additionally, studies suggest that some immune suppressed patients, especially those with immune-mediated inflammatory diseases and those on B cell-depleting therapies, remain susceptible to poor outcomes even after vaccination (8, 9). Therefore, when a high vaccination coverage has been achieved, patients with immune dysfunctions may represent a substantial proportion of hospitalized and deceased patients.

In this study, we analyzed clinical data collected from patients hospitalized for COVID-19 at the "Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico" in Milan (Lombardy, Northern Italy) since February 2020–when SARS-CoV-2 was first recognized in Italy–until the end of 2022. Lombardy was one of the first non-Asian areas with sustained SARS-CoV-2 transmission, the first epicenter of the European epidemic, and the Italian region with the highest COVID-19 clinical burden in early 2020 (10–12). In fact, in March–May 2020, Lombardy experienced a 111.8% increase in all-cause deaths compared with the same period in the quinquennia 2015–2019 (excess deaths due to all causes), being one of the heaviest contributors to the Italian overall 31.7% increase in excess mortality (10, 13). Afterward, following global trends, cycles of infection peaks and dips occurred in Lombardy as different variants characterized by diverse degrees of transmissibility and pathogenicity spread and became prevalent during different periods (14–16).

The main scope of this retrospective observational study was to evaluate the contribution of immune suppression to hospitalization during the various stages of the COVID-19 epidemic, which were characterized by the circulation of different variants and different degrees of vaccination coverage, by studying a cohort of patients hospitalized for COVID-19 in one Hospital in Milan between the end of February 2020 and November 2022. Additionally, we investigated whether immune suppression contributed to severe outcomes and death and assessed whether vaccination reduced severe outcomes and death also in immune suppressed patients.

2. Materials and methods

2.1. Data collection

This was an observational cohort study (COVID-19 Network Registry). The study population consisted of patients aged >17 years who were hospitalized at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan and who were positive for SARS-CoV-2 based on real-time PCR. Patients that were directly admitted to the

intensive care unit (ICU) were excluded. The study was approved by the Medical Ethics Committee of the Fondazione IRCCS Ca′ Granda Ospedale Maggiore Policlinico (EC approval 241_2020, 17 March 2020). The need to obtain informed consent was waived by the Medical Ethics Committee in cases where it was not possible to obtain informed consent, due to severe illness or death. In all other cases, written informed consent was obtained. Ethnicity was retrieved from medical charts. Study data were collected and managed using Research Electronic Data Capture (REDCap®) (17).

2.2. Study population, inclusion and exclusion criteria, variable definition

The study included 1727 SARS-CoV-2-positive nonminor (>17 years of age) patients that had been admitted to the hospital "Fondazione IRCCS Ca′ Granda Ospedale Maggiore Policlinico," Milan (Lombardy, Italy), between the end of February 2020 and November 2022. As information about hospitalization in other facilities for transferred patients could not be obtained, we considered only the period between the admission to and discharge from the COVID-19 unit of this hospital and all included patients that remained hospitalized for at least 1 day (distinct dates of admission and discharge). For all patients, sex at birth, ethnicity, and smoking status were recorded. Patients were divided into 3 age classes (18–50 years, 51–70 years, and > 70 years), according to what is routinely done in the epidemiological reports of the Istituto Superiore di Sanita', the National Health agency monitoring the epidemiology of SARS-CoV-2 in Italy (18).

Information about the immune status of all participants was available and patients were divided into two groups. We considered as immune suppressed (exposure) those patients with at least one of the following conditions: (i) history of any connective tissue disease, autoimmune disease, and/or primary immunodeficiency; (ii) history of an active solid or hematologic tumor; (iii) neutropenia; (iv) diagnosis of HIV or acquired immunodeficiency syndrome (AIDS); (v) history of splenectomy, solid organ transplantation, and/or hematopoietic stem cell transplantation; (vi) ongoing treatment with biological drugs during the six months prior to admission or with corticosteroids, chemotherapy, and/or other immune suppressive agents during the 3 months prior to admission. All other patients were considered immune competent.

In consideration of the fact that patient history also included the presence of co-morbidities other than immune suppression, we divided patients co-morbidities other than the ones considered above into six categories: (i) respiratory diseases: chronic obstructive pulmonary disease (COPD), asthma, interstitial lung disease, bronchiectasis; (ii) cardiovascular diseases: heart failure, atrial fibrillation, myocardial infarction, hypertension, pulmonary hypertension; (iii) nephropathies: chronic kidney disease, dialysis; (iv) gastrointestinal diseases, including gastroesophageal reflux disease, and liver diseases, including liver cirrhosis, hepatitis C virus (HCV) infection, and the presence of markers for hepatitis B virus (HBV) infection (HBsAg, HBcAb, HBsAb); (v) metabolic diseases: diabetes, dyslipidemia, malnutrition, and obesity; (vi) neurologic diseases: stroke, transient ischemic attack, and dementia.

Patient vaccination status was also considered. Since complete information (number of doses and dates of administration) about vaccination was available only for a small subset of subjects, patients were considered vaccinated if they received at least one dose of a COVID-19 vaccine (any brand) prior to hospital admission or non-vaccinated if they were hospitalized prior to March 2021 (when the first vaccine doses were made available in Italy) or, for later periods, if they declared not to have been vaccinated against SARS-CoV-2.

We considered a COVID-19 severe outcome when a patient suffered from pneumonia, acute respiratory distress syndrome (ARDS), septic shock, was admitted to an intensive care unit (ICU), or was subjected to mechanical ventilation (intubation). Death during hospitalization caused by SARS-CoV-2 as a main factor or as a co-factor was also considered a severe outcome. All other outcomes were considered favorable outcomes.

2.3. Statistical analyses

Continuous measurements were expressed as medians and compared using the permutation-based Mann–Whitney test. Categorical variables were expressed in percentages or proportions and were compared using the Chi-square test or, in case of a small sample size and where appropriate, Fisher's exact test. Confidence on observed proportions is expressed as 95% normal intervals while for medians the 25th and 75th percentiles are indicated (inter quartile range–IQR). Simple and multiple logistic regression models were used to estimate severe outcome risk factors; odds ratios (OR) with relative 95% intervals of confidence (95% IC) were considered as the measure of effect and precision, respectively. Potential predictors were age and number of co-morbidities (1, 2, 3, >3)–included as continuous variables–and sex, immune status, vaccination, and period (before and since December 2021) – included as nominal variables. The Wald test was used to assess the significance of the regression beta coefficients. Two-sided p-values <0.05 were considered statistically significant and Bonferroni correction was applied as appropriate and where indicated. A network analysis was performed with Past using the Rho similarity index with an edge cut-off of 5%. The clustering analysis was also performed in Past using the neighbor-joining algorithm with 1,000 bootstrap resamplings to assess branch robustness.

Analyses were conducted using Past 4.08 (19) and JASP 0.17.1 (20). Final image editing was performed with Inkscape (21).

3. Results

3.1. Population description

The investigated population of 1727 hospitalized subjects included 1,131 (65.5%) males and 596 females with a median age of 65 (range: 19–100) years. Patient characteristics are summarized in Table 1. Among all considered patients, 1,406 (81.4%, CI: 79.6–83.2%) were immune competent, while 321 (18.6%, CI: 16.8–20.4%) presented one or more factors of immune suppression. As shown in Table 2, the most frequent immune suppression condition was the presence of active cancer (169/321, 52.6%), with an equal presence of solid and hematologic cancers ($p = 0.5$). Drug-induced immune suppression (168/321, 52.3%), including biological drugs, chemotherapy, corticosteroids, or other drugs, was the second most frequent

TABLE 1 Characteristics of the studied population and sub-populations.

		Total		Immune competent		Immune suppressed		p^1
		N	%	*N*	%	*N*	%	
	Total	1,727	–	1,406	81.4	321	18.6	
Sex	Males	1,131	65.5	949	67.5	182	56.7	**< 0.001**
	Females	596	34.5	457	32.5	139	43.3	
Age	18–50 years	335	19.4	287	20.4	48	15.0	**0.038**
	51–70 years	723	41.9	590	42.0	133	41.4	
	>70 years	668	38.7	528	37.6	140	43.6	
Ethnicity	Caucasian	1,301	88.1	1,039	87.2	262	92.3	0.20
	Hispanic	77	5.2	68	5.7	9	3.2	
	Asian	49	3.3	42	3.5	7	2.5	
	Arab	29	2.0	27	2.3	2	0.7	
	African descent	12	0.8	10	0.8	2	0.7	
	Other	8	0.5	6	0.5	2	0.7	
Smoker	No	806	71.8	666	72.9	140	67.3	0.18
	Past	233	20.8	180	19.7	53	25.5	
	Current	83	7.4	68	7.4	15	7.2	
Co-morbidities[2]	None	424	24.6	424	30.2	63	19.6	**< 0.001**
	At least one	1,303	75.4	982	69.8	258	80.4	
	Cardiovascular diseases	902	52.2	721	51.3	181	56.4	0.098
	Metabolic diseases	628	36.4	503	35.8	125	38.9	0.28
	GI/liver diseases[3]	292	16.9	197	14.0	95	29.6	**< 0.001**
	Respiratory diseases	243	14.1	185	13.2	58	18.1	**0.022**
	Nephropathies	163	9.4	108	7.7	55	17.1	**< 0.001**
	Neurologic diseases	162	9.4	132	9.4	30	9.3	1
	More than 1 category[4]	825	47.8	567	40.3	167	52.0	**< 0.001**
Vaccination[5]	No	1,291	82.8	1,109	87.3	182	63.0	**< 0.001**
	At least 1 dose	268	17.2	161	12.7	107	37.0	

[1]*p* values for statistically significant differences calculated between the two groups of immune competent and immune suppressed patients are highlighted in bold.
[2]Presence of at least one condition of one of the considered categories and excluding immune suppression for immune suppressed patients.
[3]GI, gastrointestinal.
[4]Presence of at least one condition of more than one of the considered categories.
[5]History of vaccination against SARS-CoV-2 (any brand).

condition, followed by connective tissue disease (17.4%) and organ transplant (16.5%). The proportions of all other conditions were below 10%. A network and a clustering analysis of factors of immune suppression for the studied population are shown in Supplementary Figure S1.

While ethnicity distribution and smoking habits were similar between immune competent and immune suppressed patients, immune suppressed subjects were slightly older ($p = 0.038$) and the proportion of males was significantly lower among immune suppressed patients (67.5% vs. 56.7%, $p < 0.001$) (Table 1). Additionally, immune suppressed subjects were more likely to have other co-morbidities ($p < 0.001$), and differences were statistically significant for co-morbidities belonging to the categories of gastrointestinal and liver diseases, respiratory diseases, and nephropathies. Finally, the vaccination rate was higher in immune suppressed subjects (37% vs. 12.7%, $p < 0.001$), likely because vaccination was offered earlier to this sub-population.

Comparing different sub-populations of immune suppressed patients to the immune competent population, however, revealed some group-specific differences (Supplementary Table S1). Particularly, patients with cancer and those undergoing chemotherapy, categories tightly connected in the network and clustering analyses (Supplementary Figure S1), were older while transplant recipients and individuals with HIV infection were younger. Additionally, the proportion of females was higher in most sub-groups, but the difference was significant only for cancer patients, those with connective tissue diseases, and individuals taking immune suppressant drugs. Respiratory co-morbidities were more frequent in patients taking corticosteroids and biological drugs; nephropathies were particularly prevalent in transplant recipients and patients taking corticosteroids, which were two tightly connected categories in the correlation analysis; gastrointestinal and hepatic problems were frequent in most immune suppressed groups, except in those with connective tissue diseases.

TABLE 2 Conditions causing immune suppression in the 321 immune suppressed patients considered in this study.

Condition	N. patients	Proportion (%)	95% confidence interval
All tumors	169	52.6	47.1–58.1
Solid tumors	85	26.5	21.7–31.3
Hematologic tumors	94	29.3	24.3–34.3
All immune suppressants	168	52.3	46.8–57.8
Biological drugs[1]	53	16.5	12.4–20.6
Chemotherapeutics[2]	69	21.5	17.0-26.0
Corticosteroids and other drugs[2]	92	28.7	23.8–33.7
All connective tissue diseases	56	17.4	13.3–21.6
Rheumatoid arthritis	19	5.9	3.3–8.5
Other connective tissue diseases	38	11.8	8.3–15.3
Transplant recipient	53	16.5	12.4–20.6
Neutropenia	22	6.9	4.1–9.7
HIV/AIDS	18	5.6	3.1–8.1
Asplenia	10	3.1	1.2–5.0
Aplastic anemia	9	2,8	1.0–4.6
Other autoimmune diseases	5	1.6	0.2–3.0
A/Hypogammaglobulinemia	4	1.2	0.0–2.4

[1]During the six months prior to admission.
[2]During the three months prior to admission.

3.2. Temporal trends

To assess whether immune suppression contributed differently to hospitalization during the various phases of the pandemic, we evaluated the proportion of immune suppressed patients among all hospitalizations over time. This proportion remained stable below 20% (mostly between 10 and 20%) until November 2021 but increased to 30% around December 2021 and remained high (30–50%) until the end of the studied period (Figure 1A).

This change coincided with a shift in age distribution as, during the second period, we observed an increase in the proportion of older patients (>70 years: 36.5 vs. 48.7%, $p < 0.001$) and a decrease in the proportion of younger patients (18–50 years: 20.5% vs. 14.4%, $p = 0.017$), while the proportion of patients in the middle age category did not change significantly (51–70: 43.1% vs. 36.9%, $p = 0.051$) (Figure 1B). Older patients presented significantly more immune suppression factors compared to the youngest age group (21.0% vs. 14.3%, $p = 0.011$, with a significance cut-off of 0.025 due to Bonferroni correction) but not compared to the middle age class (18.4%, $p = 0.23$). Nonetheless, similar increasing trends in the proportion of subjects with immune suppression were observed in all three considered age groups (Supplementary Figure S2A) and the proportion of immune suppressed patients in the period from December 2021 until the end was significantly higher than the one in the period from the end of February 2020 to November 2021 in all age groups ($p < 0.001$; Supplementary Table S2).

Similar observations were made for co-morbidities. Around December 2021 the proportion of patients that had co-morbidities in more than one category increased while the proportion of patients with less co-morbidities decreased (Figure 1C). This increase was statistically significant, both considering and excluding immune suppression as a category of co-morbidity (Supplementary Table S3).

In summary, the characteristics of hospitalized patients were very different in these two different periods as patients from December 2021 onwards were older, presented a higher number of co-morbidities, and a higher proportion of them had immune suppression-related factors. In December 2021, Italy reached a COVID-19 first-dose vaccination coverage of 80% (22). The vaccination status of the investigated population is illustrated in Supplementary Figure S3. Additionally, based on national data about variant circulation (15), we observed that December 2021 also corresponded to the moment when the Omicron variants started to become the most prevalent (Supplementary Figure S2B). Overall, we could not definitely conclude whether the noted shifts were due to the reached high immunity coverage, the spread of the Omicron variant, or both.

3.3. Infection outcome

Overall, severe outcomes (including death) were observed in 843 patients, and COVID-19-associated deaths were documented in 254 (30.1%) of these individuals. Among immune competent patients, severe outcomes and death were observed in 47.9% (674/1406) and 13.5% (190/1406) of cases, respectively, while they were observed in 52.7% (169/321) and 19.9% (64/321) of immune suppressed subjects, respectively. Only mortality was significantly higher in immune suppressed patients compared to immune competent subjects ($p = 0.003$). Similarly, considering the various conditions of immune suppression separately, a severe outcome was recorded significantly more frequently, compared to immune competent subjects, only among patients treated with biological drugs and patients with connective tissue diseases (Table 3).

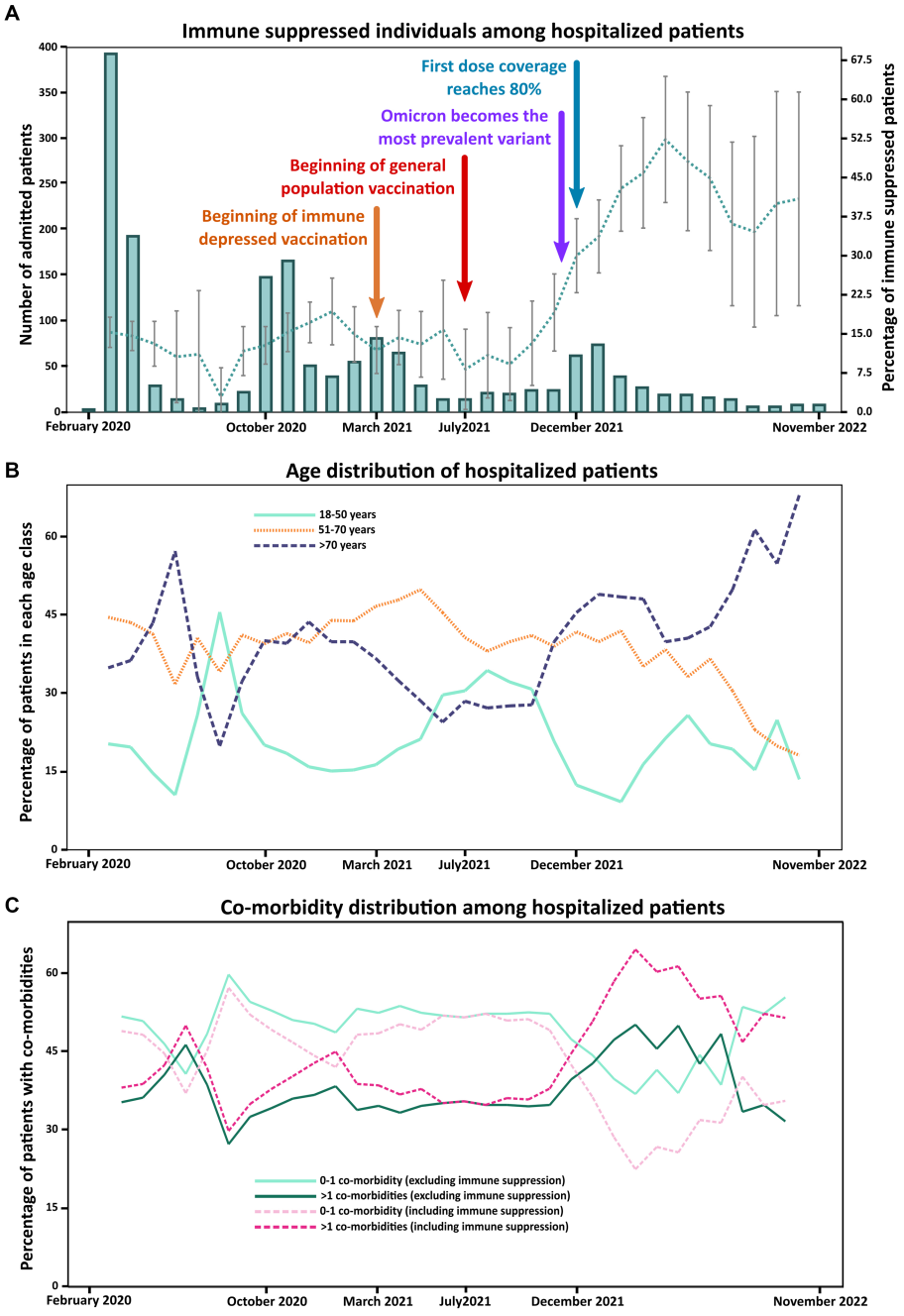

FIGURE 1
Temporal trends in patient characteristics throughout the study period. The graph in **(A)** represents the proportion of immune suppressed subjects among hospitalized patients. The bar graph (scale on the left) shows the number of hospitalized patients during each month while the dotted line (scale on the right) corresponds to the proportion of immune suppressed patients (for each timepoint the data of three months – the indicated timepoint ± 1 month – were used) with the confidence interval indicated by vertical lines. Timepoints corresponding to key events regarding vaccination or variant circulation are indicated by arrows. The graph in **(B)** shows the relative proportion of patients belonging to the three indicated age classes at the same three-month timepoints. The graph in **(C)** illustrates the proportions of patients hospitalized with no co-morbidities or co-morbidities in one considered category and of subjects with co-morbidities in more than one considered category at the same three-month timepoints; proportions calculated both considering and excluding immune suppression as a co-morbidity category are shown.

The proportion of severe outcomes in the two groups (immune competent and immune suppressed) was the highest at the beginning of the pandemic (>50%) and then fluctuated over time with lower proportions observed during the summer months (Supplementary Figure S4). Some high peaks observed in the immune suppressed group were likely caused by the extremely low numbers of patients (between 1 and 6) in the summer months. However, the proportion of cases with serious outcomes started to decrease steadily in February 2022 and remained around or below 20% since April 2022. The moment when a higher proportion of favorable outcomes started to be recorded was delayed by two months with respect to other identified trend changes (increase in age and co-morbidities,

TABLE 3 Patients with severe outcomes stratified by type of immune suppression and period of hospitalization.

Status/Condition	Patients with severe outcomes					
	Overall		Before December 2021		Since December 2021	
	N (%)	*p*	*N* (%)	*p*	*N* (%)	*p*
Immune competent	674 (47.9)	Reference	611 (50.3)	Reference	60 (32.8)	Reference
All immune suppressed	169 (52.6)	0.13	132 (64.7)	**< 0.001**	37 (32.2)	0.91
All tumors	90 (53.3)	0.19	71 (67.0)	**0.001**	19 (30.6)	0.76
Solid tumors	48 (56.5)	0.13	40 (65.6)	**0.02**	8 (34.8)	0.82
Hematologic tumors	49 (52.1)	0.43	37 (68.5)	**0.009**	12 (30.0)	0.85
All immune suppressants	87 (51.8)	0.35	60 (63.2)	**0.016**	27 (37.5)	0.48
Biological drugs[1]	34 (64.2)	0.02	25 (71.4)	**0.016**	9 (50)	0.19
Chemotherapeutics[2]	38 (55.1)	0.25	27 (71.1)	**0.013**	11 (35.5)	0.84
Corticosteroids and other drugs[2]	46 (50.0)	0.7	29 (60.4)	0.17	17 (39.5)	0.4
All connective tissue diseases	37 (66.1)	**0.008**	31 (72.1)	**0.005**	6 (46.2)	0.34
Rheumatoid arthritis	12 (63.2)	0.25	10 (76.9)	**0.091**	2 (33.3)	1
Other connective tissue diseases	25 (65.8)	**0.03**	21 (70.0)	**0.041**	4 (50.0)	0.45
Transplant recipient	16 (30.2)	**0.011**	9 (50.0)	1	7 (20.0)	0.16
Neutropenia	10 (45.5)	0.83	5 (71.4)	0.45	5 (33.3)	1
HIV/AIDS	12 (66.7)	0.15	10 (90.9)	**0.012**	2 (28.6)	1
Asplenia	4 (40.0)	0.76	4 (44.4)	0.75	1 (100.0)	0.33
Aplastic anemia	5 (55.6)	0.75	4 (57.1)	1	1 (50.0)	0.55
Other autoimmune diseases	2 (40.0)	1	1 (33.3)	0.62	1 (50.0)	0.55
A/Hypogammaglobulinemia	2 (50.0)	1	2 (66.7)	1	0 (0.0)	1

[1]During the six months prior to admission.
[2]During the three months prior to admission.
N: number of subjects with severe outcomes; %: percentage of subjects with severe outcomes. Significantly different *p* values (vs. immune competent) are in bold.

TABLE 4 Immune suppressed patients among all subjects hospitalized stratified by outcome and hospitalization time.

	Immune suppressed patients		
	N	%	*p*
Before December 2021			
Patients with favorable outcomes (*N* = 675)	72	10.7	Reference
Patients with severe outcome[1] (*N* = 743)	132	17.8	**< 0.001**
Patients who died of COVID-19 (*N* = 223)	51	22.9	**< 0.001**
Since December 2021			
Patients with favorable outcome (*N* = 201)	78	38.8	Reference
Patients with severe outcome[1] (*N* = 97)	37	38.1	0.91
Patients who died of COVID-19 (*N* = 29)	13	44.8	0.55

p values for frequencies that are significantly different are in bold (with a significance cut-off of 0.02 due to Bonferroni correction). N: number of immune suppressed subjects; %: percentage of immune suppressed subjects.
[1]Includes septic shock, intubation, ICU admission, ARDS/pneumonia, and death.

including immune suppression). Nonetheless, considering the two different periods identified before (end of February 2020–November 2021 and December 2021–November 2022) the overall proportion of patients with severe outcomes decreased significantly from 52.4% (743/1418) to 32.6% (97/298, $p < 0.001$).

As for the whole population, in patients with severe outcomes the proportion of immune suppressed patients among subjects hospitalized since December 2021 was significantly higher than the proportion of immune suppressed subjects among patients hospitalized before that date (Supplementary Table S2). However, a higher proportion of immune suppressed individuals was noted among patients with a severe outcome, compared to those with a favorable outcome, only when considering subjects hospitalized before December 2021 (Table 4).

Finally, as shown in Table 3, in the period before December 2021, severe outcomes were observed significantly more frequently among immune suppressed compared to immune competent individuals both overall as well as for some sub-categories, including cancer

TABLE 5 Outcomes among immune suppressed compared to immune competent patients stratified by vaccination status.

	Immune competent					Immune suppressed						
	Non vaccinated (*N* = 1,109)		Vaccinated (*N* = 161)			Non vaccinated (*N* = 182)			Vaccinated (*N* = 107)			
Outcome												
	N	%	*N*	%	$p^{1,2}$	*N*	%	$p^{1,3}$	*N*	%	$p^{1,4}$	p^{5}
Favorable	534	48.2	119	73.9	**<0.001**	64	35.2	**0.0011**	71	66.4	**<0.001**	**<0.001**
Severe[6]	575	51.8	42	26.1		118	64.8		36	33.6		
Septic shock	20	1.8	4	2.5	0.53	1	0.5	0.34	1	0.9	1	1
Intubation	72	6.5	0	0.0	**<0.001**	6	3.3	0.13	6	5.6	0.84	0.37
ICU admission	89	8.0	5	3.1	0.023	8	4.4	0.095	8	7.5	1	0.29
Death	165	14.9	18	11.2	0.21	51	28.0	**<0.001**	10	9.3	0.12	**<0.001**
ARDS/ Pneumonia	522	47.1	36	22.4	**<0.001**	102	56.0	0.025	28	26.2	**<0.001**	**<0.001**

Hospitalization length								
	Median	Median	$p^{1,2}$	Median	$p^{1,3}$	Median	$p^{1,4}$	$p^{1,5}$
	14	10	**<0.001**	16	**0.012**	14	0.33	0.25

p values for frequencies and medians that are significantly different are in bold. N: number of subjects; %: percentage of subjects; ICU: intensive care unit; ARDS: acute respiratory distress syndrome.
[1]Significance cut-off of 0.02 due to Bonferroni correction.
[2]Vaccinated immune competent vs. non-vaccinated immune competent.
[3]Non-vaccinated immune suppressed vs. non-vaccinated immune competent.
[4]Vaccinated immune suppressed vs. non-vaccinated immune competent.
[5]Vaccinated immune suppressed vs. non-vaccinated immune suppressed.
[6]Includes septic shock, intubation, ICU admission, death, and ARDS/pneumonia.

TABLE 6 Patients with severe outcomes among vaccinated and non-vaccinated subjects with specific immune suppression conditions.

	Number of vaccinated (%)		Number of non-vaccinated (%)		*p*
	Severe outcome	Favorable outcome	Severe outcome	Favorable outcome	
All tumors	16 (29.6)	38 (70.4)	66 (66.7)	33 (33.3)	**< 0.001**
Biological drugs	11 (55.0)	9 (45.0)	19 (70.4)	8 (29.6)	0.36
Chemotherapeutics	12 (42.9)	16 (57.1)	23 (67.7)	11 (32.3)	0.072
Other connective tissue diseases	6 (60.0)	4 (40.0)	17 (70.8)	7 (29.2)	0.69
HIV/AIDS	1 (16.7)	5 (83.3)	11 (91.7)	1 (8.3)	**0.004**

p values for frequencies that are significantly different are in bold. %: percentage of subjects.

patients, patients treated with chemotherapeutics or biological drugs, those with connective tissue diseases, and patients with HIV/AIDS. Patients with other immune suppression-associated conditions also presented elevated percentages of severe outcomes, but the statistical analyses did not evidence significant differences, likely because of the low number of patients in these groups. Nonetheless, all these differences disappeared in the period December 2021–November 2022.

3.3.1. Outcome and vaccination status

Table 5 shows the outcomes in the studied sub-populations stratified by vaccination status. Compared to non-vaccinated immune competent subjects, a higher percentage of unvaccinated immune suppressed individuals experienced severe outcomes, including deaths, and longer hospitalization times. On the contrary, vaccinated patients (both immune suppressed and immune competent groups) showed a lower proportion of severe outcomes, particularly pneumonia, and the median hospitalization length among vaccinate immune competent subjects was lower. Among immune suppressed patients, vaccinated subjects experienced less severe outcomes, particularly for what concerns pneumonia and death. In the period February 2020–November 2021, a more severe outcome was still noted for both non-vaccinated groups (Supplementary Table S4), while no significant differences were observed in the period December 2021–November 2022 (Supplementary Table S5).

Finally, within the various categories of immune suppression that were statistically associated with severe outcomes, vaccination showed a significant beneficial effect only for cancer patients and subjects with HIV/AIDS as the proportion of patients with severe outcomes was significantly higher in the non-vaccinated groups for these patient categories (Table 6). We also attempted at assessing the effect of the vaccination in the two separate periods, but the number

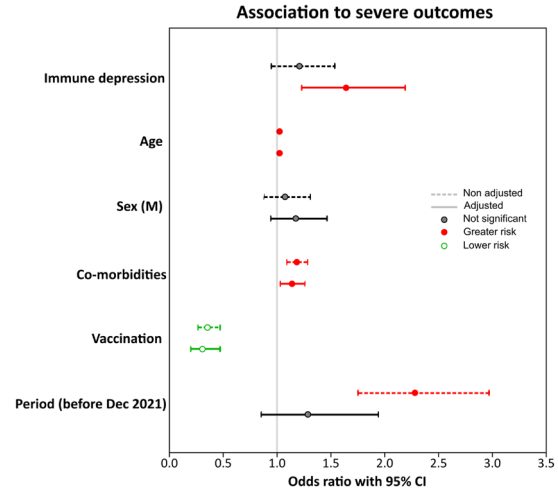

FIGURE 2
Potential predictors of severe outcomes. Odds ratios (dots) and 95% confidence intervals (lines) are indicated for each potential predictor of severe outcome when evaluated separately (simple logistic regression, dotted line) or after adjusting for the other variables (multiple logistic regression, continuous line). Results are labeled according to statistical significance as indicated in the legend. The vertical line indicates the cut-off for statistical significance.

of patients was too small to render differences detectable (Supplementary Tables S6, S7).

3.3.2. Multivariate analyses

To evaluate the contribution of immune suppression and other factors to the severity of the outcome, a logistic regression was performed considering as potential predictors all variables identified to be associated with severe outcomes in this study (age, immunological status, vaccination, number of co-morbidities, hospitalization period, and sex). As the distribution of co-morbidities differed between patients with severe and favorable outcomes ($p = 0.002$), the number of co-morbidities was assessed as a continuous variable.

The potential association of each variable to a severe outcome was evaluated individually as well as adjusted by all the other variables (Figure 2 and Supplementary Table S8, crude and adjusted models). While age and the presence of co-morbidities (other than immune suppression) were significantly associated with a severe outcome and vaccination resulted to have a protective effect against severe outcomes in both crude and adjusted models, immune suppression was a significant predictor of severe outcomes only after adjusting for the other variables. Immune suppression was associated with a 64% increase in the odds of severe outcome after adjusting for sex, age, number of co-morbidities, vaccination, and period of hospital admission (OR: 1.64; 95% CI: 1.23–2.19). Conversely, there was a 70% decrease in the odds of a severe outcome with vaccination (OR: 0.31; 95% IC: 0.20–0.47). The period of hospital admission, which was significantly associated with a severe outcome when analyzed as a single predictor, did not modify the protective effect of the vaccination and was not associated with an increased risk after adjusting for the other variables. This is likely due to the fact that the majority of patients during the first period were not vaccinated, while the opposite was true in the second period.

4. Discussion

During the three years of pandemic alert, the epidemiological and clinical features of SARS-CoV-2 kept changing while the virus was adapting to its host and spreading globally among an initially fully naïve human population that progressively acquired immunity (14). While different viral variants emerged, each characterized by different degrees of pathogenicity and transmissibility, the scientific and medical communities learned how to deal with the new disease and slowly acquired the knowledge necessary to fight it (14, 23). During this time, we collected clinical data from SARS-CoV-2-infected patients that were admitted to the COVID-19 unit of an hospital in Lombardy, the Italian region where COVID-19 initially had the heaviest impact and the first epicenter of the European epidemic (10–12). In this study, we evaluated the contribution of immune suppression to COVID-19 hospitalization and severe disease outcome to identify clinical and epidemiological trends during the three years of the SARS-CoV-2 epidemic.

4.1. The two different epidemiological and clinical phases of the COVID-19 epidemic

Over the whole study period, our analyses revealed the presence of two clearly different phases, which were distinguished by the different epidemiological and clinical features characterizing hospitalized subjects. During the first phase, starting at the beginning of the study (end of February 2020) and lasting until December 2021, patients were younger and had a lower number of co-morbidities, a smaller proportion of them presented immune suppression, and severe outcomes were frequent (approximately 52%). In the period that followed (December 2021–November 2022), the characteristics of the hospitalized patients changed reflecting a milder disease (subjects were older and had more co-morbidities) and the clinical picture changed with a lower proportion of severe outcomes (approximately 33%). Strikingly, the percentage of immune suppressed individuals in the second period increased dramatically, from 10–20% to 30–50%. These results may be partially influenced by the fact that, during the first few months of the epidemic, some elderly patients were not admitted to hospitals because of the heavy impact of COVID-19 on hospitals and bed scarcity. Nonetheless, although in the second period patients were older and these older patients presented significantly more immune suppression factors compared to the younger age groups, the proportion of immune suppressed subjects in the second period was significantly higher in all age groups. This rules out the possibility that the observed trend of increase in the proportion of patients with immune suppression was exclusively due to the shift in the age of the subjects.

The proportion of patients with severe outcomes was the highest at the beginning of the pandemic in both immune competent and immune suppressed groups and started to decrease around February 2022, two months after the other trends started to change. Nevertheless, a higher proportion of immune suppressed individuals among patients with severe outcomes was observed during the first period, when severe outcomes and deaths were also significantly more frequent in the groups of immune suppressed subjects. These differences disappeared in the period December 2021–November 2022.

In December 2021, Italy reached a COVID-19 first dose vaccination coverage of 80% and we can assume that, after almost 2 years of sustained SARS-CoV-2 transmission, natural immunity was also contributing to increase the strength of the immune response of the general population against the virus (22, 24). This could indicate that, from this point in time onwards, immune suppressed subjects and weaker members of the community were much more susceptible to the disease because they were not able to build an immune response strong enough to fight the infection, even after vaccination or previous exposure. Moreover, Omicron started to become the most prevalent variant in Italy around December 2021 and the shift we observed could have also been caused by the reduced pathogenicity of this viral variant (15). Indeed, while the variant Omicron is characterized by a higher transmission rate compared to previous variants, it has been shown to cause milder symptoms and to be associated with better hospital outcomes. This seems to be the case even if the vaccine effectiveness against severe illness, hospitalization, and mortality and high vaccination coverages make evaluations of its virulence more complicated (25, 26). Likewise, we could not definitely conclude whether the noted shifts were due to the reached high immunity coverage, the spread of the Omicron variant, or both.

The observed trend is nonetheless consistent with the recent decision of the World Health Organization (WHO) that COVID-19 no longer constitutes a public health emergency of international concern (PHEIC). This decision was driven by the high population-level immunity, the low virulence of the currently circulating Omicron sub-lineages, and the improved clinical case management that, all together, resulted in a decline in COVID-19-related deaths, hospitalizations, and intensive care need (27).

4.2. Immune suppression is associated with severe COVID-19 disease outcomes among hospitalized patients

While there are contradictory data about the association between immune suppression and severe COVID-19 outcome, possibly also due to different definitions of immune suppression in the various studies, in our population we identified a statistically significant association between them. When considering the data for the whole period, mortality was significantly higher among immune suppressed patients (20%) compared to immune competent subjects (14%). Moreover, a statistically significant increase in the frequency of severe outcomes (including mortality) was observed between the groups of immune suppressed and immune competent when we analyzed the patients of the first period alone (50% vs. 65%). Strikingly, the regression analysis showed an OR of 1.64 (1.23–2.19) for immune suppression, after adjusting for age, sex, time of hospitalization, vaccination status, and other co-morbidities.

Even though we cannot draw strong conclusions as the low number of patients in each category of immune suppression limited the power of these analyses and we could not perform a regression analysis for specific categories of immune suppression, we could observe an association between some immune suppression conditions and severe outcomes. Over the whole period, worse outcomes were noticed among patients treated with biological drugs and patients with connective tissue diseases while during the first period alone, severe outcomes were significantly more frequent also in some other categories. Finally, no differences in outcome among the various sub-populations were observed in the second period.

An association between worse outcomes among cancer patients was observed during the first period and this is consistent with published literature showing that COVID-19 is more severe in cancer patients. Additionally, previous studies have shown that hematologic cancer patients and subjects with lung cancer experience more severe COVID-19 (28, 29). In our study, we observed a worsened outcome in subjects with both hematologic and solid cancers, as well as in patients undergoing chemotherapy, and there was no difference between the two groups in terms of outcome (data not shown). Unfortunately, we could not conclude specifically about patients with lung cancer, a category particularly at high risk of severe disease (30), as this information was recorded only for a few subjects.

Patients that took biological drugs during the six months prior to hospitalization showed a worse outcome both when considering the whole period as well as when investigating the first period alone. In literature, the effect of biological drugs on COVID-19 outcomes is not entirely clear. While some studies found that immune suppressive therapies before hospitalization were not associated with in-hospital mortality, a worse outcome has been clearly documented for patients undergoing B cell-depleting therapies, including rituximab (3, 9, 31). Given the small number of patients, we could not evaluate this aspect in more detail.

While connective tissue diseases other than rheumatoid arthritis were associated with a worse outcome in our study, rheumatoid arthritis was not. As mentioned before, specific data on therapies were not considered and it is possible that we failed in identifying a correlation because worse outcomes in patients with rheumatoid arthritis seem to be therapy-dependent (i.e., rituximab and Janus kinase inhibitors) (9).

A worse outcome was observed in patients with HIV/AIDS, which were also significantly younger compared to immune competent patients. These results are in agreement with literature data (3, 6). Finally, surprisingly, we did not find more severe COVID-19 outcomes in transplant recipients, contrarily to what was observed in other studies, which recorded higher mortality in this group (3, 6). The reason for this discrepancy is not clear.

4.3. Considerations about natural and vaccine-mediated immunity against SARS-CoV-2

Unfortunately, data about COVID-19 vaccination were incomplete for most patients and information about the number and the dates of the received doses was available only for a few individuals. Therefore, for this study, we considered as vaccinated all patients who received at least one vaccine dose at any time prior to hospitalization. Since timing and number of doses are crucial in determining the severity of the outcome (32–34), our results may be biased as some of the vaccinated patients may not have reached a protective level of immunity at the moment of hospitalization, making the effect of vaccination less evident. Additionally, some immune depressed patients may not develop a protective response after vaccination (35, 36). Nonetheless, we observed a higher frequency of less severe outcomes, particularly for pneumonia and ARDS, in all vaccinated individuals, regardless of their level of immune competence.

Additionally, the regression analysis showed a 70% decrease in the odds of a severe outcome following vaccination, after adjusting for the other investigated variables. These results confirm that vaccination has a strong protective effect, also on immune suppressed individuals. Vaccination was also associated with a reduction of COVID-19-related fatalities among immune suppressed subjects. Interestingly, vaccination reduced significantly the severity of the outcome specifically for oncologic patients and subjects with HIV/AIDS, as also previously reported (37, 38).

While non-vaccinated immune suppressed patients were hospitalized for longer periods compared to non-vaccinated immune competent subjects, vaccination reduced hospitalization times in the immune competent group. However, as information about hospitalization in other facilities for transferred patients could not be retrieved, this data must be interpreted with caution as, especially at the beginning of the emergence, patients were transferred frequently between facilities.

We also documented a positive effect of the vaccination when analyzing the first period separately, although it was of weaker intensity. Nonetheless, we need to consider that during the first period, only a small proportion of patients was vaccinated (4.4%), limiting the power of the analysis. On the other hand, no strong effect due to the vaccination was detected during the second period but, during later stages of the epidemic, many of the non-vaccinated subjects may have had naturally acquired immunity against SARS-CoV-2. The impossibility of controlling for previous infections (no data on antibody levels were available for this investigation) made it impossible to discriminate between first infections and reinfections and the frequency of reinfections was likely higher in the second period. A high level of background natural immunity would make outcome measurements in non-vaccinated and vaccinated groups similar since previous immunity is effective in protecting against severe forms of COVID-19 (32). Therefore, we postulate that the lack of differences in outcomes between vaccinated and non-vaccinated patients during the second period was due to a high percentage of non-vaccinated subjects possessing naturally acquired antibodies against SARS-CoV-2.

Finally, we could not properly evaluate vaccine-related disease outcomes in sub-groups of subjects with different types of immune suppression in the two periods. This is due to very low numbers of vaccinated subjects in the first period and of non-vaccinated subjects in the second period, impeding a meaningful assessment. One also needs to consider that only 17% of the subjects included in this study were vaccinated and these were mostly hospitalized during the second period.

In any case, in regression analyses, vaccination was always protective against severe outcomes, independently of whether the period of hospital admission was included or not in the model (OR of approximately 0.3 with upper bound CI < 0.5). On the other hand, the period of hospitalization was a significant predictor of severe outcomes only when included in a model that did not consider vaccination status. This suggests that the differences in outcome between the two periods can be explained by the different vaccination status of the two sub-populations.

4.4. Conclusion

Despite some limitations, including the relatively low number of patients in some sub-populations (particularly for what regards specific immune suppression conditions and vaccinated and non-vaccinated subjects during the first and second period, respectively) and the unavailability of some important data, such as details on times and doses of vaccination or specific information for certain types of immune suppression, this study has the strength of including data collected from patients hospitalized since the very beginning of the COVID-19 hospitalization insurgence. This allowed us to detect shifts in epidemiological and clinical characteristics of hospitalized patients throughout almost three years and draw conclusions about the clinical significance of immune suppression during the various stages of the epidemic.

During the first part of the COVID-19 epidemic, hospitalized patients were younger, had fewer co-morbidities and a lower proportion of them had factors of immune suppression. After adjusting for other factors, immune suppression was responsible for an overall 64% increase in the odds of severe outcomes and different conditions seemed to contribute differently to the severity of the outcome. While the spread of the less pathogenic Omicron variant may have been partly responsible for the reduced severity of the disease, a higher level of (natural and vaccine-induced) immunity in the general population significantly contributed to the observed shift in the characteristics of the hospitalized patients. Metanalyses or studies with a larger number of patients will be required to draw stronger conclusions for specific categories of immune suppression and determine their influence on COVID-19-related hospitalizations and severe outcomes throughout the various stages of the epidemic. Nonetheless, our results show that immune suppression is still a relevant co-morbidity in the clinical course of COVID-19 patients.

Data availability statement

The datasets presented in this article are not readily available due to the nature of the research, because of ethical reasons and of the sensitive nature of the research data, supporting data is not available. Requests to access the datasets should be directed to AB, alessandra.bandera@policlinico.mi.it.

Ethics statement

The studies involving humans were approved by the Medical Ethics Committee of the Fondazione IRCCS Ca′ Granda Ospedale Maggiore Policlinico (EC approval 241_2020, 17 March 2020). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft. MM: Supervision, Writing – review & editing. CB: Data curation, Resources, Writing – review & editing. AM: Data curation, Resources, Writing – review & editing. TM: Data curation, Validation, Writing – review & editing. SB: Validation, Writing – review & editing. FB: Data curation, Resources, Writing – review &

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Statistical explanation of the protective effect of four COVID-19 vaccine doses in the general population

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Objectives: To assess the effectiveness of four doses of the vaccine against SARS-CoV-2 in the general population and the impact of this on the severity of the disease by age group.

Methods: By using data from the health authority public data base, we build statistical models using R and the GAMLSS library to explain the behavior of new SARS-CoV-2 infections, active COVID-19 cases, ICU bed requirement total and by age group, and deaths at the national level.

Results: The four doses of vaccine and at least the interaction between the first and second doses were important explanatory factors for the protective effect against COVID-19. The R^2 for new cases per day was 0.5644 and for occupied ICU beds the R^2 is 0.9487. For occupied ICU beds for >70 years R^2 is 0.9195 and with the interaction between 4 doses as the main factor.

Conclusions: Although the increase in the number of vaccine doses did not adequately explain the decrease in the number of COVID-19 cases, it explained the decrease in ICU admissions and deaths nationwide and by age group.

KEYWORDS

COVID-19, vaccination, ICU hospitalizations, explanatory model, GAMLSS

Introduction

The pandemic caused by SARS-CoV-2 between 2020 and 2022 has caused a major public health burden with large number of deaths worldwide (1). As of May 23, 2023, a total of 676,609,955 cases and 6,881,955 deaths have been recorded (2). Globally, an estimated 68.4% of the population has received at least one dose of one of the available COVID-19 vaccine (1). With vaccination, face mask usage and quarantines, contagion rates were reduced. However, new SARS-CoV-2 variants of concern (VOC) have emerged further increasing virus spread and need of a global health emergency declaration by the World Health Organization (WHO) (3). The Omicron variant (B.1.1.529) has caused worldwide concern due to its high transmissibility and the reduced protection generated by vaccines against infection with this variant (4). Fortunately, the beneficial effect of herd immunity produced by mass vaccination has led to a reduction of severe COVID19 cases worldwide allowing WHO to declare the end of sanitary emergency on May 2023 (5).

Longitudinal studies of efficacy and effectiveness for various vaccines have reported a decrease in neutralizing antibodies during follow-up, which generated the need to introduce booster doses in the population (6–8). In Chile, Phase 3 studies in adults immunized with two doses of an inactivated SARS-CoV-2 vaccine (CoronaVac®) separated by 14 or 28 days showed that immunization with this vaccine induced robust humoral and cellular immunity and that the 28-day schedule induced a stronger humoral immune response than did the 14-day schedule (9–12). Further, a fourth dose of a homologous scheme with CoronaVac® managed to reestablish the neutralizing antibodies and maintain the cellular response against the wild type (WT) strain and Delta and Omicron (B.1.1.529) variants of SARS-CoV-2 (13). However, recent evidence is consistent in showing that the immune response triggered by original vaccines is lower for the Omicron variant and its subvariants as compared to the Wuhan SARS-CoV-2 strain (14). Therefore, booster vaccination campaigns for COVID-19 continue to be a priority for global public health (15, 16). When evaluating the effectiveness of vaccination against intensive care unit (ICU) admission, two-dose vaccination is less effective than three-dose vaccination, and the effectiveness drops from 68 to 36% if more than 2 months have passed since the last vaccination (17).

Chile has been one of the countries with the highest rates of vaccination against SARS-CoV-2 and, currently, 79.9% of this population has received a second booster (18). Previously, we built a model that explained the behavior of the pandemic data as a function of the vaccination, which at that time consisted of only two doses, and gave a central role to the number of doses administered and the interaction between these two doses (19). The hypothesis of this work is that the total number of doses of SARS-CoV-2 vaccine administered to the Chilean population contributed to the control of the pandemic. This was evaluated on the basis of the models developed in the previous work, with the objective of analyzing how the data behaved as the number of doses in the total population of Chile increased.

Materials and methods

Public data provided by the Ministry of Health of Chile and the Ministry of Science and Technology of the same country were analyzed for this study (18). As an initial analysis, models were used to examine the evolution of key pandemic-related variables; (1) daily number of new COVID-19 cases; (2) daily active COVID-19 cases; (3) daily ICU bed occupancy; and (4) daily COVID-19-related deaths. The different models generated for each response variable were constructed based on successive combinations of the doses administered with the vaccines, including their interactions or the total amount of vaccines administered. That is, for each variable studied, a model was developed that considered the total number of vaccines administered without discrimination by dose. Other models were also generated for each of the following situations: with only the first dose, with the first and second doses, with the first, second and third doses, and with the four doses administered to the population. In addition, the various interactions that could occur between the different doses administered were included. In the analysis of each variable studied, models were generated with the different combinations of doses described above. This was done for the variable new cases per day. However, in the case of active cases, in addition to considering the different models with the doses administered, new cases per day was introduced as an additional factor. In the context of ICU bed occupancy, both new and active cases were added as factors. Furthermore, in the models explaining deaths, additional variants were generated that included new cases, active cases, and ICU bed occupancy as influential factors in their dynamics. These analyzes were performed considering the total population.

A second considered the population according to different age groups for the variables in which this information was available. The models were made using different combinations of de variables age groups (3–39 yo, 40–49 yo, 50–59 yo, 60–69 yo, and 70 and over yo), the total cumulative number of vaccines administered, the cumulative daily number of vaccines administered and of first, second, third, and fourth dose of the vaccine. All these factors were adjusted to weekly counts because of the periodicity with which the data were uploaded to the public database. All outcome variables were normalized to counts per 100,000 population. This comprehensive approach made possible to address the relationship between vaccination and key epidemiologic variables, taking into account both the doses administered and other factors influencing the dynamics of the pandemic, but did not take into account other factors such as hospitalization measures, other health measures, or comorbidities of individuals in intensive care or deceased, as these types of data were not available in the source from which they were obtained.

All generated models and their respective analyses are available in the Github repository https://github.com/Aujeszky/vaccination_with_4_doses.

For our outcome variables of interest, we used generalized additive models for location, scale, and shape (GAMLSS) using a Gamma distribution (which is appropriate for continuous variables, as is the case for the normalized outcome variables used here). The processing and analysis of the national dataset was automated in scripts written in the R programming language (20) and the models generated were analyzed using the GAMLSS library (21). Previously published criteria were used to select the best model (19). To analyze the evolution of the models and their influence on the different variables have influenced them, the best model for each case was taken and compared with its similar models, iterating over each day to obtain the Akaike Information Criterion (AIC) and R^2 of each model. The R^2-value was maintained, but the AIC values were normalized to the best model to facilitate comparison.

Results

Four vaccine doses reduce infection severity

In the Chilean population, 91.92% have received at least one dose of the SARS-CoV-2 vaccine, 87.03% have received at least two doses, 79.9% have received three doses and 59.72% have received four doses (Supplementary Figure 1A). The vaccine formulations administered in the Chilean population were those

produced by Sinovac, Pfizer, Moderna, CanSino, and AstraZeneca. Out of these vaccines, Sinovac was the most massively administered vaccine for the first and second doses, and Pfizer vaccines were the most frequently administered for the third and fourth doses (Supplementary Figure 1B). To correctly analyze the results derived from the models, it must be considered that the national mass vaccination campaign started on February 3, 2021 and the administration of the second dose started 28 days later, in March 2021, with only 0.019% of the population vaccinated with the first dose; this group corresponds mainly to older adults. On August 11th of the same year, immunity was reinforced with a third vaccine dose, by which time 72.24 and 63.04% of the population had been vaccinated with the original first and second doses, respectively (22). One year after the start of the national vaccination campaign, in February 2022, the second booster (fourth vaccine dose) was administered to the population, at which time 89.8% of the population had been vaccinated with the first dose, 83.65% with the second dose and 64.1% with the first booster (or third vaccine dose) (Supplementary Figures 1A, B). When observing the number of cases per day, a peak was observed in January 2022, which coincided with the start of the second booster dose (Supplementary Figure 2A). On the contrary, the peak did not coincide with the number of occupied ICU beds, observing a slight increase in December 2022 that did not exceed the previous increases (Supplementary Figure 2C). Deaths associated with COVID-19 infection had dropped since October 2021, however, an increase was observed in February 2022 (Supplementary Figure 2E).

Booster immunization led the decline in ICU bed occupation and deaths

To understand the impact of vaccination on the Chilean population throughout the pandemic, explanatory models were generated based on GAMLSS, evaluating the number of new COVID-19 cases, active cases, occupied ICU bed number and deaths, based on models generated with the same data provided by (18). As described previously (19), the best model at national level for each response variable always includes the doses administered and the interaction between the original doses. However, since a larger number of doses we given, new models are required to analyze the effect of all four doses and the statistical interaction that may occur between all four doses. Therefore, the variable "total vaccines administered" was also included in the models, which consists of evaluating the vaccination as a whole and not by dose, and no model that adequately explained the behavior of the data included this variable. The best model to explain the behavior of new cases from the start of the national vaccination campaign until February 8th, 2023, is the one that includes the four doses administered to the population over 3 years of age and the statistical interaction between the first and second dose. This model has an $R^2 = 0.5644$ (Figure 1A), which decreases to 0.3866 when the interaction between the first and second dose is removed as an explanatory factor. The most important variables in this model are the third doses, followed by the interaction between first and second dose (Figure 2A). Active cases are explained by new cases, the four doses given and the interaction between the first two doses, although the model that includes new cases is very close to this model. The R^2 of this model is 0.7921 (Figure 1B) and drops to 0.7305 when the interaction factor is removed. In addition, the performance of the model drops significantly when new cases are excluded, resulting in an $R^2 = 0.3224$. The most important factors in the explanation of the behavior of active cases are, in order of importance: new cases and the third dose (Figure 2B). For the occupied ICU beds variable, the best model explaining the behavior of the data is the one that includes active cases, new cases, the four doses of vaccine and the interaction between the first three doses. This model has an $R^2 = 0.9489$ and shows a downward trend from the beginning (Figure 1C). The most important factors in this model are the fourth dose and the interaction between doses one, two, and three (Figure 2C). To explain the number of deaths due to COVID-19 in Chile, active cases, ICU beds occupancy, the four vaccine doses and the interaction between the first three vaccines are the factors that best explain the behavior of the data, giving an $R^2 = 0.8415$ (Figure 1D). Within this model, the most important factors are the interaction between first three doses and active COVID-19 cases (Figures 2A–D).

Interaction between doses explains reduced ICU bed occupancy based on age range

At a national level, the model explaining the behavior of ICU bed occupancy due to COVID-19 has the best fit, so we wanted to use GAMLSS models to break down how the data behave according to the age range of ICU patients. The data used to generate the models only included new cases per week, total vaccines and vaccines administered to each age group, as daily data were not available, so the data were analyzed on a weekly basis. In the age group corresponding to persons under 39 years, the most parsimonious model includes as factors the new cases within the same group, the four doses of vaccine and the interaction between the first and second dose. The R^2 of this model was equal to 0.9634 (Figure 3A) and has as the most important factor the interaction between the doses, followed by the new cases (Supplementary Figure 3A). For those aged 40–49, as for those under 39, the best model includes new cases, the four vaccine doses and the interaction between the first and second doses. This model has an $R^2 = 0.9376$ (Figure 3B), which drops to 0.922 when the interaction is removed from the explanatory factors, but the drop is radical when only new cases are considered, resulting in a an $R^2 = 0.001$ (Supplementary Figure 3B). The trend of the previous models was maintained in the 50–59 age group, with new cases, the four vaccine doses and the interaction between the first two doses. This model also explains the variability of the data very well, with an $R^2 = 0.9457$ (Figure 3C), which decreased to 0.0001 when only new cases were considered as an explanatory factor (Supplementary Figure 3C). The scenario begins to change for people aged between 60 and 69, as the new cases and the four doses are still present in the best models, but now the interaction between the four doses explains the behavior of the data better than the interaction between the

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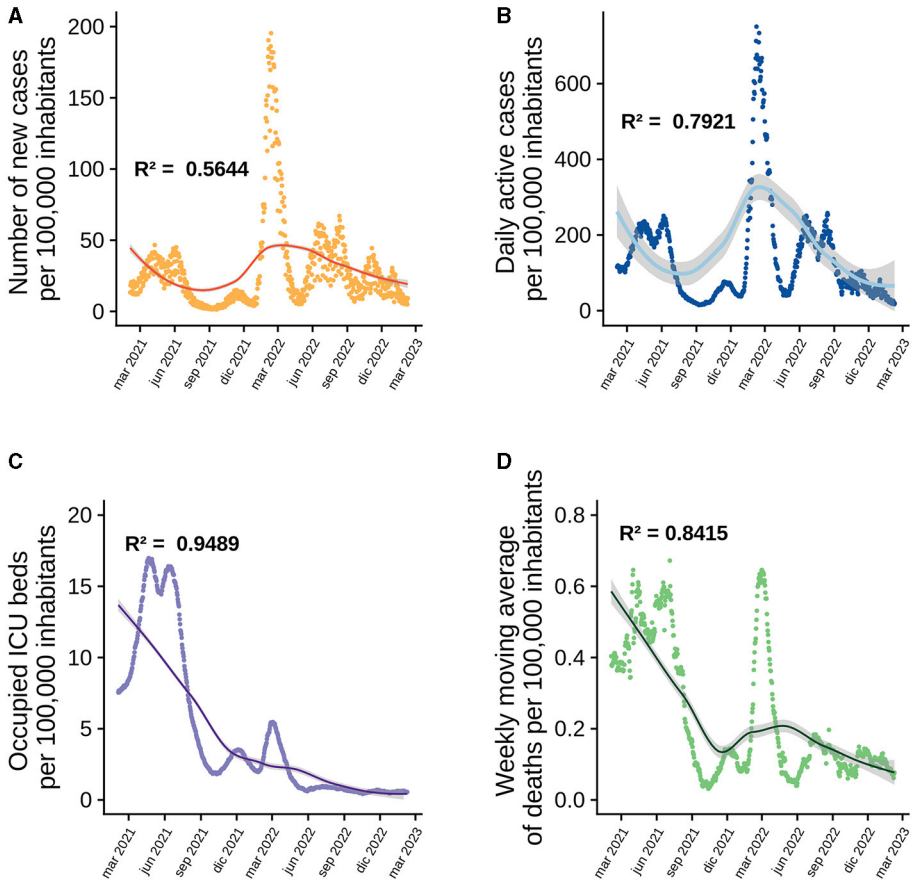

FIGURE 1
Behavior of national level data analyzed since the beginning of the national vaccination campaign using GAMLSS. In each graph the points correspond to daily count data per 100,000 population, the curves represent the model fit and the shaded area is the standard error of the model, each graph shows its corresponding R^2. **(A)** New cases increase in 100,000 inhabitants over time. **(B)** Active cases. **(C)** ICU beds occupied by patients with COVID-19. **(D)** Deaths due to COVID-19.

first two doses, giving an $R^2 = 0.9322$ (Figure 3D). When the interaction was removed from the model, the R^2 drops to 0.837, indicating the importance of the interaction at this level between the different vaccine doses (Supplementary Figure 3D). Something similar to the case for people aged 60–69 was observed for people aged 70 and more: the best model included the new cases, the four vaccine doses and the interaction between them, but the R^2 was only 0.9195 (Figure 3E), the most important factor in the model was the interaction between the doses (Supplementary Figure 3D).

The interaction between the doses explains the behavior of all variables over time

The R^2 obtained for each model, both at the national level and in the ICU bed models by age group, can be explained in the context of the start of the national vaccination campaign until February 8th, 2023. However, the analysis of each case does not explain how the model itself evolved over time and whether there were models with different variables that performed better in explaining the behavior of the data at certain points in time and, more importantly, how the interactions between the different doses explain the increase or decrease in performance of each model. For the national models, analyzing both new and active COVID-19 cases, all four doses are present in the model, but only the interaction between the first two doses gave the best model. The situation was similar for ICU bed occupancy and deaths, except that the interaction between the first three doses replaced the interaction with two doses. As time progressed, the models with more doses of vaccine differed from the others, but they were always accompanied by the interaction between the first and second dose for new and active cases, and the first three doses for ICU bed occupancy and deaths. It is also noteworthy that over time, models with few or no doses, and therefore fewer interactions, lose fitness, evaluated with the AIC, and ability to explain the dispersion of the data (R^2) compared to themselves at the beginning of the national vaccination campaign. Finally, it can be seen that the model with the interaction between the four doses is not yet equal to the model with the interaction between the first three doses (Figure 2; Supplementary Figure 3). It can be added that in the models with more than three doses, the regression coefficients were similar, but the AIC was very pronounced, making it clear which is the best model, and this is more easily seen as the age range increases, suggesting the importance of the vaccination plan with a fourth dose in older adults (Figure 4; Supplementary Figure 4).

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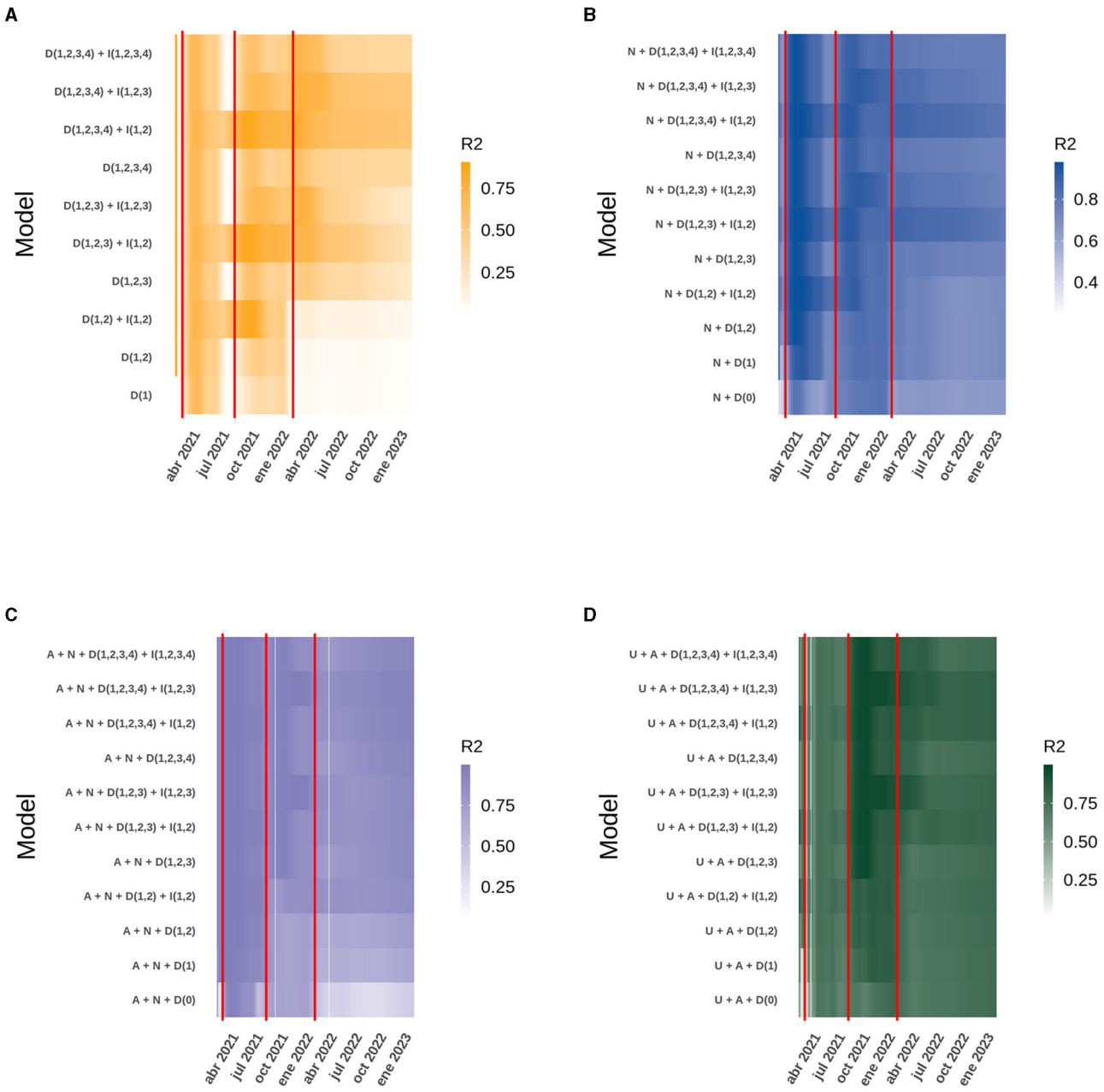

FIGURE 2
Heatmap of the daily R^2 obtained from the best model compared to similar models for the data analyzed at the national level. The red vertical lines correspond to the dates on which the second, third and fourth doses of the vaccine were started. The letter code corresponds to: U (Daily ICU beds), A (Daily active cases), N (Daily new cases), D (Number of doses), I (Interaction between doses). **(A)** New cases. **(B)** Active cases. **(C)** ICU beds occupied by patients with COVID-19. **(D)** Deaths due to COVID-19.

Discussion

All the models presented in this report include the four vaccine doses as explanatory factors and support to their importance in reducing the severity of COVID-19 cases. All the selected models also include the factor of interaction between other variables. A statistical interaction is understood as a situation in which the effect of one causal variable on an outcome depends on the state of a second causal variable, i.e., when the effects of the two causes are not additive (23). In the context of the immunization of a population, we explain the presence of the four doses as the immediate and protective effect of vaccination through the production of antibodies and effector immune cells. However, there is also the factor of interaction between doses of vaccines, which we understand as the development of an immunological memory specific for viral antigens through immunization, and because this process establishes later in the development of an immune response. We have developed here models that consider four COVID19 vaccine doses given to the population and showed that only the interaction between the first two or three doses affected

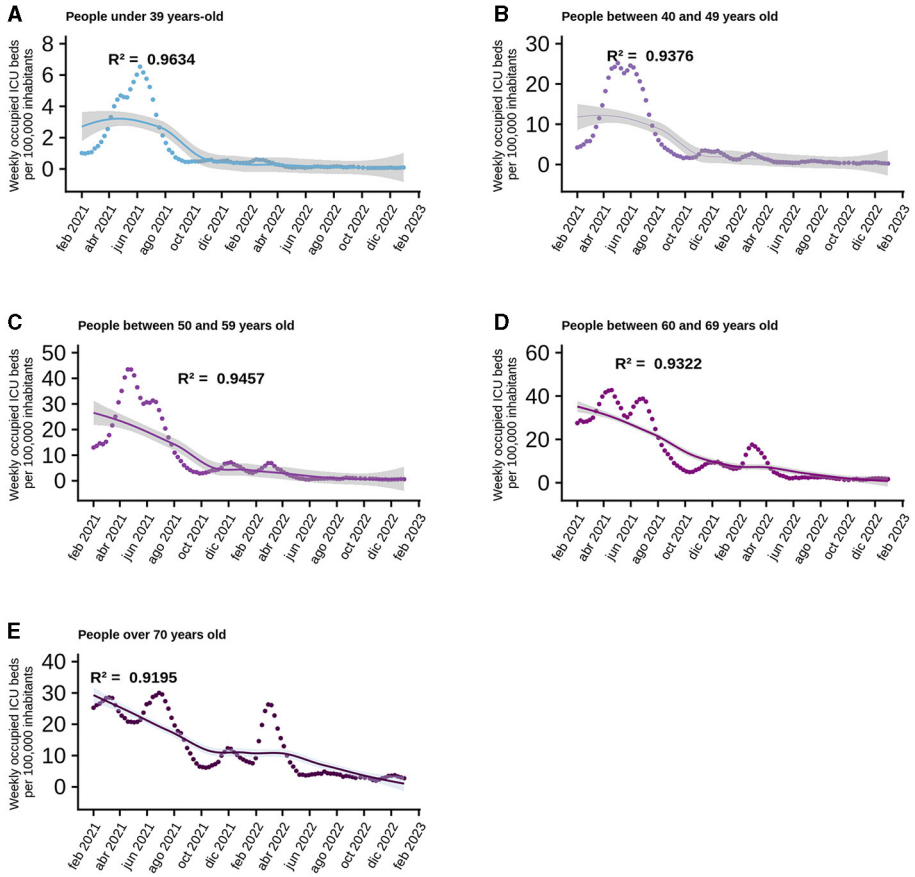

FIGURE 3
Behavior of normalized data per 100 thousand inhabitants of ICU beds according to age range. Analyzed since the beginning of the national vaccination campaign, the points correspond to the data provided by MINSAL on a weekly basis and the curve corresponds to the best model generated and the shaded area is the standard error of the model. Each graph shows the R^2 for each of them: **(A)** Persons under 39 years-old. **(B)** People between 40 and 49 years old. **(C)** People between 50 and 59 years old. **(D)** People between 60 and 69 years old. **(E)** People over 70 years old.

the data handling (Figures 2, 4). The interaction of the fourth dose is not present in the models of new cases, active cases and ICU beds occupied by patients between 3 and 59 years of age, due to the fact that people within this age range do not have great coverage with the fourth dose of vaccine preventing it from interacting at the population level with the other doses, but it is expected as the number of people vaccinated with this last dose is suspected, the interaction of the fourth dose appeared as an explanatory factor.

The model of new cases at national level shows that vaccination alone does not satisfactorily explain the decrease in cases, because the model does not include factors such as sanitary measures or variations in population mobility, and none of the models includes the different variants of the coronavirus circulating in each period.

With this work, we cannot confirm the mechanism by which heterologous vaccination works in the population, but based on studies, it has been seen that in the mouse model, the humoral and cellular immune response was poor when immunized with two doses of an inactivated virus vaccine, but the amount of neutralizing antibodies improved when a booster with an mRNA or adenoviral vector-based vaccine was applied (24), a heterologous adenoviral and mRNA vaccine schedule is better at developing a Th1 response in conjunction with cytotoxic T lymphocytes than a homologous vaccine schedule (25), and a recombinant BCG-based vaccine for the nucleoprotein has shown an increase in the number of $CD4^+$ and $CD8^+$ lymphocytes, and the parameters studied are related to a trained immunity profile (26). In Germany, a study showed that heterologous immunization with a first dose of the Oxford-AstraZeneca vaccine and a booster at 9–12 weeks with the Pfizer vaccine produced a higher level of neutralizing antibodies than homologous immunization with either vaccine (27), this is because the Pfizer vaccine induces a high production of antibodies, while the AstraZeneca vaccine induces a stronger cellular response, which when mixed together produces a much greater effect than vaccination with a single formulation (28). On the other hand, people who had two doses of CoronaVac® and received a booster from Pfizer had higher levels of neutralizing antibodies specific to the beta, gamma and delta variants of SARS-CoV-2 than people who had a booster with CoronaVac® again (29).

In Chile, a two-dose vaccination schedule with CoronaVac® in children under 17 years of age was shown to be safe, with an increase in antibody titers and $CD4^+$ lymphocyte activation 4 weeks after the second dose, although antibody titers against the Delta and Omicron (B.1.1.529) variants were lower than those against the D614G strain (30, 31). In a homologous schedule with a

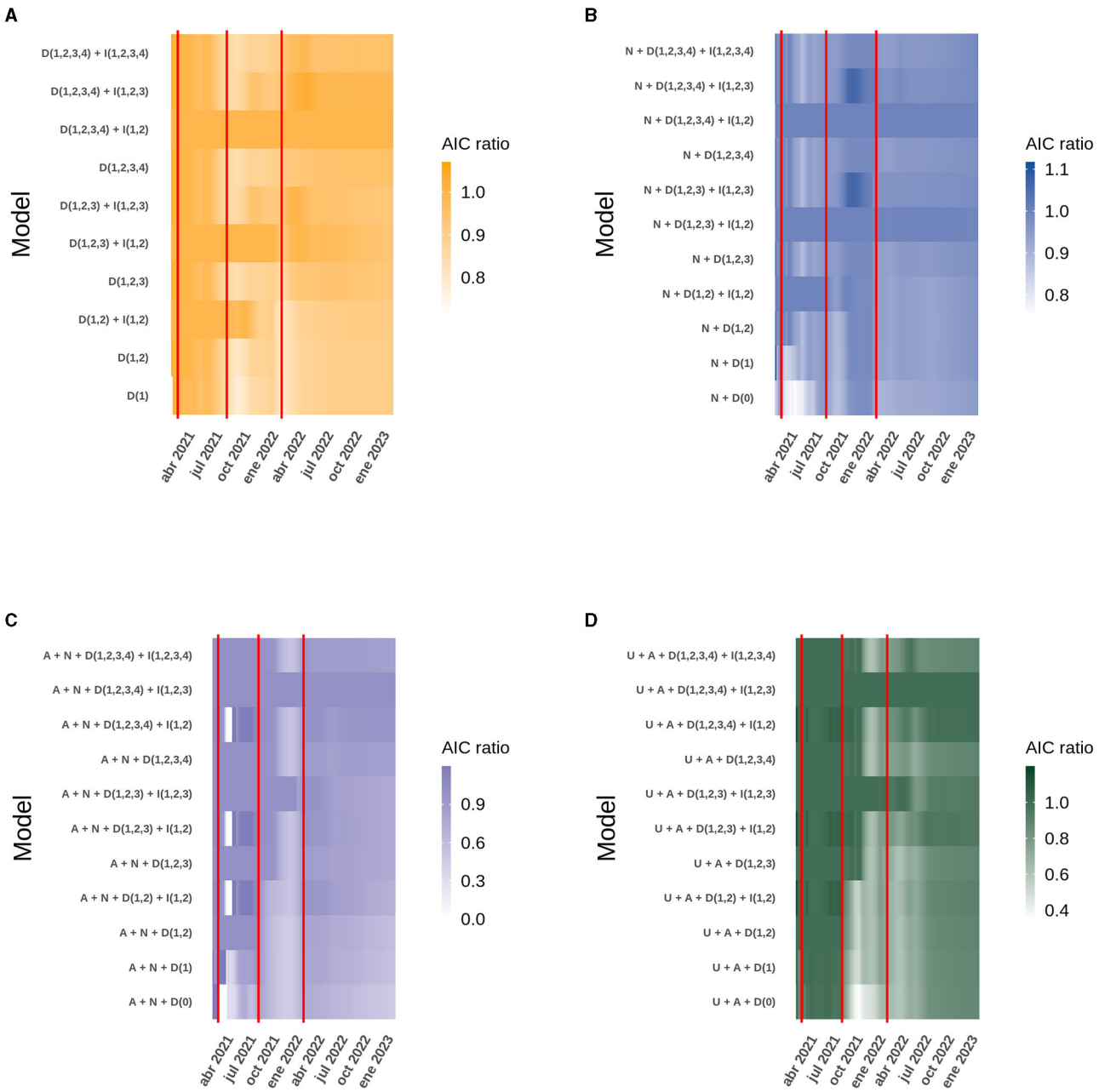

FIGURE 4
Heatmap of the AIC obtained daily from the best model compared to similar models for the data analyzed at the national level. The red vertical lines correspond to the dates on which the second, third and fourth doses of the vaccine were started. The letter code corresponds to: U (Daily ICU beds), A (Daily active cases), N (Daily new cases), D (Number of doses), I (Interaction between doses). **(A)** New cases. **(B)** Active cases. **(C)** ICU beds occupied by patients with COVID-19. **(D)** Deaths due to COVID-19.

booster dose following two doses of CoronaVac® vaccine in adults, an increase in neutralizing antibodies was observed 4 weeks after the booster dose, and an increase in anti-SARS-CoV-2 specific T cells was also observed, peaking 4 weeks after the booster dose (32). In addition, the immune response generated showed activity against Delta and Omicron (B.1.1.529) variants (33).

Our model is consistent with the study by Jara et al., which suggests that a homologous or heterologous booster dose for individuals with a complete primary vaccination schedule with CoronaVac® provides a high level of protection against COVID-19, including severe disease and death. Heterologous boosters showed greater vaccine efficacy than homologous boosters for all outcomes (34).

A heterologous vaccination schedule has been shown to be more effective than a homologous vaccination, leading to the development of new vaccination schedules against this or other pathogens, and may also reduce the use of drugs for comorbidities (35).

The nature of these models is only explanatory but looking at how the models have behaved over time, we can predict that the models with the four doses and the interaction between the first, second and third dose will tend to be better than those already shown in this work, as long as the population completes its vaccination schedule with the four doses.

Policy implications

This work highlights the importance of achieving full vaccination status and reinforces the notion that heterologous vaccination confers greater protection. The trends observed may also support the inclusion of seasonal vaccination program for vulnerable individuals. These data could guide other countries in their vaccination campaigns.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

HR: Data curation, Formal analysis, Methodology, Visualization, Writing—original draft, Writing—review and editing. CM: Visualization, Writing—original draft, Writing—review and editing. AK: Funding acquisition, Resources, Supervision, Writing—original draft, Writing—review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1253762/full#supplementary-material

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Relationship between clinical-epidemiological parameters and outcomes of patients with COVID-19 admitted to the intensive care unit: a report from a Brazilian hospital

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Background: People in low-income countries, especially those with low socio-economic conditions, are likelier to test positive for SARS-CoV-2. The unequal conditions of public health systems also increase the infection rate and make early identification and treatment of at-risk patients difficult. Here, we aimed to characterize the epidemiological profile of COVID-19 patients in intensive care and identify laboratory and clinical markers associated with death.

Materials and methods: We conducted an observational, descriptive, and cross-sectional study in a reference hospital for COVID-19 treatment in the Southern Region of Bahia State, in Brazil, to evaluate the epidemiological, clinical, and laboratory characteristics of COVID-19 patients admitted to the intensive care unit (ICU). Additionally, we used the area under the curve (AUC) to classify survivors and non-survivors and a multivariate logistic regression analysis to assess factors associated with death. Data was collected from the hospital databases between April 2020 and July 2021.

Results: The use of bladder catheters (OR 79.30; $p < 0.0001$) and central venous catheters (OR, 45.12; $p < 0.0001$) were the main factors associated with death in ICU COVID-19 patients. Additionally, the number of non-survivors increased with age ($p < 0.0001$) and prolonged ICU stay ($p < 0.0001$). Besides, SAPS3 presents a higher sensibility (77.9%) and specificity (63.1%) to discriminate between survivors and non-survivor with an AUC of 0.79 ($p < 0.0001$).

Conclusion: We suggest that multi-laboratory parameters can predict patient prognosis and guide healthcare teams toward more assertive clinical management,

better resource allocation, and improved survival of COVID-19 patients admitted to the ICU.

KEYWORDS

COVID-19, SARS-CoV-2, biomarkers, epidemiology, SAPS3, in-hospital mortality, intensive care unit, catheter

1. Introduction

The global impact of the coronavirus disease 2019 (COVID-19) is unquestionable. Concerning deaths, 68% were concentrated in 10 countries: Brazil, Egypt, India, Indonesia, Mexico, Peru, Russia, South Africa, Turkey, and the United States (1). The first COVID-19 case in Brazil was confirmed on February 26, 2020. The disease rapidly spread in the capital and countryside regions, and within a month, community transmission was documented in Brazilian cities. Bahia is Brazil's fourth most populous State and the sixth state in cumulative deaths as of 2022, with the first case confirmed on March 6, 2020, through reverse transcription-quantitative polymerase chain reaction (RT-qPCR) (2–4).

By December 2022, Brazil had an incidence coefficient of 17,152, with an incidence rate of 11,738 per 100,000 inhabitants in Bahia. One of the main cities in the Southern Region of Bahia State, Ilhéus, has an incidence rate of 17,129 per 100,000 inhabitants, which is higher than that of Bahia. While Brazil's lethality rate is 1.9%, Bahia's rate is 1.8%, and Salvador, Vitória da Conquista, Feira de Santana, and Ilhéus have the highest number of deaths (2, 5).

Generally, the infection can manifest in a varied clinical spectrum ranging from asymptomatic to critical presentations. In addition to respiratory symptoms, severe cases may present with extrapulmonary complications or multiple organ failure, and early identification and treatment of at-risk patients are essential to prevent mortality (6–8). From an epidemiological perspective, a profile analysis of severe COVID-19 cases indicates that males have higher mortality rates than females do. Furthermore, comorbidities such as hypertension, diabetes, heart disease, malignancy, and immunodeficiency are more prevalent in individuals with severe COVID-19, irrespective of sex (9–13). However, in the Southern region of Bahia State, at the beginning of the pandemic, males with comorbidities were more likely to test positive for SARS-CoV-2 (14).

Early indicators of death in hospitalized patients guide clinical decision-making and include blood pressure, respiratory rate, D-dimer levels, international normalized ratio (INR), **and Simplified** Acute Physiology Score 3 (SAPS 3), which are predictors of in-hospital mortality (15–20). Multiple biomarkers are necessary to assess disease progression and an individual's response to clinical interventions (21–23). Notably, well-established biomarkers include interleukin-6 (IL-6) and C-reactive protein levels (24–26).

Nonetheless, the profile of SARS-CoV-2 infection changes as new variants emerge, increasing the infection rate, mortality, and symptomatic profile (27–29). People in low-income countries, especially those with low socio-economic conditions, are more likely to test positive for SARS-CoV-2, with higher mortality rates (30). Accordingly, a study conducted in South America showed high seropositivity in individuals with low socio-economic status (31). Similarly, the unequal conditions of public health systems increase the infection rate and make early identification and treatment of at-risk patients difficult (32–34).

We aimed to characterize the clinicopathological profile of hospitalized patients with COVID-19 admitted to the intensive care unit (ICU) of a reference hospital for COVID-19 in the Southern Region of Bahia State, in Brazil, between April 2020 and July 2021. Additionally, we analyzed the data to identify the laboratory and clinical markers associated with death in patients admitted to the ICU.

2. Materials and methods

2.1. Ethical considerations

The study was submitted to the Research Ethics Committee of the State University of Santa Cruz and approved under protocol number CAAE:40671720.4.0000.5526 on February 22, 2021.

2.2. Study design, data collection, and curation

We conducted an observational, descriptive, and cross-sectional study at a reference hospital for COVID-19 treatment in the Southern Region of Bahia State, Brazil. Data from individuals admitted to the ICU with COVID-19, confirmed using RT-qPCR for SARS-CoV-2 RNA, were collected between April 2020 and July 2021. The care and clinical observations of the patients were performed by a multidisciplinary team at the hospital, and a registered nurse entered the epidemiological, clinical, and complete laboratory information into the Epimed Monitor System database as a hospital routine. The Epimed Monitor System is a cloud-based registry of clinical and administrative data for managing intensive care unit patients.

The patient data were collected from the Epimed Monitor System database. No patient identification was accessed; instead, patients were identified through numerical coding, ensuring the confidentiality and anonymity of participants. The inclusion criteria for this study were as follows: individuals who entered the ICU between April 2020 and July 2021, adults (18 years or older), positivity for SARS-CoV-2 RNA by RT-qPCR, at least 1 day (24 h) of ICU stay, and availability of clinical and epidemiological data in the Epimed Monitor System database. The exclusion criteria included: patients aged <18 years, those who tested negative or inconclusive for SARS-CoV-2 RNA by RT-qPCR, and those who stayed in the ICU for less than 24 h. The clinical data considered for the analysis included arterial hypertension, diabetes, vasopressor use, renal injury, and respiratory failure. Laboratory data included the fraction of inspired oxygen (FiO_2), partial pressure of

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carbon dioxide ($PaCO_2$)/FiO_2, serum lactate, arterial pH, serum creatinine (CR), serum urea (SR), and white blood cell count (measured as white blood cell,WBC, count × 1,000/mm^3). Additionally, invasive procedures associated with severe cases, such as mechanical ventilation and catheter use, were included in the analysis. We considered all COVID-19-positive individuals admitted to the ICU whose epidemiological, clinical, and laboratory data were available during the study period.

In total, 501 individuals were included in the analysis. We excluded individuals who tested negative for SARS-CoV-2 ($n = 92$) and those with suspected or unconfirmed detection ($n = 45$) by RT-qPCR. Individuals with incomplete data on comorbidities ($n = 97$), physiological data ($n = 17$), or laboratory data ($n = 32$) were excluded from the analysis (**Figure 1**). In total, 218 individuals were included in this study.

2.3. Breakdown of variables for the study

Categorical variables: Hypertension, diabetes, vasopressors, renal injury, respiratory failure, invasive and non-invasive mechanical ventilation, and use of catheters are represented as absolute frequencies (n), percentages (%), odds ratios (ORs), and 95% confidence intervals (95% CIs) with respective *p*-values. Continuous variables included: blood urea nitrogen (BUN), CR, age, lactate, white blood cells (WBCs), greater $PaCO_2$, greater $PaFiO_2$, greater $PaO_2/PaFiO_2$, greater arterial pH, hospital stay, ICU stay, SAPS3, and urea, are shown as individual values, mean ± standard deviation, minimum, maximum, and median values, with respective *p*-values. For the logistic regression model, data were represented as ORs and 95% confidence intervals (CI associated with individual *p*-values).

2.4. Statistical analyses

Continuous variables were evaluated for normality using the Shapiro–Wilk test. Variables that assumed a normal distribution and those that did not were analyzed using Student's *t*-test and the Mann–Whitney *U* test, respectively.

We classified survivors and non-survivors using the area under the curve (AUC) from the Wilson/Brown method, with sensitivity (Se, %), specificity (Sp, %), and 95% CI values associated with the respective *p*-values. First, the cut-off point of the variables (discriminant value) was established as the value associated with maximum sensitivity and specificity (28). The statistical significance of the cut-off point was then selected by analyzing the sensitivity and specificity, AUC, value of *p*, and 95% CI values (35).

We used Pearson's chi-square test (X^2) and Fisher's exact test (36) to analyze the association between the frequency of each categorical variable and the participants' clinical outcomes (ICU discharge and death). Statistical analyses were performed using the GraphPad Prism software (version 9.0; GraphPad Prism Software, San Diego, CA,

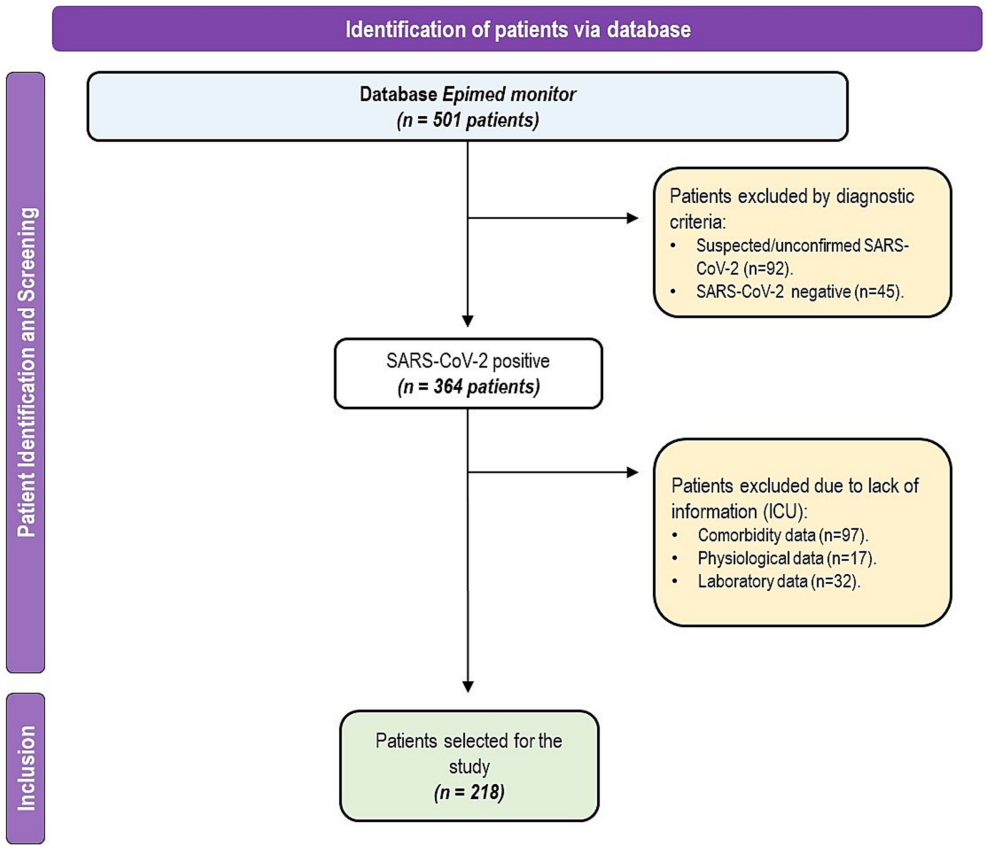

FIGURE 1
Participant selection flowchart in the reference hospital database.

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United States) at a significance level of 5%. Therefore, $p < 0.05$ were considered statistically significant.

We used a bivariate analysis with a significance level of $p < 0.20$ to identify candidate variables to fit in the logistic regression analysis in a multivariate model. Moreover, a *stepwise backward* (conditional) elimination method was used, and the best model was defined as one that included statistically significant variables ($p < 0.05$) and minimized the value of the Akaike Information Criteria (AIC). All the statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 26.0 (IBM Corporation, Armonk, NY, United States). Los Angeles, CA, United States.

3. Results

3.1. Clinical profile of COVID-19 patients associated with patient outcome

Between April 2020 and July 2021, 501 SARS-CoV-2 positive individuals from the Southern Region of Bahia State were admitted to the ICU of a referred hospital for COVID-19 treatment. After screening the data, 218 individuals were included in our analysis:141 (64, 68%) were discharged from the ICU, and 77 (35, 32%) died. The average age of patients was 64.37 ± 15.19 years, and males comprised the majority of our population ($n = 123$, 56.4%). Sex did not increase the odds of death (OR 1.58; 95% CI 0.89–2.81; $p = 0.112$), while patients with advanced age were more likely to die (Figure 2A); accordingly, the concentration of non-survivors was higher among patients older than 66 years of age (Se 70.1; Sp 61.7; AUC 0.74; 95% CI 0.678–0.811; $p < 0.0001$; Figure 2B). Furthermore, among clinical requirements in hospitalized patients, the use of vasopressors (OR 6.28; 95% CI 3.08–12.56; $p < 0.0001$) and mechanical ventilation (OR 5.56; 95% CI 3.05–10.15; $p < 0.0001$) increased the odds of death (Table 1). We also observed that the use of a bladder catheter ($p < 0.0001$), central venous catheter ($p < 0.0001$), and arterial by 79.30, 45.12, and 16.11, respectively. On the other hand, the use of non-invasive mechanical ventilation decreased the chance of death in hospitalized patients (OR 0.34; 95% CI 0.18–0.60; $p = 0.0003$).

3.2. Time of ICU stay and clinical score can be used to discriminate survivors and non-survivors with COVID-19 in the ICU

Hospitalized patients presented an average ICU stay of 14.8 ± 13.18 days. Differences between the length of ICU stay of the patient discharged (13.77 ± 14.51) and death (16.71 ± 10.11) were observed ($p < 0.0001$; Figure 3A, left). Furthermore, individuals with an ICU stay of >11.5 days were more likely to die (Se 67.5; Sp 57.4; AUC 0.66, 95% CI 0.587–0.735; $p < 0.0001$; Figure 3A, right).

The SAPS3 is a scoring system widely used to predict in-hospital mortality and uses pertinent variables of acute physiological derangements, current conditions, interventions, and health status before ICU admission to predict mortality (24, 25). The highest concentration of deaths due to COVID-19 was in ICU participants who had SAPS3 > 51.5 (Se 77.9; Sp 63.1; AUC 0.79; 95% CI 0.727–0.855; $p < 0.0001$; Figure 3B). Moreover, PaO_2/FiO_2 was used to determine the need for invasive or non-invasive mechanical ventilation in the hospital setting and was associated with death. Participants with FiO_2 greater than 57.5% were more likely to die (Se, 70.1; Sp, 58.1; AUC, 0.67; 95% CI 0.602–0.747; $p < 0.0001$; Figure 3C). Regarding the PaO_2/FiO_2 ratio, which represents the degree of lung injury, participants with a higher PaO_2/FiO_2 ratio > 139.0 were more likely to die (Se 62.3; Sp 55.3; AUC 0.60; 95% CI 0.528–0.685; $p = 0.0093$; Figure 3D). Among other markers such as Higher $PaCO_2$, PaO_2, lower diastolic blood pressure, lower systolic blood pressure, and hospital stay (days), we did not observe any statistical significance (Supplementary Figures S1A–E).

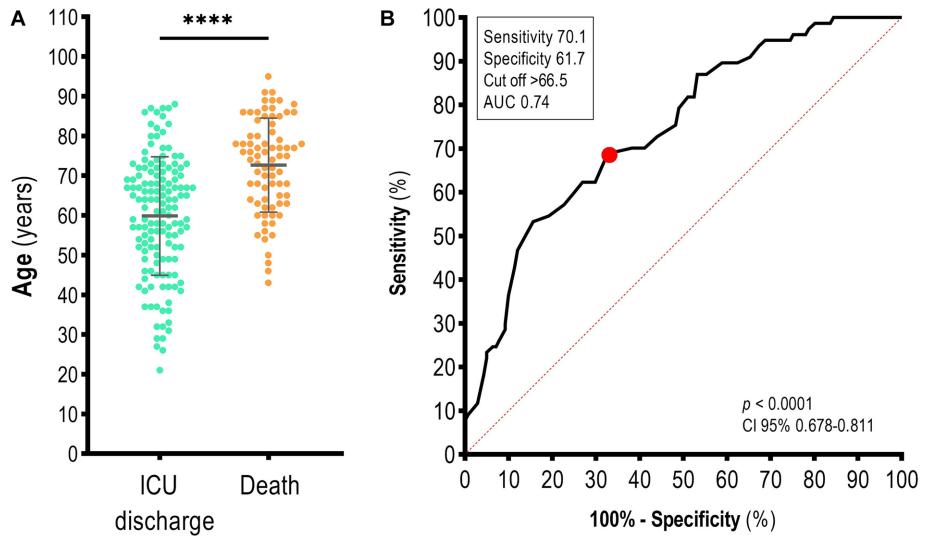

FIGURE 2
Age distribution of patients with COVID-19 in the ICU of a reference hospital in the Southern Region of Bahia State, Brazil. **(A)** Age in years and **(B)** the area under the curve (AUC) to discriminate between survivors and non-survivors. The red point indicates the cut-off value. Mann–Whitney test. Data are presented as the mean ± standard deviation. *p*-values <0.05 were considered statistically significant. ****$p < 0.0001$.

TABLE 1 Clinical characteristics of COVID-19 patients admitted to the ICU.

	COVID-19 patients			Univariate analysis		
	Total *n* =218 (%)	Death *n* =77 (%)	ICU discharge *n* =141 (%)	OR	95% CI	*p*[a]
Sex						
Male	123 (56.4)	49 (22.5)	74 (33.9)	1.58	0.89–2.81	*0.112*
Female	95 (43.6)	28 (12.9)	67 (30.7)			
Arterial hypertension						
Yes	173 (79.3)	63 (28.9)	110 (50.5)	1.27	0.61–2.61	*0.507*
No	45 (20.7)	14 (6.4)	31 (14.2)			
Diabetes						
Yes	92 (42.2)	34 (15.6)	58 (26.6)	1.13	0.66–2.00	*0.669*
No	126 (57.8)	43 (19.7)	83 (38.1)			
Vasopressors						
Yes	43 (80.3)	30 (13.7)	13 (6.0)	6.28	3.08–12.56	***<0.0001***
No	175 (19.7)	47 (21.6)	128 (58.7)			
Kidney injury						
Yes	7 (3.2)	5 (2.3)	2 (0.9)	0.21	0.04–1.01	*0.099*
No	211 (96.8)	72 (33.2)	138 (63.6)			
Respiratory failure						
Yes	208 (96.3)	76 (34.9)	132 (60.5)	5.18	0.82–57.58	*0.102*
No	10 (3.7)	1 (0.5)	9 (4.1)			
Mechanical ventilation						
Yes	78 (35.8)	47 (21.5)	31 (14.2)	5.56	3.05–10.15	***<0.0001***
No	140 (64.2)	30 (13.8)	110 (50.5)			
Non-invasive mechanical ventilation						
Yes	92 (42.2)	20 (9.2)	72 (33.0)	0.34	0.18–0.60	***<0.0003***
No	126 (57.8)	57 (26.1)	69 (31.7)			
Central venous catheter						
Yes	139 (63.8)	75 (34.4)	64 (29.4)	45.12	11.60–191.2	***<0.0001***
No	79 (36.2)	2 (0.9)	77 (35.3)			
Arterial catheter						
Yes	124 (56.9)	70 (31.1)	54 (24.8)	16.11	7.05–39.07	***<0.0001***
No	94 (43.1)	7 (3.2)	87 (39.9)			
Bladder catheter						
Yes	145 (66.5)	76 (34.9)	69 (31.6)	79.30	13.693–810.2	***<0.0001***
No	73 (33.5)	1 (0.5)	72 (33.0)			

[a]Chi-Square test (X^2) and Fisher's exact test. Highlighted values are considered statistically significant.

3.3. Laboratorial markers associated with death in COVID-19 patients in the ICU

COVID-19 patients who died in the ICU presented higher leukocyte count (14.22 ± 6.78 cells x 1,000/mm^3) than did the ICU-discharged patients (9.46 ± 4.18 cells x 1,000/mm^3; $p < 0.0001$; Figure 4A, left). Although cardiovascular complications and thromboembolism have been previously reported in COVID-19 patients (37, 38), we did not observe a difference in the platelet count between dead and discharged patients with COVID-19 in the ICU (Supplementary Figure S1F). Among the studied biomarkers, we observed higher arterial lactate ($p < 0.01$), serum creatine ($p < 0.0001$), serum urea ($p < 0.0001$), and serum urea nitrogen ($p < 0.0001$) in patients with death outcomes than in discharged patients (Figures 4B,D–F, left), whereas higher arterial pH was lower in death patients ($p < 0.001$; Figure 4C, left). Higher leukocyte count (Se 71.4; Sp 61.7; AUC 0.71; 95% CI 0.644–0.792; $p < 0.0001$; Figure 4A, right), serum creatine (Se 77.9; Sp 62.4; AUC 0.74; 95% CI 0.674–0.811; $p < 0.0001$; Figure 4E, right), and serum urea nitrogen (Se 75.3; Sp 63.1; AUC 0.74; 95% CI 0.674–0.811; $p < 0.0001$; Figure 4F,

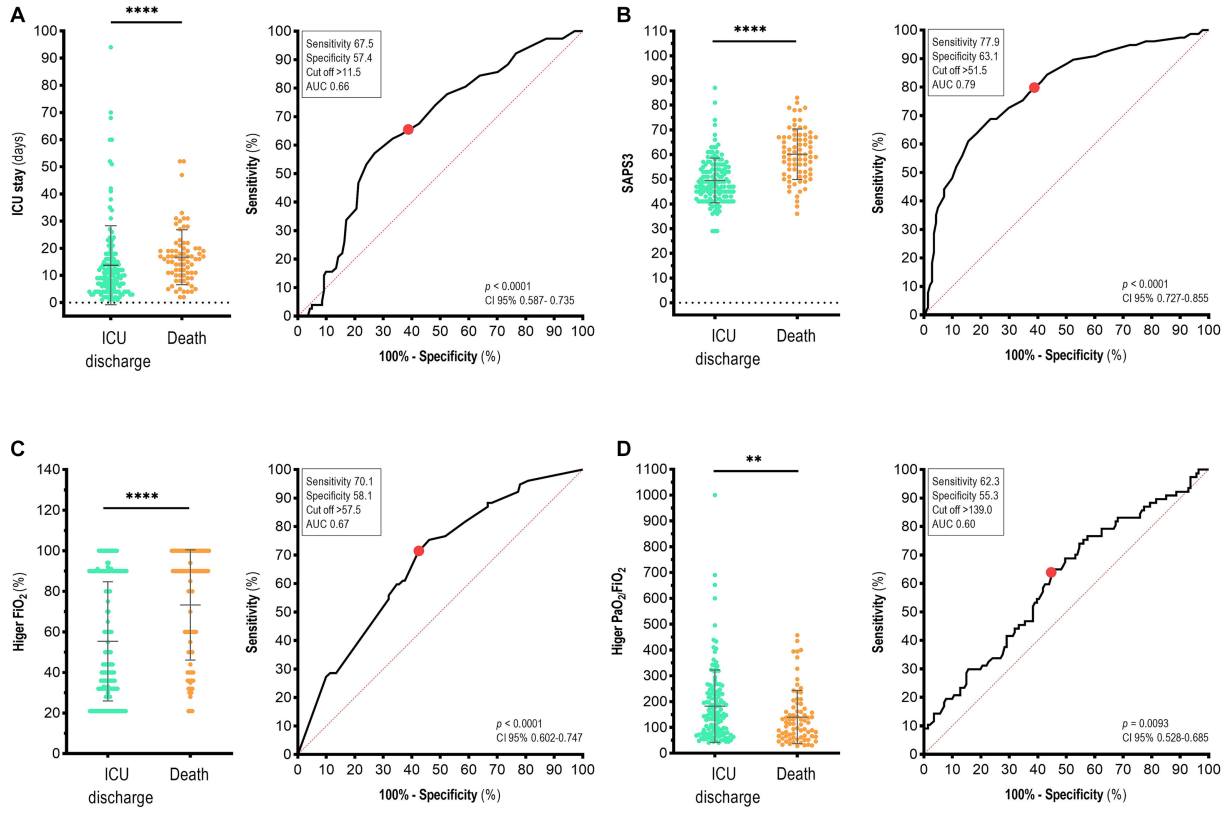

FIGURE 3
Clinical parameters used to discriminate between survivors and non-survivors with COVID-19 in the ICU of a reference hospital in the Southern Region of Bahia State, Brazil. Analysis of variables (left) and area under the curve (AUC, right) for **(A)** ICU stay, **(B)** SAPS3 – Simplified Acute Physiology Score 3, **(C)** higher FiO2, and **(D)** higher PaO2/FiO2. The red point indicates the cut-off value. Mann–Whitney test. Data are presented as the mean ± standard deviation. *p*-values <0.05 were considered statistically significant. $^{**}p < 0.01$; $^{****}p < 0.0001$.

right), were the best markers to discriminate survivors and non-survivors.

3.4. Factors associated with death from COVID-19 patients in the ICU

We performed multivariate logistic regression analysis to verify whether the significant variables described above were associated with death in COVID-19 patients. The analysis revealed that men were more likely to die from COVID-19 in the ICU (OR 2.73; 95% CI 1.15–6.46; $p = 0.022$; Table 2). Moreover, the ICU stay and PCO_2 did not increase the odds of death in our population, while bladder catheter (OR 28.09; 95% CI 2.69–292.8; $p = 0.005$) and central venous catheter (OR 12.97; 95% CI 2.25–74.74; $p = 0.004$) presented as risk factors and increased the odds to death (Table 2).

4. Discussion

Herein, we describe the epidemiological and clinical characteristics of COVID-19 patients admitted to the ICU of a hospital for COVID-19 treatment in the Southern Region of the Bahia State, Brazil. We also analyzed the factors associated with death. For example, we identified clinical parameters such as the use of mechanical ventilation, central venous catheters, arterial catheters, vasopressors, and bladder catheters related to the respiratory, cardiovascular, and urinary systems, which increased the odds of death in COVID-19 patients in intensive care.

The impact of the COVID-19 pandemic has not been homogeneous worldwide, with some countries being more affected and presenting different mortality rates (1). Social factors and precarious socio-economic conditions are drivers of increased infection and mortality rates (30–34, 39, 40). For example, the positivity of SARS-CoV-2 infection in cities in the Southern Region of Bahia State was negatively correlated with a low Human Development Index (HDI) (41) (Bahia State has a low HDI, and the average worker salary is less than US$ 600.00). Furthermore, it was also shown that individual and community risk factors for SARS-CoV-2 infection varied between the Bahia cities; for example, gender and age were not homogenous risk factors for SARS-CoV-2 infection between the 12 cities studied (42).

A retrospective study in Brazil using population-based registers demonstrated that individuals hospitalized for less than 4 days presented high odds of death (OR 2.07, 95% CI 2.05–2.10). Moreover, the odds of death were five times higher than for individuals requiring ICU admission (OR 5.19, 95% CI 5.14–5.24) (43). Notably, in our study, 35.32% of the COVID-19 patients in the ICU died. An in-hospital mortality rate of 37% for COVID-19 was reported in Brazil, and the mortality rate increased with advanced age, low

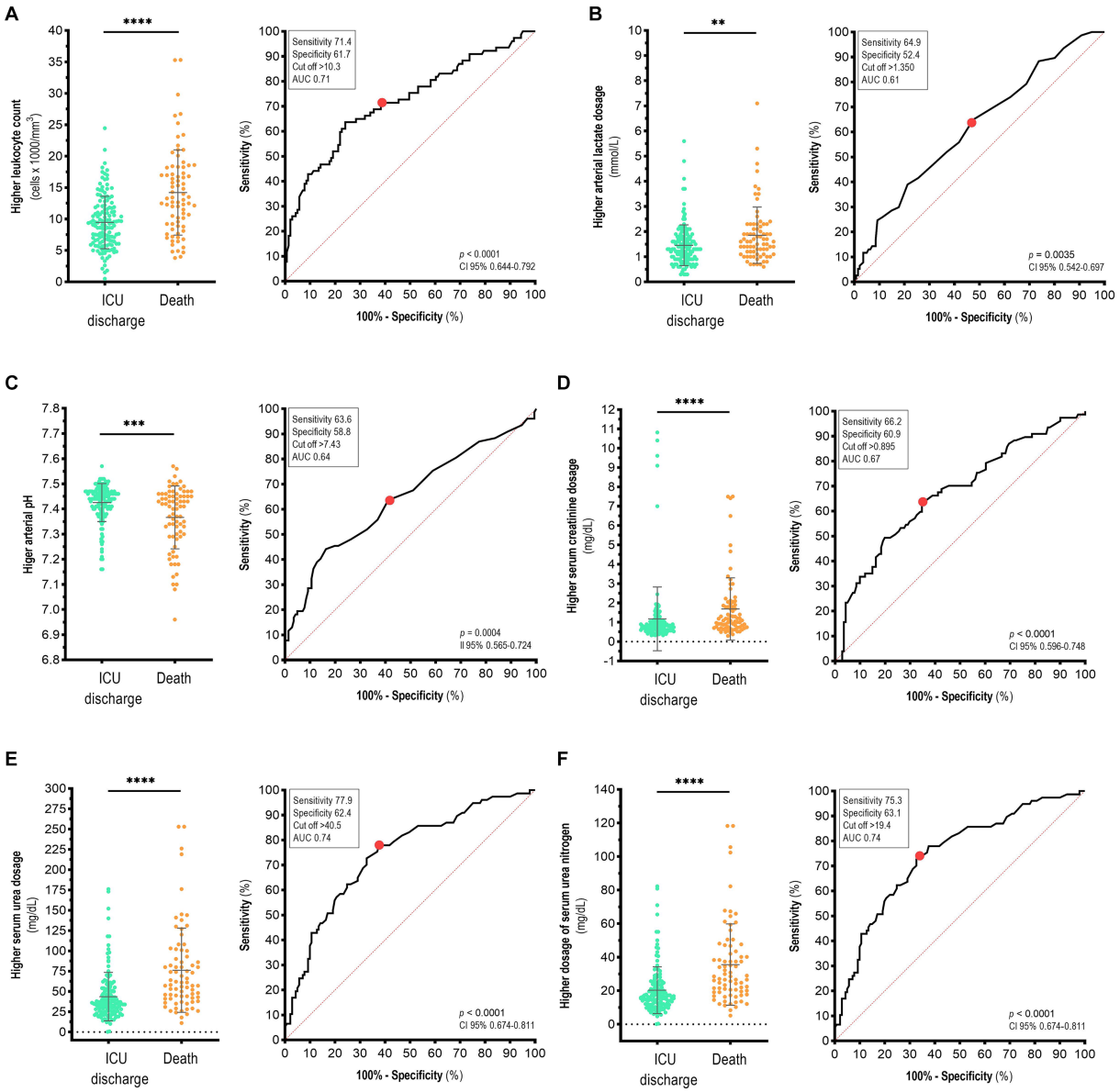

FIGURE 4
Biochemical and hematological parameters used to discriminate between survivors and non-survivors with COVID-19 in the ICU of a reference hospital in the Southern Region of Bahia State, Brazil. Analysis of variables (left) and area under the curve (AUC, right) for **(A)** higher leukocyte count, **(B)** higher arterial lactate, **(C)** higher arterial pH, **(D)** higher serum creatinine, **(E)** higher serum urea, and **(F)** serum urea nitrogen. The red point indicates the cut-off value. Mann–Whitney test. Data are presented as the mean ± standard deviation. *p*-values <0.05 were considered statistically significant. $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

education level, comorbidities, and in individuals of black/brown self-reported race (44).

Notably, determining the clinical-epidemiological and laboratory profiles of COVID-19 patients can provide valuable information for a multidisciplinary healthcare team for more assertive clinical management, better resource allocation, and improved survival of patients admitted with COVID-19 in the ICU (44, 45). In this study, when multifactorial variables were correlated using regression analysis, the male sex had a higher chance of death, consistent with previous studies (46, 47). Furthermore, male-specific variables such as hypogonadism and low testosterone levels have been linked to the development of comorbidities that increase mortality from COVID-19, including type 2 diabetes, obesity, and cardiovascular disease (46). Additionally, evidence suggests that unbalanced testosterone levels may facilitate infection and disease progression in men because of their impact on the expression of the SARS-CoV-2 receptor, angiotensin-converting enzyme-2, and major fusogenic transmembrane serine protease 2 under regular transcription by androgens (47, 48).

The average age of the ICU patients in our study was 64 years. We observed that older patients, especially those aged >66 years, were more likely to die from COVID-19. Comparing patients from wards and ICU, Pereira and coauthors showed that mortality rates increased with advanced age, according to sex, ethnic/racial background, and

TABLE 2 Logistic regression analysis of characteristics associated with death in COVID-19 patients.

	Univariate analysis			Multivariate analysis‡		
	OR	95% CI	*p*	OR	95% CI	*p*
Age, years	1.07	1.04–1.10	**0.000**	1.08	1.04–1.11	**0.000**
Sex						
Female	Reference			Reference		
Male	1.58	0.89–2.80	*0.113*	2.73	1.15–6.46	**0.022**
Bladder catheter						
No	Reference			Reference		
Yes	79.30	10.73–586.13	**0.000**	28.09	2.69–292.8	**0.005**
ICU stay, days	1.01	0.99–1.03	*0.122*	0.97	0.94–1.00	*0.106*
Central venous catheter						
No	Reference			Reference		
Yes	45.11	10.65–190.97	**0.000**	12.97	2.25–74.74	**0.004**
PCO_2, mmHg	1.01	0.99–1.03	*0.097*	0.97	0.95–1.00	*0.082*
White blood cell count, $10^3/mm^3$	1.18	1.11–1.26	**0.000**	1.17	1.07–1.27	**0.000**

‡Multivariate logistic regression model with stepwise backward (conditional) elimination method.
OR, odds ratio; 95% CI, confidence interval 95%. Highlighted values are considered statistically significant.

vaccination status (43). Moreover, previous studies have indicated that individuals aged 65 years and older have a higher risk of death from COVID-19 (49, 50). This may be attributed to an age-related decline in innate immunity and immunosenescence. Accordingly, a study conducted in 2020 in the Southern Region of Bahia State with hospitalized patients showed a higher frequency of COVID-19 among patients of advanced age (51). Furthermore, in severe cases of COVID-19, hematological changes in peripheral leukocytes reflect a compromised immune response during SARS-CoV-2 infection. These changes are early indicators of fatal outcomes and are crucial for maintaining immune homeostasis during viral infections (52).

In addition, the current study also suggests a high WBC count >10.03 cells x 1,000/mm^3 as a predictive death parameter, which was higher in patients with death outcomes than in discharged patients. These data are consistent with those of previous studies (53) and a meta-analysis examining the relationship among WBC count, COVID-19 severity, and mortality (54). The meta-analysis reported a WBC count of 0.41×10^9 /L for patients with moderate COVID-19, while the count increased significantly to 4.15×10^9 /L in patients who died (55). Another meta-analysis showed that the WBC and neutrophil counts decreased significantly in patients with mild COVID-19. However, similar to the results of the present study, higher counts were observed in severe COVID-19 (56).

Although we have shown that clinical parameters such as the use of mechanical ventilation, central venous catheters, arterial catheters, vasopressors, and bladder catheters increased the odds of death in COVID-19 patients, we also analyzed laboratory markers, including arterial lactate, serum creatine, urea, and serum urea nitrogen, which were higher in patients who died than in those discharged from the ICU. Investigators have suggested that determining changes in lactate levels can provide insights into COVID-19 pathophysiology and multisystem interactions (57). Furthermore, oxygen deprivation in tissues leads to lactate overproduction because pyruvate cannot be oxidized in the Krebs cycle. Predisposing factors for lactic acidosis, including diabetes and acute respiratory distress syndrome are common in hospitalized COVID-19 patients. In addition, COVID-19-related damage to alveolar cells may contribute to increased lactic acid (22, 58, 59).

Due to altered dyspnea and extremely low oxygen saturation, individuals with impaired respiratory metabolism are at an exceptionally high risk of death. Specifically, changes in carbon dioxide levels trigger a hypoxic threshold, resulting in lung damage. Under normal hypoxic conditions, even a slight imbalance in $PaCO_2$ levels quickly evokes significant increases in ventilation per minute and brief respiratory alkalosis, which physiologically alters blood pH (37, 46, 47).

Regarding laboratory markers, we also observed an association between COVID-19 non-survivors and urea and serum creatinine levels. These biomarkers can help evaluate kidney injury, especially the acute forms that occur in 3–29% of COVID-19 patients. According to a study of 701 patients with COVID-19, both kidney injury and acute kidney injury increased the risk of death, with elevated serum creatine and urea nitrogen levels being predictive of mortality (60). Furthermore, we observed high levels of these biomarkers in COVID-19 patients who died. Data from 95 patients, of whom 25 were admitted to the ICU, showed a short-term increase in the urea and serum creatine ratios (OR, 1.72; 95% CI, 1.20–2.66), characterizing them as independent predictors of the prognosis of death.

Finally, we observed that the odds of death were five times higher for individuals requiring mechanical ventilation in the ICU, and patients with a higher FiO_2 were more likely to die of COVID-19 in the ICU. During the COVID-19 outbreak, ICU stay and mechanical ventilation devices have been associated with respiratory failure (61–66), and the unprecedented number of patients weaned from non-invasive ventilation proved to be highly challenging. Additionally, here, the bladder catheter and central

venous catheter groups presented higher ORs for death, 79.3 and 45.12, respectively.

Invasive ventilation is an intricate procedure that requires skilled multidisciplinary teams and expensive equipment. The lack of trained professionals to administer and maintain the technique, the increased number of patients with respiratory injuries, and the shortage of materials increase the risk of infection during these procedures (67). Furthermore, the use of a bladder catheter increases the risk of catheter-associated urinary tract infection (68), and a central venous catheter is associated with mortality in chronic hemodialysis patients with COVID-19 in Brazil (69). Additionally, SAPS3, a scoring system widely used for predicting in-hospital mortality, was able to discriminate between survivors and non-survivors in our study (17–19), and the highest concentration of deaths due to COVID-19 was in ICU patients with SAPS3 > 51.5.

In summary, this study reported the clinical profile of a low-income population admitted to the COVID-19 ICU at a reference hospital in the Southern Region of Bahia State, Brazil. Our data demonstrate that the use of a catheter (central venous, arterial, or bladder) was the main factor associated with death in COVID-19 patients. Although platelet count was not associated with the death of patients in the ICU, leukocyte count and biochemical parameters were valuable indicators of death. The SAPS3 presented the highest sensitivity (77.9%) and specificity (63.1%) for discriminating between survivors and non-survivors, with an AUC of 0.79. Lastly, we suggest that multi-laboratory parameters can be used to predict patient prognosis and guide healthcare teams toward more assertive clinical management, better resource allocation, and improved survival of patients admitted to COVID-19 in the ICU.

5. Conclusion

We identified some factors (epidemiological and laboratory) associated with a higher chance of death among patients with COVID-19 treated in the ICU. For example, patients aged 65 years or older, those with a prolonged ICU stay, and those who required catheter use were more likely to die of COVID-19. Identifying predictors of death is important for choosing the best clinical management and therapeutic approaches to patients to avoid or minimize unfavorable outcomes. Moreover, it is important that epidemiological and clinical laboratory data are available for decision-making purposes. Thus, by knowing the predictors of worse prognosis and having these data, clinicians can act early and with scientific evidence.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving humans were approved by the Human Beings Research Ethics Committee of the State University of Santa Cruz, Ilhéus, Brazil. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LC, JG, CJ, and HP: conceptualization. MD'C, US, LC, and RF: methodology. RF, AC, CR, and AS: validation. MD'C and US: formal analysis. US, MD'C, and LC: data curation. MD'C, AC, CM, EG, SG, LM, and LC: original draft preparation. US, CJ, RR, RF, SG, AC, CR, and AS: writing review and editing. LC: supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1241444/full#supplementary-material

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Health surveillance for SARS-CoV-2: infection spread and vaccination coverage in the schools of Modena province, Italy

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Introduction: In Italy, over 4.8 million individuals aged 0–19 years have been infected with SARS-CoV-2. This study aims to evaluate the spread of SARS-CoV-2 within schools in Modena province and the influence of anti-SARS-CoV-2 vaccination coverage.

Methods: We performed a survey in the period 1 September-15 December 2021, involving student population aged 0–19 years and related teachers screened for SARS-CoV-2 infection using nasopharyngeal swab after the detection of an index case within their class. During the study period, vaccination against SARS-CoV-2 was actively offered to all subjects aged ≥12 years.

Results: A total of 13,934 subjects were tested, 12,534 students and 1,400 teachers (594 classes). We identified a total of 594 and 779 index and secondary cases, respectively. We found that 9.8% of students and 10.6% of teachers were positive for SARS-CoV-2. Overall at the test time, 32.5% were vaccinated with at least one dose of anti-SARS-CoV-2 vaccine. Among secondary cases, 7.8% were vaccinated compared to 34.9% among negative tested subjects. A higher secondary attack rate was for non-vaccinated subjects rather than vaccinated ones (8.1% vs. 1.4%). Higher secondary attack rates were reported for subjects attending infant and primary school (5.9 and 9.6%, respectively). Lower secondary attack rates were for those who attended middle school (4.9%) and especially high school (1.7%).

Conclusion: Our results highlight the differential spread of the infection within various educational settings and that the vaccination, available in the study period for the population aged ≥12, have mitigated SARS-CoV-2 spread in high and middle schools.

KEYWORDS

SARS-CoV-2, vaccination, schools, infection spread, health surveillance

1. Introduction

In Italy, over 4.8 million individuals aged 0–19 years have been infected by SARS-CoV-2 virus, representing about 19% of all reported cases since the beginning of the pandemic (1, 2). Schools closures, home confinement, and social distancing measures have disrupted the children daily routines and adolescents and limited their access to social activities, which can have negative effects on their mental health and well-being, with a significant decrease in quality of life (3–5). COVID-19 has strictly related to children and adolescents' development due to confinement measures (6).

Worldwide, one of the main preventive strategy against the COVID-19 pandemic was schools closure, even if, in the early stages of the pandemic, the role of children in the transmission of the virus was unknown (7). In Italy, most of the children did not present symptoms and for this reason they were quarantined at home without any molecular or antigenic tests (8). Additionally, physical distancing and specifically distance learning in school setting have been implemented in several countries, including Italy; as a consequence, no increased risk of infection was reported among workers of the education sector (9, 10).

Keeping school as a safe and accessible environment is of utmost importance and goes beyond the primary objective of the educational needs as it affects the social and mental development of children (6), and it is the primary means to reduce inequality (11). The role of school in SARS-CoV-2 spread and the effectiveness of its closure in the control of the epidemic has been long debated. Several studies showed that the prevalence of positive cases in schools is lower than in the general population when appropriate mitigation measures are implemented, as well as the number and size of clusters in educational settings are generally smaller (12–16).

The health behavioral policies adopted before the availability of the vaccination and still in use helped in mitigating the risk of viral spread in schools; particularly, contact tracing turned out to be useful to promptly isolate infected students and staff (17). Regular testing could be also a key strategy to control the epidemic in school settings characterized by lower vaccination coverage compared to the general adult population or after the waning of vaccine protection, minimizing lost days (18). A modeling study in simulated elementary and middle schools found that screening tests eased in-person schooling with limited transmission risk, and test-to-stay policies were associated with increased school attendance and only little incremental transmission. Epidemiological surveillance has been identified as a useful, low-cost option for the detection of outbreaks and identification of school environments that could benefit from increased mitigation (19).

A widespread increase in vaccination coverage for the pediatric population has been strongly recommended (20, 21). Initially, the European Medicines Agency (EMA) recommended the administration of the vaccine to children aged 12 years and above. Subsequently, this recommendation was extended to encompass children below 12 years (22). After that, on 7 December 2021, the Italian Ministry of Health extended the use of the vaccine to children aged 5–11 years (23). In Emilia Romagna Region, since 16 December 2021 vaccination against SARS-CoV-2 has been also available for subjects aged 5–11 years (24).

The acceptance of vaccination against SARS-CoV-2 among parents has a significant role: the safety of vaccination is considered the most important factor affecting vaccine hesitancy during childhood (25). Therefore, advocating the safety and efficacy of vaccines through trusted and institutional sources might help the development of a sense of confidence and security among parents and the general public (26, 27). Researchers underlined that virus circulation among students, educators, staff, and their family members is high when a highly infectious variant predominates in unvaccinated students. Nevertheless, the implementation of mitigation measures or use of vaccinations in students can substantially reduce these modeled risks (28). Especially, vaccination remains the most effective and sustainable strategy for risk reduction, thus efforts should focus on the increase of coverage and use of booster doses among eligible students and school staff (29).

Since children infected with SARS-CoV-2 are mostly without symptoms or with mild non-specific symptoms, outbreaks are difficult to record in educational settings (30, 31). Therefore, this study aims to assess the spread of SARS-CoV-2 within schools in Modena province and the influence of vaccination coverage in these settings.

2. Methods

This study was approved by the "Area Vasta Emilia Nord" Ethics Committee (approval no. AUO/0017667/20 of June 25, 2020).

2.1. Study population

We performed a survey in school settings of Modena province (Northern Italy) in the period from September 1 to December 15, 2021. We considered all students and teachers who were screened for SARS-CoV-2 infection through nasopharyngeal swab after the detection of an index case within their classroom, and the related onset of secondary cases.

According to ministerial and regional policies (32), teachers and students were tested if they attended the same class with a confirmed positive case in the 48 h prior test or symptoms onset (school contact). Differently from the general definition of close contact, the distance from the index case was not considered to define a school contact who has to be included in the screening. Screening tests were performed with molecular or antigenic tests, but in case of positive result with antigenic test, individuals needed molecular test to be considered a confirmed secondary case. Indication for quarantine varied from nursery, infant, primary and secondary (middle and high) schools, but the execution of at least one nasopharyngeal swab for SARS-CoV2 was mandatory for the re-admission to all the grades of school. Non-adherence to screening tests was very low (<5%). In the analysis, these few subjects were considered negative for SARS-CoV-2 screening. We considered as a school cluster the presence of 2 or more SARS-CoV-2 cases (students or teachers with a positive molecular SARS-CoV-2 test, regardless the occurrence of correlated symptoms) attending the same classroom within a period of 14 days. In the considered period, vaccination against SARS-CoV-2 was actively offered to all subjects aged at least 12 years, while for children under 12 years old, the use of vaccine was still not approved. All the data have been collected by the Public Health Department of Modena Local Health Authority (AUSL Modena) through an application with a specific designed format for contact tracing in the pandemic period.

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2.2. Data analysis

For continuous variables, we reported mean, standard deviation (SD), and range (min-max). For categorical variables, we reported absolute (N) and relative (%) frequencies. We performed the analyses in the entire study population and in selected subgroups. In particular, we subdivided the sample of teachers/students into not-vaccinated and vaccinated for SARS-CoV-2. This latter group was further divided according to the type of educational setting. To evaluate the spread of infection, secondary attack rate was calculated. Secondary attack rate was defined as the number of secondary cases exposed to index cases divided by total number of tested subjects exposed to index cases. We also compared the daily incidence of new cases over time occurred in the study sample with those occurred in the overall population of Modena province. Moreover, we assessed the influence of vaccination coverage on the SARS-CoV-2 spread of within schools using a logistic regression model adjusted for relevant confounders, sex, age group, type of educational setting and school role (teachers/students) for calculating the odds ratio (OR) with 95% confidential intervals (95% CI). Data analysis was performed using statistical software Stata v17.0 (StataCorp, College Station, TX, USA, 2021).

3. Results

From September 1 to December 15, 2021, 594 classes were followed by the Public Health Dept. of AUSL Modena due to the identification of an index case, with 13,934 subjects tested for SARS-CoV-2. Specifically, we included 1,400 teachers and 12,534 students within different educational settings, from nursery school up to high school. The identified clusters were 265 with a range of 2–22 total cases within the class (from 1 to 21 secondary cases). Through swab testing in the study population, 1,373 (9.9%) were identified as confirmed cases (10.6% of teachers and 9.8% of students), of whom 594 were classified as index cases and 779 as secondary cases. Among index cases, 101 (17.0%) were teachers and 493 (83.0%) were students; among secondary cases, 47 (6.0%) and 732 (94.0%), respectively.

We collected data on vaccination status. Within the study population, information on the vaccination coverage was missing for only 24 (0.2%) subjects, respectively 14 teachers and 10 students, and none of them was a confirmed case. These data are summarized in Table 1, along with socio-demographic characteristics.

Among all included subjects, 4,525 (32.5%) resulted to be vaccinated with at least one dose at the moment of exposure to virus. A higher percentage of vaccinated individuals (68.5%) could be observed considering only the population aged ≥12 years, for which the vaccination was regularly offered.

Out of 1,373 confirmed cases, only 155 (11.3%) were vaccinated, and the proportion decreased among secondary cases (7.8%), which are possibly related to school attendance.

In Tables 2, 3, we reported both secondary and index cases rate and secondary attack rate within the study population, divided into different subgroups based upon vaccination status or educational setting.

Overall, the secondary attack rate for non-vaccinated subjects was higher than for vaccinated subjects (8.1% vs. 1.4%). A similar trend was also observed among people aged ≥12 years, who represented the target population for vaccination.

TABLE 1 Socio-demographic characteristics and vaccination status of the total population ($n = 13,934$) and divided into different subgroups.

Characteristics	Male, N (%)	Female, N (%)	Age, mean ± SD (min-max)
Overall ($n = 13,934$)	6,545 (47.0)	7,389 (53.0)	13.9 ± 11.3 (0–68)
Teachers ($n = 1,400$)	209 (14.9)	1,191 (85.1)	43.7 ± 11.0 (19–68)
Students ($n = 12,534$)	6,336 (50.5)	6,198 (49.5)	10.5 ± 4.2 (0–22)
Index cases ($n = 594$)	282 (47.5)	312 (52.5)	15.8 ± 13.1 (0–63)
Secondary cases ($n = 779$)	370 (47.5)	409 (52.5)	11.1 ± 9.2 (1–65)
No infection ($n = 12,561$)	5,893 (46.9)	6,668 (53.1)	13.9 ± 11.3 (0–68)

Vaccination status		
	Yes N (%)	No N (%)
Overall ($n = 13,910$)	4,525 (32.5)	9,385 (67.5)
Teachers ($n = 1,386$)	895 (64.6)	491 (35.4)
Students ($n = 12,524$)	3,630 (29.0)	8,894 (71.0)
Students ≥12 years ($n = 5,194$)	3,630 (69.9)	1,564 (30.1)
Index cases ($n = 594$)	94 (15.8)	500 (84.2)
Secondary cases ($n = 779$)	61 (7.8)	718 (92.2)
Total cases ($n = 1,373$)	155 (11.3)	1,218 (88.7)
No infection ($n = 12,537$)	4,370 (34.9)	8,167 (65.1)
<12 years ($n = 7,330$)	–	7,330 (100.0)
≥12 years ($n = 6,580$)	4,525 (68.8)	2055 (31.2)

Information on the vaccination status was missing for 24 (0.2%) subjects, respectively 14 teachers and 10 students, and none of them were confirmed cases of SARS-CoV-2 infection. Percentages are calculated excluding missing data on vaccination status.

A logistic regression model was implemented to evaluate the vaccination coverage influence on the SARS-CoV-2 spread within schools. The results showed that being vaccinated was highly protective for risk of secondary infection within class following an index case (OR [95% CI]: 0.28 [0.20–0.40]). The analysis stratified by school role confirmed the protective effect of vaccination for students (OR [95% CI]: 0.41 [0.28–0.61]) and for teachers (OR [95% CI]: 0.09 [0.05–0.20]).

Higher secondary attack rates have been reported for subjects attending infant and elementary school (5.9 and 9.6%, respectively), compared to other types of educational settings. Indeed, lower secondary attack rates have been calculated for those who attend middle school and especially high school (4.9 and 1.7%, respectively). A low secondary attack rate (2.5%) was also seen for nursery school.

We also included data regarding the presence of symptoms in confirmed cases identified within our study population. As shown in Table 4, 662 (48.2%) confirmed subjects reported one or more symptoms, whereas 711 (51.8%) claimed to be asymptomatic. Among index cases, 391 (65.8%) subjects were symptomatic and 203 (34.2%) without symptoms. The proportion was inverted among secondary cases, 271 (34.8%) and 508 (65.2%), respectively.

Figure 1 shows the total of daily cases and index cases at school, and confirmed cases in the entire population of the province of Modena. The trends nearly overlapped during our study period.

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TABLE 2 Secondary/index cases rate and secondary attack rate within the study population, divided into different subgroups based on vaccination status.

	Total N	Index cases N (%)	Tested subjects following the index case N	Secondary cases N (%)**	No infection N (%)**	Secondary and index cases rate	Secondary attack rate
Vaccinated	4,525	94 (2.1)	4,431	61 (1.4)	4,370 (98.6)	0.65	1.4%
Number of doses							
1 dose	613	22 (3.6)	591	8 (1.4)	583 (98.6)	0.36	1.4%
2 doses*	3,804	72 (1.9)	3,732	52 (1.4)	3,680 (98.6)	0.72	1.4%
3 doses	108	-	108	1 (0.9)	107 (99.1)	-	0.9%
Non-vaccinated	9,385	500 (5.3)	8,885	718 (8.1)	8,167 (91.9)	1.44	8.1%
Overall	13,934	594 (4.3)	13,340	779 (5.8)	12,561 (94.2)	1.31	5.8%
Age groups							
<12 years	7,330	292 (4.0)	7,038	629 (8.9)	6,409 (91.1)	2.15	8.9%
≥12 years	6,604	302 (4.6)	6,302	150 (2.4)	6,152 (97.6)	0.50	2.4%
Vaccination status							
Vaccinated	4,525	94 (2.1)	4,431	61 (1.4)	4,370 (98.6)	0.65	1.4%
Non-vaccinated	2055	208 (10.1)	1847	89 (4.8)	1758 (95.2)	0.43	4.8%
Overall	13,934	594 (4.3)	13,340	779 (5.8)	12,561 (94.2)	1.31	5.8%

Percentages are calculated excluding missing data on vaccination status.
*Among those vaccinated with 2 doses, 48 are vaccinated > 6 months and 3,756 are vaccinated < 6 months.
**Percentages are calculated out of tested subjects.

4. Discussion

This study aims to evaluate the spread of SARS-CoV-2 within schools of various types and grades in Modena province, from September to December 2021, further to rate the influence of vaccination in this environment.

The secondary attack rate was found higher in non-vaccinated subjects compared with value in vaccinated subjects (8.1% vs. 1.4%). Furthermore, the lowest secondary attack rate has been found for those who attended high school (1.7%).

Our results underline the effective protection of vaccination against SARS-CoV-2 as 88.7% of overall cases were not vaccinated. Particularly, the percentage of non-vaccinated subjects is 92.2% among secondary cases, which are those infections possibly related with school attendance. Therefore, vaccination shows a relevant impact in preventing transmission of SARS-CoV-2 within educational settings suggesting the collective beneficial effect of extensive vaccination in school population to reduce the outbreaks probability and the size (18). As a matter of that, comparison of secondary attack rates shows higher values for non-vaccinated subjects than vaccinated ones (8.1% vs. 1.4%). These data confirm that non-vaccinated subjects have a higher risk of contracting SARS-CoV-2 infection (33–35). As further demonstration for vaccination efficacy, lower secondary attack rate is reported for subjects with three doses of vaccine compared to those with only one dose (0.9% vs. 1.3%), suggesting that high levels of protection might be re-established through booster doses (36).

Based on different type of educational settings, lowest secondary attack rates were depicted for subjects attending middle school and especially high school (4.9 and 1.7%, respectively) compared to other settings. This difference may be explained by the observation that during the study period vaccination anti-SARS-CoV-2 was approved for people aged ≥12 years only. Our results show that having at least one vaccine dose is protective against transmission of virus, as reported by ECDC (20). In literature, widespread vaccine coverage is confirmed to be very important, especially among adolescents. Indeed, if the vaccine were not available to high school students, they would be expected to be at a higher risk of contracting the infection than primary school students, due to age-specific epidemiological characteristics and contact types (7).

It is interesting that a low secondary attack rate (2.5%) has been found also in nursery school. Possible explanations may be the different measures of isolation and quarantine adopted in that educational settings (32), the only one in which, during the study period, quarantine was disposed for all children of the same classroom, as a consequence of only one confirmed case. Moreover, analyzing transmission of respiratory disease in schoolchildren of different ages, in Japan Matsuda et al. have found a higher percentage of primary schools students with influenza than nursery or kindergarten children (23.4% *vs* 18.9%) over 5 influenza seasons (37).

It is also important to notice that many mitigation measures and health behavioral policies were adopted during our study period, according to ministerial and regional specific protocols (32): wearing a face mask was always mandatory, except for children aged <6 years and subjects with specific pathologies; periodic room ventilation and social distancing rules had to be guaranteed; the use of outdoor spaces was encouraged wherever possible.

Data regarding the symptoms presence in confirmed cases show a higher percentage of symptomatic individuals among index cases in comparison to secondary cases (65.8% vs. 34.8%). We can explain this difference due to the reason to perform swab testing within these two subgroups. Index cases were usually diagnosed with a positive swab test after the onset of symptoms. Instead, secondary cases underwent

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TABLE 3 Secondary/index cases rate and secondary attack rate within study population, divided into different subgroups based on educational setting.

Type of educational setting		Total N	Index cases N	Tested subjects following the index case N	Secondary cases N	Secondary and index cases rate	Secondary attack rate
Nursery school (0–3 years)	Overall	420	27	393	10	0.37	2.5%
	Teachers	66	6	60	3		
	Students	354	21	333	7		
Infant school (3–5 years)	Overall	966	45	921	54	1.20	5.9%
	Teachers	138	18	120	7		
	Students	828	27	801	47		
Elementary school (6–10 years)	Overall	5,233	219	5,014	479	2.19	9.6%
	Teachers	622	37	585	29		
	Students	4,611	182	4,429	450		
Middle school (11–13 years)	Overall	3,732	154	3,578	176	1.14	4.9%
	Teachers	297	18	279	6		
	Students	3,435	136	3,299	170		
High school (14–19 years)	Overall	3,583	149	3,434	60	0.40	1.7%
	Teachers	277	22	255	2		
	Students	3,306	127	3,179	58		
Total	Overall	13,934	594	13,340	779	1.31	5.8%
	Teachers	1,400	101	1,299	47		
	Students	12,534	493	11,802	732		

TABLE 4 Presence of symptoms in confirmed cases identified within study population between September 1 and December 15.

	Symptom presence	
	Yes N (%)	No N (%)
Index cases (n = 594)	391 (65.8)	203 (34.2)
vaccinated (*n* = 94)	67 (71.3)	27 (28.7)
- 1 dose (*n* = 22)	13 (59.1)	9 (40.9)
- 2 doses *(*n* = 72)	54 (75.0)	18 (25.0)
non-vaccinated (*n* = 500)	324 (64.8)	176 (35.2)
Secondary cases (n = 779)	271 (34.8)	508 (65.2)
vaccinated (*n* = 61)	17 (27.9)	44 (72.1)
- 1 dose (*n* = 8)	2 (25.0)	6 (75.0)
- 2 doses*(*n* = 52)	15 (28.8)	37 (71.2)
- 3 doses (*n* = 1)	–	1 (100.0)
non-vaccinated (*n* = 718)	254 (35.4)	464 (64.6)
Total cases (n = 1,373)	662 (48.2)	711 (51.8)
vaccinated (*n* = 155)	84 (54.2)	71 (45.8)
- 1 dose (*n* = 30)	15 (50.0)	15 (50.0)
- 2 doses* (*n* = 124)	55 (44.4)	69 (55.6)
- 3 doses (*n* = 1)	–	1 (100.0)
non-vaccinated (*n* = 1,218)	578 (47.4)	640 (52.6)

Data are number (n) and percentage (%) for total cases (n = 1,373), and divided into different subgroups.
*Among those vaccinated with 2 doses, only 5 are vaccinated > 6 months and all those 5 subjects are symptomatic index cases.

swab testing for screening protocol, as established by the Dept. of Public Health regardless of the symptoms presence (32).

According to our results, a comparison between scholastic cases and total cases in the overall population shows similar overlapping trends. This finding suggests that the SARS-CoV-2 transmission within schools was quite similar to the general virus circulation in Modena province over the same period; therefore, school contacts do not seem to have played a relevant role in the spread of the pandemic neither they have represented a higher risk factor for virus transmission, in line with the literature (12). Similarly, other studies revealed low rates of infection in school contacts, and SARS-CoV-2 circulation in schools was found to be much limited compared to the general population (17, 38).

A limitation of our investigations is that we did not have detailed data on symptoms but detailed information was not collected. However, considering the still limited evidence available on this topic, our findings expand the knowledge regarding the SARS-CoV-2 spread within schools and the impact of vaccination coverage in these settings. Some strengths should also be outlined. The epidemiological surveillance carried out by the Local Health Authorities were mandatory during the study period, thus occurrence of selection bias can be ruled out. Furthermore, the epidemiological investigation on the entire involved classes allowed a more in-depth study of transmission in different type of educational settings.

5. Conclusion

Vaccination against SARS-CoV-2 in children aged 12 years and older showed effectiveness in preventing virus transmission

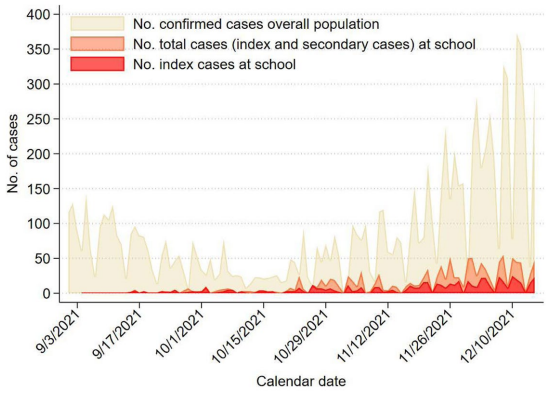

FIGURE 1
The daily number of confirmed cases in the overall population of Modena province, the number of total cases and of index cases in the scholastic population of Modena province from September 1 to December 15, 2021.

in school settings after the detection of an index case within their classroom. Indeed, a higher secondary attack rate was found among non-vaccinated subjects compared with vaccinated subjects. Furthermore, the lowest secondary attack rate has been found for those who attended high school. In conclusion, our findings highlight the importance of widespread anti-SARS-CoV-2 vaccination to reduce virus circulation also in school settings.

Data availability statement

The datasets presented in this article are not readily available due to privacy and legal restrictions. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by "Area Vasta Emilia Nord" Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants and participants' legal guardians/next of kin.

Author contributions

SP, LB, and TF: conceptualization. SP and TF: methodology and funding acquisition. SP, MF, AD'A, and TF: formal analysis. SP, LB, AB, and TF: investigation. LI, AG, EF, GC, ZK, and SZ: resources and recruitment. SP, MF, LB, AD'A, LI, ZK, and SZ: data curation. SP, MF, and TF: writing—original draft preparation. SP, MF, LB, AD'A, LI, AG, EF, GC, ZK, SZ, AB, and TF: writing—review and editing. SP, TF, and AB: project administration. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Prevalence and impact of long COVID-19 among patients with diabetes and cardiovascular diseases in Bangladesh

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Introduction: Co-prevalence of long-COVID-19, cardiovascular diseases and diabetes is one of the major health challenges of the pandemic worldwide. Studies on long-COVID-19 and associated health outcomes are absent in Bangladesh. The main aim of this study was to determine the prevalence and impact of long-COVID-19 on preexisting diabetes and cardiovascular diseases (CVD) on health outcomes among patients in Bangladesh.

Methods: We collected data from 3,250 participants in Bangladesh, retrospectively. Multivariable logistic regression model was used to determine the odds ratio between independent and dependent variables. Kaplan-Meier survival curve was used to determine the cumulative survival.

Results: COVID-19 was detected among 73.4% (2,385 of 3,250) participants. Acute long-COVID-19 was detected among 28.4% (678 of 2,385) and chronic long-COVID-19 among 71.6% (1,707 of 2,385) patients. CVD and diabetes were found among 32%, and 24% patients, respectively. Mortality rate was 18% (585 of 3,250) among the participants. Co-prevalence of CVD, diabetes and COVID-19 was involved in majority of fatality (95%). Fever (97%), dry cough (87%) and loss of taste and smell (85%) were the most prevalent symptoms. Patients with co-prevalence of CVD, diabetes and COVID-19 had higher risk of fatality (OR: 3.65, 95% CI, 2.79–4.24). Co-prevalence of CVD, diabetes and chronic long-COVID-19 were detected among 11.9% patients.

Discussion: Risk of hospitalization and fatality reduced significantly among the vaccinated. This is one of the early studies on long-COVID-19 in Bangladesh.

KEYWORDS

long-COVID-19, comorbidity, diabetes, cardiovascular disease, Bangladesh

1. Introduction

In Bangladesh, COVID-19 have become one of the major public health concerns since the first report on March, 2020. COVID-19 has spread faster and affected people of all aspects in Bangladesh (1, 2). Nearly 2 038 129 people have been infected with COVID-19 and 29 446 deaths have been reported till April 24, 2023 in Bangladesh (3, 4). About 88.5% of the total population of Bangladesh has received at least one dose and 48% got 3rd dose of vaccine (1, 3). Three peaks of COVID-19 pandemic have been identified in Bangladesh since 2020. One peak was apparently confined within the period of March, 2021 to May, 2021, and another one during June, 2021 to September, 2021 and the last one during January, 2022 to March, 2022 (3, 4). Though vaccination is ongoing, breakthrough cases and variants with escape mutations are continuously contributing to the increase of cases of COVID-19 in Bangladesh. As of daily update on 24th April, 2023 the number of active cases were 10 197 and 254 of them had critical health conditions (3–5).

Clinical features of post-COVID-19 and health conditions among the patients are highly heterogenous, complex and not well-characterized (6). According to Fernández-de-las-Peñas et al. long post-COVID-19 is defined as cases having symptoms from week 12 to week 24 after (6, 7). According to WHO, "post COVID-19 or long COVID condition refers to long-term symptoms after having COVID-19". However, the current knowledge suggests drawback of this definition. Based on the fluctuation of post-COVID-19 symptoms on patients, long COVID-19 should be monitor for newer and persistent symptoms over weeks, months and years. According to the most accepted definition of long COVID-19, post-acute sequelae is defined as any symptoms of COVID-19 after recovery of infection and lasting for more than 5 to 12 weeks and chronic post-COVID-19 symptoms lasting for more than 12 weeks (6, 7). A significant proportion of COVID-19 patients have been suffering from acute and chronic long-COVID-19 (6–8). Existing data suggests that presence of pre-existing diseases including diabetes, hypertension, cardiovascular diseases (CVD), autoimmune diseases, obesity, and chronic obstructive pulmonary disease (COPD) among COVID-19 patients contribute to serious health outcome, hospitalization and ICU admission (9–11). Further, patients with long COVID-19 and preexisting health conditions have poor prognosis and high fatality rate (8–10). Studies suggest that COVID-19 has the ability to influence the onset of specific type of diabetes among non-diabetic patients (11, 12). Presence of long-COVID-19 have worsened the health condition and disease prognosis of diabetes, CVD and COPD over long time (8–11). Prevalence of diabetes among COVID-19 patients varied between 9 and 45% and CVDs from 7 to 45% in Bangladesh, UK, China, USA, India and Italy (12–19). Studies have suggested that COVID-19 patients with diabetes, COPD and CVDs have higher risk of fatality and developing severe health condition (12–19).

Studies on the prevalence of acute and chronic long-COVID-19 and their relation with pre-existing health conditions on the outcome are lacking in Bangladesh. Early studies on the impact of COVID-19 and comorbidities on the health outcome among patients in Bangladesh also suggest significant relationship of these health conditions. Therefore, we conducted this study to investigate the impact of long-COVID-19 among patients with diabetes and CVDs on health outcomes in Bangladesh.

2. Materials and methods

2.1. Study design and population

A retrospective study was designed. Data was collected from 3,250 participants from seven divisions in Bangladesh during 01 January, 2022 and 31 December, 2022. The age of the participants ranged from 20 years to 78 years. According to the guidelines of the World Health Organization, the diagnosis was conducted by RT-PCR method (20). Data were collected in four sampling frames. Data on the report of hospitalization, ICU admission and discharge were collected directly from the patients and hospital authorities. Death reports were collected from the authorities and confirmed from the relatives of the patients.

2.2. Ethical approval

This study was ethically approved by the Biosafety, Biosecurity and Ethical Committee (BBEC) of Jahangirnagar University. Informed consent was taken from patients or relatives of the patients. The protocol number approved by the ethics committee is BBEC, JU/M 2021/COVID-19/(2)1.

2.3. Data collection

According to the guidelines of the World Health Organization, nasal or pharyngeal swab specimens were collected and used for the test in the hospitals/clinics (20). A positive outcome was defined by a positive laboratory test in the real-time reverse-transcriptase– PCR (RT-PCR) assay for SARS-CoV-2 and confirmed by high throughput sequencing (20). Data on the sociodemographic and economic conditions including sex, age, origin, monthly income, residing place, occupation, medical history, complication, treatment received (antiviral, antibiotic, steroid therapies, immune therapy, plasma therapy, respiratory support by mechanical ventilation and ICU support) were collected from the patients. Any pre-existing health conditions (defined by the International Classification of Diseases, 10th Revision, Clinical Modification), and outcome were included in this study (16, 19). Data on the pre-existing health conditions including diabetes mellitus, cardiovascular disease (CVD), hypertension, hyperlipidemia, chronic obstructive pulmonary disease (COPD), malignancy, obesity and autoimmune disease were taken from the patients and examined by two experts.

2.4. Outcomes

The primary outcome was long time illness associated with COVID-19 infection. Secondary outcomes included hospitalization, admission to ICU, requirement of mechanical

ventilation and fatality. Presence of chronic long-COVID-19 have worsened the clinical outcomes of pre-existing CVD, diabetes and COPD. Cardiovascular disease, malignant arrhythmia, diabetes and acute myocardial injury were defined based on the published works (16–19).

2.5. Statistical analyses

Percentage, rate and frequency were used for representing the categorical variables. Mean and standard deviation were used for representing central tendency of continuous variables. Independent sample *t*-tests were performed with 95% confidence intervals. $P < 0.05$ was considered statistically significant. The relationship between comorbidities and long COVID-19 were determined. Multivariable logistic-regression analysis was conducted to determine the impacts of sociodemographic factors and comorbidities on health outcome including fatality, hospitalization, ICU and long COVID-19. With 95% confidence intervals, adjusted odds ratios were determined. For the pre-existing comorbidities, Charlson Comorbidity Index (CCI) were computed. We determined the Kaplan-Meier survival estimate by considering different age, sex, and comorbidities among patients with long COVID-19. All of the statistical analyses were performed by International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) version 28.0 (Chicago, IL, USA) and Microsoft Excel 2021.

3. Results

3.1. Sociodemographic characteristics of the participants with COVID-19

This study included 3,250 participants from seven divisions in Bangladesh. Nearly 73.4% (2,385 of 3,250) of the participants were COVID-19 positive. The mean (SD) age of the study population was 49 ± 3.6 years. Majority of the participants (66.5%) aged above 40 years (Table 1). The ratio of male to female was 2,340:910 (about 2.6:1). Majority of the participants (65.1%) were from semi-urban and rural areas with poor health facilities. About 96% of the population were from native Bangladeshi. Majority of the participants (68.9%) had a monthly income below 50,000 Bangladeshi taka (500 USD). The availability of health facility and effective treatment varied significantly on monthly income and place of residence in Bangladesh (Table 1).

3.2. Prevalence of acute long-COVID-19 and chronic long-COVID-19 in Bangladesh

According to the definition of acute long-COVID-19 and chronic long-COVID-19, we determined the prevalence among the patients. Data were collected from the patients during 2nd week to 24th week after the first appearance of RT-PCR positive test for COVID-19. Acute long-COVID-19 (symptoms within 5–12 weeks) was detected among 28.4% (678 of 2,385) of the COVID-19 positive participants. Acute long-COVID-19 was most prevalent among patients aged 30–39 years (189 of 531) followed by 40–49 years (137 of 528) and 20–29 years (131 of 445), respectively (Table 2). Chronic long-COVID-19 (symptoms after 12 weeks) was found among 71.6% (1,707 of 2,385) patients with COVID-19. Longitudinal analyses showed that after recovery from infection, symptoms of COVID-19 persisted for 13–24 weeks among 29%, 25–48 weeks among 25.6% and >48 weeks among 17% of the patients (Table 2).

3.3. Characterization of clinical symptoms and pre-existing health conditions

Clinical symptoms were analyzed by three physicians, recorded separately and compiled. Collected symptoms were cross-checked and evaluated for correction. Fever (97%, 2,313 of 2,385) was the most prevalent symptom followed by dry cough (87%, 2,074 of 2,385), loss of taste or smell (85%, 2,027 of 2,385), fatigue (81%, 1,932 of 2,385), sore throat (79%, 1,884 of 2,385), body aches (72%, 1,717 of 2,385), and chest pain or pressure (56%, 1,335 of 2,385), respectively (Figure 1A). Co-prevalence of multiple symptoms and duration of illness increased with increasing age among the patients. Reappearance of symptoms after 2 weeks of negative of COVID-19 infection were common among the participants (73.4%, 2,385 of 3,250). Male patients had two times greater risk of developing different symptoms associated with COVID-19 than female.

Among the pre-existing health conditions, cardiovascular (CVD) disease was the most prevalent (32%, 1,040 of 3,250) followed by diabetes (24%, 780 of 3,250; Type 2 diabetes mellitus was 63.7% and Type 1 was 36.3%). We detected at least 532 (16.4%) patients of COVID-19 had both CVD and diabetes at the same time (Table 3). Health complications associated with CVD increased among 673 of 1,040 (64.7%) patients and diabetes among 429 of 780 (55%) after patients getting COVID-19 infection. Co-prevalence of CVD, diabetes and acute long-COVID-19 was found among 11% (359 of 3,250) patients. Further, co-prevalence of CVD, diabetes and chronic long-COVID-19 were detected among 11.9% (387 of 3,250) patients (Table 3). Distribution of CVD, diabetes, acute long-COVID-19 and chronic long-COVID-19 were higher among male than female (Figure 1B). Nearly, 7.1% (231 of 2,130) patients with CVD and diabetes had problem to take proper treatment during COVID-19 infection. Mortality rate was 18% (585 of 3,250) among the participants. About 64% (374 of 585) of the fatalities were found in patients with CVD and COVID-19 followed by 42% (246 of 585) in patients with diabetes and COVID-19. We found that about 96% of the participants had taken 1st dose, 88% 2nd dose and 41% 3rd dose of COVID-19 vaccine (Table 3).

Symptoms were analyzed among patients with acute long-COVID-19, CVD and diabetes (Figure 1C) and chronic long-COVID-19, CVD and diabetes (Figure 1D). Fever (576 of 678) was the most frequent symptoms among patients with acute long-COVID-19 and CVD followed by dry cough (513 of 678), loss

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TABLE 1 Socio-demographic characteristics of study participants.

Variables	Male (%)	Female (%)	Total (%)
Study population	2,340/3,250 (74)	910/3,250 (26)	3,250/3,250 (100)
Age (in years)			
20–29	290/408 (71)	118/408 (29)	408/3,250 (12)
30–39	510/680 (75)	170/680 (25)	680/3,250 (21)
40–49	460/639 (72)	179/639 (28)	639/3,250 (20)
50–59	382/502 (76)	120/502 (24)	502/3,250 (15)
60–69	358/543 (66)	185/543 (36)	543/3,250 (17)
Above 70	340/478 (71)	138/478 (29)	478/3,250 (15)
Origin			
Bangladeshi	2,243/3,125 (72)	882/3,125 (28)	3,125/3,250 (96)
Non-Bangladeshi	97/125 (78)	28/125 (22)	125/3,250 (4)
Monthly income (Bangladeshi Taka)			
<20,000	974/1,284 (76)	310/1,284 (24)	1,284/3,250 (39)
20,000–49,999	639/956 (67)	317/956 (33)	956/3,250 (29)
50,000–100,000	492/665 (74)	173/665 (26)	665/3,250 (21)
>100,000	235/345 (68)	110/345 (32)	345/3,250 (11)
Employment			
Employed	1,365/1,780 (77)	415/1,780 (23)	1,780/3,250 (55)
Unemployed	975/1,470 (66)	495/1,470 (34)	1,470/3,250 (45)
Residence			
Urban	823/1,128 (73)	305/1,128 (27)	1,128/3,250 (35)
Semi-urban	754/924 (82)	170/924 (18)	924/3,250 (28)
Rural	965/1,198 (81)	233/1,198 (19)	1,198/3,250 (37)

The percentage values represented male and female distributed in different groups.

TABLE 2 Duration of symptoms after recovery from COVID-19 infection among patients of different age group in Bangladesh.

Age in years	5–12 weeks (%)	13–24 weeks (%)	25–48 weeks (%)	>48 weeks (%)	Total (%)
20–29	131/445 (29.4)	130/445 (29.2)	114/445 (25.6)	70/445 (15.7)	445/2,385 (18.7)
30–39	189/531 (35.6)	141/531 (26.6)	117/531 (22.0)	84/531 (15.8)	531/2,385 (22.3)
40–49	137/528 (25.9)	132/528 (25.0)	137/528 (25.9)	122/528 (23.1)	528/2,385 (22.1)
50–59	95/293 (32.4)	81/293 (27.6)	65/293 (22.2)	52/293 (17.7)	293/2,385 (12.3)
60–69	85/352 (24.1)	111/352 (31.5)	103/352 (29.3)	53/352 (15.1)	352/2,385 (14.8)
Above 70	41/236 (17.4)	97/236 (41.1)	74/236 (31.4)	24/236 (10.2)	236/2,385 (9.9)
Total	678/2,385 (28.4)	692/2,385 (29.0)	610/2,385 (25.6)	405/2,385 (17.0)	
	Acute long-COVID-19 (28.4)	Chronic long-COVID-19 (71.6%)			

The percentage values in bracket represented the persistence of symptoms among the participants distributed in different age group.

of taste or smell (511 of 678) and body aches (345 of 678), respectively (Figure 1C). Similarly, among patients with chronic long-COVID-19, fever (1,695 of 1,707) was the most prevalent followed by dry cough (1,408 of 1,707), loss of taste and smell (1,345 of 1,707) and fatigue (1,234 of 1,707) were most commonly reported (Figure 1D).

3.4. Multivariable logistic regression analysis

Multivariable logistic-regression model was used to determine the relationship of different variables with outcome of COVID-19. Independent variables of hospitalization, ICU admission

and fatality among patients with COVID-19 and comorbidities and respective odds ratios with 95% confidence intervals were calculated and represented in Table 4. The major predictors of the higher odds of severe health outcome were age >40 years, sex-male, presence of CVD, diabetes, having Charlson Comorbidity Index (CCI) >3, co-prevalence of CVD, diabetes and COVID-19. The highest odds ratio for hospitalization was detected among patients with CCI >3 (OR: 5.53, 95% CI, 5.15–6.14; *p*-value 0.003), followed by positive COVID-19 (OR: 4.63, 95% CI, 3.42–5.37; *p*-value 0.001) and co-existence of COVID-19 and CVD (OR: 4.23, 95% CI, 3.65–5.18; *p*-value 0.002), respectively (Table 4). The risk of ICU admission and fatality was significantly higher among patients with COVID-19 (OR: 4.74, 95% CI, 3.83–5.63; *p*-value 0.001) followed by presence of >3 symptoms (OR: 4.46, 95% CI, 4.02–5.14; *p*-value 0.001), co-prevalence of CVD, diabetes and COVID-19 (OR: 3.65, 95% CI, 2.79–4.24; *p*-value 0.001), and respectively (Table 4). The risk of hospitalization reduced significantly among the vaccinated participants (OR: 0.27, 95% CI, 0.1–0.95; *p*-value 0.001). Notably, vaccinated participants also had lower risk of ICU admission and fatality (OR: 0.38, 95% CI, 0.17–0.74; *p*-value 0.003).

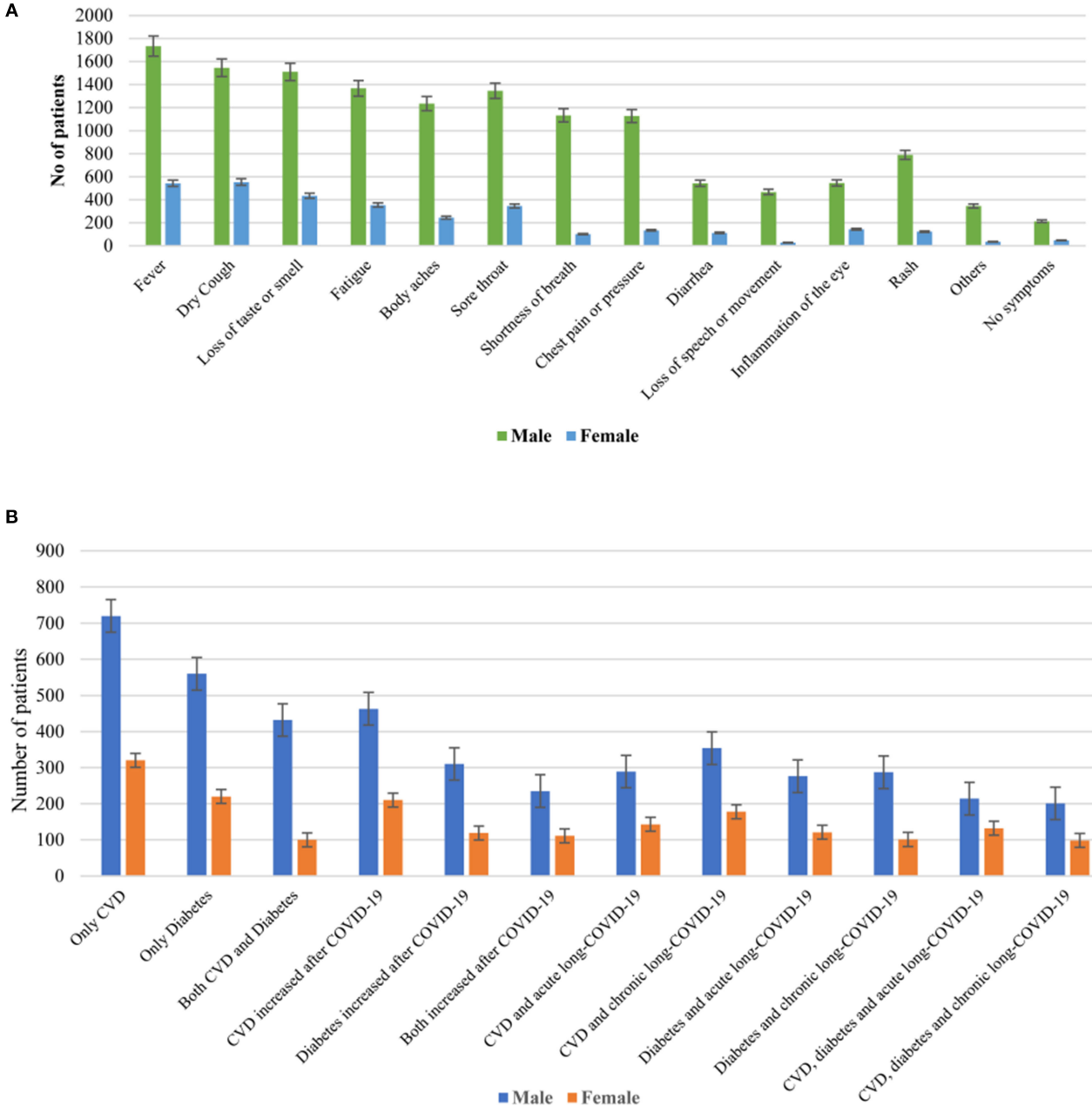

FIGURE 1
(Continued)

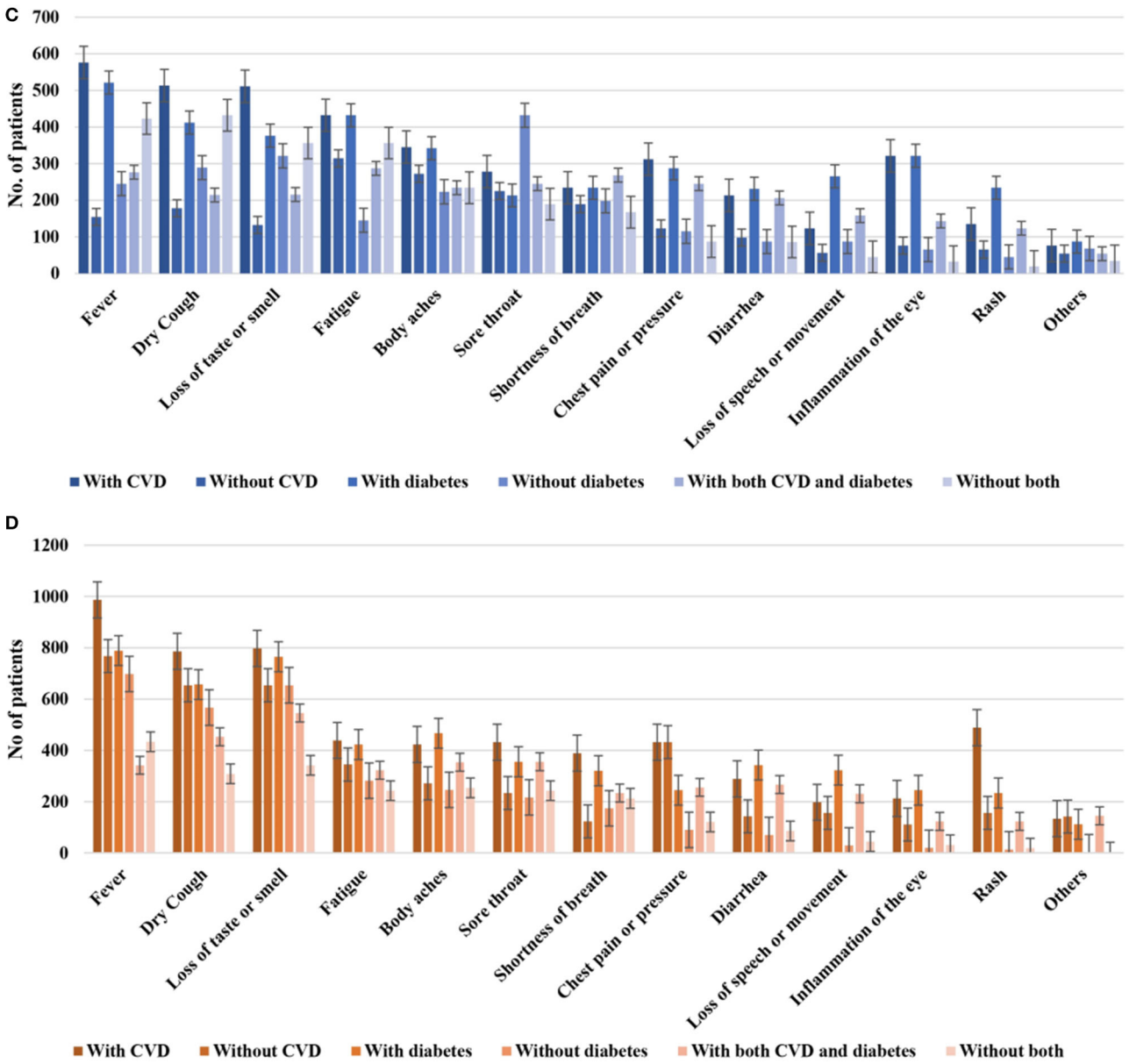

FIGURE 1 (Continued)
Frequency distribution of **(A)** different clinical symptoms in male and female, **(B)** different comorbidities in male and female patients with COVID-19 in Bangladesh, **(C)** distribution of clinical symptoms among patients with acute long-COVID-19, **(D)** patients with chronic long-COVID-19. Lines in each bar represented confidence intervals.

3.5. Survival rate analysis

Cumulative survival analysis of the study population was determined by using the Kaplan-Meier model. The cumulative survival rate of the participants was plotted against the time duration of survival or recovery. The analysis was conducted from week 0 to 25. The Kaplan-Meier model was applied for the data of the patients aged >40 years. We represented the findings for male and female separately. The cumulative survival rate of the male patients without COVID-19 remained above 0.5. Among female patients without COVID-19 the cumulative survival rate was also above 0.5. The cumulative survival rate gradually decreased to 0.0 in 24th week from 1.0 in 1st week among male patients with CVD, diabetes and COVID-19 (Figure 2). The cumulative survival rate among both male and female patients with COVID-19 decreased to 0.2 in the 24th week from 1 in the 1st week. Similarly, the survival rate among female patients with CVD, diabetes and COVID-19 was the lowest 0.1 in the 25th week. Male patients with CVD, diabetes and chronic long-COVID-19 had lower survival rate than female patients at the same time period (Figure 2).

TABLE 3 Distribution of diabetes and cardiovascular disease in different sex and age groups among the study participants.

Variables	Age groups in years (%)						$N = 3,250$	P value
	20–29	30–39	40–49	50–59	60–69	Above 70		
Suffering from cardiovascular diseases (CVD)								
Yes	48/1,040 (4.6)	216/1,040 (20.8)	222/1,040 (21.3)	265/1,040 (25.5)	143/1,040 (13.8)	135/1,040 (14.0)	1,040/3,250 (32.0)	0.005
No	360/2,210 (16.3)	464/2,210 (21.0)	417/2,210 (18.9)	237/2,210 (10.7)	400/2,210 (18.1)	332/2,210 (15.0)	2,210/3,250 (68.0)	
Suffering from diabetes								
Yes	27/780 (3.3)	145/780 (18.6)	142/780 (18.2)	154/780 (19.7)	167/780 (21.4)	145/780 (18.6)	780/3,250 (24.0)	0.005
No	381/2,470 (15.4)	535/2,470 (21.7)	497/2,470 (20.1)	348/2,470 (14.1)	376/2,470 (15.2)	333/2,470 (13.5)	2,470/3,250 (76.0)	
Suffering from both diabetes and CVD								
Yes	15/532 (2.8)	103/532 (19.4)	96/532 (18.0)	105/532 (19.7)	123/532 (23.1)	90/532 (16.9)	532/3,250 (16.4)	0.004
No	393/2,718 (14.5)	577/2,718 (21.2)	543/2,718 (20.0)	397/2,718 (14.6)	420/2,718 (15.5)	388/2,718 (14.3)	2,718/3,250 (83.6)	
Complication related with CVD increased after COVID-19 infection								
Yes	19/673 (2.8)	105/673 (15.6)	141/673 (21.0)	135/673 (20.1)	131/673 (19.5)	142/673 (21.1)	673/3,250 (20.7)	0.005
No	389/2,577 (15.1)	575/2,577 (22.3)	498/2,577 (19.3)	367/2,577 (14.2)	412/2,577 (16.0)	336/2,577 (13.0)	2,577/3,250 (79.3)	
Complication related with diabetes increased after COVID-19 infection								
Yes	5/524 (1.0)	43/524 (8.2)	124/524 (23.7)	78/524 (14.9)	35/524 (6.7)	21/524 (4.0)	429/3,250 (14.4)	0.001
No	405/1,824 (22.2)	468/1,824 (25.7)	304/1,824 (16.7)	215/1,824 (11.8)	237/1,824 (13.0)	195/1,824 (10.7)	1,824/3,250 (85.6)	
Symptoms of CVD and diabetes worsen after COVID-19 infection								
Yes	19/320 (5.5)	60/320 (17.3)	81/320 (23.4)	75/320 (21.7)	64/320 (18.5)	47/320 (13.6)	346/3,250 (10.6)	0.005
No	389/2,904 (13.4)	620/2,904 (21.3)	558/2,904 (19.2)	427/2,904 (14.7)	479/2,904 (16.5)	431/2,904 (14.8)	2,904/3,250 (89.4)	
Co-prevalence of CVD, diabetes and acute long-COVID-19								
Yes	11/359 (3.1)	57/359 (15.9)	75/359 (20.9)	92/359 (25.6)	68/359 (18.9)	56/359 (15.6)	359/3,250 (11.0)	0.001
No	397/2,891 (13.7)	623/2,891 (21.5)	564/2,891 (19.5)	410/2,891 (14.2)	475/2,891 (16.4)	422/2,891 (14.6)	2,891/3,250 (89.0)	
Co-prevalence of CVD, diabetes and chronic long-COVID-19								
Yes	9/387 (2.3)	56/387 (14.5)	79/387 (20.4)	92/387 (23.8)	105/387 (27.1)	46/387 (11.9)	387/3,250 (11.9)	0.005
No	399/2,863 (13.9)	624/2,863 (21.8)	560/2,863 (19.6)	410/2,863 (14.3)	438/2,863 (15.3)	432/2,863 (15.1)	2,863/3,250 (88.1)	

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TABLE 3 (Continued)

Variables	Age groups in years (%)						$N = 3,250$	P value
	20–29	30–39	40–49	50–59	60–69	Above 70		
Fatality in patients with CVD, diabetes and COVID-19								
Yes	4/235 (1.7)	26/235 (11.1)	43/235 (18.3)	67/235 (28.5)	43/235 (18.3)	52/235 (22.1)	235/3,250 (7.2)	0.005
No	404/3,015 (13.4)	654/3,015 (21.7)	596/3,015 (19.8)	435/3,015 (14.4)	500/3,015 (16.6)	426/3,015 (14.1)	3,015/3,250 (93.3)	
Taken 1st dose vaccine against COVID-19								
Yes	360/3,125 (11.5)	656/3,125 (21.0)	619/3,125 (19.8)	491/3,125 (15.7)	527/3,125 (16.9)	472/3,125 (15.1)	3,125/3,250 (96.2)	0.001
No	48/125 (38.4)	24/125 (19.2)	20/125 (16.0)	11/125 (8.8)	16/125 (12.8)	6/125 (4.8)	125/3,250 (3.8)	
Taken 2nd dose vaccine against COVID-19								
	322/2,856 (11.3)	603/2,856 (21.1)	581/2,856 (20.3)	431/2,856 (15.1)	507/2,856 (17.8)	412/2,856 (14.4)	2,856/3,250 (87.9)	0.005
	86/394 (21.8)	77/394 (19.5)	58/394 (14.7)	71/394 (18.0)	36/394 (9.1)	66/394 (16.8)	394/3,250 (12.1)	
Taken 3rd dose vaccine against COVID-19								
Yes	87/1,347 (6.5)	131/1,347 (9.7)	165/1,347 (12.2)	383/1,347 (28.4)	344/1,347 (25.5)	237/1,347 (17.6)	1,347/3,250 (41.4)	0.002
No	321/1,903 (16.9)	549/1,903 (28.8)	474/1,903 (24.9)	119/1,903 (6.3)	199/1,903 (10.5)	241/1,903 (12.7)	1,903/3,250 (58.6)	
Treatment problems among patients with CVD and diabetes during COVID-19								
Yes	11/231 (4.8)	23/231 (10.0)	31/231 (13.4)	36/231 (15.6)	58/231 (25.1)	72/231 (31.2)	231/3,250 (7.1)	0.002
No	397/3,019 (13.2)	657/3,019 (21.8)	608/3,019 (20.1)	466/3,019 (15.4)	485/3,019 (16.1)	406/3,019 (13.4)	3,019/3,250 (92.9)	

$P < 0.05$ was considered statistically significant.

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TABLE 4 Multivariable logistic regression analyses to determine the odds of hospitalization and severe outcome among patients with COVID-19 in Bangladesh.

Variables	Odds ratio (95% confidence intervals)	*P*-value	Odds ratio (95% confidence intervals)	*P*-value
Age, >60 years vs. <60 years	4.14 (3.76–5.29)	0.001	3.23 (2.54–4.78)	0.003
Sex, male vs. female	3.34 (2.75–4.58)	0.005	2.64 (1.85–3.43)	0.001
Unemployed vs. employed	1.43 (0.55–2.65)	0.001	1.63 (0.45–2.42)	0.002
CVD and diabetes together vs. CVD alone	3.27 (2.34–4.58)	0.001	2.39 (1.43–3.62)	0.001
CVD and diabetes together vs. diabetes alone	2.43 (1.19–3.53)	0.005	2.14 (1.05–3.79)	0.005
COVID-19 positive vs. COVID-19 negative	4.63 (3.42–5.37)	0.001	4.74 (3.83–5.63)	0.001
CVD and COVID-19 vs. CVD	4.23 (3.65–5.18)	0.002	3.45 (2.63–4.27)	0.001
Diabetes and COVID-19 vs. diabetes	2.26 (1.56–3.14)	0.001	3.15 (2.64–4.02)	0.005
CVD, diabetes and COVID-19 vs. CVD and diabetes	3.19 (2.27–4.69)	0.003	3.65 (2.79–4.24)	0.001
Acute long-COVID-19 vs. chronic long-COVID-19	2.46 (1.76–3.23)	0.001	2.35 (1.54–3.21)	0.007
Acute-long COVID-19, CVD and diabetes vs. CVD and diabetes	3.27 (2.35–4.65)	0.001	3.58 (2.76–4.32)	0.005
Chronic long COVID-19 and CVD vs. CVD	1.56 (0.86–2.41)	0.002	2.31 (1.83–3.23)	0.001
Chronic long COVID-19, CVD and diabetes vs. CVD and diabetes	2.72 (1.75–3.57)	0.003	1.48 (0.9–2.35)	0.048
Vaccinated vs. unvaccinated	0.27 (0.1–0.95)	0.001	0.38 (0.17–0.74)	0.003
Two doses vs. one dose	0.45 (0.13–0.97)	0.002	0.14 (0.09–0.95)	0.001
Three doses vs. two doses	0.56 (0.15–0.99)	0.001	0.68 (0.16–1.21)	0.005
>3 symptoms vs. <3 symptoms	3.45 (2.63–4.67)	0.003	3.25 (2.74–4.11)	0.005
Worse access to health facilities vs. better access to health facilities	2.76 (2.24–3.53)	0.001	2.53 (2.17–3.26)	0.005
CCI >3 vs. CCI <3	5.53 (5.15–6.14)	0.005	4.46 (4.02–5.14)	0.001

$P < 0.05$ was considered statistically significant. OR, odds ratio; COPD, chronic obstructive pulmonary disease; CCI, Charlson comorbidity index.

4. Discussion

The severity and longtime impact of the COVID-19 pandemic have adversely affected the global health system (8–11). After the onset of the pandemic, it has remained one of the leading causes of global health burden among people. Among many health effects, post-COVID-19 sequelae in the infected is a major problem (11). In this study, we determine the prevalence of patients with acute long-COVID-19 and chronic long-COVID-19 in Bangladesh, and impact of COVID-19 on pre-existing cardiovascular disease and diabetes among them. We specified the predictors associated with poor health outcome, hospitalization, ICU admission and fatality among patients with COVID-19. We found that 73.4% of the participants were positive for COVID-19. About 28.4% of the patients were suffering from acute long-COVID-19 and 71.6% from chronic long-COVID-19 in Bangladesh. The prevalence of long COVID-19 reported in this study is higher than any of the previous studies (12–20). The probable reason might be the study cohort had pre-existing comorbidities, high prevalence of COVID-19, circulation of omicron variant (90%) during the study, and higher population density in the study regions. Nearly 29% of the patients had symptoms of COVID-19 for 13–24 weeks, 25.6% for 25–48 weeks and 17% for >48 weeks. These findings are one of the first reports of long COVID-19 in Bangladesh. The prevalence of COVID-19 is also high in this study compared to the previous studies (8–12, 16, 18–20).

In the comorbidity analysis, we found that cardiovascular (CVD) disease was the most prevalent (32%) followed by diabetes (24%; Type 2 diabetes mellitus was 63.7% and Type 1 was 36.3%). Previous studies have reported the prevalence of diabetes between 10% and 100% among patients with COVID-19 (12–19, 21–24). Reported median glycaemia was 9.3 mmol/L (IQR 7·2–10·7) among the patients with COVID-19, which is in good agreement with previous studies (12, 16, 19). Previous

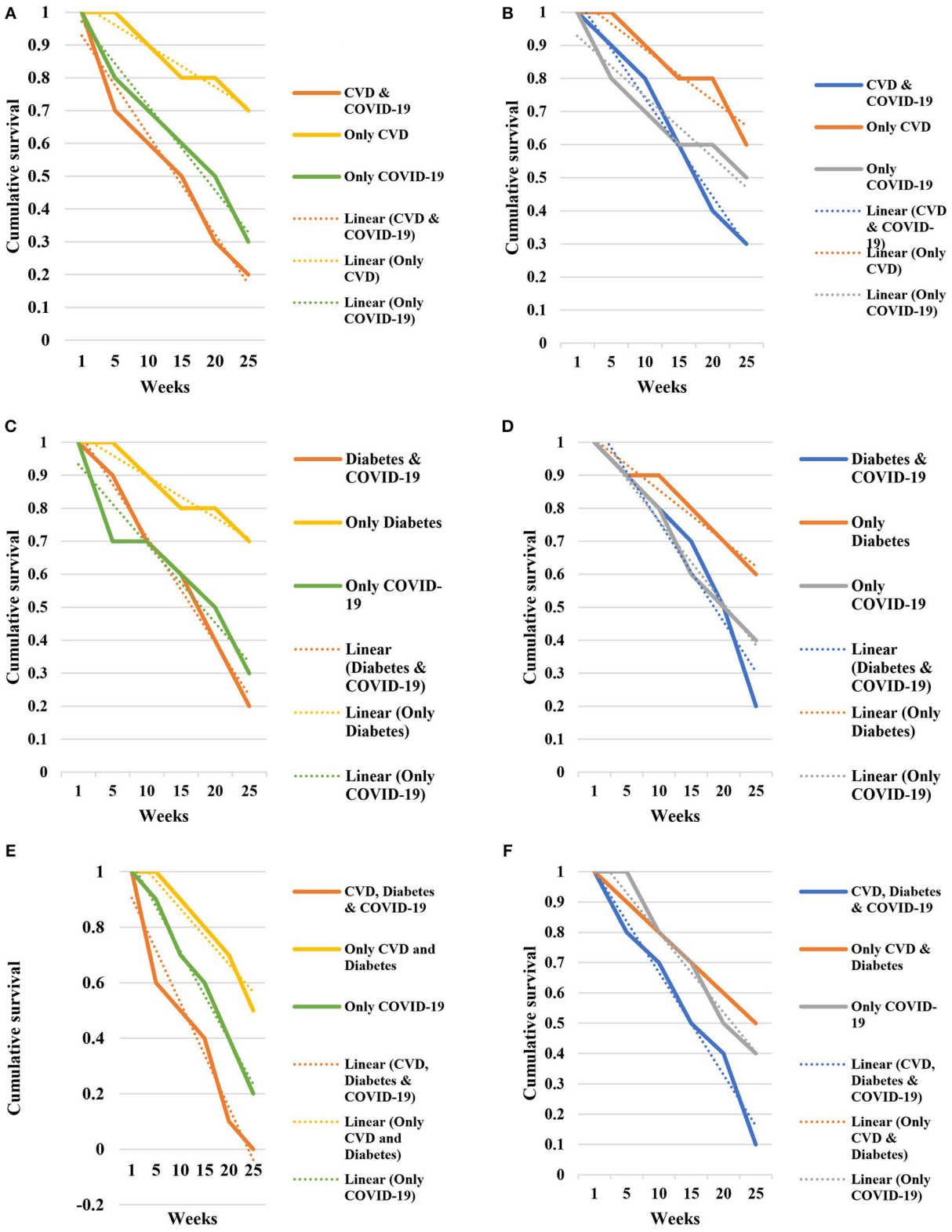

FIGURE 2
Cumulative survival rate among **(A)** male patients with CVD and COVID-19, **(B)** female patients with CVD and COVID-19, **(C)** male patients with diabetes and COVID-19, **(D)** female patients with diabetes and COVID-19, **(E)** male patients with CVD, diabetes and COVID-19, **(F)** female patients with CVD, diabetes and COVID-19 in Bangladesh.

studies have reported the prevalence of cardiovascular disease between 2% and 40% in patients with COVID-19 in different countries (16, 19, 21–25). Coronary artery disease was the most frequent cardiovascular disease followed by hypertension, cardiac arrhythmia and congestive heart failure, respectively. These findings are in good agreement with previous studies in Bangladesh, Saudi Arabia and China (11–19, 21–27). Co-prevalence of CVD, diabetes and COVID-19 was involved in majority of fatality (95%) reported among the participants, which is in good agreement with previous studies in Bangladesh, China and other countries (16–19, 22–28). We detected 16.4% patients of COVID-19 had both CVD and diabetes. Health complications associated with CVD increased among 64.7% patients and diabetes among 55% patients after getting COVID-19 infection. These findings are supported by the previous studies in different countries (16, 18, 19, 22–31). Co-prevalence of CVD, diabetes and acute long-COVID-19 was found among 11% and co-prevalence of CVD, diabetes and chronic long-COVID-19 were detected among 11.9% patients. These findings will add knowledge on long-COVID-19 and comorbidity in Saudi Arabia for the first time. This study reflected previous studies focusing single and combined impacts of comorbidities like CVD, diabetes, COPD and demographic factors like age and sex on the outcome including hospitalization, ICU admission and fatality (11, 13–19, 22, 24–32).

Symptoms associated with acute and chronic long-COVID-19 were analyzed. Among the patients with acute long-COVID-19, fever (97%) was most prevalent followed by sore throat (89%), loss of taste or smell (86%) and dry cough (79%), respectively. Further, fever (95%) was the most prevalent symptoms followed by dry cough (88%), loss of taste and smell (83%) and fatigue (78%) among the patients with chronic long-COVID-19. Reappearance of different of symptoms of COVID-19 after recovery from infection had prolonged health impact on the patients with CVD and diabetes (8–12). However, the distribution of prevalence of different symptoms were similar among the COVID-19 patients and those with long-COVID-19. These findings will add new knowledge on the clinical spectrum of COVID-19 in Bangladesh.

We detected the highest odds ratio for hospitalization among patients with CCI >3 (OR: 5.53, 95% CI, 5.15–6.14) and COVID-19 (OR: 4.63, 95% CI, 3.42–5.37). Patients with COVID-19 (OR: 4.74, 95% CI, 3.38–5.63) and co-prevalence of CVD, diabetes and COVID-19 (OR: 3.65, 95% CI, 2.79–4.24) had higher risk of ICU admission and fatality. We also found that vaccinated people had lower risk of hospitalization (OR: 0.27, 95% CI, 0.1–0.95; *p*-value 0.001), ICU admission and fatality (OR: 0.38, 95% CI, 0.17–0.74; *p*-value 0.003). These findings are in good similarity with previous studies (11–19, 22–28, 31–33). In a similar study in Bangladesh, the authors also reported higher odds of fatality and ICU admission among patients with CVD, diabetes and COVID-19 (16, 19). We found higher risk of fatality in male patients aged above 40 years suffering from CVD, diabetes and COVID-19 (OR: 3.41, 95% CI, 2.62–4.83). These findings are in similarity with previous reports worldwide (11–19, 24–33). Presence of acute and chronic long-COVID-19 also increased the risk of hospitalizations, ICU admission and fatality among the participants. In similar with previous studies, we also found that female and participants aged below 30 years had lower risk of developing long-COVID-19 associated severity (16, 19). These might be due to their stronger immunity against viral infection, which needs detail analysis in future (16, 19).

We found that presence of COVID-19 contributed to the development of serious health outcome among patients with CVD and diabetes in Bangladesh. Majority of the fatality were associated with COVID-19, CVD and diabetes among the patients. However, we found lower fatality rate among the patients with chronic long-COVID-19 compared with patients with acute long-COVID-19. These findings are relatively new for data of Bangladesh. Presence of symptoms of COVID-19 for longer period and reappearance after certain time might affect the pre-existing CVD and diabetes. Studies have found that infection of COVID-19 have contributed to development of diabetes for certain period among the patients (16–19, 21–28). Further, studies have also reported that infection with COVID-19 might worsen the existing cardiovascular disease in patients. Certain medicines used to treat cardiovascular disease might have roles in poor health outcome among the COVID-19 patients (16, 19, 24–29, 31–33). Presence of previous CVD and diabetes increased the risk of poor health outcome and fatality rate after COVID-19 infection. Inversely, infection of COVID-19 has also triggered different adverse health impact in patients with CVD and diabetes. Previous studies have confirmed that COVID-19 infection has increased incidence of cardiac arrest, cardiomyopathy, myocardial infarction, and cardiac arrhythmias (13–19, 23, 26–33).

The main limitation of the study was the limited size of the population. Further, we could not include data on real-time hemoglobin A1c (HbA1c) and CVD disease conditions of the patients. Data on the clinical manifestations were missing for some of the patients and some of the data were self-reported.

This is one of the first report of long-COVID-19 and associated health outcome in Bangladesh. In this study we reported high prevalence of chronic long-COVID-19 among patients in Bangladesh. Co-existence of CVD, diabetes and COVID-19 have significantly contributed to hospitalization, ICU admission and fatality among the participants. This study will add knowledge in understanding the long-time impact of COVID-19 and health burden of preexisting CVD and diabetes. Further, these findings will provide baseline data for future studies to reveal the exact mechanism of complicated health outcome and impact of COVID-19 among patients with CVD and diabetes.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Biosafety, Biosecurity, and Ethical Committee (BBEC) of Jahangirnagar University, Savar, Dhaka-1342, Bangladesh. The protocol number approved by the Ethics Committee is BBEC,

JU/M 2021/COVID-19/(2)1. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

NadS: conceptualization (lead), data curation (lead), formal analysis (lead), investigation (equal), methodology (lead), project administration (lead), software (lead), validation (lead), writing—original draft (lead), and writing—review and editing (lead). NazS: data curation (equal), methodology (equal), investigation (equal), methodology (lead), project administration (lead), software (lead), validation (lead), writing—original draft (lead), and writing—review and editing (lead). AK: validation (equal), writing—original draft (supporting), data curation (equal), and investigation (equal). IH: writing—review and editing (equal) and formal analysis (supporting). FA: software (lead) and writing—review and editing (equal). KA: writing—original draft preparation (supporting) and methodology (supporting). ID: data curation (equal), project administration (equal), software (equal), and validation (equal). DV: data curation (equal), software (equal), and validation (equal). AC: data curation (equal), project administration (equal), software (equal), and validation (equal). AP: software (lead) and validation (lead). SD: project administration (lead), software (lead), validation (lead), and writing—review and editing (lead). All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1222868/full#supplementary-material

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Adverse reactions following COVID-19 vaccine among healthcare professionals working in Ethiopia: a facility-based cross-sectional study

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Background of the study: One of the best medical approaches for halting the spread of infectious diseases is vaccination. During the COVID-19 pandemic, healthcare workers (HCWs) were a high-risk population. Due to their susceptibility in terms of their working environment, front-line healthcare personnel should receive vaccinations before others.

Objective: The purpose of this study was to assess the adverse reactions to COVID-19 vaccines among Ethiopian healthcare professionals in 2022.

Methods: A facility-based cross-sectional study design was conducted in Addis Ababa Health Facilities, Ethiopia. A total of 290 health professionals who were vaccinated during the study period were involved. Data entry was done by Epidata (version 3.1) and analyzed using SPSS software version 26. Bivariable analysis was conducted and a *p* value of less than 0.25 was selected for further multivariable analysis. A *p* value of 0.05 was considered statistically significant at a 95% confidence level.

Results: A total of 277 study participants were successfully involved in the study, yielding a response rate of 95.5%. The study participants comprised 123 (44.4%) women and 154 (55.6%) men. The majority of them (202, 72.9%) had received the Oxford AstraZeneca vaccine. Among the 277 study participants, 142 (51.3%) had developed adverse reactions associated with vaccination. Of these, 81 (29.2%) had moderate adverse reactions. Only 2 (0.7%) had developed adverse reactions that led to hospitalization. The most reported short-term adverse reactions were injection site pain (151, 54.5%), headache (114, 41.2%), fever (104, 37.5%), fatigability and tiredness (94, 33.9%), chills (92, 33.2%), muscle pain (79, 28.5%), and decreased sleep quality (34, 12.3%). The multivariable logistic regression showed that the odds of having an adverse reaction were 1.501 times higher among women than men (AOR = 1.501, 95% CI [1.08, 2.754]).

Conclusion and recommendations: This study revealed that adverse effects following the COVID-19 vaccine were moderate in magnitude and minimal in severity. This study showed that adverse reactions that led to hospitalization were rare. Based on the findings of this study, it is recommended that national, multicenter, prospective, and randomized studies be conducted to assess the independent association of each vaccine.

KEYWORDS
COVID-19 vaccine, healthcare professionals, vaccine, Oxford AstraZeneca, corona virus

Introduction

One of the best medical approaches for halting the spread of infectious diseases is vaccination. To protect communities from COVID-19 and prevent further economic hardship, safe and effective SARS-CoV-2 vaccinations are required (1).

A 94.1% efficacy of the SARS-CoV-2 vaccine (mRNA-1273) has been confirmed, and the first human clinical study of the vaccine began in March 2020 in the United States. However, the SARS-CoV-2 vaccine's global uptake is still insufficient for herd immunity (2).

Healthcare workers (HCWs) are a high-risk population during the COVID-19 pandemic. This subpopulation has a 9–11 times higher infection risk than the general population (3). In China, a total of 1,433 healthcare workers (HCWs) received vaccinations, and 135 of them reported adverse reactions (9.4%) (4).

According to a study done in India, 98.2% of people experienced adverse effects following immunization. In this study, generalized weakness, local pain, or swelling at the injection site were some of the side effects that were frequently experienced after vaccination. In this study, women (67.7%) were more likely than men (32.3%) to experience detrimental impacts when working as healthcare professionals (5).

The Centers for Disease Control and Prevention (CDC) and other studies have shown that symptoms at the injection site (swelling, pain, and redness) and systemic effects (back pain, fatigue, headache, muscle pain, joint pain, chills, fever, and nausea) were connected to post-COVID-19 vaccination (6).

According to a Chinese study, the two most common complaints were weakness (74, 5.2%) and headache/dizziness (58, 4.0%). The most often reported side effects associated with the COVID-19 vaccination include headache, weariness, muscle and joint pain, fever and chills, and soreness at the injection site (7).

According to a study from Nigeria, participants who had previously experienced an adverse reaction to a medication or vaccination were younger (40 years old), had received two doses, and reported experiencing symptoms more frequently. Approximately 71.1% of the 295 vaccine recipients in Nigeria who participated in the trial experienced at least one side effect (8).

Another study conducted on Ghanaian healthcare workers showed that 528 (80.7%) of the participants reported having adverse reactions. The most common adverse effects among Ghanaian healthcare workers were generalized weakness (32.0%), headache (27.3%), and fever (19.1%) (9). According to a study, healthcare workers aged 35–39 and 40–44 had reduced probabilities of adverse reactions compared to those aged 25–29. Analgesics used by medical personnel before immunization reduced the risk of negative reactions (9).

A study done in Togo revealed that out of 1,639 medical professionals, 71.6% of participants reported at least one adverse effect (10). According to a study done in Ethiopia, 510 (75.7%) medical professionals who received the vaccination reported injection site symptoms of pain (65.48%) and discomfort (57.9%) (11).

Since evidence of the adverse effects of all vaccines given in Ethiopia is scarce, this study was conducted to quickly document adverse events to reassure the population. This study was intended to assess adverse reactions following COVID-19 vaccination and their associated factors among healthcare professionals working in Ethiopia.

Materials and methods

Study design and setting

A facility-based cross-sectional study was carried out among healthcare professionals working in Addis Ababa Public Health Facilities, Ethiopia from February 10, 2022 to June 10, 2022.

Sample size determination

According to a previous study conducted in Ethiopia, 75.8% of healthcare professionals who received the Oxford AstraZeneca vaccine reported injection site symptoms of pain and tenderness (11). Based on this assumption, the minimum sample size required for this study was determined using the single population proportion formula.

$$\left(\mathrm{ni} = \left(Z\alpha / 2\right)2\,\mathrm{p}\left(1-\mathrm{p}\right) / \mathrm{d}2\right)$$

Taking $p = 75.8\%$, 5% level of precision (d) with a 95% confidence interval, and a 10% non-response rate was added. Since the source population was 4,471, the population correction formula was utilized. The final sample size was = 290.

Sampling procedures and techniques

From a total of 11 Governmental Hospitals in Addis Ababa, three were selected by simple random sampling technique (lottery method). The selected Governmental Hospitals included St. Paul's Millennium Medical College, Yekatit 12 Hospital Medical College, and Eka Kotebe General Hospital. Based on the data from the Addis Ababa Health Bureau and the Federal Ministry of Health, the total number of healthcare professionals working in Addis Ababa governmental hospitals was 4,471.

A systematic random sampling technique was employed after using proportional allocation. The sampling fraction was: 4,471/290 = 15. The first sample was selected using a simple random sampling technique. Then, every 15 healthcare professionals were included in the study from each of the governmental hospitals until the calculated sample size was achieved.

Abbreviations: ADRs, Adverse drug reactions; AEFI, Adverse events following immunization; AEs, Adverse effects; CDC, Center for Disease Control and Prevention; CVST, Cerebral venous sinus thrombosis; EMA, European medicines agency; HCPs, Health care professional; NDVP, National deployment and vaccination plan; WHO, World Health Organization.

Study variables

Dependent variable

Adverse reactions following COVID-19 vaccine.

Independent variables

Socio-demographic factors (age, sex, and educational status), behavioral factors (alcohol drinking, cigarette smoking, khat chewing, and drugs used for any other chronic illnesses), and other factors (type and dose of vaccine, presence of chronic illnesses, and COVID-19 result before or after vaccination).

Data collection tools and procedures

Data were collected using a structured questionnaire adapted from different literature. The questionnaire includes four parts; socio-demographic characteristics, medical history, behavioral factors, and vaccination status. The questionnaire was prepared in the English language and translated into Amharic and then back into English. Five BSc health professionals were recruited to collect the data and two BSc/MSc health professionals supervised the data collection process. Timely supervision was undertaken by the principal investigator during the data collection period.

Operational definition

Adverse reactions: unintended pharmacologic reactions that occur when medication or vaccine is administered correctly.

Mild adverse reaction: HCPs who stayed at home to rest and who also took painkillers.

Moderate adverse reaction: HCPs who attended health institutions but did not require hospitalization.

Severe adverse reaction: HCPs who were admitted to hospital and received the required health care services.

Data processing and analysis

The data were entered into EPI data manager version 3.3 and analyzed using IBM SPSS Statistics version 22. Model fitness was also checked using the Hosmer and Lemeshow test. A summary of descriptive statistics was computed for most variables. A binary logistic regression analysis model was utilized. A point estimate of Odds ratio (OR) with a 95% confidence interval (CI) was determined to assess the strength of association between independent and dependent variables. For all statistically significant tests, value of $p < 0.05$ was used as a cut-off point.

Results

Out of 290 study participants, 277 were successfully involved in the study, yielding a response rate of 95.5%. The study participants comprised 123 (44.4%) women and 154 (55.6%) men. The study participants' ages ranged from 22 to 54 years, with mean and standard deviations of 31 and ± 6.46 years, respectively. Most of the participants (127, 45.8%) were nurses in the profession. The majority (266, 96%) had no chronic diseases (Table 1).

TABLE 1 Sociodemographic characteristics of the study participants, Addis Ababa, Ethiopia, 2022.

Variables	Frequency (%)
Gender	
Men	154 (55.6)
Women	123 (44.4)
Age	
<30	172 (62.1)
30–50	101 (36.5)
>50	4 (1.4)
Job category	
Doctors	72 (26)
Health officers	56 (20.2)
Nurses	127 (45.8)
Midwives	10 (3.6)
Medical laboratories	12 (4.3)
Suffering from chronic diseases	
Yes	11 (4)
No	266 (96)

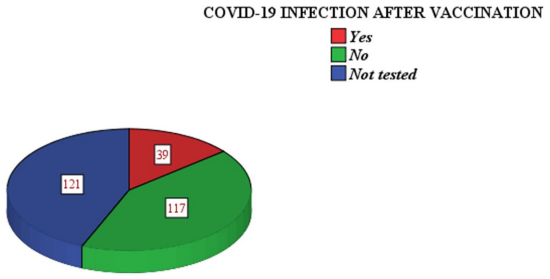

FIGURE 1
COVID-19 infection after vaccination among health professionals in Addis Ababa, Ethiopia.

COVID-19 vaccination status

The majority (159, 57.4%) of study participants' previous COVID-19 results were negative. All of the study participants were vaccinated. Among the vaccinated, 39 (14.1%) were infected by the virus (Figure 1).

The majority of them (202, 72.9%) had received the Oxford AstraZeneca vaccine. Only 11 (4%) took Sinopharm. Most of the participants (208, 75.1%) received two doses of the COVID-19 vaccine. A total of 249 had no allergies to any types of food or medicines. Only 5 (1.8%) had used substances (Figure 2).

Prevalence of adverse reactions following COVID-19 vaccine

Among the 277 study participants, 142 (51.3%) had developed adverse reactions associated with vaccination. Of these, 81 (29.2%) had moderate adverse reactions. Only 2 (0.7%) had developed adverse reactions that led to hospitalization (Figure 3). Among those who had

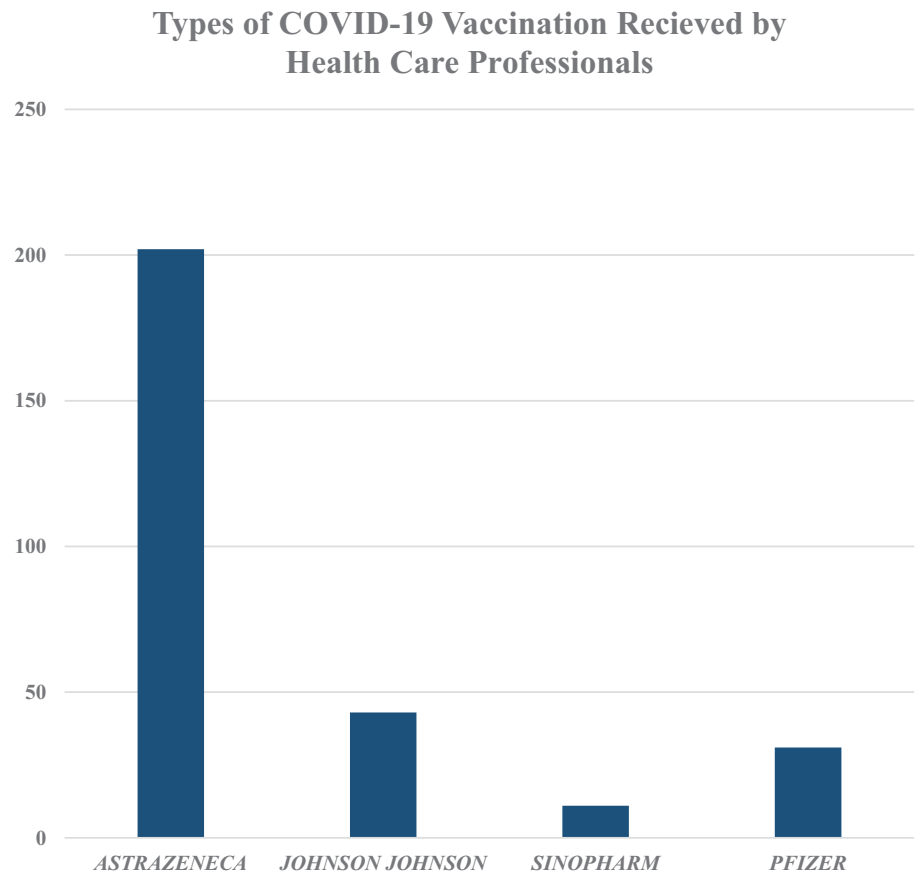

FIGURE 2
Types of COVID-19 vaccination received by healthcare professionals working in Addis Ababa Public Health Facilities, Ethiopia.

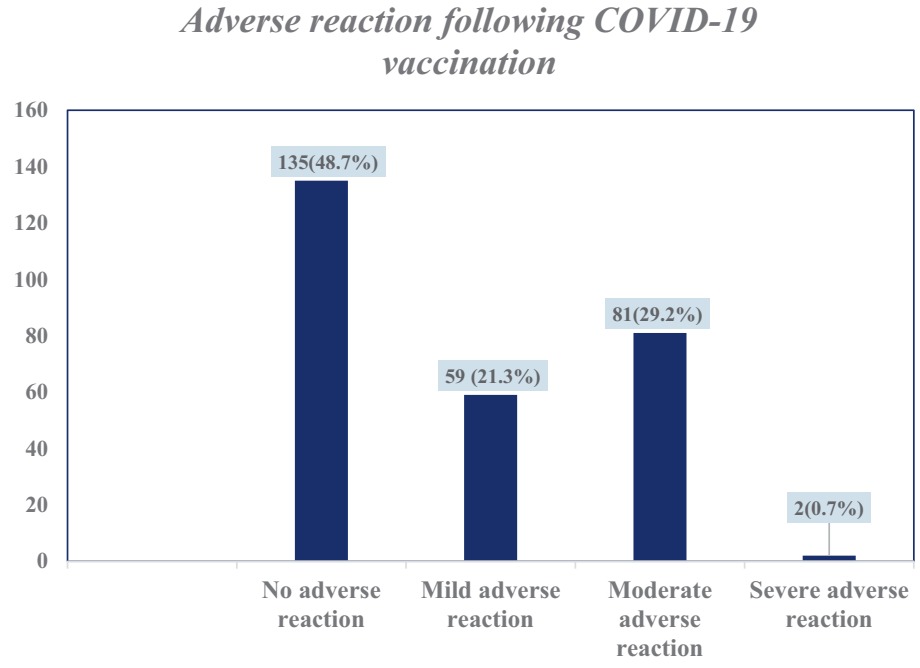

FIGURE 3
Categories of adverse reactions following COVID-19 vaccination among health care professionals in Addis Ababa, Ethiopia.

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adverse reactions, 70 (25.3%) developed adverse reaction symptoms within 5–8 h of vaccine administration. In the majority (79, 28.5%), the symptoms lasted for 1–3 days. Of the study participants who had received the COVID-19 vaccine, only 2 (0.7%) were diagnosed with thrombosis. Most of the study participants (245, 88.4%) recommended having the COVID-19 vaccine to others.

The most reported short-term adverse reactions were injection site pain 151 (54.5%), headache 114 (41.2%), fever 104 (37.5%), fatigability and tiredness 94 (33.9%), chills 92 (33.2%), muscle pain 79 (28.5%), and decreased sleep quality 34 (12.3%; Table 2).

TABLE 2 Adverse reaction after COVID-19 vaccine administration among health care professionals in Addis Ababa, Ethiopia.

No	Adverse reactions following COVID-19	Frequency
1.	Did you experience any adverse reactions?	
	Yes	142 (51.3%)
	No	135 (48.7%)
2.	Adverse reactions experienced after vaccine administration	
	Injection site pain	151 (54.5%)
	Headache	114 (41.2%)
	Fever	104 (37.5%)
	Fatigability and tiredness	94 (33.9%),
	Chills	92 (33.2%)
	Muscle pain	79 (28.5%)
	Decreased sleep quality	34 (12.3%)
	Nausea and vomiting	24 (8.7%)
	Irritation and skin reaction	19 (6.9%)
	Body sweating	12 (4.3%)
	Runny nose	25 (9%)
	Dyspnea	8 (2.9%)
	Chest pain	14 (5.1%)
	Sore throat	21 (7.6%)
	Cough	28 (10.1%)
3.	How soon the symptoms appeared after injection with a COVID-19 vaccine	
	Up to 4 h	17 (6.1%)
	5–8 h	70 (25.3%)
	9–12 h	41 (14.8%)
	After 24 h	14 (5.1%)
4.	How long the symptoms lasted	
	Less than 1 day	29 (10.5%)
	1–3 days	79 (28.5%)
	4–7 days	34 (12.3%)
	More than 7 days	0
5.	Have you been diagnosed with any types of thrombosis (blood clots)?	
	Yes	2 (1.4%)
	No	140 (98.6%)
6.	Would you recommend the vaccine that you received to others?	
	Yes	245 (88.4%)
	No	32 (11.6%)

Factors associated with the occurrence of adverse reactions following COVID-19

All sociodemographic characteristics were entered into bivariate logistic regression. Age group (p value = 0.23), gender (p value = 0.017), job category (p value = 0.031), and underlying chronic diseases (p value = 0.320). Based on a binary regression result, the odds of having adverse reactions were 1.799 times higher among women than men (COR = 1.799, 95% CI [1.13, 2.906]). The odds of adverse reactions were 66% less likely among study participants who had received two doses of the COVID-19 vaccine than among those who had received it once (COR = 0.337, 95% CI [0.117, 0.975]).

There was no statistically significant association with adverse reactions related to the specific types of COVID-19 vaccine [Oxford AstraZeneca (COR = 1.385, 95% CI [0.814, 2.359]), Johnson & Johnson (COR = 0.90, 95% CI [0.469, 1.727]), Sinopharm (COR = 0.00), and Pfizer (COR = 2.169, 95% CI [0.981, 4.797])].

Multivariable logistic regression analysis has shown that the odds of having adverse reactions were 1.501 times higher among women than men (AOR = 1.501, 95% CI [1.08, 2.754]; Table 3).

Discussion

During the COVID-19 pandemic, healthcare professionals were among the high-risk populations. Due to their susceptibility in terms of their working conditions, front-line healthcare personnel were given priority when it came to vaccination (3). Evidence of the adverse effects of the COVID-19 vaccines administered in Ethiopia is scarce.

Among the study participants, 51.3% had developed adverse reactions associated with vaccination. The current study's findings are lower than those of other studies conducted in Ghana, which showed that the prevalence of adverse reactions among study participants was 80.7% (9); in Togo, it was 71.6% (10); and in UAE, it was 64.8% (12). These differences in the prevalence of adverse reactions could be due to variation in sample size and socioeconomic status.

The major adverse effects reported by the COVID-19 vaccine recipients were pain at the site of injection (47%), fatigue and drowsiness (28.2%), and joint/muscle pain (23.1%), followed by headache (17.7%) and fever (14.4%). A survey based on a mobile self-report questionnaire to assess the prevalence and characteristics of adverse reactions following the first dose of the ChAdOx1 nCoV-19 Vaccine and the BNT162b2 vaccine was conducted among healthcare workers in South Korea. Of the 5,589 healthcare workers in the ChAdOx1 nCoV-19 group, the overall adverse reaction rate was 93%.

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TABLE 3 Factors associated with the occurrence of adverse reactions among healthcare professionals in Addis Ababa, Ethiopia.

Variables	*COVID-19 adverse reaction*				
	Yes	No	COR (CI)	AOR (CI)	*p* value
Gender					
Men	69 (44.8)	85 (55.2)	1	1	0.01*
Women	73 (59.3)	50 (40.7)	1.79 (1.13–2.906)	1.50 (1.08–2.75)	
Age category					
<30	95 (55.2)	77 (44.8)	1.23 (0.17–8.96)	0.88 (0.10–7.31)	0.9
30–50	45 (44.6)	56 (55.4)	0.80 (0.10–5.93)	0.61 (0.07–5.28)	0.66
>50	2 (50)	2 (50)	1	1	
Suffering chronic diseases					
Yes	4 (36.4)	7 (63.6)	0.53 (0.15–1.85)	0.63 (0.12–3.25)	0.58
No	138 (51.9)	128 (48.1)	1	1	
COVID-19 vaccine types					
AstraZeneca (reference is No)	108 (53.5)	94 (46.5)	1.38 (0.81–2.36)	1.12 (0.75–2.25)	0.13
Johnson (reference is No)	23 (53.5)	20 (46.5)	0.90 (0.46–1.72)	0.65 (0.35–1.46)	0.22
Sinopharm (reference is No)	0	11 (100)	0.05 (0.01–0.23)	0.03 (0.01–0.18)	0.38
Pfizer (reference is No)	21 (67.7)	10 (32.3)	2.16 (0.98–4.79)	0.88 (0.75–4.23)	0.11
COVID-19 vaccine received					
One time	17 (35.4)	31 (64.6)	0.34 (0.12–0.97)	0.24 (0.04–1.30)	0.09
Two times	112 (53.8)	96 (46.2)	0.71 (0.28–1.80)	0.73 (0.20–2.61)	0.63
More than two times	13 (61.9)	8 (38.1)	1	1	
Allergy to foods or medicines					
Yes	19 (67.9)	9 (32.1)	2.16 (0.94–4.96)	2.56 (1.86–7.59)	0.04*
No	123 (49.4)	126 (50.6)	1	1	
Substance use					
Yes	2 (40)	3 (60)	1.43 (0.23–8.73)	0.98 (0.10–8.94)	0.99
No	139 (51.1)	133 (48.9)	1	1	
Recommend for others					
Yes	122 (49.8)	123 (50.2)	0.59 (0.28–1.27)	0.62 (0.27–1.42)	0.26
No	20 (62.5)	12 (37.5)	1	1	

*Indicate significantly associated variables.

Approximately, half of the ChAdOx1 nCoV-19 group reported moderate or severe grade events (13).

In the current study, only 0.7% had developed adverse reactions that led to hospitalization. Comparable findings were noted in a study conducted in Southern Ethiopia, which showed that 1.1% had severe adverse reactions (14), and in Togo, where 1% were found to have been hospitalized (10). These comparable findings from different studies might implicate the rare occurrence of severe adverse reactions associated with the COVID-19 vaccine.

The most reported short-term adverse reactions were headache (41.2%), fever (37.5%), fatigability and tiredness (33.9%), chills (33.2%), muscle pain (28.5%), and decreased sleep quality (12.3%). These findings are comparable with other studies conducted in Ghana (9), Togo (10), and Southern Ethiopia (14). The similarities could be due to the wide scale use of the AstraZeneca vaccine in this population.

The present study revealed that only 1.4% had been diagnosed with thrombosis (blood clots). This finding contradicts a study conducted in Ethiopia, which showed none of the study participants reported laboratory-confirmed blood clotting problems (11). However, a systematic review and meta-analysis study indicated that venous thrombosis due to the COVID-19 vaccine was 28 per 100,000 doses (15). Similarly, other systematic reviews and exploratory analysis studies indicated the presence of venous thrombosis due to the COVID-19 vaccine (16). According to this systematic review and exploratory analysis study, the pathophysiology behind venous thrombosis is explained as follows: "New experimental studies have assumed that thrombosis is related to a soluble adenoviral protein spike variant, originating from splicing events, which cause important endothelial inflammatory events, and binding to endothelial cells expressing ACE2" (16) (p.2).

Multivariable logistic regression analysis showed that the odds of experiencing adverse reactions were 1.501 times higher among women than men. Similar findings were noted in a study conducted in Togo (10). This result may be explained by a greater immunological response brought on by estrogen (6) or other unidentified immunologic differences between men and women (10).

This study revealed no statistically significant correlation between the different COVID-19 vaccination types and associated adverse reactions. These results suggest that unfavorable reactions to a vaccine are not influenced by the type of vaccine.

Conclusion

In this study, adverse reactions following the COVID-19 vaccine were moderate in magnitude and minimal in severity. This study revealed that 51.3% of participants had developed adverse reactions associated with vaccination. The majority of the study participants (72.9%) had received the AstraZeneca vaccine. The most reported short-term adverse reactions following vaccination were headache, fever, fatigability and tiredness, chills, and muscle pain. Less than 1% (0.7%) had developed adverse reactions that led to hospitalization. The present study revealed that the occurrence of thrombosis (blood clots) was rare. In the current study, the odds of having adverse reactions were higher among women than men. The type of COVID-19 vaccine had no significant association with adverse reactions.

Based on our findings, we recommend health professionals receive any of the COVID-19 vaccines without fear or hesitancy since severe adverse reactions were found to be rare. Future national, multicenter, prospective, and randomized study should be conducted to assess the independent association of each vaccine with adverse reactions. Our results show that women were more likely to develop adverse reactions than men. Therefore, future randomized control studies should investigate this association clearly.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was conducted after obtaining ethical approval letters from the (IRB) of the College of Medicine, Asrat Woldeyes Health Science Campus, Debre Berhan University, and Addis Ababa Health Bureau complied with the Declaration of Helsinki. A permission letter was obtained from the study hospitals. The data were collected after obtaining written informed consent from the study participants. To keep confidentiality, codes were used and unauthorized persons did not have access to the data.

Author contributions

AA contributed to conception or design, data collection, acquisition, analysis, or interpretation, drafted the manuscript, and critically revised the final manuscript. ND contributed to conception or design and data collection, and drafted the manuscript. AB, FT, and DM contributed to acquisition, analysis, or interpretation, drafted the manuscript, and critically revised the final manuscript. BG drafted the manuscript and critically revised the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The impact of SARS-CoV-2 on healthcare workers of a large University Hospital in the Veneto Region: risk of infection and clinical presentation in relation to different pandemic phases and some relevant determinants

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Aim: The aim of this study is to evaluate the incidence of SARS-CoV-2 infection and the prevalence of COVID-19-related symptoms in relation to pandemic phases and some relevant variables in a cohort of 8,029 HCWs from one of the largest Italian University Hospitals.

Methods: A single-center retrospective study was performed on data collected during SARS-CoV-2 infection surveillance of HCWs. Cox's multiple regression was performed to estimate hazard ratios of SARS-CoV-2 infection. Logistic multivariate regression was used to assess the risk of asymptomatic infections and the onset of the most frequent symptoms. All analyses were adjusted for sociodemographic and occupational factors, pandemic phases, vaccination status, and previous infections.

Results: A total of 3,760 HCWs resulted positive (2.0%–18.6% across five study phases). The total incidence rate of SARS-CoV-2 infection was 7.31 cases per 10,000 person-days, significantly lower in phase 1 and higher in phases 4 and 5, compared to phase 3. Younger HCWs, healthcare personnel, and unvaccinated subjects showed a higher risk of infection. Overall, 24.5% were asymptomatic infections, with a higher probability for men, physicians, and HCWs tested for screening, fully vaccinated, and those with previous infection. The clinical presentation changed over the phases in relation to vaccination status and the emergence of new variants.

Conclusion: The screening activities of HCWs allowed for the early detection of asymptomatic cases, limiting the epidemic clusters inside the hospital wards. SARS-CoV-2 vaccination reduced infections and symptomatic cases, demonstrating again its paramount value as a preventive tool for occupational and public health.

KEYWORDS

COVID-19, healthcare personnel, health protection measures, asymptomatic infection, vaccines, contact tracing, biological risk, SARS-CoV-2 variants

Introduction

The outbreak of the novel coronavirus, "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2), began in Wuhan, Hubei Province, China, in December 2019. The resulting pandemic has caused significant morbidity and mortality, with over 764 million infections and over 6.9 million deaths reported globally as of 27 April 2023 (1). Italy was one of the first countries affected: since the start of the epidemic, over 25,9 million cases have been diagnosed and reported to the COVID-19 integrated surveillance system, with over 188,000 deaths (2). The global COVID-19 health emergency required unprecedented measures to control the spread of the virus, primarily through social distancing and mass quarantine, until vaccines against COVID-19 became available. Vaccination campaigns against SARS-CoV-2 began in several countries, including Italy, in December 2020. Priority was given to healthcare workers (HCWs) because of two main reasons. One reason relates to the fact that HCWs are at high risk of infection (3, 4), and infection among HCWs represents a matter of public health concern because they may have a role in spreading the disease among patients or colleagues, resulting in increased transmission in the community. In fact, in the early phase of the COVID-19 epidemic, several outbreaks of nosocomial transmission of SARS-CoV-2 infection have been documented involving patients, HCWs and other hospital staff, and subjects of the general population who came into close contact with hospital cases (5). Second, a significant transmission of infection among HCWs and their absence from work can also lead to a shortage of skilled personnel, given the increased demand for HCWs and hospital care during the pandemic (6). To date, over 479,835 cases have been diagnosed among Italian HCWs, with over 12,354 hospital admissions (7). For HCWs, the symptoms of SARS-CoV-2 infection, as well as those of the general population, were initially more severe and mainly involved the respiratory tract (5). The development of effective vaccines at the end of 2020 had a major impact on the clinical burden of COVID-19, reducing the cases of infection, preventing progression to serious and symptomatic forms of the disease, and reducing mortality (8–10). In Italy, during the COVID-19 pandemic, Law 76 of 28 May 2021 made vaccination mandatory for all HCWs. If they did not comply, they could be suspended from their profession (11, 12). Despite the positive impact of COVID-19 vaccines, the emergence of variants of concern with particular regard to Delta and Omicron since 2021 remains a challenge in controlling the spread of the virus and limits the efficacy of the vaccines (13). Some studies investigated the incidence of SARS-CoV-2 breakthrough infections (BIs) in HCWs and their determinants (14, 15). Earlier studies (13, 14, 16) found that previous SARS-CoV-2 infection and the standardized antibody titer were inversely related to the risk of BI. In particular, individuals with chronic diseases such as hypertension or cardiovascular diseases may have a lower serological response to vaccines administered for SARS-CoV-2 (17) and thus an increased risk of BI. Instead, the risk of BIs after a booster dose is significantly reduced by previous infection, heterologous vaccination, and older ages. Time elapsed from the booster affects BI severity, confirming the public health usefulness of the booster (18). To date, vaccines are still associated with a lower rate of hospitalization and milder forms of the disease, frequently leading to paucisymptomatic infection (14, 19). Since the beginning of the pandemic with vaccination, immunity from previous infection, and the evolution of new variants that cause less intense acute infection, the presentation of symptoms has evolved (20). To the best of our knowledge, there are no studies that have investigated the trend of the SARS-CoV-2 infection and clinical presentation in HCWs for a long period covering different pandemic phases (i.e., from 17 February 2020 to 06 June 2022), in relation to some relevant determinants. The analysis of the trend of SARS-CoV-2 infections in HCWs over a long time across different pandemic phases could help to understand better and evaluate the role of some infection prevention and control measures such as hospital screening activities, contact tracing, and vaccination, which could be useful to implement, for example, in future epidemic exacerbations, to reduce the spread of contagion in working and living environments. Thus, this study aimed to evaluate the incidence of SARS-CoV-2 infection in a cohort of HCWs from a large University Hospital in the Veneto Region, northeastern Italy, and the probability of occurrence of asymptomatic infections among positive HCWs in relation to some demographic and occupational characteristics, different pandemic phases, vaccination status, and previous infections. The prevalence of different COVID-19-related symptoms was also investigated in relation to the aforementioned variables.

Materials and methods

Study design

A single-center retrospective observational study was performed on data collected during the risk management of SARS-CoV-2 infection and surveillance of HCWs from Azienda Ospedale-Università Padova (AOUP).

Setting

AOUP is one of the largest University Hospitals in Italy, with 1,700 beds, 70,000 recovery, and 7 million outpatient specialist procedures performed every year in close collaboration with the University of Padova. AOUP employs more than 8,000 operators, including physicians, residents, nurses, allied health professionals, and technical and administrative staff, who assist in more than 100 different units. During COVID-19, AOUP was identified as a regional emergency hub. In early February 2020, the Hospital Direction of AOUP activated a crisis unit and, based on the rapid evolution of the epidemiological scenario, undertook a major reorganization to increase the wards' capacity to admit COVID-19 patients and the availability of dedicated

healthcare staff. A detailed description of the organizational and management measures implemented by AOUP in relation to the COVID-19 pandemic has been previously reported (21).

Study period, sample and data collection, and inclusion/exclusion criteria

The information systematically collected during the HCWs' surveillance of SARS-CoV-2 infection during the period 24 February 2020–06 June 2022 was retrospectively analyzed. AOUP personnel have been subjected to periodic screening tests for SARS-CoV-2 since 18 March 2020, with timing determined by the hospital management based on the epidemiological trend of the pandemic and the recommendations from the Regional Directorate of Prevention, Food Safety—Public Health of the Veneto Region. SARS-CoV-2 infections were diagnosed by positive real-time reverse-transcriptase polymerase chain reaction (rt-PCR) on nasopharyngeal swabs and from August 2021 on saliva samples alternatively described elsewhere (22, 23). Based on the epidemiological trend of the infections, the introduction of vaccination, and the emergence of the variant of concerns, five study phases have been identified as follows: the first between 17 February 2020 and 19 July 2020, the second between 20 July 2020 and 31 January 2021, the third between 01 February 2021 and 31 October 2021, the fourth between 01 November 2021 and 28 February 2022, and the fifth between 01 March 2022 and 06 June 2022. The beginning of the second phase was identified with the resumption of cases after a period of absence of infections among HCWs (the last case of the first phase was on 10 May 2020); the beginning of the third phase was identified with the start of the administration of the second dose of vaccine for SARS-CoV-2 (the vaccination campaign started in AOUP on 27 December 2020 with the administration of the Comirnaty Pfizer m-RNA vaccine-Biontech); the fourth phase was defined in relation to the predominance of the Delta variant and the administration of the booster dose of the vaccine (third dose); and the fifth was associated with the spread of the Omicron variant.

HCWs were included in the study if routinely tested at the phases in which they were present, while those absent for the entire period and those not yet vaccinated (at least one dose) at the end of the study period were excluded from the analysis. Unvaccinated HCWs, according to Italian legislation (Law 76 of 28 May 2021), could not have a job position that presented a risk of spreading the infection.

During the SARS-CoV-2 infection surveillance of HCWs, a 24-h telephone triage was carried out to provide information support to HCWs, trace close contacts of suspected or confirmed COVID-19 cases according to international, national, and local guidelines (24), and collect some other information on symptoms and vaccination status. Additional sociodemographic information was retrieved from hospital databases made available to occupational physicians who carry out the health surveillance activities of HCWs according to current Italian legislation (Legislative Decree 81/08). The following information was obtained from these databases and by contact tracing activity: sex, age, job-title, working in a COVID area, presence of clusters, swab motivation (by contact with an infected patient or colleague, outwork contact, and test performed for screening or any symptom), vaccination status, and pandemic phase. We categorized the job titles as physicians, residents, nurses, allied health professionals, other healthcare personnel (e.g., radiology technicians and laboratory technicians), and other non-healthcare personnel (e.g., administrative staff and others).

For SARS-CoV-2 positive HCWs, information on symptomatic infection (yes/no), type of symptoms, and previous infections were also collected. HCWs without symptoms at the time of the positive swab and who continued to remain symptom-free during the isolation period were considered asymptomatic. During contact tracing activity, the following symptoms were referred by positive HCWs: fever, sore throat, cough, dyspnea, rhinorrhea and nasal obstruction, headache, ageusia/anosmia, asthenia, myalgia/arthralgia, nausea/vomiting, diarrhea, anorexia, chest pain, and mental confusion.

We categorized the timing of previous infection as "no previous infection," "≤12 months," and "12+ months."

We considered as vaccinated those individuals who received the first vaccine dose 14 or more days before infection.

Data were anonymized and entered into an *ad hoc* database. The research was performed following the 1964 Declaration of Helsinki standards and its later amendments and was approved by both the Ethics Committee of the Italian National Institute of Infectious Diseases (INMI) Lazzaro Spallanzani and the local Ethics Committee (288n/AO/22).

Statistical analysis

A descriptive analysis was conducted on HCWs' demographic, occupational, and clinical data. Data were presented as percentages for categorical variables or as means ± standard deviation (SD) for continuous variables. The continuous variables were compared using Student's t-test for unpaired data, performing *a priori* test for equality of variances. To evaluate the incidence of SARS-CoV-2 infection among HCWs, a Cox's multiple regression model was performed. For each subject, their follow-up was computed as the number of days that elapsed between the entry date (starting date of the study period or the work) and the exit date (date of infection or ending date of the study period or drop-out from follow-up, whichever came first). The incidence rate was calculated by dividing the number of positive tests by the total person-time expressed per 10,000 person-days. Thus, Cox's regression analysis was used to estimate hazard ratios (HR) of SARS-CoV-2 infection in the study period, considering the presence of an infection as the dependent event and adjusting for potential confounding factors such as sex, age, occupational characteristics (job title, working in a COVID area, and presence of clusters), pandemic phases, and vaccination status. The adjusted HRs (adj) and 95% confidence intervals (95%CI) were estimated. To assess the risk of asymptomatic/symptomatic infections among positive HCWs and to assess the risk of the onset of the most frequent symptoms, a logistic multivariate regression was performed. The following covariates were considered: sex, age, job title, working in a COVID area, presence of clusters, swab motivation, vaccination status, previous SARS-CoV-2 infection, and pandemic phase. Regarding symptoms, the covariates included in the model were sex, age, vaccination status, previous infection, and pandemic phase. The adjusted ORs (adj) and 95% confidence intervals (95%CI) were estimated. A value of p of <0.05 was accepted as statistically significant. Statistical analyses were performed using SPSS Statistics, version 28.0.

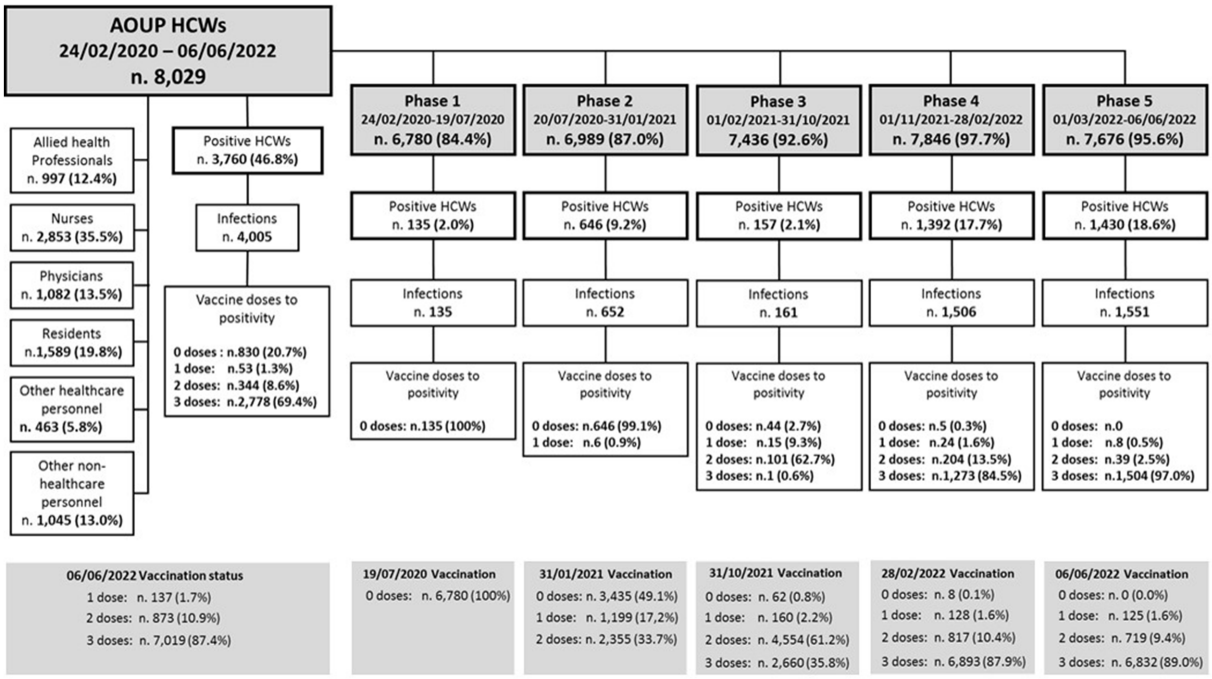

FIGURE 1
Distribution of AOUP HCWs by job title, pandemic study phases, vaccination status, and SARS-CoV-2 infections.

Results

A total of 8,029 HCWs permanently employed in AOUP and routinely tested for SARS-CoV-2 in the study period were included in the analysis. The distribution of HCWs by job title, study phases, prevalence of SARS-CoV-2 infections, and vaccination status to positivity are shown in Figure 1. Physicians, residents, and nurses represented more than two-thirds of HCWs tested for SARS-CoV-2. The remaining personnel included allied health professionals (12.4%) and employed in other jobs (18.8%), identified as "other healthcare personnel" and "other non-healthcare personnel" (i.e., administrative staff and technical workers). Key characteristics of the study population are included in Table 1. More than three-quarters of the study population were over 30 years old, and women represented more than two-thirds of HCWs included in the analysis (which reflects the HCWs demographics in Italy). Overall, the mean age was 43.7 ± 12.3 years, with a slightly but significantly lower mean age in men (42.7 ± 13.1 vs. 44.2 ± 11.9 years; $p < 0.001$). At the end of the study period, most HCWs were vaccinated with one dose (1.7%), two doses (10.9%), and three doses (87.4%). In particular, during phase 1, no HCWs were vaccinated as no vaccine was available. During phase 2, 50.9% of HCWs were vaccinated (17.2% with one dose and 33.7% with two doses), and nearly all (99.2%) by the end of phase 3 (61.2% with two doses and 35.8% with three doses). At the end of phases 4 and 5, 87.9 and 89.0% of HCWs were vaccinated with three doses, respectively (Figure 1). Overall, 3,760 HCWs (i.e., 46.8% of HCWs tested) resulted positive in a total of 4,005 infections in the study period. The number of HCWs with multiple infections was 240, for a total of 485 infections. Of these, 236 had a second positivity with a mean elapsed time of 372 days, while three subjects tested positive three times and one subject four times. In phase 1, positive HCWs accounted for 2.0%, in phase 2 for 9.2%, in phase 3 for 2.1%, in phases 4 and 5 for 17.7 and 18.6%, respectively (Figure 1; Table 1). At the time of their first positivity, 20.7% of HCWs were not vaccinated, 1.3% had received a single dose of vaccine, 8.6% had two doses of vaccine, and 69.4% also had the booster dose (Figure 1). Overall, there was an incidence rate of SARS-CoV-2 infections of 7.31 cases per 10,000 person-days, without significant sex differences. In particular, the lowest incidence rate was recorded in phase 3 and among workers with two doses of vaccine, while the highest incidence rate was recorded in phases 4 and 5 (Table 1). Multivariate analysis showed a significant risk of infection for younger workers and healthcare personnel (i.e., allied health professionals, nurses, physicians, and residents). With regard to the pandemic phase, the risk of infection increased over the study period, resulting in significantly lower in phase 1 and higher in phases 4 and 5, compared to phase 3. Interestingly, the risk of infection for HCWs with two doses of vaccine was significantly lower compared to unvaccinated workers. In addition, HCWs who had received the booster dose also showed a significantly lower risk of infection compared to the unvaccinated HCWs. However, this reduction in the risk of infection was lower in HCWs with booster doses compared to HCWs with two doses of vaccine.

In Table 2, the totality of SARS-CoV-2 infections is stratified for the different characteristics of interest. More than three-quarters of the 4,005 infections presented at least one symptom attributable to COVID-19, while 24.5% were asymptomatic infections. Multivariate analysis showed a significant increase in the probability of being asymptomatic for men, physicians, HCWs tested for screening, primary-cycle vaccinated HCWs and booster dose recipients, and those HCWs who have already had previous infection (≤12 months and >12 months). With regard to the pandemic phase, a higher

TABLE 1 Incidence rates of SARS-CoV-2 infections among HCWs according to selected characteristics.

	HCWs (n.8,029)		Positive HCWs (n.3,760)		Follow-up (days-person)	Incidence × 10,000	adjHR (IC95%)
	N	(%)	*N*	(%)			
Gender							
M	2,512	(31.3)	1,175	(31.3)	1,561,179	7.53	0.99 (0.92–1.06)
F	5,517	(68.7)	2,585	(68.8)	3,583,499	7.21	(ref)
Age groups							
≤30	1,896	(23.6)	1,143	(30.4)	931,189	12.27	**2.34 (2.10–2.60)**
31–49	2,914	(36.3)	1,415	(37.6)	1,879,032	7.53	**1.51 (1.39–1.63)**
50+	3,219	(40.1)	1,202	(32.0)	2,334,457	5.15	(ref)
Job title							
Allied health professionals	997	(12.4)	495	(13.2)	647,331	7.65	**1.62 (1.41–1.85)**
Nurses	2,853	(35.5)	1,359	(36.1)	1,937,190	7.02	**1.28 (1.14–1.44)**
Physicians	1,082	(13.5)	489	(13.0)	706,054	6.93	**1.35 (1.18–1.55)**
Residents	1,589	(19.8)	868	(23,1)	801,632	10.83	**1.23 (1.06–1.41)**
Other healthcare personnel	463	(5.8)	179	(4.8)	319,697	5.60	1.01 (0.84–1.21)
Other non-healthcare personnel	1,045	(13.0)	370	(9.8)	732,774	5.05	(ref)
Pandemic phase							
Phase 1	6,780	(84.4)	135	(3.6)	968,365	1.39	**0.35 (0.26–0.49)**
Phase 2	6,989	(87.0)	646	(17.2)	1,279,045	5.05	1.11 (0.84–1.487)
Phase 3	7,436	(92.6)	157	(4.2)	1,727,458	0.91	(ref)
Phase 4	7,846	(97.7)	1,392	(37.0)	734,465	18.95	**16.79 (13.32–21.17)**
Phase 5	7,676	(95.6)	1,430	(38.0)	435,334	32.85	**29.84 (23.55–37.80)**
Vaccination status (n. of doses)							
0	7,090	(88.3)	821	(21.8)	2,282,299	3.60	(ref)
1	6,286	(78.3)	36	(1.0)	155,919	2.31	**0.38 (0.26–0.57)**
2	6,723	(83.7)	301	(8.0)	1,720,784	1.75	**0.14 (0.11–0.19)**
3	6,174	(76.9)	2,602	(69.2)	985,665	26.40	**0.36 (0.26–0.49)**

Cox's multiple regression model. Bold indicates statistically significant results. adjHR, adjusted Hazard Ratio; (IC95%), 95% Confidence Interval; ref, reference.

probability of being asymptomatic was found during phase 1, compared to phase 5. However, phases 2, 3, and 4 showed a significant increase in the probability of being asymptomatic compared to phase 5, although lower than that is seen in phase 1.

Table 3 shows the distribution of HCWs' self-reported source of contact and exposure circumstances. Approximately one-third (35.4%) of the total SARS-CoV-2 infections occurred outside of the workplace, while 13% were by contact with a positive patient or a positive colleague (4.9% and 8.1%, respectively). The source of contact was unknown for 51.6% of infections and emerged during the hospital screening activity (29.1%) or tests executed in symptomatic HCWs (22.5%). Interestingly, the percentage of HCWs infected outside of the workplace increased from 15.6% in phase 1 up to 48.4% in the next phases, while the percentage of HCWs infected in the workplace (by contact with a positive colleague or with a positive patient) was higher in phase 1 and progressively decreased in the next phases (Table 3).

Multivariate analysis (see Supplementary Table S1) showed a significant increase in the risk of infection from contact with positive patients in workers in the ≤30 years age class. HCWs in the 31–49 years and +50 years age classes were more likely to be infected out of the workplace, instead. Regarding the job title, allied health professionals had a significantly higher probability of infection by contact with positive patients, while nurses were from contacts out of the workplace. Residents and other non-healthcare personnel showed a significant increase in the infection risk by contact with a positive colleague. In addition, the probability of infection by contact with a positive patient or colleague was significantly higher within a cluster. Finally, the probability of infection by contact with a positive colleague was also significantly higher in the non-COVID area and during phase 1 (Supplementary Table S1).

The most frequent symptoms reported by HCWs during the acute phase of infection were fever (37.2%), sore throat (37.4%), cough (33.7%), and rhinorrhea (33.7%), followed by headache (19.0%), myalgia/arthralgia (16.0%), and nasal obstruction (14.7%; see Supplementary Table S2), some with significant variations between pandemic study phases. Multivariate analysis showed (Table 4) a significant risk of presenting ageusia/anosmia in all study phases, compared to phase 5, with a decreasing trend from phase 1 to phase

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TABLE 2 Distribution of HCWs testing positive for SARS-CoV-2 and multivariate logistic regression analysis investigating some relevant characteristics and symptoms.

	HCWs (n. 3,760)		Positivity to SARS-CoV-2	Asymptomatic HCWs (n.981)		adjOR (IC95%)
	n.	(%)	(n. 4,005)	n.	(%)	
Gender						
Male	1,175	(31.3)	1,236	369	(29.9)	**1.39 (1.17–1.65)**
Female	2,585	(68.8)	2,769	612	(22.1)	(ref)
Age groups						
≤30	1,143	(30.4)	1,203	290	(24.1)	0.80 (0.62–1.04)
31–49	1,414	(37.6)	1,527	382	(25.0)	1.01 (0.83–1.22)
50+	1,203	(32.0)	1,275	309	(24.2)	(ref)
Job title						
Allied health professionals	495	(13.2)	548	122	(22.3)	(ref)
Nurses	1,359	(36.1)	1,460	317	(21.7)	1.17 (0.91–1.52)
Physicians	489	(13.0)	514	159	(30.9)	**1.71 (1.26–2.33)**
Residents	868	(23.1)	912	241	(26.4)	**1.61 (1.15–2.243)**
Other healthcare personnel	179	(4.8)	189	46	(24.3)	1.30 (0.85–2.00)
Other non-healthcare personnel	370	(9.8)	382	96	(25.1)	1.33 (0.95–1.86)
Swab motivation						
Contact with positive HCW	314	(8.4)	324	94	(29.0)	**1.37 (1.01–1.86)**
Contact with positive patient	183	(4.9)	196	50	(25.5)	1.27 (0.87–1.84)
Outwork contact	1,327	(35.3)	1,417	308	(21.7)	(ref)
Screening	1,079	(28.7)	1,167	493	(42.2)	**2.90 (2.42–3.48)**
*Non screening**	857	(22.8)	901	36	(4.0)	**0.17 (0.12–0.24)**
Cluster						
Yes	551	(14.7)	574	139	(24.2)	**0.70 (0.55–0.89)**
NO	3,209	(85.3)	3,431	842	(24.5)	(ref)
COVID area						
Yes	553	(14.7)	589	151	(25.6)	0.94 (0.75–1.17)
NO	3,207	(85.3)	3,416	830	(24.3)	(ref)
Vaccination status to positivity (N. of doses)						
0	821	(21.8)	830	245	(29.5)	(ref)
1	36	(1.0)	53	15	(28.3)	1.69 (0.65–4.40)
2	301	(8.0)	344	99	(28.8)	**2.56 (1.10–5.93)**
3	2,602	(69.2)	2,778	622	(22.4)	**2.49 (1.04–5.99)**
Previous infections						
None	3,520	(93.6)	3,760	905	(24.1)	(ref)
≤12 months	82	(2.2)	86	29	(33.7)	**1.80 (1.09–2.98)**
12+ months	158	(4.2)	159	47	(29.6)	**1.51 (1.02–2.23)**
Pandemic study phase						
Phase1	135	(3.6)	135	65	(48.1)	**14.03 (5.35–36.84)**
Phase2	646	(17.2)	652	176	(27.0)	**6.03 (2.45–14.82)**
Phase3	157	(4.2)	161	41	(25.5)	**2.61 (1.53–4.44)**
Phase4	1,392	(37.0)	1,506	456	(30.3)	**2.20 (1.82–2.67)**
Phase5	1,430	(38.0)	1,551	243	(15.7)	(ref)

*Test performed for symptoms; bold indicates statistically significant results. adjOR, adjusted Odds Ratio; (IC95%), 95% Confidence Interval; ref, reference.

TABLE 3 Distribution of HCWs self-reported source of contact and swab motivation.

	Phase 1		Phase 2		Phase 3		Phase 4		Phase 5		Total	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
Outwork contact	21	15.6	208	31.9	78	48.4	602	40.0	508	32.8	1,417	35.4
Contact with positive patient	15	11.1	83	12.7	8	5.0	52	3.5	38	2.5	196	4.9
Contact with positive HCW	49	36.3	72	11.0	8	5.0	107	7.1	88	5.7	324	8.1
Screening	32	23.7	151	23.2	32	19.9	510	33.9	442	28.5	1,167	29.1
*Non-screening**	18	13.3	138	21.2	35	21.7	235	15.6	475	30.6	901	22.5
Total	135	100.0	652	100.0	161	100.0	1,506	100.0	1,551	100.0	4,005	100.0

*Test performed for symptoms.

5. The probability of presenting fever was significantly higher in phase 5 and in non-vaccinated HCWs, while rhinorrhea/nasal obstruction in phases 2 and 3 and sore throat in phases 4 and 5 and among younger HCWs. The risk of presenting headache and myalgia/arthralgia was significantly higher in women, and changed among phases; in addition, the risk of myalgia/arthralgia was higher in the 50+ years age class.

Overall, nine HCWs were hospitalized in phase 1 and six in phase 2, while no hospitalizations occurred in the other phases. Among the hospitalized subjects in phase 1, seven were admitted to a non-critical area for a limited period of time, one required semi-intensive therapy, and the last, affected by comorbidities (65-year-old men, suffering from hypertension and type II diabetes), developed severe acute respiratory failure (ARDS) and was admitted to the intensive care unit. Among the hospitalized HCWs of phase 2, five were hospitalized in non-critical areas and a 40-year-old worker, with no significant comorbidities, required a semi-intensive therapy for interstitial pneumonia, which evolved into ARDS. No deaths were reported in AOUP HCWs.

Discussion

This study analyzed the risk of SARS-CoV-2 infection and the probability of having an asymptomatic infection among HCWs belonging to one of the largest Italian University Hospital (25) for a long pandemic period stretching up to 27 months, in relation to different pandemic phases (from 17 February 2020 to 06 June 2022) and some relevant determinants.

The main results of this analysis revealed that the prevalence of infection in AOUP HCWs varied across study phases, ranging from 2.0% to 18.6%. The incidence of SARS-CoV-2 infection was significantly lower in phase 1 and higher in phases 4 and 5, compared to phase 3. Younger HCWs (≤30 year age class), healthcare personnel, and unvaccinated subjects showed a higher risk of infection. Approximately a quarter of positive HCWs presented an asymptomatic infection that was influenced in this study population by the following determinants: being of male gender, physician, personnel tested for screening, primary-cycle vaccinated HCWs, booster dose recipients, and subjects with previous infection. A higher probability of being asymptomatic was found in phase 1, compared to phase 5. The clinical presentation of positivity changed over the study phases in relation to vaccination status and the emergence of new variants.

Overall, 35.4% of the total SARS-CoV-2 infections occurred outside of the workplace, while only a small part occurred in the workplace (i.e., 13%% by contact with a positive patient or by contact with a positive colleague), and more than half of the infections had an unknown source of contact. We can speculate that a considerable proportion of these cases with unknown sources of contact probably occurred outside of the workplace. Interestingly, during phase 1, the source of contact resulted unknown for 37% of infections. These cases emerged, for the most part, during the hospital screening activity that was promptly implemented for all AOUP HCWs on 18 March 2020. In fact, AOUP already had an emergency plan in place in early February 2020 and was able to activate the crisis unit as soon as the first positive case of COVID-19 was confirmed on 21 February 2020. To adapt to COVID-19's rapid spread, the hospital has been reorganized to meet the key objectives, as described previously (21). During the early stages of phase 1, in relation to the occurrence of some SARS-CoV-2 clusters within some operating units and the analysis performed to reconstruct the transmission chains of the infections (see Supplementary Figure S1), strong evidence emerged that asymptomatic and pre-symptomatic subjects represented a significant risk for transmission. This was also observed by others both in the healthcare setting (26–28) and in the general population (22, 29). This observation prompted the application of infection control policies to protect HCWs and patients. In addition to the availability of protective devices and the implementation of safety protocols, the major challenge in preventing the spread of nosocomial is the prompt detection and isolation of asymptomatic individuals by screening campaigns. Despite the control measures taken during phase 1, AOUP HCWs showed to be more infected in the workplace (47.4% of the total number of contagions in this phase), due to the aforementioned clusters occurring in AOUP, and only 15.6% out of the workplace, probably in relation to the introduction of the lockdown measures in our country (in the period 09 March 2020–03 May 2020). However, in this phase, the prevalence of infection in AOUP (i.e., 2%) was lower than those reported in other Italian hospitals (30, 31). It should be noted that the lack of personal protective equipment (PPE) suffered in the early stages of the pandemic never occurred in AOUP, which always guaranteed them, at least in risky activities. Thus, the prevalence in AOUP was also the lowest among the hospitals of the Veneto Region (in which the mean prevalence was 5.5%) (32). At the University Hospital of Verona, where periodic screening of all HCWs was performed as per AOUP, the prevalence of infection was 4% (33). Lahner et al. (34) recorded a prevalence of

TABLE 4 Distribution of COVID-19 symptoms and multivariate logistic regression analysis investigating some relevant characteristics.

	Symptomatic HCWs	Fever (n.1,125)			Cough (n.1,020)			Sore throat (n.1,132)			Anosmia/ageusia (n.152)		
	(n.3,024)	*N*	%	adjOR (95%CI)	*N*	%	adjOR (95%CI)	*N*	%	OR adjOR (95%CI)	*N*	%	adjOR (95%CI)
Gender													
Male	867	321	(37.0)	0.96 (0.81–1.13)	273	(31.5)	ref	320	(36.9)	ref	48	(5.5)	1.17 (0.80–1.71)
Female	2,157	804	(37.3)	ref	747	(34.6)	1.17 (0.99–1.39)	812	(37.6)	1.07 (0.90–1.26)	104	(4.8)	ref
Age groups													
≤30	913	338	(37.0)	1.15 (0.95–1.40)	322	(35.3)	1.12 (0.92–1.36)	379	(41.5)	**1.22 (1.01–1.48)**	42	(4.6)	ref
31–49	1,145	434	(37.9)	1.13 (0.94–1.36)	377	(32.9)	0.99 (0.83–1.19)	418	(36.5)	1.03 (0.86–1.24)	60	(5.2)	0.95 (0.61–1.47)
50+	966	353	(36.5)	ref	321	(33.2)	ref	335	(34.7)	ref	50	(5.2)	0.77 (0.49–1.22)
N. of doses to positivity													
0	585	331	(56.6)	**3.19 (1.45–7.02)**	189	(32.3)	0.94 (0.40–2.18)	94	(16.1)	0.60 (0.23–1.61)	115	(19.7)	**6.49 (1.54–27.31)**
1	38	10	(26.3)	0.87 (0.40–1.86)	13	(34.2)	1.16 (0.57–2.37)	17	(44.7)	1.49 (0.74–3.01)	1	(2.6)	2.17 (0.26–18.28)
2	245	87	(35.5)	1.32 (0.94–1.84)	83	(33.9)	1.15 (0.83–1.60)	68	(27.8)	**0.67 (0.48–0.93)**	19	(7.8)	**6.50 (2.88–14.72)**
3	2,156	697	(32.3)	ref	735	(34.1)	ref	953	(44.2)	ref	17	(0.8)	ref
Previous infections													
None	2,855	1,077	(37.7)	ref	958	(33.6)	ref	1,056	(37.0)	ref	149	(5.2)	ref
≤12 months	57	18	(31.6)	0.79 (0.44–1.41)	20	(35.1)	1.01 (0.58–1.77)	22	(38.6)	0.96 (0.55–1.69)	2	(3.5)	0.81 (0.18–3.64)
1 + months	112	30	(26.8)	0.74 (0.48–1.14)	42	(37.5)	1.14 (0.77–1.68)	54	(48.2)	1.23 (0.84–1.80)	1	(0.9)	0.63 (0.08–4.76)
Pandemic study phase													
Phase 1	70	52	(74.3)	2.41 (0.94–6.21)	28	(40.0)	1.58 (0.59–4.14)	9	(12.9)	0.88 (0.29–2.65)	17	(24.3)	**10.19 (1.92–54.25)**
Phase 2	476	255	(53.6)	0.96 (0.43–2.12)	151	(31.7)	1.09 (0.46–2.58)	79	(16.6)	1.18 (0.49–2.85)	94	(19.7)	**7.81 (1.59–38.42)**
Phase 3	120	54	(45.0)	1.31 (0.78–2.20)	34	(28.3)	0.80 (0.47–1.37)	20	(16.7)	ref	12	(10.0)	**3.78 (1.08–13.27)**
Phase 4	1,050	297	(28.3)	ref	338	(32.2)	ref	424	(40.4)	**2.49 (1.34–4.61)**	22	(2.1)	**2.52 (1.01–6.27)**
Phase 5	1,308	467	(35.7)	**1.48 (1.23–1.77)**	469	(35.9)	**1.21 (1.01–1.44)**	600	(45.9)	**3.0 (1.59–5.64)**	7	(0.5)	ref

(Continued)

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TABLE 4 (Continued)

	Symptomatic HCWs	Rhinorrhea/nasal obstruction (n.1,406)			Headache (n.576)			Myalgia/arthralgia (n.483)			Asthenia (n.395)		
	(n.3,024)	*N*	%	adjOR (95%CI)	*N*	%	adjOR (95%CI)	*N*	%	adjOR (95%CI)	*N*	%	adjOR (95%CI)
Gender													
Male	867	392	(45.2)	ref	132	(15.2)	ref	115	(13.3)	ref	116	(13.4)	1.04 (0.82–1.32)
Female	2,157	1,014	(47.0)	1.10 (0.93–1.29)	444	(20.6)	**1.42 (1.15–1.76)**	368	(17.1)	**1.30 (1.03–1.64)**	279	(12.9)	ref
Age groups													
≤30	913	443	(48.5)	1.16 (0.96–1.39)	151	(16.5)	ref	110	(12.0)	ref	111	(12.2)	ref
31–49	1,145	541	(47.2)	1.13 (0.94–1.34)	225	(19.7)	1.17 (0.93–1.47)	184	(16.1)	1.28 (0.98–1.66)	142	(12.4)	1.00 (0.77–1.31)
50+	966	422	(43.7)	ref	200	(20.7)	1.21 (0.96–1.54)	189	(19.6)	**1.54 (1.19–2.01)**	142	(14.7)	1.19 (0.91–1.56)
N. of doses to positivity													
0	585	176	(30.1)	**0.24 (0.11–0.54)**	148	(25.3)	0.88 (0.37–2.11)	179	(30.6)	1.38 (0.57–3.35)	114	(19.5)	1.60 (0.55–4.68)
1	38	17	(44.7)	0.63 (0.31–1.25)	7	(18.4)	0.79 (0.32–1.94)	7	(18.4)	1.08 (0.44–2.67)	1	(2.6)	0.21 (0.03–1.56)
2	245	126	(51.4)	0.87 (0.63–1.19)	57	(23.3)	1.01 (0.71–1.56)	42	(17.1)	ref	27	(11.0)	0.93 (0.55–1.55)
3	2,156	1,087	(50.4)	ref	364	(16.9)	ref	255	(11.8)	**0.63 (0.41–0.96)**	253	(11.7)	ref
Previous infections													
None	2,855	1,321	(46.3)	ref	552	(19.3)	ref	459	(16.1)	ref	381	(13.3)	ref
≤12 months	57	26	(45.6)	0.94 (0.55–1.62)	10	(17.5)	0.96 (0.47–1.93)	5	(8.8)	0.48 (0.18–1.22)	5	(8.8)	0.73 (0.29–1.87)
12+ months	112	59	(52.7)	1.14 (0.77–1.67)	14	(12.5)	0.65 (0.37–1.16)	19	(17.0)	1.34 (0.80–2.25)	9	(8.0)	0.68 (0.34–1.36)
Pandemic study phase													
Phase 1	70	9	(12.9)	ref	14	(20.0)	1.50 (0.52–4.35)	9	(12.9)	ref	14	(20.0)	1.21 (0.36–4.05)
Phase 2	476	154	(32.4)	**3.21 (1.55–6.64)**	123	(25.8)	2.09 (0.84–5.18)	161	(33.8)	**3.55 (1.71–7.35)**	92	(19.3)	1.18 (1.40–3.46)
Phase 3	120	68	(56.7)	**3.73 (1.43–9.70)**	37	(30.8)	**2.43 (1.33–4.44)**	23	(19.2)	2.02 (0.73–5.55)	17	(14.2)	1.26 (0.57–2.77)
Phase 4	1,050	498	(47.4)	1.47 (0.50–4.28)	199	(19.0)	**1.26 (1.01–1.57)**	127	(12.1)	1.93 (0.60–6.19)	113	(10.8)	ref
Phase 5	1,308	677	(51.8)	1.71 (0.58–5.04)	203	(15.5)	ref	163	(12.5)	2.16 (0.66–7.04)	159	(12.2)	1.13 (0.87–1.47)

Bold indicates statistically significant results. adjOR, adjusted Odds Ratio; (IC95%), 95% Confidence Interval; ref, reference.

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2.7% among all employees tested at University Hospital in Lazio, a region that was less affected than Veneto in the early stages of the pandemic (35). Moreover, an infection prevalence of 4.8% was reported at Cambridge University Hospital (36), 11.9% at a University Hospital in Madrid (37), and 9.0% at a hospital in Cleveland, Ohio (38). In addition, infections that occurred by confirmed contact with a positive patient in phase 1 (11.1%) were lower than those recorded during the same period in the hospitals of Turin (47.8%) (39), Milan (50%) (40), and Trieste (51.3%) (41). Overall, these data confirm the efficacy of the measures introduced in AOUP to limit the nosocomial spread of SARS-CoV-2 among HCWs. The next phases were characterized by a progressive decrease in viral transmission in the workplace and an increase in infections occurring outside of the workplace. It should be kept in mind that HCWs were exposed to the virus outside the workplace since the lockdown was no longer declared. In fact, phase 2 was signed by the rapid resumption of cases after a period of absence of infections among HCWs in a pre-vaccination era. In AOUP, the vaccination campaign started on 27 December 2020 (with the administration of the Comirnaty Pfizer m-RNA vaccine–BioNTech) and continued with the administration of the second dose at the end of phase 2 and in phase 3. In compliance with legislative decree 81/08, occupational physicians participated in this campaign, vaccinating HCWs (11). Italy, with Law 76/21, decided to make this vaccination mandatory for HCWs, following a different approach compared to many other European countries (12). In our study population, the lowest incidence rate of SARS-CoV-2 infections was recorded during phase 3 and among workers who received two doses of the vaccine. Indeed, vaccination reduced the transmission rates of SARS-CoV-2, particularly in the first 4–6 months after the vaccination, due to a more rapid decline in viral load and decreased viability of the virus shed by vaccinated individuals; indeed, it was less likely to raise a virus culture from swabs of these subjects (19). AOUP HCWs who had received the booster dose showed a less impressive reduction of the risk of infection compared to those with two doses of vaccine, although still significant compared with unvaccinated HCWs. Several reasons may be taken into account for this result. In fact, the continued emergence of new viral variants with different traits has both extended viral transmission and threatened the effectiveness of vaccines, boosting the risk of BI (13). Thus, the risk of testing positive was significantly higher in phases 4 and 5 compared to phase 3 due to the spread of the highly contagious variants (the Delta and the Omicron variants, respectively), despite the administration of the booster dose of the vaccine during phase 4. However, taking the period covering phases 1, 2, and 3, the prevalence of SARS-CoV-2 infections in AOUP was lower than those reported in the same period at the Trieste University Hospital, North East of Italy (42), and in line with those reported at the University Health Agency Giuliano-Isontina (ASUGI) that, however, analyzed data from 1 March 2020 to 31 May 2022 (43). Furthermore, during phases 2 and 3, the infection prevalence in AOUP was lower than that estimated in a multicenter study among HCWs of 105 secondary care health organizations in the UK, between the beginning of September 2020 and the end of April 2021 (44). However, by January 2022, HCWs were exposed to a new variant (i.e., Omicron) that from December 2021 spread aggressively worldwide among the vaccinated healthcare force, rapidly becoming dominant and increasing the risk of re-infections (45).

To date, literature studies investigating the role of significant determinants on SARS-CoV-2 infections in HCWs have conflicting results on the possible role of age (13, 44). Our results show an increased risk of infection for ≤30 year age class HCWs, in line with other studies (42, 46–48), and this is consistent with younger people having more intense social relationships and higher rates of contact. Another possible reason that could explain this result is that younger HCWs might be more likely to be on the frontline and be more likely to be in charge of direct caregiving of patients (42). However, in our hospital, we did not have evidence that younger HCWs were more involved in direct patient care than the other workers. In addition, younger HCWs, despite having received the same training as all HCWs, could still be more at risk due to less work experience, as also suggested in other studies (48).

Our HCWs population did not show any significant differences between sexes. This is consistent with the results reported in other studies (49, 50).

Regarding the job title, a slight but significant increase in the risk of infection was identified for the HCWs (allied health professionals, nurses, physicians, and residents), whose work activity usually involves direct contact with patients, compared to other healthcare and non-healthcare personnel. This result is in agreement with those recently reported in a systematic review and meta-analysis of 54 studies that showed an increased risk of being positive for frontline HCWs (51).

Moving on to the clinical presentation, most HCWs showed mild SARS-CoV-2 infection with few hospitalizations (0.4%), limited to the first two phases of the pre-vaccination era, and no deaths occurred. In particular, during phase 1, 6.7% of positive HCWs were hospitalized, a higher percentage than that reported by other authors (28, 30, 34) but lower than that recorded (8.6%) among the HCWs from the others Regione Veneto health authorities in that period (32). In a meta-analysis of 97 studies that assessed infection among HCWs, 5% of COVID-19 cases in HCWs had severe complications, and 0.5% of HCWs died (5). In phase 2, hospitalizations in AOUP amounted to 0.9%. A study conducted in nine European countries from 31 January 2020 to 13 January 2021 showed an increased adjusted risk of COVID-19 requiring hospitalization or ICU admission in HCWs compared to non-HCWs, respectively, of 1.8 (95% CI 1.2–2.7) and 1.9 (95% CI 1.1–3.2) (52).

Overall, 24.5% of total infections were asymptomatic. Interestingly, our results showed significant differences among study phases with the higher probability of being asymptomatic during phase 1, with a percentage of asymptomatic cases (48%) in line with literature data (53), perhaps, at least partially, justified in the early pandemic stages also by a lower knowledge and awareness of COVID19-related symptoms by both workers and occupational physicians who collected clinical information. A meta-analysis conducted between 01 January 2020 and 02 April 2021 estimated 35.1% of asymptomatic infections in more than 350 studies and 38.5% in 81 studies carried out in healthcare facilities (54). Data from the literature (55) highlighted that centers adopting a screening approach with frequent testing and fast turnaround, such as our center, were more likely to detect a higher number of asymptomatic infections.

Multivariate analysis showed a higher probability of being asymptomatic for men, primary-cycle vaccinated and with a booster dose of vaccine, and those who have already had previous infection. Indeed, vaccinated HCWs are known to have a significantly lower

incidence of symptomatic and asymptomatic SARS-CoV-2 infections compared with unvaccinated HCWs (56). In addition, previous SARS-CoV-2 infections are known to reduce the risk of BI (13). As in our study, Methi et al. (57) found that asymptomatic cases had a higher chance of being men. The authors speculated that this result could be an example of men having a higher threshold of reporting symptoms (58).

Fever, upper airway symptoms, myalgia/arthralgia, and headache were the more frequently reported acute phase symptoms. Headache and myalgia/arthralgia were significantly more frequent in women. Indeed, several studies on the long COVID syndrome have identified headache, myalgia (i.e., muscle/body pain), and joint pain as frequently reported symptoms among women (59–61). Unvaccinated HCWs developed more systemic symptoms, e.g., fever, than the vaccinated ones. HCWs vaccinated with the booster dose had a significant reduction in the occurrence of myalgia/arthralgia compared to the two-dose vaccinated. These data are in line with those from the literature that showed vaccine effectiveness against symptomatic infection and severe COVID-19 (62). Overall, during the study period, the symptoms reported by HCWs changed significantly among the five study phases, confirming that the clinical presentation in symptomatic SARS-CoV-2 infections has evolved (20). Indeed, vaccination, immunity from prior infection, and the emergence of the Omicron variant seem to cause a milder clinical presentation (63). However, surveillance of HCWs in AOUP is still going on to evaluate possible post-acute and long-term sequelae of SARS-CoV-2 infection among HCWs (64). Recent studies showed significant long-term persistent symptoms and functional impairment, even in non-hospitalized patients with COVID-19 (65) and occupational settings (66), highlighting the central role of the occupational physician in monitoring workers more closely in the months following primary COVID-19 illness.

Strengthens and limitations

This study has some strengthens. To the best of our knowledge, this is the first study that analyzed the risk of infection and the clinical presentation of SARS-CoV-2 in HCWs for up to 27 months (i.e., from 15 March 2020 to 06 June 2022), focusing on different pandemic phases related to vaccination and emergence of viral variants. This single-center study involves one of the largest Italian University Hospitals with a large sample of HCWs (health and non-health personnel) routinely and stringently tested for SARS-CoV-2, thus providing reliable estimates of infection rates. In addition, we believe that our data are robust because they emerged from tests always carried out with the rt-PCR method (nasal-pharyngeal or salivary). In fact, when the rapid test was used for the SARS-CoV-2 antigen detection, the confirmation by subsequent rt-PCR test was always performed. This study differentiated between occupational and non-occupational infections by contact tracing, as stated by the Italian occupational compensation scheme. Furthermore, for the contact tracing activity, we did not use a questionnaire intrinsically affected by recall bias, but we performed the activity by direct phone contact with HCWs.

This study has also some limitations. First, since this is a single-center study, it could have limited generalizability issues. Second, those HCWs absent for the entire period and those not yet vaccinated (with at least one dose) at the end of the study period were excluded from the analysis. However, according to the Italian legislation related to the pandemic period, unvaccinated HCWs were suspended from work and remained at home, thus presenting a different risk of infection than that of other health professionals. Third, some HCWs may have intentionally overlooked some source of SARS-CoV-2 infection outside the workplace to access the occupational compensation scheme. However, in the case of coexistent exposures (both in and out of the workplace), our approach was to treat all these infections as occupational. Another limitation was regarding the analysis of the dominant SARS-CoV-2 variant during the study period due to the limited capacity of the DNA sequencing facilities in our center that were mainly dedicated to the analyses of clusters. However, for the considerations presented in this study, the knowledge of the circulating and prevalent variants in a given period/phase was taken into account, as derived from the data regularly communicated at the national and local levels.

Conclusion

Our analyses provided accurate information on the risk and the determinants of SARS-CoV-2 infection among AOUP HCWs in relation to the different pandemic phases. Our data point out that, besides the availability of protective devices and the implementation of safety protocols, the screening activity on all hospital staff, in particular in the presence of high viral circulation, allowed for the early detection of asymptomatic infected subjects, thus limiting the presence and spreading of clusters inside the hospital wards. However, the control of exposure outside of the workplace also appears to be necessary to limit the nosocomial spread of SARS-CoV-2 among HCWs. The risk of infection was influenced by age, job title, vaccination status, previous infections, and specific pandemic phases that were related to the emergence of new viral variants. During the study period, the clinical presentation in positive HCWs has evolved in relation to vaccination status and the spread of different variants causing less severe disease. Indeed, SARS-CoV-2 vaccination reduced infection spread in working and living environments and the probability of symptomatic COVID-19, demonstrating again its paramount value as a preventive tool for occupational and public health. The results of this study conducted over a long time period across different pandemic phases, characterized by the vaccination campaign and the emerging of new variants, allow us to better identify how the different determinants of infection vary over time. Therefore, based on the aforementioned considerations, hospital administrations will be able to promptly activate proper preventive measures and infection surveillance in future epidemic exacerbations to reduce the spread of COVID-19, especially in vulnerable environments such as hospitals, where HCWs play a critical role in the overall community.

Data availability statement

The datasets presented in this article are not readily available because the data are not publicly available due to ethical and legal restrictions, as participants of this study did not agree for their data to be shared publicly. Requests to access the datasets should be directed to filippo.liviero@unipd.it.

Ethics statement

The study was approved by the Italian National Institute of Infectious Diseases (INMI) Lazzaro Spallanzani and the local Research Ethics Committee: Comitato Etico Territoriale Area Centro—Est Veneto (CET-ACEV)—Azienda Ospedale Università di Padova (288n/AO/22). Date were collected during routine health surveillance carried out in compliance with Legislative Decree 81/08 and European Community Directive 90/679. Patient consent was waived since according to Italian privacy law (Legislative Decree 101/2018) patients' data routinely collected by the Italian National Health Service (NHS) can be used for scientific purposes within the frame of approved studies/protocols, provided sensitive information is anonymized. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

FL, MS, and AM: conceptualization. PF, CC, SC, and VB: methodology. PF: formal analysis. AV, FF, MB, AB, LF, PM, FL, and MS: investigation. AV, FF, MB, AB, LF, PM, FL, and MS: data curation. FF, MB, AB, LF, PF, FL, and MS: writing—original draft preparation. FL, MS, VB, and AM: writing—review and editing. AM: supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1250911/full#supplementary-material

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Willingness to vaccinate among adults, and factors associated with vaccine acceptance of COVID-19 vaccines in a nationwide study in Poland between March 2021 and April 2022

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Introduction: Despite the availability, safety and effectiveness of COVID-19 vaccines, Poland remains one of the six countries of the European Union with the lowest cumulative uptake of the vaccine's primary course in the general population. This study examined willingness to vaccinate and the associated factors in samples of unvaccinated and vaccinated adults between March 2021 and April 2022.

Methods: Data were collected using OBSER-CO, a nationwide, repeated cross-sectional study, conducted at four different time points (rounds). Data on willingness to vaccinate among the unvaccinated (at all rounds) and willingness to receive another dose in the vaccinated (at 2 rounds-after booster introduction), reasons for reluctance, sociodemographic, health, and behavioral factors were collected using a uniform questionnaire via computer-assisted telephone interviewing. In each round, more than 20,000 respondents were interviewed. To assess associations between factors and willingness to vaccinate, separate multivariable logistic regression models were fitted for each factor at each round and adjusted for confounders.

Results: Between rounds 1 and 4 (March 2021–April 2022), in the unvaccinated, willingness to vaccinate declined from 73 to 12%, whereas in the vaccinated, willingness to receive another dose declined from 90 to 53%. The highest magnitude of decline between subsequent rounds occurred during the Omicron wave. Overall, concerns about side effects, effectiveness, and vaccine adverse effects were common but decreased over time. Age, gender, employment, place of residence, COVID-19 diagnosis or exposure, hospitalization, and participation in social activities were among the factors associated with willingness. However, associations changed over rounds highlighting the influence of different pandemic waves and variants.

Conclusion: We observed a declining and multifactorial willingness to vaccinate in Poland, with vaccine attitudes dynamically changing across subsequent rounds.

To address vaccine concerns, sustained health communication about COVID-19 vaccines is essential, especially after the emergence of new variants.

KEYWORDS

vaccine hesitancy, SARS-CoV-2, Omicron variant, Delta variant, attitudes, COVID-19 waves, booster, vaccination campaign

Introduction

Early on during the pandemic, vaccines became one of the key preventive measures against COVID-19 (1, 2). In the last weeks of 2020, mass vaccination campaigns against COVID-19 were introduced in the European Union. In Poland, vaccination of individuals aged 60–64 years began in the last week of March 2021, and as of April 12, 2021, individuals younger than 60 could register to receive the vaccine. Additionally, on August 27, 2021, the Medical Council of Poland recommended administering the third (booster) dose of the vaccine to immunocompromised individuals. Vaccination with the booster dose began on September 1, 2021. As of September 23, 2021, booster vaccination was administered to individuals over 50 years of age, and as of November 2, 2021, to all adults. In the European Union (EU), Comirnaty, Valneva, Jcovden, Nuvaxovid, Spikevax, Vaxzevria, Bimervax, and VidPrevtyn Beta have been authorized for use by the European Medicines Agency (EMA). In Poland, most of the vaccinated population have received an mRNA vaccine.

Despite high availability of the vaccines through the National COVID-19 Immunization Program, as of 7th March 2023 in Poland, only 59.9% had completed the primary course (two doses), 33% had received the first, and 7.3% the second booster dose (3). The vaccine uptake in Poland is below the EU/EEA average in all vaccine categories (EU/EEA average for primary course: 73%; first booster: 54.7%; second booster: 14.1%). The difference in vaccination rates grows even bigger when compared with EU countries with the highest vaccination coverage, such us Portugal (86.4; 68.4; 30.3%, respectively) and Denmark (81.9; 62.8; 32.7% respectively) (3). Since February 2022, uptake of the primary course in Poland has remained stable, while uptakes of the first and second booster doses have been increasing at a very slow rate since their introduction (3).

Although vaccines have been proven to be effective against COVID-19 (4), willingness to vaccinate remains moderate worldwide and particularly in Poland (5, 6). Willingness to vaccinate is multifactorial and varies across countries (7). It can be influenced by demographic, psychological, and social/cultural factors (7–11). It has been shown consistently that being a woman, unemployment, and no prior COVID-19 infection are associated with higher reluctance to vaccinate (8, 9). Similarly, these factors affect uptake of booster doses. According to a recent meta-analysis of data from 23 different countries, age, gender, COVID-19 infection, work status, income, and health status were all predictors of willingness to receive a booster vaccine (12). Furthermore, concerns about the safety and effectiveness of vaccines have been prevalent in Poland since the beginning of the vaccination campaign (13). These concerns can impact an individual's decision to get vaccinated, and particularly it has been demonstrated that fear of side effects of COVID-19 vaccines and concerns about the speed of development or low trust in the effectiveness of the vaccine can negatively influence willingness to vaccinate in the adult population (7, 12, 14). In addition, since the start of the pandemic, the spread of misinformation and conspiracy theories about COVID-19 vaccines have hindered vaccine uptake and willingness to vaccinate (13, 15). As a result of the multifactorial nature of willingness to vaccinate and varying results across countries it is difficult to provide a single explanation behind the driving forces of willingness to vaccinate.

Importantly, these factors are unlikely to remain stable. Different COVID-19 variants and pandemic waves can heighten concerns and mistrust about vaccines (6). The severity of each variant (i.e., Delta, Omicron) can influence public opinion on the necessity of vaccination (16–18). While studies examining willingness to vaccinate/vaccine acceptance usually addressed the problem in one point in time, less is known about longitudinal changes in attitudes toward COVID-19 vaccines across different pandemic waves. To better understand the drivers behind willingness to vaccinate, it is important to disentangle how multifactorial associations evolved over the course of the COVID-19 pandemic. And more importantly, because of the unsatisfactory vaccination uptake in Poland, it is essential to gain insight into the reasons for reluctance to vaccinate over time. Findings can advise actions to boost vaccination but can also benefit health communication and vaccination campaigns, which can adjust their message according to the evolving concerns and specific characteristics of the population.

Therefore, this study aimed to examine willingness to vaccinate among unvaccinated individuals in four different time points (March 2021–April 2022; at least 2 months apart) and willingness to receive another dose of a COVID-19 vaccine among vaccinated individuals in two time points (November 2021–April 2022; after introduction of the booster vaccination campaign). Additionally, we aimed to investigate the reasons for reluctance to vaccinate in both vaccinated and unvaccinated participants, as well as the factors associated with willingness to vaccinate at 4 time points in unvaccinated participants in Poland. We focused on this period to examine the impact of different pandemic waves on willingness to vaccinate, especially Delta and Omicron waves. Due to the dynamic nature of this pandemic, beliefs and attitudes toward vaccinations were constantly shifting. Furthermore, we aimed to include key time points for COVID-19 vaccination, such as the introduction of COVID-19 primary and booster vaccination campaigns, and explore how their introduction influenced attitudes toward vaccination.

Materials and methods

Study design and participants

OBSER-CO is a nationwide, repeated cross-sectional study aiming to examine seroprevalence of COVID-19 antibodies, vaccination status and willingness to vaccinate in Poland. This study was based on the standardized protocol published by the World Health Organization (WHO) *"Population-based age stratified seroepidemiological investigation protocol for coronavirus 2019 (COVID-19) infection"* (WHO Unity studies) (19). This protocol provided guidelines for the investigation of the seroprevalence of COVID-19 antibodies and infection rates in the general population (19). However, each country could adjust the protocol according to specific country characteristics and additional research objectives. Details on study design, recruitment, and sampling can be found elsewhere (19, 20). Data collection took place at four different rounds, starting from March 2021 until April 2022. In particular, round 1 was carried out between 29th March and 14th May 2021, round 2 from 27th July to 7th September 2021, round 3 from 16th November to 23rd December 2021 and round 4 from 14th March 2022 to 26th April 2022.To monitor changes over time, the distance between former and next round was set to be at least 2 months. Sampling and recruitment of participants were performed by IPSOS. Participants were recruited randomly by Random Digit Dialing (RDD). Once the random sample was selected, it was stratified according to age and population distribution of each administrative region. For each region we aimed to recruit participants representative of the age distribution of the region. During random dialing if a prospective participant was part of an age group that we had already recruited the necessary number of participants, then this individual would not be invited to participate in the study.

In each round, data were collected through a telephone interview by trained interviewers (21). After the initial contact and once the individual had agreed to participate, a computer assisted telephone interview (CATI) was conducted. During the CATI, participants were asked about their willingness to vaccinate as well as demographics, household size, COVID-19 diagnosis, symptoms, sick leave, general and COVID-19 related hospitalization, exposure to COVID-19, and vaccination status. In comparison to the questionnaire supplied through the WHO protocol, we added items on demographic characteristics, vaccination status, willingness to vaccinate for both vaccinated and unvaccinated and reasons for reluctance to vaccinate in both subgroups. In our study, the questionnaire was developed by a research group based at the Department of Epidemiology and Surveillance of the National Institute of Public Health based on the questionnaire appended to the WHO Unity Protocol (20). Although the questionnaire was not validated, the items included were gathered from existing tools or have been already used in previous seroprevalence studies. Additionally, questions on reasons for reluctance to vaccinate were informed by published studies examining reasons for vaccine hesitancy of COVID-19 vaccines in Poland and worldwide. Furthermore, after the first two rounds we added questions about reinfections to account for repeated COVID-19 infections in individuals. Overall, after emergence of each variant questions were revised to ascertain that they reflected disease characteristics of each variant/pandemic wave. The detailed questionnaires used in round 1 and subsequent rounds can be found in Supplementary Text 1. At rounds 2, 3, and 4, alongside the recruitment of new individuals, participants from previous rounds were also invited to participate, resulting in a sample of new and panel participants. In round 1 data from 25,202 participants from the telephone interview were available; in round 2 from 21,503; in round 3 from 20,958; and in round 4 from 20,942 participants. The study was conducted according to the Declaration of Helsinki. Participants provided informed consent for their participation in the telephone interview. The study protocol was approved by the Bioethics Committee of the National Institute of Public Health NIH - National Research Institute (No. 5/2021 of 02/03/2021).

Measures

Willingness to vaccinate among unvaccinated

To assess willingness to receive any COVID-19 vaccine at each round, participants were asked whether they were planning to get vaccinated. This question was asked only among unvaccinated individuals during the telephone interview. Available responses were yes or no. In rounds 2, 3, and 4 unvaccinated participants who responded no, were asked further about the reasons for their reluctance to vaccinate. They could choose one response from a set of reasons including: (1) I am concerned about the side effects/I am afraid of allergic reactions, (2) the vaccine was developed too quickly, it can't be safe, (3) the vaccine will be effective only for a short time and it will not protect against COVID-19 variants, (4) I do not vaccinate as a rule; I do not trust pharmaceutical companies (5) I got sick with COVID-19, (6) I faced difficulties enrolling at a vaccination center near my residence and I will not try again, (7) I faced difficulty reaching the vaccination center on my own; I am sick/unhealthy/unable to move, (8) I do not consider COVID-19 a dangerous disease, (9) I have a doctor's contraindication to vaccination, (10) I believe that getting sick is more effective than getting vaccinated, and (11) other reason. In round 4, participants could also opt out of responding to this question.

Willingness to receive another dose of a COVID-19 vaccine

Between rounds 2 and 3 the booster dose was recommended in the adult population. To estimate willingness to receive another dose of the COVID-19 vaccine, vaccinated participants were asked the following question: "*Are you planning to get vaccinated with another dose of the COVID-19 vaccine?*" in rounds 3 and 4. Participants could respond yes or no. Those responding no, were asked about the reasons for their reluctance to receive another dose. Participants were provided with the following reasons: (1) I got infected with COVID-19 despite being vaccinated, (2) I stopped believing in the effectiveness of the vaccine, (3) I felt bad after the previous vaccine dose (adverse effects), (4) The vaccine is only effective for a short period of time and it will not protect against variants, (5) I faced difficulties enrolling at a vaccination center near my residence, (6) I had difficulty reaching the vaccination center on my own; I am sick/unhealthy, (7) the doctor did not qualify me for

vaccination due to health reasons, and (8) other reason. Participants could choose one reason.

Factors

Sociodemographic

In all four rounds, sociodemographic factors included age, gender, work status, remote work, household size, and place of residence. Age consisted of four groups 20–39, 40–59, 60–69, and ≥70 years, and gender included man or woman. Work status comprised employed, and unemployed, whereas remote work was classified as remote or hybrid/stationary. For household size, participants were asked about the number of people included in their household, which ranged from 1 to ≥5 members. The participant's place of residence was based on population size and consisted of four levels: village, city up to 50,000 residents, city of 50,000–100,000 residents, and city of >100,000 residents. Additionally, in rounds 3 and 4, education was measured and consisted of low (primary, junior high school, basic vocational), medium and high (university degree, engineer degree, master's degree) level.

Infection with COVID-19

In all rounds, participants were asked whether they had received a positive COVID-19 test (PCR, antigen) since March 2020 (yes/no). In rounds 3, and 4 participants were additionally asked whether they had received more than one positive COVID-19 test results to account for new infections or re-infections (yes/no).

Exposure to COVID-19

Participants were asked whether they were in direct contact for at least 15 min with a person diagnosed with COVID-19 during the infectious period. Contacts with infectious individuals while wearing a mask, i.e., at least a FFP2 (N95) mask were not included in the contact group. Only contacts with infected individuals when wearing a cloth mask or only a face shield were included. Available responses consisted of yes, once; yes, multiple times; or no contact.

Symptoms

Participants were asked whether, in the previous 6 months, they had experienced any of the following symptoms: fever, cough, dyspnea, loss of smell or taste, sore throat, rhinorrhea, myalgia, fatigue, headache, abdominal pain, nausea or vomiting, diarrhea, rash, conjunctivitis, chills, loss of appetite, epistaxis (nosebleed), confusion, and other neurological symptoms. In rounds 3, and 4, other neurological symptoms were excluded, and instead participants were asked whether they experienced hearing problems. Participants could choose more than one symptoms. Then, a continuous variable for the number of symptoms was created, ranging from 0 to 18.

Sick leave, general hospitalization, hospitalization due to COVID-19

For sick leave, participants were asked whether they were on sick leave due to these symptoms (yes, no, or not applicable). For general hospitalization, participants were asked whether they had been hospitalized since March 2020 (yes/no). If participants were hospitalized for any reason, then they would be further asked about COVID-19 related hospitalization. To assess hospitalization due to COVID-19, participants were asked whether they were hospitalized due to COVID-19 or a respiratory infection (pneumonia, bronchitis). Available responses were yes, no, or not applicable. This question was not asked to participants who did not report general hospitalization.

Participation in activities

Participants were asked three separate questions about participation in specific social activities. Individuals were asked whether, since March 2020 (rounds 1 and 2) or May 2021 (rounds 3, and 4), they took part in events such as weddings, communions, baptisms, and/or funerals (yes/no). Similarly, participants were asked whether they regularly participated in sports, religious, artistic groups, or similar activities/meetings, not related to work (yes/no). Finally, individuals were asked if they participated in organized trips (i.e., trip or camping, business trip, sports trip) (yes/no).

Statistical analysis

Prevalence of willingness to vaccinate was defined as the percentage of participants, who responded that they were planning to get vaccinated or receive another dose of COVID-19 vaccine at each round. Demographic variables as well as reasons for reluctance to vaccinate were summarized as proportions with percentages. Aside from symptoms which was coded as a continuous variable (number of symptoms), all other factors were categorical (binary, nominal, ordinal). To analyze the factors associated with willingness to vaccinate in unvaccinated participants we performed the following steps. Factors of interest were chosen according to previous research examining variables associated with COVID-19 disease characteristics, vaccination and willingness to vaccinate. These factors included age, gender, place of residence, work status, remote work, COVID-19 diagnosis, exposure to COVID-19, general hospitalization, hospitalization due to COVID-19, participation in events, participation in social groups, and participation in organized trips. Secondly, we performed univariate analysis for the selected factors in each round separately. Factors which were not associated with willingness to vaccinate in univariate analysis were not examined further. Finally, multiple multivariable logistic regression models were created to examine these associations. At each round, for each of the selected factors a separate regression model was fitted, which was adjusted for a different set of confounders. A detailed list of confounders for each regression model can be found in Supplementary Table 1. Each set of confounders was selected according to previous literature about COVID-19 in general and COVID-19 vaccination behaviors. We

TABLE 1 Characteristics of unvaccinated participants according to willingness to vaccinate in rounds 1–4.

	Willingness to vaccinate															
	Round 1 (n = 15,885)				Round 2 (n = 4,006)				Round 3 (n = 3,044)				Round 4 (n = 4,023)			
	Yes (n = 11,596, 73%)		No (n = 4,289, 27%)		Yes (n = 1,482, 37%)		No (n = 2,524, 63%)		Yes (n = 974, 32%)		No (n = 2,070, 68%)		Yes (n = 483, 12%)		No (n = 3,540, 88%)	
	N	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)
Age in years																
20–39	3,759	(65)	1,996	(35)	614	(34)	1,196	(66)	439	(29)	1,060	(71)	217	(12)	1,567	(88)
40–59	4,857	(76)	1,524	(24)	504	(38)	817	(62)	293	(34)	571	(66)	184	(13)	1,250	(87)
60–69	2,135	(79)	563	(21)	236	(42)	329	(58)	178	(41)	258	(59)	67	(12)	483	(88)
≥70	810	(77)	241	(23)	131	(42)	179	(58)	80	(33)	165	(67)	35	(13)	229	(87)
Gender																
Man	5,770	(73)	2,124	(27)	736	(36)	1,336	(64)	484	(29)	1,172	(71)	229	(11)	1,828	(89)
Woman	5,791	(72)	2,200	(28)	749	(39)	1,185	(61)	506	(36)	882	(64)	274	(14)	1,701	(86)
Place of residence																
Village	3,312	(70)	1,422	(30)	588	(42)	816	(58)	373	(36)	654	(64)	182	(14)	1,164	(87)
City up to 50,000 inhabitants	2,810	(72)	1,120	(28)	394	(37)	661	(63)	227	(29)	544	(71)	132	(13)	898	(87)
City 50,000–100,000 inhabitants	897	(72)	351	(28)	117	(34)	224	(66)	84	(29)	203	(71)	36	(11)	293	(89)
City >100,000 inhabitants	4,542	(76)	1,431	(24)	386	(32)	820	(68)	306	(32)	653	(68)	153	(12)	1,174	(88)
Size of household																
1	1,911	(74)	661	(26)	283	(40)	429	(60)	213	(36)	380	(64)	118	(15)	673	(85)
2	3,401	(76)	1,054	(24)	361	(35)	676	(65)	224	(31)	499	(69)	110	(12)	799	(88)
3	2,432	(74)	871	(26)	323	(39)	510	(61)	200	(32)	422	(68)	107	(13)	695	(87)
4	2,357	(71)	967	(29)	281	(35)	512	(65)	188	(31)	420	(69)	87	(11)	727	(89)
5 or more	1,460	(65)	771	(35)	237	(38)	394	(62)	165	(33)	333	(67)	81	(11)	635	(89)
Work status																
Employed	6,281	(72)	2,448	(28)	733	(35)	1,368	(65)	469	(30)	1,105	(70)	236	(11)	1,913	(89)
Unemployed	1,047	(65)	555	(35)	211	(41)	308	(59)	144	(37)	244	(63)	96	(18)	435	(82)

(Continued)

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TABLE 1 (Continued)

	Willingness to vaccinate															
	Round 1 (n = 15,885)				Round 2 (n = 4,006)				Round 3 (n = 3,044)				Round 4 (n = 4,023)			
	Yes (n = 11,596, 73%)		No (n = 4,289, 27%)		Yes (n = 1,482, 37%)		No (n = 2,524, 63%)		Yes (n = 974, 32%)		No (n = 2,070, 68%)		Yes (n = 483, 12%)		No (n = 3,540, 88%)	
	N	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)
Pensioner																
Yes	2,756	(79)	753	(21)	366	(42)	502	(58)	248	(38)	399	(62)	111	(14)	697	(86)
No	8,805	(71)	3,571	(29)	1,119	(36)	2,019	(64)	742	(31)	1,655	(69)	392	(12)	2,832	(88)
COVID-19 diagnosis																
Yes	2,091	(79)	554	(21)	253	(49)	267	(51)	768	(31)	1,710	(69)	39	(13)	264	(87)
No	9,470	(72)	3,770	(29)	1,232	(35)	2,254	(65)	222	(39)	344	(61)	464	(12)	3,265	(88)
Contact with infected individual																
Once	1,363	(75)	446	(25)	131	(43)	173	(57)	86	(32)	187	(68)	63	(13)	414	(87)
Multiple times	2,047	(72)	784	(28)	188	(28)	484	(72)	101	(27)	273	(73)	76	(8)	888	(92)
No contact	8,151	(72)	3,094	(28)	1,166	(38)	1,864	(62)	803	(34)	1,594	(66)	364	(14)	2,227	(86)
General hospitalization																
Yes	1,118	(78)	317	(22)	171	(41)	246	(59)	144	(40)	218	(60)	87	(17)	411	(83)
No	10,443	(72)	4,007	(28)	1,314	(37)	2,275	(63)	846	(32)	1,836	(68)	416	(12)	3,118	(88)
Hospitalization due to COVID-19																
Yes	180	(88)	25	(12)	29	(56)	23	(44)	17	(50)	17	(50)	25	(30)	58	(70)
No	11,381	(73)	4,299	(27)	1,456	(37)	2,498	(63)	973	(32)	2,037	(68)	478	(12)	3,471	(88)
Participation in events																
Yes	3,637	(69)	1,597	(31)	593	(32)	1,278	(68)	413	(28)	1,054	(72)	155	(9)	1,597	(91)
No	7,924	(74)	2,727	(26)	892	(42)	1,243	(58)	577	(37)	1,000	(63)	348	(15)	1,932	(85)
Participation in social groups																
Yes	2,288	(65)	1,249	(35)	383	(29)	955	(71)	285	(26)	819	(74)	154	(9)	1,507	(91)
No	9,273	(75)	3,075	(25)	1,102	(41)	1,566	(59)	705	(36)	1,235	(64)	349	(15)	2,022	(85)
Participation in organized trip																
Yes	1,597	(68)	744	(32)	222	(27)	600	(73)	179	(25)	540	(75)	70	(8)	821	(92)
No	9,964	(74)	3,850	(26)	1,263	(40)	1,921	(60)	811	(35)	1,514	(65)	433	(14)	2,708	(86)

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TABLE 2 Characteristics of vaccinated participants according to willingness to receive another dose of a COVID-19 vaccine in rounds 3-4.

	Willingness to receive another dose of a COVID-19 vaccine							
	Round 3 (n = 12,573)				Round 4 (n = 7,105)			
	Yes (n = 11,347, 90%)		No (n = 1,226, 10%)		Yes (n = 3,775, 53%)		No (n = 3,330, n = 47%)	
	N	(%)	*N*	(%)	*N*	(%)	*N*	(%)
Age in years								
20–39	3,557	(85)	643	(15)	1,505	(51)	1,463	(49)
40–59	3,254	(91)	331	(9)	1,338	(54)	1,156	(46)
60–69	3,108	(96)	136	(4)	581	(56)	458	(44)
≥70	1,428	(92)	116	(8)	351	(58)	351	(42)
Gender								
Man	5,439	(89)	679	(11)	1,857	(51)	1,753	(49)
Woman	5,908	(92)	547	(8)	1,918	(55)	1,577	(45)
Place of residence								
Village	3,177	(90)	354	(10)	1,047	(52)	970	(48)
City up to 50,000 inhabitants	2,946	(91)	301	(9)	907	(52)	580	(48)
City 50,000–100,000 inhabitants	959	(92)	87	(8)	325	(56)	259	(44)
City >100,000 inhabitants	4,265	(90)	484	(10)	1,496	(54)	1,251	(46)
Size of household								
1	2,118	(91)	221	(9)	697	(52)	639	(48)
2	3,842	(93)	307	(7)	1,049	(56)	836	(44)
3	2,230	(89)	273	(11)	771	(54)	654	(46)
4	1,911	(87)	289	(13)	755	(51)	724	(49)
5 or more	1,246	(90)	136	(10)	503	(51)	477	(49)
Work status								
Employed	5,372	(88)	736	(12)	2,097	(55)	1,993	(60)
Unemployed	712	(89)	87	(11)	364	(10)	250	(7)
Pensioner								
Yes	4,120	(95)	230	(5)	857	(57)	655	(43)
No	7,227	(88)	996	(12)	2,918	(52)	2,675	(48)

(Continued)

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TABLE 2 (Continued)

	Willingness to receive another dose of a COVID-19 vaccine									
	Round 3 (n = 12,573)				Round 4 (n = 7,105)					
	Yes (n = 11,347, 90%)		No (n = 1,226, 10%)		Yes (n = 3,775, 53%)		No (n = 3,330, n = 47%)	
	N	(%)	*N*	(%)	*N*	(%)	*N*	(%)
Positive COVID-19 test								
Yes	2,084	(91)	197	(9)	391	(60)	265	(40)
No	9,263	(90)	1,029	(10)	3,384	(52)	3,065	(48)
Participation in organized trip								
Yes	3,195	(28)	408	(33)	844	(22)	861	(26)
No	8,152	(71)	818	(67)	2,931	(78)	2,469	(74)
Number of vaccine doses received								
1 dose	1,041	(78)	318	(23)	532	(42)	724	(22)
2 doses	10,306	(92)	908	(8)	3,243	(55)	2,606	(45)

also used the maximum likelihood estimate of the model to check how well each confounder fitted the regression model. We selected this approach instead of a single multivariable model to build the most appropriate model for each exposure, accounting for the fact that each exposure can be influenced by different confounders (22). Additionally, we performed separate analyses for each round, to observe the effects of different COVID-19 variants and subsequent pandemic waves.

Effect estimates are presented as odds ratios (OR), crude for univariate and adjusted for the multivariable analysis, with corresponding 95% confidence intervals (CI). Regression analyses were bootstrapped (1,000 repetitions) and the Bonferroni correction was applied to correct confidence intervals for multiple comparisons. Analyses were performed using STATA 14 (College Station, TX: StataCorp LP).

Results

In total, 92,607 CATI interviews were conducted. In round 1 (R1), 63% were unvaccinated (n = 15,885), and 27% were vaccinated (n = 9,317). In round 2 (R2), 18.6% were unvaccinated (n = 4,006), whereas 81.4% were vaccinated (n = 17,497). In round 3 (R3), 14.5% were unvaccinated (n = 3,044) and 85.5% vaccinated (n = 17,914). Finally, in round 4 (R4), 19.3% were unvaccinated (n = 4,032) and 80.7% were vaccinated (n = 20,942).

Characteristics of unvaccinated participants according to willingness to vaccinate

By round, among unvaccinated participants, there were respectively 7,991 (59%), 1,934 (17%), 1,388 (12.9%), 1,975 (15.4%) women with median age 47 years (40–62) in R1, 47 (36–61) in R2, 44 (34–62) in R3, and 45 (35–60) in R4. Characteristics of unvaccinated study participants according to their willingness to vaccinate at each round are presented in Table 1. Among unvaccinated individuals, willingness to vaccinate was 73% in R1. In R2, 3 months after vaccination became available to all adults, willingness fell to 37%. In R3, willingness decreased slightly to 32%, and in R4, after the emergence of Omicron, willingness to vaccinate fell to 12%.

Across all sociodemographic and other groups studied, willingness to vaccinate followed a declining pattern from R1 to R4. In R1, R2, and R3 middle-aged and older individuals were more likely to express willingness to vaccinate than younger participants. However, in R4, there were no differences among age groups. In the first 2 rounds, willingness to vaccinate was similar between men and women. However, in R3 (Delta wave/booster vaccination), and R4 (Omicron) women reported willingness to vaccinate more often than men. No clear pattern was observed for place of residence or size of household. For work status, in R1 72% of employed participants reported willingness to vaccinate compared to 65% of unemployed. Nevertheless, from

round 2 onwards, unvaccinated, unemployed individuals were more likely to be willing to get vaccinated compared to employed. Additionally, a clear pattern was present for pensioners in all four rounds. Pensioners were consistently more willing to get vaccinated compared to non-pensioners. However, the size of the difference decreased from R3 to R4, after the emergence of the Omicron variant. A similar pattern was observed for COVID-19 diagnosis. Those with a positive COVID-19 test were more likely to be willing to vaccinate in the first three rounds (R1: 79%; R2: 49%; R3:39%) compared to those without a COVID-19 diagnosis (R1: 72%; R2: 35%; R3: 31%). But in R4, no difference between group levels was present. In R2, R3, and R4 those who had multiple contacts with an infected person reported a lower willingness to vaccinate compared to those who had no or just one contact. Overall, in all rounds prevalence of willingness to vaccinate was higher in those hospitalized (general or COVID-19 related). Finally, in all four rounds, individuals who did not participate in events, social groups, and organized trips, were more likely to express willingness to vaccinate than those who participated.

Reasons for reluctance to vaccinate among unvaccinated participants

Figure 1 presents the reasons for reluctance to vaccinate among unvaccinated participants at R2, R3, and R4. In R2, 26.5% of participants expressed concern about the side effects of the COVID-19 vaccine as the reason for reluctance to vaccinate. In R3, concerns about the side effects and allergic reactions declined but remained the most common reason with 24.1%. Similarly, in R4, the percentage of participants expressing concerns about side effects or allergic reactions continued to decline but these concerns remained one of the most prevalent. Furthermore, in R2, 19.5% mentioned that the vaccine was developed too quickly and expressed concerns about its safety. In R3, 16.7% reported this reason, which further declined in R4 to 13.9%. Additionally, in R2, 8.2% of participants reported a doctor's diagnosis of contraindication, 7.4% said that they did not consider COVID-19 a dangerous disease, 5.7% cited COVID-19 infection, 4.7% responded that they do not trust pharmaceutical companies in general, and 2.7% thought that getting infected is more effective than vaccination. In R3, more people chose COVID-19 infection (7.5; 1.8% increase since R2), 7.3% responded that getting sick is more effective than being vaccinated (4.6% increase since R2), 6.9% chose medical contraindications, and 7.4% lacked trust in pharmaceutical companies and vaccines in general (2.7% increase since R2). In R4, 9.9% of respondents reported that getting sick is more effective than the vaccine, a reason that has become more prevalent since R3. Additionally, 7.8% of participants mentioned getting sick with COVID-19, 8% of people mentioned lack of trust against pharmaceuticals (further increase since R3), and 5.5% did not consider COVID-19 dangerous. In R2, R3, and R4 a significant proportion (R2: 21.5%; R3: 20.3%; R4: 15.6%) of participants chose "other reason". In R4, 6% of participants did not want to answer this question.

Characteristics of vaccinated participants according to willingness to receive another dose of a COVID-19 vaccine

There were 17,914 vaccinated individuals in R3 and 20,942 in R4, including 9,347 (52.2%) and 10,868 (51.9%) women with median age 62 years (43–70) and 57 (41–68) in R3 and R4, respectively. Table 2 presents the characteristics of respondents according to willingness to receive another dose in R3 and R4. A decline in willingness to receive another dose of a vaccine was observed from R3 to R4. In R3, 90% of vaccinated participants reported that they were willing to receive another dose. Among those, 91% had so far received 2 doses and 9% one dose of a COVID-19 vaccine. In R4, willingness decreased to 53%. Among those, 86% had so far received two doses and 14% one dose of a COVID-19 vaccine. Across all factors, willingness among the vaccinated declined substantially from R3 to R4. For age, willingness to receive another dose was the lowest among 20–39 years (R3: 85%; R4: 51%). Those $\geq$60 years reported the highest willingness to receive another dose in both rounds. Women consistently expressed higher willingness to receive another dose than men, while there was no clear pattern for place of residence. For work status, there was no big difference between employed and unemployed in R3 (88 and 89%, respectively), however this changed in R4. While willingness to vaccinate declined in both groups, it remained higher among the unemployed. Furthermore, vaccinated pensioners were more likely to express willingness than non-pensioners in both rounds. Furthermore, in R3, no differences were present in willingness according to COVID-19 diagnosis. However, in R4, those with a positive COVID-19 test reported higher willingness to receive another dose (60%) when compared to 52% of those without a positive test.

Reasons for reluctance to receive another dose of a COVID-19 vaccine among vaccinated participants

Reasons for reluctance to receive another dose of a COVID-19 vaccine are presented in Figure 2. In R3, 16% of participants chose vaccine adverse effects (feeling bad after the previous vaccine dose). In R4, the prevalence of vaccine adverse effects declined to 14%. Additionally, in R2, 13% believed that the vaccine is only effective for a short period of time and will not protect against variants, while 10% stopped believing in the effectiveness of the vaccine altogether. In R4, more participants believed that the vaccine would only effective for a short period of time (17%), while 12% reported that they did not believe in the vaccine's overall effectiveness. In R3, only 2% of participants mentioned that they were reluctant to receive another dose because they were infected with COVID-19 after vaccination. This reason became more prevalent in R4 and increased to 5%. In R3, 55% of respondents chose "other reason" whereas in R4, there was a small decline with 47% citing "other reason".

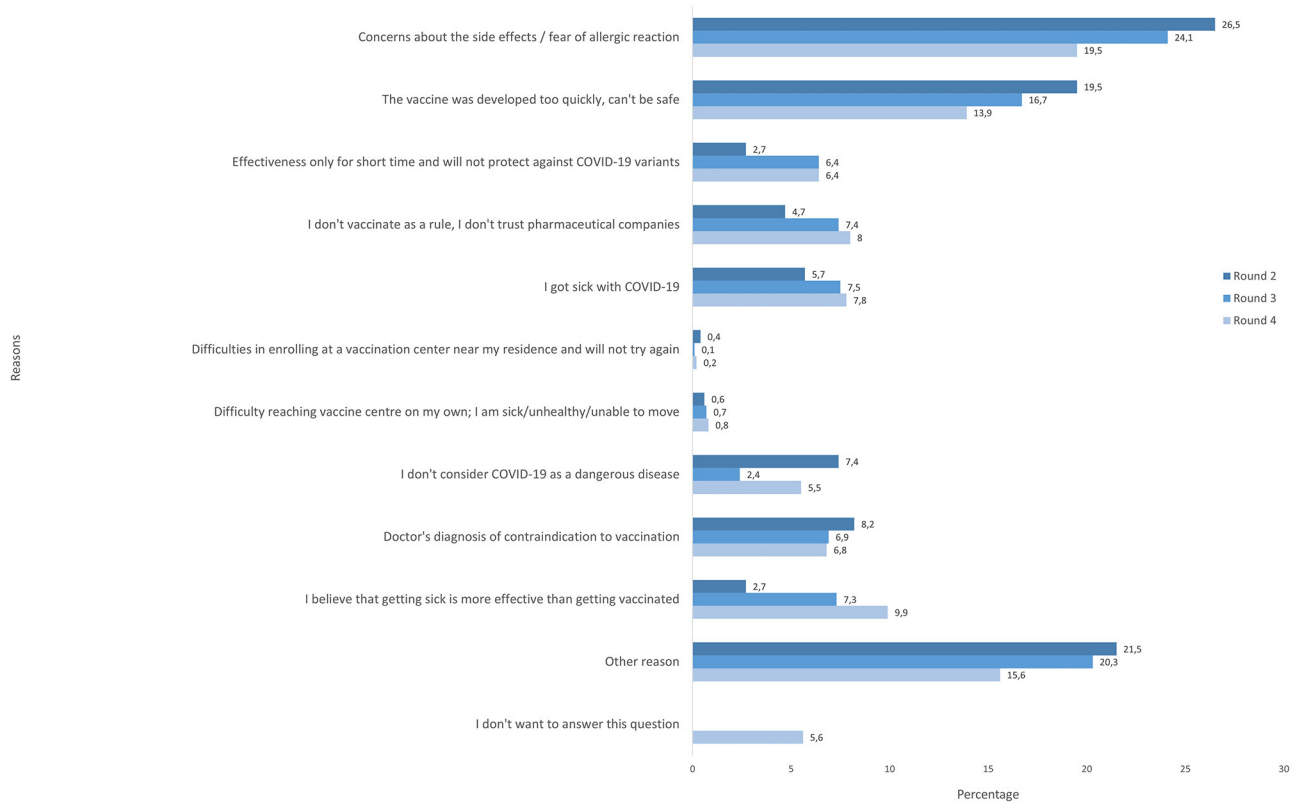

FIGURE 1
Reasons for reluctance to vaccinate among unvaccinated participants in rounds 2–4.

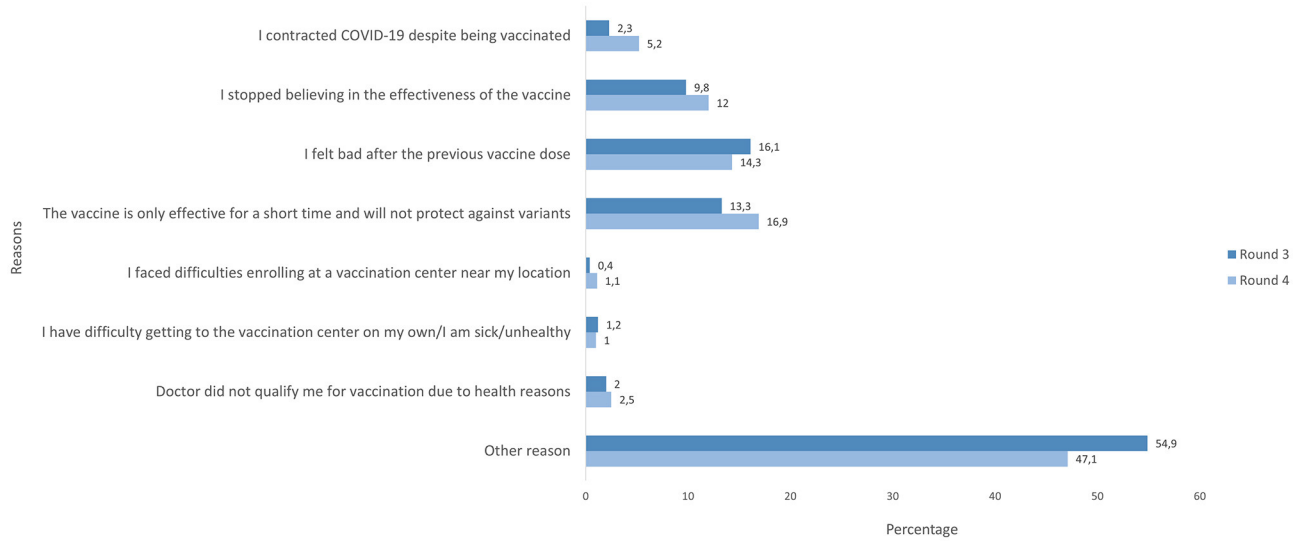

FIGURE 2
Reasons for reluctance to receive another dose of a COVID-19 vaccine among vaccinated participants in rounds 3–4.

Factors associated with willingness to vaccinate among unvaccinated participants

Fully adjusted odds ratios (OR) and 95% CIs of associations of age, place of residence, gender, work status, and remote work with willingness to vaccinate among unvaccinated participants are presented in Figure 3. Detailed crude and fully adjusted ORs and 95% CIs can be found in Supplementary Table 2. Estimates for the association between age and willingness to vaccinate varied over time (rounds) and age groups. In R1, 40–59 years vs. 20–39 (reference), was associated with increased odds of willingness to vaccinate (OR = 1.79, 95% CI: 1.62–1.99) in the fully adjusted

model. This association was attenuated in R2, R3, and R4. Furthermore, 60–69 years was associated with increased odds of willingness at R1 (start of primary vaccination) and R3 (booster vaccination) (OR = 1.98, 95% CI: 1.69–2.31; OR = 1.47, 95% CI: 1.10–1.95, respectively). At R2, and R4 no associations were observed between this age group and willingness to vaccinate. Finally, for those ≥70 years, a positive association was reported only in R1 (OR = 1.60, 95% CI: 1.28–1.99), with the association attenuating over the next 3 rounds.

In R1, living in a city of >100,000 residents (vs. a village) was associated with a higher willingness to vaccinate (OR = 1.27, 95% CI: 1.12–1.45); however this association reversed in the next three rounds with those living in big cities having decreased odds of willingness (R2, OR = 0.65, 95% CI: 0.53–0.79; R3, OR = 0.82, 95% CI: 0.64–1.04; R4: OR = 0.82, 95% CI: 0.60–1.12). Similar associations were observed from R2–R4 in those living in (a) cities up to 50,000 residents and (b) cities from 50,000 to 100,000 residents when compared to living in a village, after adjustment for confounders.

In R1, being a woman was associated with decreased odds of willingness (OR = 0.89, 95% CI: 0.82–0.96) when compared to men, after adjustment. In R2 we did not observe any association between gender and willingness. However, in R3, women had higher odds of willingness to vaccinate than men (OR = 1.25, 95% CI: 1.06, 1.48). But, in R4, this association was attenuated (OR = 1.11, 95% CI: 0.91–1.36). For work status, a negative association was observed for unemployed participants (vs. employed) in R1 (OR = 0.90, 95% CI: 0.78– 1.03). However, this association was reversed in the following 3 rounds, with unemployed participants reporting increased odds of willingness to vaccinate. Moreover, remote work was associated with increased odds of willingness to vaccinate when compared to hybrid/stationary work in R1 (OR = 1.79, 95% CI: 1.63–1.98). However, the association was attenuated in the next 3 rounds.

Fully adjusted ORs and 95% CIs of associations of COVID-19 diagnosis, exposure to COVID-19, general hospitalization, hospitalization due to COVID-19, participation in events, social groups, and organized trips with willingness to vaccinate are presented in Figure 4. Detailed crude and fully adjusted ORs and 95% CIs can be found in Supplementary Table 2. COVID-19 diagnosis was consistently associated with higher odds of willingness to vaccinate when compared to no diagnosis in all four rounds after adjustment for confounders (R1, OR = 1.35, 95% CI: 1.21–1.50; R2, OR = 1.55, 95% CI: 1.26–1.92; R3, OR = 1.38, 95% CI: 1.13–1.70; R4, OR = 1.28, 95% CI: 1.02–1.59). Moreover, having had contact once with an infected individual was positively associated with willingness to vaccinate in R1 (OR = 1.17, 95% CI: 1.02–1.34) in the fully adjusted model. A similar association was observed in R2 but was further attenuated in subsequent rounds. For multiple contacts with infected individual(s), associations changed from R1 (OR = 1.07, 95% CI: 0.95–1.20) to R2 (OR = 0.67, 95% CI: 0.53–0.83). In R3 the association was attenuated, but became stronger in R4 (OR = 0.62, 95% CI: 0.46–0.85). Moreover, general hospitalization and hospitalization due to COVID-19 were associated with increased odds of willingness to vaccinate in R1 (OR = 1.26, 95% CI: 1.10–1.44; OR = 1.77, 95% CI: 1.13–2.76, respectively). However, associations were attenuated in R2. In R3 the odds ratio for general hospitalization was equal to 1.31 (95% CI: 1.04–1.65) and for COVID-19-related hospitalization to 1.50 (0.73–3.12). In R4 those hospitalized for any reason had an OR of 1.46 (95% CI: 1.12–1.91) and those hospitalized because of COVID-19 had an OR of 2.36 (95% CI: 1.40–3.97). Lastly, participation in events, social groups, and organized trips were consistently associated with decreased odds of willingness to vaccinate in all 4 rounds after adjustment for confounders.

Discussion

Our study documents the change in attitudes toward getting vaccinated or receiving an additional dose of a COVID-19 vaccine from March 2021 to April 2022 in Poland. Willingness to vaccinate among unvaccinated participants exceeded 70% in the first round but declined substantially in the next 3 rounds. Among those who remained unvaccinated until April 2022, only 12% planned to vaccinate in the future. This may be explained on one hand by the fact that the individuals, who planned to vaccinate already had a chance to do so, and on the other hand by decreasing overall interest to get vaccinated. The latter is also supported by the fact that, among vaccinated individuals, willingness to receive an additional dose of a COVID-19 vaccine also declined, although not to such a large extent. In round 3, November-December 2021, 90% of vaccinated participants intended to receive another dose, but after 4 months, in round 4, this percentage decreased to 53%. The decline in willingness to vaccinate over the study period among both vaccinated and unvaccinated individuals was observed across all sociodemographic, health, and behavioral factors examined.

The initial percentage of unvaccinated participants who intended to vaccinate against COVID-19 in our study (73%) was higher than reported by other authors. A study examining data from 2020 showed that Poland had one of the lowest vaccine acceptance rates (56.3%) (5), while other Polish studies reported vaccine hesitancy or reluctance to vaccinate varying between 31 and 49.2% in 2021 (7, 23). Although our study followed a random digit dialing recruitment the respondents who agreed to participate were clearly more inclined to vaccinate as the proportion of vaccinated in our study exceeded the population statistics. For example, in rounds 2–4 over 80% of participants were vaccinated with at least one dose, but in the official statistics this percentage reached only slightly above 60%, which is why we focused on separate analysis of vaccinated and unvaccinated cohorts. On the other hand differences in data collection (time, sample size, population characteristics) can potentially explain differences as compared with other research studies. A previous study, conducted at the start of the vaccination program reported increasing trend in willingness to vaccinate (14) so it is possible that our first round occurred at the time of the highest acceptance of the COVID-19 vaccines, which declined afterwards.

Of note, studies in the US conducted between 2020–2021, and 2021–2022 indicate that it is possible to maintain an increasing trend in willingness to vaccinate, although in contrast to our study this analysis included both the vaccinated and planning to get vaccinated as willing to vaccinate (24, 25). Their findings also indicate the positive impact of a number of interventions such as

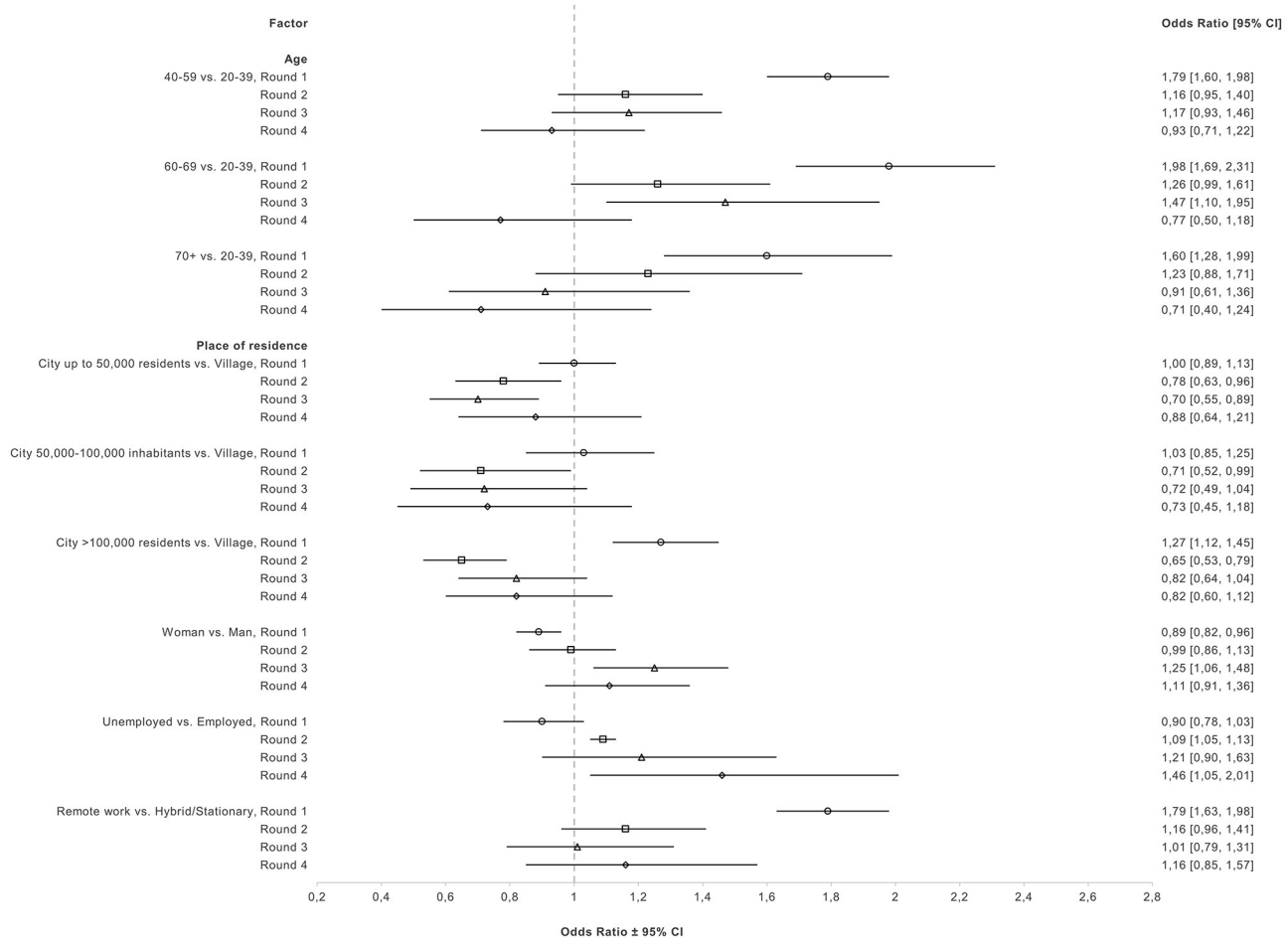

FIGURE 3
Fully adjusted odds ratios and 95% CI for associations of sociodemographic factors with willingness to vaccinate among unvaccinated participants in rounds 1–4.

releasing restrictions and mask mandates for vaccinated individuals (24). Poland did not fully implement such approach and possibly in effect the pressure to vaccinate was less (26).

What is more, with longer duration of the COVID-19 emergency situation, the intention to vaccinate may be undermined by pandemic fatigue (6, 27). This could partially explain the decreasing trend in willingness to vaccinate observed in our study along with evolution of specific concerns regarding the vaccines and the infection itself. We note that the concerns were fueled by the increase of misinformation around the safety and efficacy of COVID-19 vaccines in the EU, giving rise to conspiracy theories (11), which then negatively influenced perceptions of vaccines. It is highly possible that the decreasing levels of willingness to vaccinate over time in our study also reflected the impact of the COVID-19 infodemic, especially driven by social media in Poland (7).

In rounds 2–4, we were able to collect data on reasons why the participants were reluctant to vaccinate confirming common themes of social media discourse (13). In accordance with previous studies in Poland, concerns about side effects/allergic reactions (28) and speed of development/safety were the most prevalent reasons for reluctance to vaccinate (14), listed by 26.5 and 19.5% of vaccine hesitant participants in round 2, respectively. However, there was a decreasing trend in these concerns over the study period. As time passed and more data became available about the safety of COVID-19 vaccines and in conjunction with communication efforts by public health authorities, concerns subsided, but nevertheless remained significant. In round 4 still 19.5% of participants stated concerns about side effects and 13.9% stated quick vaccine development/safety as key reasons for reluctance to vaccinate.

Furthermore, the belief that the effectiveness of the vaccine is limited and will not protect against new variants became more popular. The proportion of unvaccinated reluctant to vaccinate due to this reason changed from 2.7 to 6.6% between round 2 and 4 and the proportion of vaccinated not willing to take additional dose—from 13.3 to 16.9%. Interestingly, the rate of decrease of willingness to vaccinate was the largest after the Omicron wave across all factors. Moreover, in unvaccinated participants, after the emergence of the Omicron variant (R4) differences among levels of several factors disappeared, which could suggest strong influence of the Omicron wave on attitudes toward COVID-19 vaccination. The Omicron epidemic wave was characterized by very high transmission rates in combination with lowered vaccine effectiveness against mild infection (29–31). This could have contributed to increasing beliefs of lack of effectiveness or

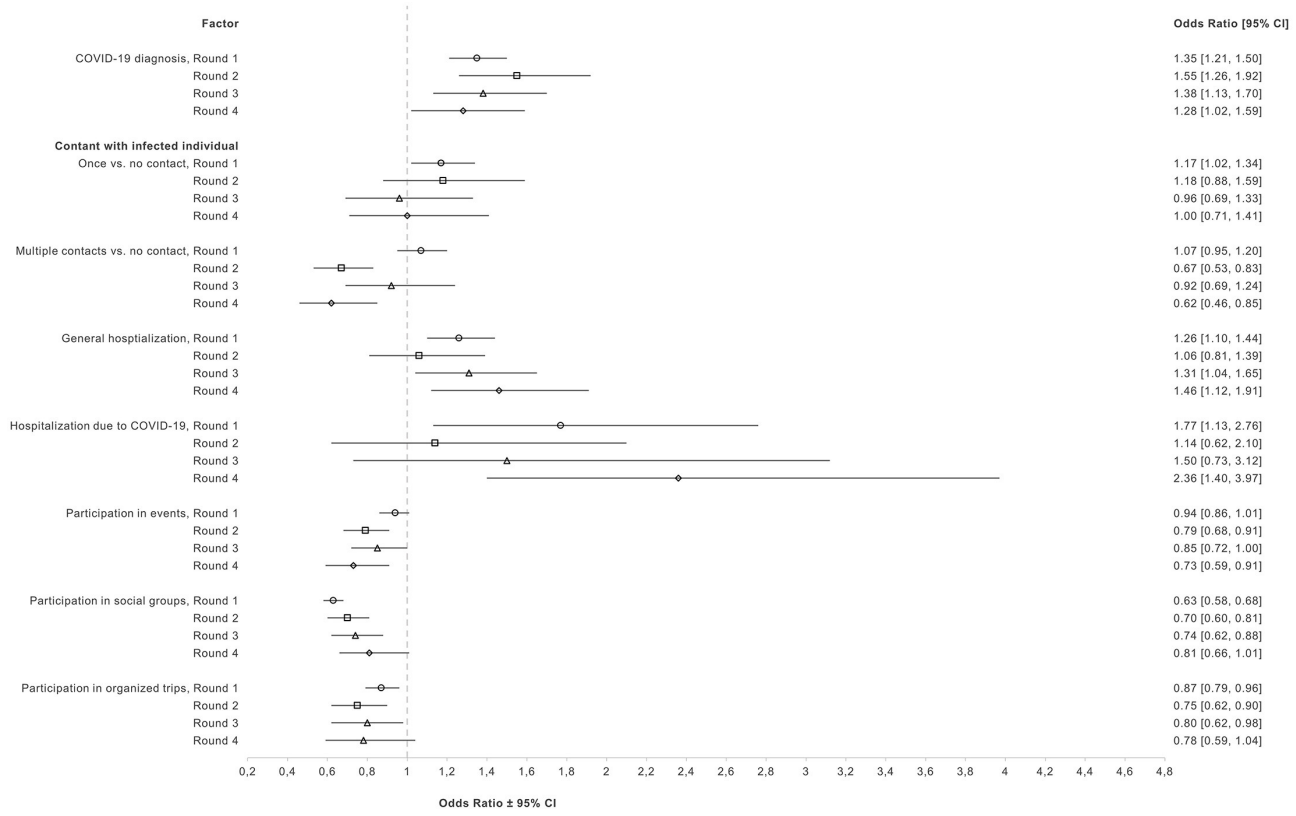

FIGURE 4
Fully adjusted odds ratios and 95% CI for associations of health, and behavioral factors with willingness to vaccinate among unvaccinated participants in rounds 1–4.

only short-lived effectiveness of the COVID-19 vaccine observed in our study, despite the fact of clear evidence of high vaccine efficacy against severe disease.

Additionally, the belief that the Omicron variant was not as severe as previous variants could also explain the increasing proportion of participants in our study believing that getting sick is more effective than getting vaccinated. Concerns about vaccine effectiveness were also identified as crucial for vaccine acceptance in other studies (10, 23, 32). Similarly, vaccinated participants in our study cited frequently vaccine-related adverse effects (16.1% in R3 and 14.3% in R4) and mistrust about the effectiveness of the vaccine in general (9.8% in R3 and 12.0% in R4) as reasons behind reluctance to receive another dose. Reduced effectiveness of the first mRNA vaccines against the Omicron variant and the increased number of Omicron infections in vaccinated people may be the driving forces behind these responses (10, 33). These findings highlight the importance of continuing health communication adjusted to the current concerns and incorporating new scientific developments (6). Of note, a substantial proportion of participants chose "other reason" as their response (15.6–21.5% among unvaccinated and 47.1–54.9% among vaccinated). The list of reasons provided to participants in our study, were chosen according to previous literature. The fact that so many participants did not find it sufficient underscores the dynamic nature of this pandemic and beliefs and attitudes toward vaccination, and the necessity to continuously evaluate new reasons behind reluctance in order to update the communication strategies.

Equally important, our study helps to better characterize the changing population who is reluctant to vaccinate or to receive another dose. In our study associations between age and willingness to vaccinate varied between rounds. Initially, there was a strong association with age group, with older unvaccinated individuals more likely to be willing to receive the vaccine in the future. This is in line with prior research indicating that older individuals are more likely to get vaccinated (7) and less likely to delay getting the vaccine compared to younger individuals (25). Middle-aged and older individuals tend to have higher risk perception toward COVID-19 and higher engagement with preventive measures (34), which explains the initial finding. However, another study in Poland did not report any associations between age and willingness to vaccinate (35). We observed that the difference between age groups decreased in subsequent rounds, so evolution in time of the reluctant group may explain contrasting findings reported in previous literature.

Moreover, in round 1, women had decreased odds of willingness to vaccinate, in accordance with another study in Poland (36). Women in general experience more vaccine-related adverse effects than men, which can explain the increased reluctance and could potentially reflect increased fear toward COVID-19 vaccination (37). However, by round 3 (November-December 2021; Delta/Omicron) women were more willing to

get vaccinated than men. Potentially fears of women subsided, as vaccines proved to be safe and effective, but it is also likely that women who intend to vaccinate in general, were delaying getting the vaccine, while those men who wanted to get vaccinated, did so. Similar mechanism could explain the changes of the association between place of residence and willingness to vaccinate throughout the study period. In round 1, participants living in big cities were more willing to get vaccinated than those living in villages, a finding which is in accordance with previous studies in Poland (14, 35, 36). However, we observed a reversal in associations of all levels of place of residence compared to living in a village in rounds 2–4. During all four rounds, we observed the lowest rates of vaccine uptake in villages than cities, which supports the hypothesis of delaying vaccination, possibly related to more difficult access to vaccinations centers.

During all rounds, prior COVID-19 diagnosis was associated with increased willingness to vaccinate. One previous study reported similar results where individuals without prior COVID-19 diagnosis were more hesitant and resistant toward vaccination against COVID-19 (8). It is possible that those who have not been infected with COVID-19 might be less concerned about COVID-19, which then can lead to lower willingness to vaccinate (38). In addition, severe COVID-19 can be a significant motivator for vaccination against COVID-19, with adults experiencing mild symptoms being more hesitant to vaccination (39). This is also supported by our findings, that participants who were previously hospitalized with COVID-19 reported the highest willingness to receive the vaccine. The positive association between general hospitalization and willingness to vaccinate could indicate that people with health problems and therefore vulnerable to COVID-19, were more willing to get vaccinated to protect themselves against severe outcomes (40).

Exposure to COVID-19 was positively associated with willingness in round 1, whereas multiple contacts with infected individual(s) were negatively associated with willingness in rounds 2, and 4. In round 1 there was higher risk perception and fear around contracting COVID-19 which could have led to higher vaccine acceptance (41). In round 2, after the vaccination campaign, individuals may have felt safer and therefore were less fearful of getting infected. Likewise, after summer 2021, with the relaxation of restrictions and prevention measures, and with a perceived lower risk regarding Omicron infections, unvaccinated individuals may have felt less concerned, even after being in contact with infected individuals (40).

Additionally, participation in events, social groups, or trips was associated with decreased willingness to vaccinate through all four rounds. It has been reported that individuals who did not avoid contact with other people, did not keep minimum distance, or did not cover their mouth and nose in the public were more likely to be vaccine hesitant (7). People participating in events with other individuals may feel that COVID-19 is not a dangerous disease, perceive COVID-19 as low risk and therefore are less likely to get vaccinated (7, 8).

Moreover, the risk perception of a given health behavior or advice, in this case receiving a COVID-19 vaccination, can influence decision-making of individuals (42). People who think that they have higher risk of experiencing vaccine-related side effects may be more reluctant to receive a COVID-19 vaccine, even if they are worried about COVID-19 (42, 43). In combination with evolving dynamics and information about population groups at risk it could have contributed to higher vaccine hesitancy. A previous study in medical professionals in Poland indicated that low risk perception and lack of information about vaccines can make an individual resistant to persuasion about the importance of vaccination (44). The same study also pointed out the importance of accessibility and low cost in convincing people to get vaccinated. Even though accessibility was not a prevalent reason for reluctance in our study, it should be an important element of future vaccination campaigns. Vaccine knowledge and vaccine literacy can also impact willingness to vaccinate (42, 45). Those with higher level of vaccine literacy may be more willing to receive any vaccine than those with lower levels of vaccine literacy (42). It is possible that in our study those who remained hesitant toward vaccination may have lower overall vaccine literacy and knowledge about vaccine development and safety. Nevertheless, we did not assess perceptions toward vaccines in general in our study. Finally, mandatory vaccination, although successfully implemented for other viruses, may not have been beneficial for COVID-19 vaccination uptake (46). In the context of COVID-19, mandatory vaccinations were seen as limiting personal freedom and decision-making (46). In novel vaccines compulsory vaccinations may negatively influence vaccine uptake in the general population, where it has been shown that dialogue and detailed and targeted communication can be more beneficial in improving willingness to vaccinate (46). In Poland, COVID-19 vaccination certificates allowed more freedom to enter public spaces including restaurants and lifted the quarantine obligation. Even if not mandatory, these initial strategies could have also contributed to the decreasing trend in willingness to vaccinate that we observed in our study, especially once vaccine certificates were not needed.

Strengths

This was the largest nationally representative, repeated cross-sectional study conducted in Poland to date. It collected data at four different time points after the National COVID-19 Immunization Program was introduced and spanning three different epidemic waves related to Alfa, Delta, and Omicron variants. Therefore, we were able to capture changes in attitudes toward COVID-19 vaccination as pandemic conditions were changing. Furthermore, we were able to assess changes in associations of several factors with willingness to vaccinate during this dynamic period. Stratification according to age and population distribution of each administrative region in Poland facilitated representativeness of our study sample. Apart from the addition of few questions at subsequent rounds, the same set of variables were collected in each round. This enabled us to compare findings between rounds and thus capture the impact of emerging variants, including Omicron.

Limitations

Participants were not asked about the reasons for reluctance in round 1. Additionally, many participants did not provide a

specific reason for their reluctance to vaccinate ("other reason") and we were unable to further explore this response. However, we provided participants with several reasons in our questionnaire. Moreover, in rounds 2, 3, and 4 a subset of panel participants were included (independence of observations); however, bias is unlikely due to the dynamic changes in attitudes during the COVID-19 pandemic. Although we adjusted our analyses for several variables, we have not included all potential confounders. Therefore, residual confounding may still be present. Moreover, in our study, most participants were of Polish nationality, thus we were unable to examine willingness to vaccinate in other nationalities or ethnic minorities in Poland. Finally, it is possible that vaccinated and/or health-conscious participants were more willing to participate in the study and therefore our estimates may have been underestimated.

Conclusions

We observed a decline in willingness to vaccinate among unvaccinated and vaccinated participants. Concerns around side effects, safety, overall effectiveness and against COVID-19 variants were the most prevalent reasons for reluctance to vaccinate. Several factors were associated with willingness to vaccinate, with COVID-19 diagnosis, and participation in social activities being consistently associated with willingness to vaccinate in all rounds. The Omicron wave significantly influenced attitudes toward vaccination. This study underscores the critical role of public health messaging based on ongoing monitoring of attitudes and the need for constant health communication about COVID-19 vaccines. Future research should also examine the influence of misinformation on vaccine attitudes over time and how it influences different groups of people, especially vulnerable and vaccine resistant groups.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Bioethics Committee of the National Institute of Public Health NIH - National Research Institute (No. 5/2021 of 02/03/2021). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because data collection for this study took place during a telephone interview. Before the interview participants were asked whether they consented to participate in the study. No identifiable data were collected in this study.

Author contributions

EK developed the research proposal, conducted the statistical analysis, and wrote the manuscript. MR, MS, MC, and MS-T developed and contributed to the study protocol and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1235585/full#supplementary-material

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The increase in SARS-CoV-2 lineages during 2020–2022 in a state in the Brazilian Northeast is associated with a number of cases

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SARS-CoV-2 has caused a high number of deaths in several countries. In Brazil, there were 37 million confirmed cases of COVID-19 and 700,000 deaths caused by the disease. The population size and heterogeneity of the Brazilian population should be considered in epidemiological surveillance due to the varied tropism of the virus. As such, municipalities and states must be factored in for their unique specificities, such as socioeconomic conditions and population distribution. Here, we investigate the spatiotemporal dispersion of emerging SARS-CoV-2 lineages and their dynamics in each microregion from Sergipe state, northeastern Brazil, in the first 3 years of the pandemic. We analyzed 586 genomes sequenced between March 2020 and November 2022 extracted from the GISAID database. Phylogenetic analyses were carried out for each data set to reconstruct evolutionary history. Finally, the existence of a correlation between the number of lineages and infection cases by SARS-CoV-2 was evaluated. Aracaju, the largest city in northeastern Brazil, had the highest number of samples sequenced. This represented 54.6% (320) of the genomes, and consequently, the largest number of lineages identified. Studies also analyzed the relationship between mean lineage distributions and mean monthly infections, daily cases, daily deaths, and hospitalizations of vaccinated and unvaccinated patients. For this, a correlation matrix was created. Results revealed that the increase in the average number of SARS-CoV-2 variants was related to the average number of SARS-CoV-2 cases in both unvaccinated and vaccinated individuals. Thus, our data indicate that it is necessary to maintain epidemiological surveillance, especially in capital cities, since they have a high rate of circulation of resident and non-resident inhabitants, which contributes to the dynamics of the virus.

KEYWORDS

phylogenetic analysis, SARS-CoV-2 genomes, epidemiology, genomic surveillance, Sergipe

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China in late 2019 and rapidly spread across the globe, leading the World Health Organization (WHO) to declare a pandemic state on 11 March 2020 (1, 2). The virus has been widespread, causing waves of infections in almost all regions of the world (3). The first cases were confirmed in the state of São Paulo in February 2020. After that, actions were taken by the Ministry of Health in order to contain the emerging epidemic (4). As of today, 37.9 million cases in Brazil have resulted in 706,531 deaths, representing a mortality rate of 441.3 individuals per 100,000 inhabitants (accessed on 28 October 2023; available in https://covid.saude.gov.br//). This high mortality rate is related to the lack of a national policy against the disease, the increasing population mobility, especially in large urban centers, the return of face-to-face work activities, difficulties in implementing individual and community preventive measures to reduce the spread of COVID-19, and delays in vaccination have contributed to the emergence and spread of SARS-CoV-2 variants of concern (VOCs) across the country over time (5).

In Brazil, the pandemic was characterized by the co-circulation of multiple variants over time (6). The emergence of new variants was directly related to adaptive mutations in the viral genome that modified the pathogenic potential of SARS-CoV-2. A single amino acid change can dramatically affect a virus's ability to evade the immune system and complicate the clinical status of infected individuals (7). Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and omicron (B.1.1.529) lineages were important variants associated with greater transmissibility or virulence, reduced neutralization by antibodies obtained through natural infection or vaccination, ability to avoid detection, and/or decreased therapeutic or vaccination efficacy (8).

Monitoring SARS-CoV-2 was possible due to recent technological and scientific advances in genome sequencing and bioinformatics tools, allowing almost real-time genomic surveillance and tracking the emergence and replacement dynamics of variant emergence and prevalence among populations (9). Several studies focusing on genomic surveillance have provided crucial information to understand the dynamics of SARS-CoV-2 lineages in the states or regions of Brazil due to the large differences between inter- and intra-state population sizes, concentration, and dynamics of human movement (10, 11). This proposal has been shown to be relevant to determine the spread of the virus based on the specific characteristics of the state in a refined resolution (11). In the Brazilian Northeast, 7.4 million cases of COVID-19 and 136,000 deaths have already been reported. Bahia, Ceará, and Pernambuco are the states with the highest incidence of cases and deaths in the region. In Sergipe state, 363,329 individuals were diagnosed with COVID-19, resulting in 6,539 deaths (accessed on 28 October 2023; available at https://covid.saude.gov.br//). At the moment, a single study has been identified in the literature related to genomic surveillance in Sergipe, and this analyzed genomes sequenced between March 2020 and February 2021 (5). This demonstrates the necessity to implement new research aimed at understanding the effects of the pandemic.

Therefore, this study aimed to assess the dynamics of SARS-CoV-2 variants from 2020 to 2022 in the state of Sergipe within Brazil. Knowledge gained would identify viral evolutionary patterns and behavior as it relates to epidemiological impacts.

Methods

Study area

Sergipe is located in northeastern Brazil and has a land area of 21,938,188 km^2 and an estimated population of 2,338,474 inhabitants. The state has 75 municipalities and is divided into 13 microregions (Agreste de Itabaiana, Agreste de Lagarto, Aracaju, Baixo Cotinguiba, Boquim, Carira, Cotinguiba, Estância, Japaratuba, Nossa Senhora das Dores, Propriá, Sergipana do Sertão do São Francisco, and Tobias Barreto) (Figure 1). The microregion of Aracaju is made up of the capital (Aracaju), and the municipalities of Barra dos Coqueiros, Nossa Senhora do Socorro, and São Cristóvão, forming the metropolitan region of Aracaju, which represents approximately 36% of the state population[1].

Data collection

Full-length SARS-CoV-2 genomes from February 2020 to November 2022 were obtained from the GISAID database[2]. Only complete genomes and complete collection data were used. The sequences were evaluated individually, considering the lineage, which was determined by the Pangolin software[3], municipality, and collection date. Soon after, the genomes were separated by year, giving rise to three data sets. The Circos program (12) was used to visualize the distribution of the genomes by strains and municipalities.

In order to correlate the number of lineages of SARS-CoV-2 with the average of infections by months, daily cases, daily deaths, and admissions of vaccinated and unvaccinated patients, the data were uploaded to a cross-country database of COVID-19[4] (13, 14). The Pearson correlation test was performed using a native stats (V.4.0.3) package available in R software, and the grouped stacked bars with the abundance of lineage between months were performed and represented using the ggplot2 package (15, 16) and the correlation matrix was performed using *corrplot* package (17). All the differences with p-values <0.05 were considered statistically significant.

Phylogenetic analyses

Multiple sequence alignment was carried out using MAFFT v.7 with auto and add fragments parameters (18). The sequence from Wuhan-Hu-1 (NC_045512.2) was then added as an outgroup. Subsequently, the maximum likelihood (ML) phylogenetic trees were built using IQ-TREE v2.1.2 (19). The nucleotide substitution models TN + F, GTR + F + I + I + R4, and TIM + F + I + I + R3 were selected using ModelFinder in IQ-TREE2 v2.1.2 for the SARS-CoV-2 genomes of 2020, 2021, and 2022, respectively (20). Clade support was estimated using 1,000 replicates of bootstrap. The tree was visualized and edited using the iTOL v.4 tool (21). The haplotype network was created with PopART software version 1.7

1 https://censo2022.ibge.gov.br/
2 https://www.gisaid.org/
3 https://cov-lineages.org/resources/pangolin.html
4 https://globalepidemics.org/

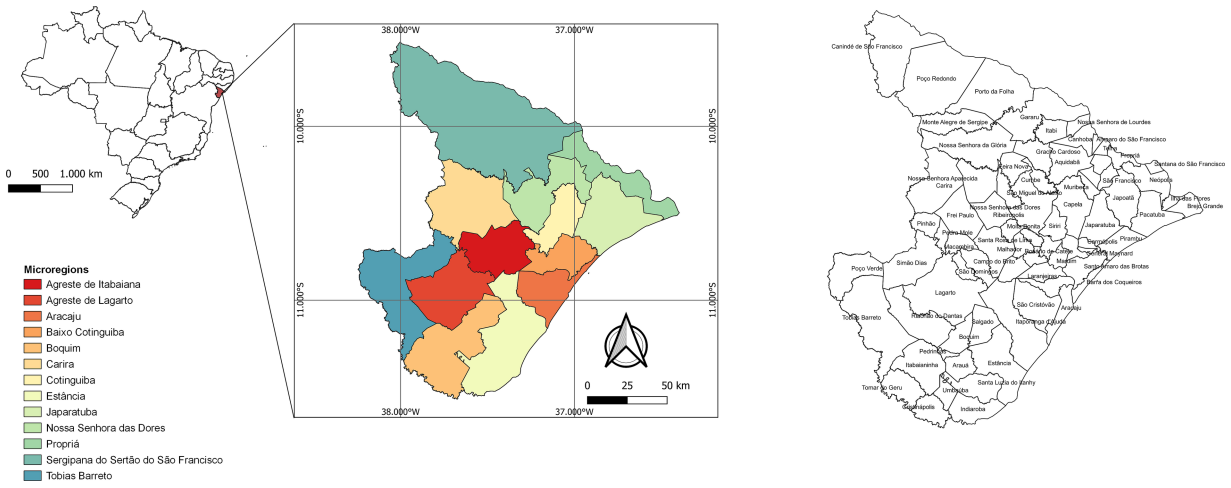

FIGURE 1
State map of Brazil with emphasis on Sergipe state, showing the 13 microregions.

(22) using the median-joining method to identify the existence of shared haplotypes.

Spatial analysis

The maps to represent the spatial distribution of SARS-CoV-2 lineages were constructed using the QGIS software version 3.18.2, with the cartographic projection corresponding to the Universal Reference System SIRGAS 2000. The cartographic projection used corresponded to the Universal Transverse Mercator (UTM) system, Terra Datum horizontal model (SIRGAS 2000) to segment by municipalities and states were collected from the databases of the Brazilian Institute of Geography and Statistics (IBGE).

Results

Genomic surveillance of SARS-CoV-2 variants in Sergipe

For this analysis, 586 SARS-CoV-2 viral genomes were evaluated and classified into 36 variant lineages (Figure 2). Sequences have been distributed in 47 municipalities, representing 62.7% of the total. Most of the genomes obtained from the GISAID database have their origin in the Aracaju microregion, as can be seen in Table 1.

In 2020, five lineages were detected circulating in Sergipe, B.1 (11 sequences, 36.7%) was the most frequent, followed by B.1.1 (6 sequences, 20%), B.1.1.33 (6 sequences, 20%), B.1.1.28 (4 sequences, 13.3%), and B.1.212 (3 sequences, 10%) (Figure 2). A total of 30 genomes were available on the GISAID database. In total, 19 of those 30 genomes were related to samples from Aracaju (Supplementary Figure S1). Genomic sequences have also been observed in 10 other municipalities (Figure 3). At first, B.1 was identified in the state on 12 March 2020 during the first wave. This sample belongs to an individual who resided in Aracaju with a travel history to Europe (Spain).

For 2021, 406 sequences were used to create the datasets and subsequently classified into 16 viral variant lineages. In total, 212 samples were identified as the P.1 gamma variant, representing approximately 52.2% (Figure 2). Initially, the circulation of this variant was registered on 17 January 2021 in the municipality of Aracaju during the second wave. Delta sequences have been registered in Sergipe between January and September. P.1.2 (56 sequences, 13.8%) and P.2 Zeta variant (50 sequences, 12.3%) were also highly represented (Figure 4). This variant was predominant in infection cases from September and December. A total of 57 genomes of the AY.* lineages were found in the GISAID database. This is distributed in four strains (AY.34.1.1, AY.99.1, AY.99.2, and AY.101). AY.99.2 (45 sequences, 11.1%) was prevalent during this period. Lineages AY.34.1.1, AY.99.1, and AY.101 represented approximately 2.9% of the total genomes (Figure 2). All other strains identified in 2021 represent approximately 10.6% (43 sequences). Aracaju was the municipality with the highest number of strains circulating when compared to other localities (Figure 4). Lineages were also identified in 37 other cities (Supplementary Figure S2).

An alignment with 150 genomes was created using the genomes of 2022, and it was possible to identify 18 lineages distributed in 27 municipalities (Figure 5) (Supplementary Figure S3). In January, nine sublineages of the Omicron variant were identified as circulating. The first variant sample detected was on 3 January 2022. The lineage BA.1.1 (33 sequences, 22%) was the most frequent during the third wave, followed by BA.1 (28 sequences, 18.7%), BA.5.2 (21 sequences, 14%), and BA.5.2.1 (18 sequences, 12%). All other lineages identified represented approximately 33.3% (50 sequences) (Figure 2).

Evolutionary analysis of SARS-CoV-2 lineages

The maximum likelihood phylogenetic tree was constructed to confirm the SARS-CoV-2 variant classification that circulated between February 2020 and November 2022 in the state of Sergipe. Considering the sequences from 2020, the phylogenetic analysis suggested five distinct well-supported groups (B.1, B.1.1, B.1.212, B.1.1.28, and

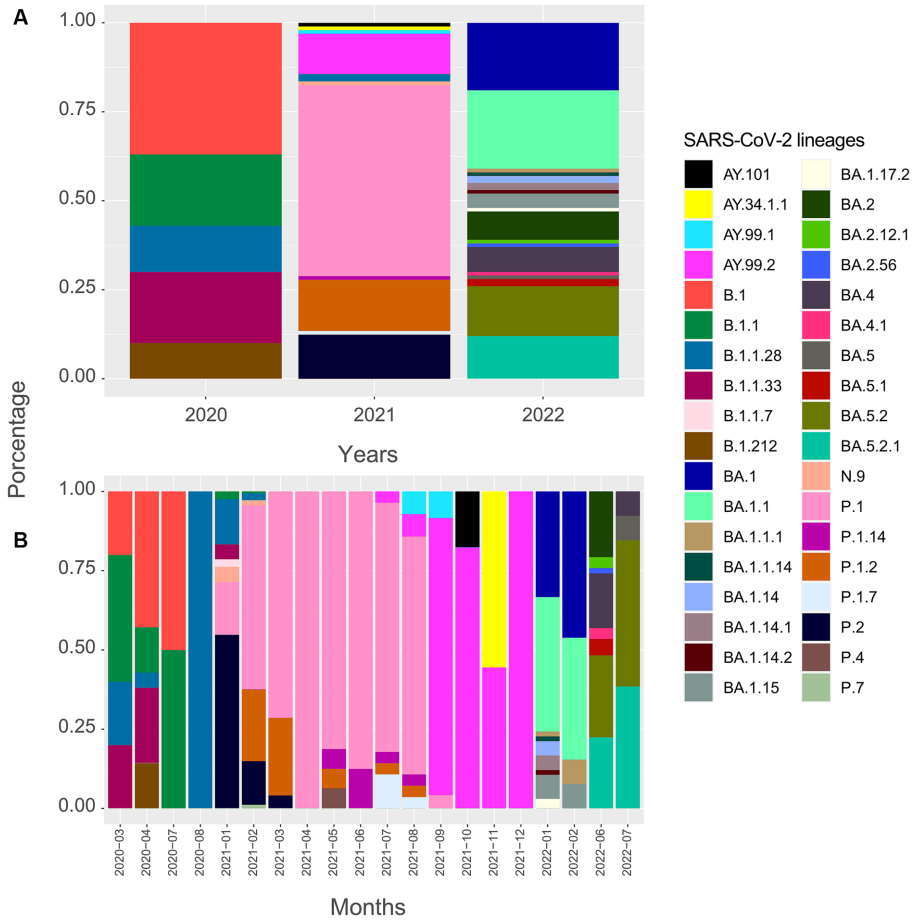

FIGURE 2
Timeline of genomes sequenced for the SARS-CoV-2 virus in Sergipe state, Brazil (SARS-CoV-2 lineages in the state from March 2020 to November 2022, obtained from GISAID database).

B.1.1.33) (Figure 6). The haplotype network has been constructed with the purpose of characterizing the ancestral relationships maintained between the lineages. Five well-defined clades (B.1, B.1.1, B.1.212, B.1.1.28, and B.1.1.33) were identified. Notably, haplotype sharing was not observed among the sequences from these different strains (Figure 7).

For the genomes from 2021, the ML tree revealed five main well-supported clades. One clade was composed only of the delta variant. N.9, B.1.1.28, and P.2 lineages were divided into different clades with significant support values (Figure 8). B.1.1.28 was identified as a common ancestor of P.1 and P.2. A clade represented by sublineages relative to P.1 (P.1.7, P.1.14, and P.1.2) was observed. However, sequences belonging to lineages B.1.1, B.1.1.33, and P.4 have not demonstrated significant bootstrap values. P.7 clade showed high support value, and its genetic pattern is associated with the P.2 lineage. The haplotype network revealed five heterogeneous clusters, where P.1 was associated with P.1.2, P.1.14, and P.1.7. Another cluster was observed with P.2 and P.7 lineages. N.9, B.1.1.28, and all AY.* remained isolated in the phylogenetic tree (Figure 9).

Analyzing the genomes from 2022, it was possible to observe differences, revealing two distinct clades (Figure 10); both groups were significantly supported. A clade was formed by genomes from lineages BA.5, BA.5.1, BA.5.2, BA.5.2.1, BA.2, BA.2.12.1, BA.2.56, BA.4, and BA.4.1, and another clade was formed by genomes from lineages BA.1, BA.1.1, BA.1.14, BA.1.14.1, BA.1.14.2, BA.1.15, BA.1.17.2, BA.1.1.1, and BA.1.1.14. The haplotype network also suggested two clusters. One cluster was composed by BA.5, BA.5.1, BA.5.2, BA.5.2.1, BA.2, BA.2.12.1, BA.2.56, BA.4, and BA.4.1. On the other hand, the other cluster was formed by lineages BA.1, BA.1.1, BA.1.14, BA.1.14.1, BA.1.14.2, BA.1.15, BA.1.17.2, BA.1.1.1, and BA.1.1.14. In addition, it was observed genomes from different lineages sharing haplotypes, such as BA.1.1|10,322,472, BA.1.1|10,322,464, BA.1.14|10,322,474, BA.5.2.1|15,279,654, BA.5.2.1|15,202,013, BA.5.2.1|15,802,440, and BA.5.2.1|15,802,439 (Figure 11). In the BA.1.1|10,322,472 genome, genetic patterns associated with BA.1 and BA.1.1 have been identified, suggesting the maintenance of ancestral relationship.

Correlation analysis of SARS-CoV-2 lineages and infection cases in Sergipe

A correlation matrix analysis was employed to examine the relationship between the average distributions of viral lineages and the average number of individuals infected, as indicated by registered cases reported by global epidemics (Figure 12). Furthermore, we explored relationships among the averages of hospital admissions

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TABLE 1 Distribution of SARS-CoV-2 genomes obtained from the GISAID database divided by microregions of Sergipe.

Microregions	Municipalities	2020	2021	2022	%
Aracaju	Aracaju	19	252	49	69.6%
	Barra dos Coqueiros	02	11	22	
	São Cristovão	–	02	12	
	Nossa Senhora do Socorro	01	25	13	
Estância	Estância	01	05	–	2.6%
	Itaporanga D'ajuda	01	07	01	
Agreste de Lagarto	Lagarto	01	01	05	2.2%
	Riachão do Dantas	–	02	04	
Baixo Cotinguiba	Laranjeiras	01	04	–	1.9%
	Riachuelo	01	01	–	
	Maruim	–	01	–	
	Santo Amaro das Brotas	–	01	–	
	Carmópolis	–	–	02	
Propriá	Telha	01	01	–	1.9%
	Amparo de São Francisco	–	01	–	
	Canhoba	–	01	–	
	Cedro de São João	–	03	–	
	Nossa Senhora de Lourdes	–	01	01	
	Propriá	–	02	–	
Boquim	Tomar do Geru	01	02	–	4.4%
	Boquim	–	06	02	
	Cristinápolis	–	02	–	
	Itabaianinha	–	06	–	
	Salgado	–	03	03	
	Umbaúba	–	–	01	
Nossa Senhora das Dores	Aquidabã	–	02	–	0.5%
	Nossa Senhora das Dores	–	–	01	
Sergipana do Sertão do São Francisco	Canindé de São Francisco	–	14	–	4.8%
	Nossa Senhora da Glória	01	05	–	
	Gararu	–	01	–	
	Monte Alegre de Sergipe	–	03	–	
	Porto da Folha	–	04	–	
Cotinguiba	Capela	–	04	03	1.9%
	Divina Pastora	–	–	04	
Carira	Carira	–	01	–	3.4%
	Frei Paulo	–	03	03	
	Ribeirópolis	–	08	04	
	Pinhão	–	–	01	
Agreste de Itabaiana	Itabaiana	–	04	09	2.9%
	Areia Branca	–	–	02	
	Macambira	–	–	01	
	Malhador	–	–	01	
Tobias Barreto	Simão Dias	–	11	01	3.4%
	Tobias Barreto	–	04	02	
	Poço Verde	–	01	01	
Japaratuba	Pirambu	–	01	–	0.5%
	Japaratuba	–	–	02	
Total		30	406	150	

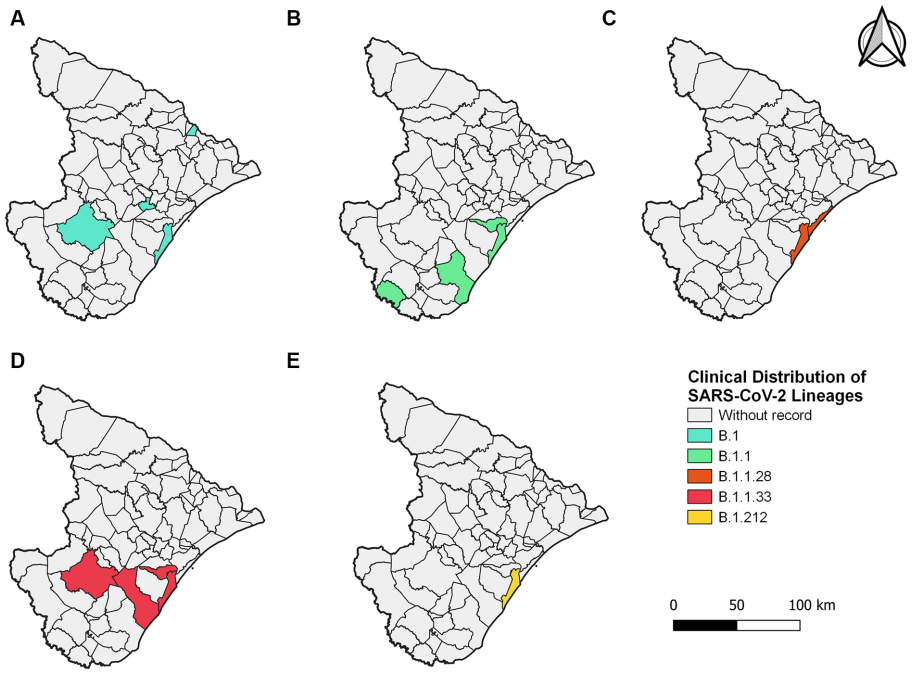

FIGURE 3
Sergipe state map showing the clinical distribution of the SARS-CoV-2 lineages in 2020 by municipality. Colors represent the municipality and lineages downloaded from the GISAID database.

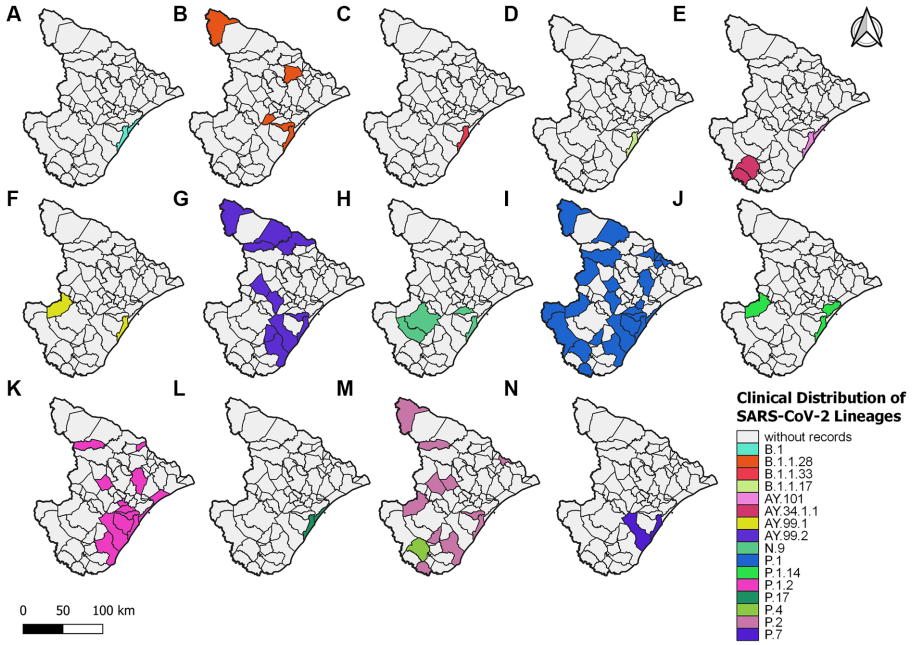

FIGURE 4
Sergipe state map showing the clinical distribution of the SARS-CoV-2 lineages in 2021 by municipality. Colors represent the municipality and lineages downloaded from the GISAID database.

in vaccinated and unvaccinated patients as well as the averages of daily deaths and daily cases (Figure 12). Notably, the averages of registered cases exhibited a correlation cluster among all reported cases as well as between vaccinated and unvaccinated patients. Additionally, the averages of daily cases and deaths showed a direct correlation. Hospital admissions in vaccinated patients were associated with clusters of registered cases, while hospital admissions in unvaccinated patients were linked to daily deaths and daily cases. The observed increase in the number of variant lineages during the study period was directly correlated with the averages of infections.

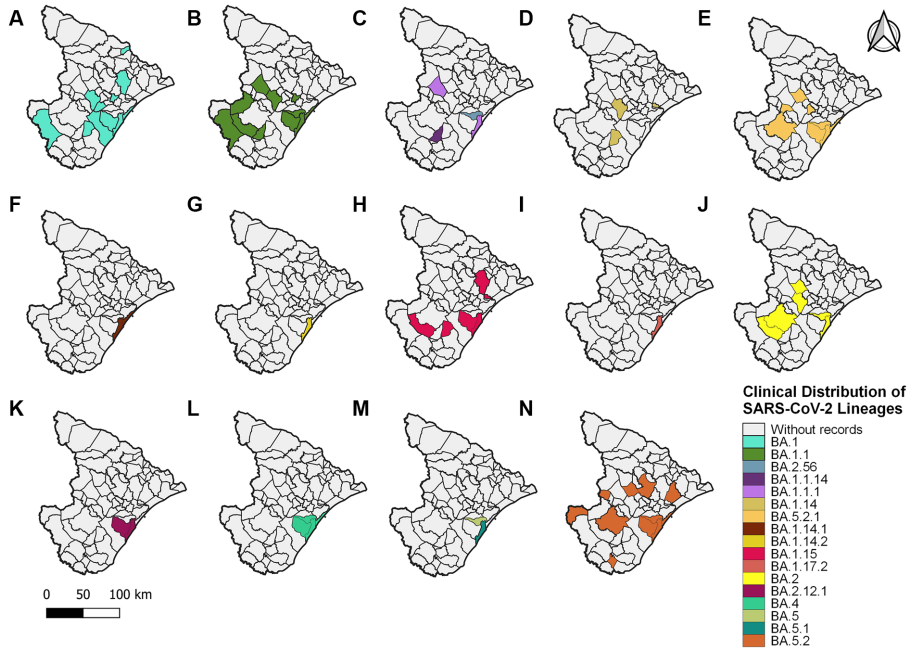

FIGURE 5
Sergipe state map showing the clinical distribution of the SARS-CoV-2 lineages in 2022 by municipality. Colors represent the municipality and lineages downloaded from the GISAID database.

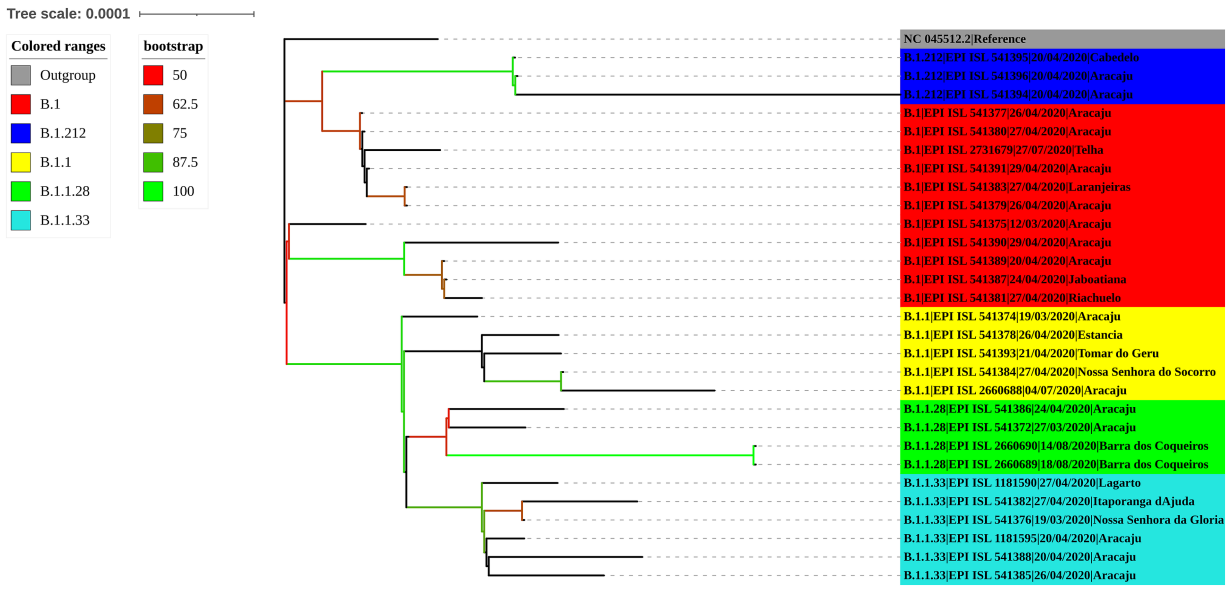

FIGURE 6
Maximum likelihood tree of 30 SARS-CoV-2 whole-genome sequences available at the GISAID database using the TN + F evolutive model. The colors assigned to the branches are related to their respective lineages and the samples are labeled according to their sampling dates and virus lineages.

Discussion

This is a pioneering study of the state of Sergipe. The evolutionary history of circulating SARS-CoV-2 genomes over the last 3 years was reconstructed using phylogenetic analyses based on ML and the median-joining method. Our data support that B.1 was the first lineage detected in Sergipe (as recorded in Aracaju on 12 March 2020). However, Gurgel et al. (23) report in their study that the first case of COVID-19 in the state of Sergipe may have occurred a few months earlier, as samples from asymptomatic individuals sent for blood tests between the months of January and April 2020 by reasons unrelated to COVID-19 showed the presence of SARS-CoV-2 immunoglobulins (IgM and IgG) before the notification of clinical cases in the state. The country registered its first COVID-19 case in

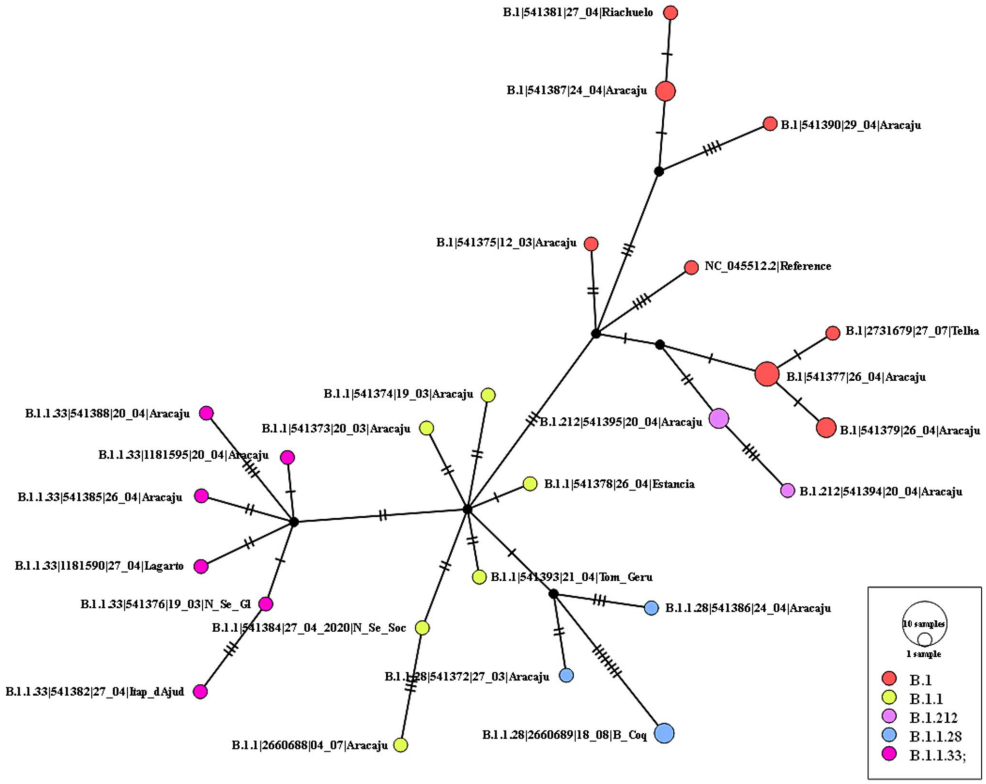

FIGURE 7
Haplotype network, obtained with PopART software, showing relationships among haplotypes of the SARS-CoV-2 genomes available in 2020. Each circle represents one haplotype. Diameters of the circles correspond to the frequencies of the respective haplotypes. The numbers of dashes display mutational steps (each dash stands for one single nucleotide mutation). Small black circles represent hypothetical (missing) haplotypes.

late February in São Paulo (4). In Bahia, the first confirmed SARS-CoV-2 infections occurred on 28 February, being the first case in the Northeast region[5].

The lineages B.1, B.1.1, B.1.1.33, B.1.1.28, and B.1.212 were prevalent until mid-August 2020. In an analysis carried out by Dos Santos et al. (5), B.1 (58.5%), B.1.1.33 (17.1%), B.1.1.119 (12.2%), B.1.1.28 (9.8%), and B.1.212 (2.4%) were dominant from March to August 2020 in Sergipe. In late 2020, the variants zeta (P.2) (24) and gamma (P.1) (25), descendants of lineage variants B.1.1.28, emerged and were associated with the second phase of the pandemic. P.1 was identified in the state of Amazonas in mid-December 2020, with a proposed emergence around November (25, 26).

P.1 was dominant in infection cases by SARS-CoV-2, having their circulation on 17 January 2021. Some studies proposed that the emergence of three mutations E484K, N501Y, and K417T in the Spike protein allowed the virus to escape from the host immune response (27–29). In mid-January 2021, samples from 11 suspected cases and their contact reporting a travel history to/from Amazonas state were screened at the Central Laboratory of Health of the Bahia state (LACEN-BA). Genetic evidence has confirmed for the first time the circulation of the P.1 in the Brazilian Northeast (30). The study conducted by Dos Santos et al. (5) also confirms that P.1 circulated in Aracaju (Sergipe) on 17 January 2021 in a sample belonging to a resident from the city of Manaus (Amazonas) who traveled to Sergipe to visit his family (5).

Our results from the evolutionary analysis showed that B.1.1.28 is a common ancestor of P.1 and P.2. In the studies conducted by Varela et al. (31) and Harvey et al. (32), it was indicated that P.1 and P.2 descend from B.1.1.28 although they have different times of appearance and share the S: E484K mutation. A cluster formed by P.1, P.1.1, P.1.2, and P.1.7 demonstrates the ancestral relationship between these lineages, as reported by Varela et al. (31) and Machado et al. (11). In the present study, a shared ancestry between P.2 and P.7 was also observed similar to that suggested by Lamarca et al. (33).

Assessing the results, it was suggested that AY.99.2 (11.1%) became dominant in cases from September to December 2021. There are signs that the delta variant emerged in October 2020 on the Asian continent as has been classified by WHO (33). In Brazil, the first community-sustained transmission chains of the delta variant were registered in June 2021 in the state of Rio de Janeiro (34), and it has been widely detected in other Brazilian states over time (see footnote 5). Among the delta variants, the AY.99.2 was the most dominant, reaching 58% of all sublineages sampled during the period (35). Some evidence demonstrate that AY.99.2 emerged in Brazil; the first SARS-CoV-2 genomes from this lineage available in the GISAID database are from samples collected in April 2021 in the northeastern state of Ceará (36). Studies have identified mutations in the spike protein of the delta sublineages found in Brazil, and the most common mutations

5 www.genomahcov.fiocruz.br/gisaid/

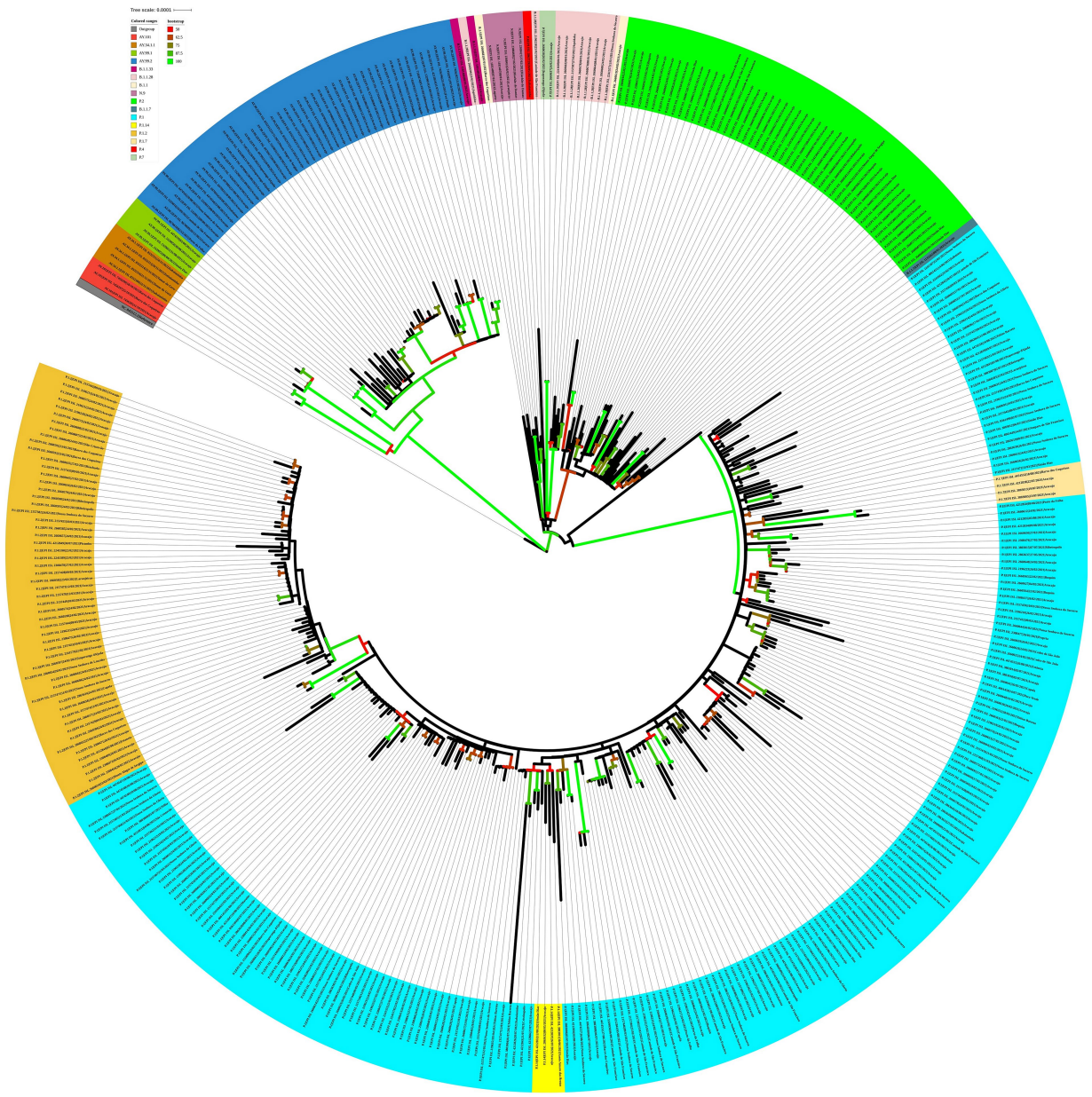

FIGURE 8
Maximum likelihood tree of 406 SARS-CoV-2 whole-genome sequences available at the GISAID database using the GTR + F + I + I + R4 evolutive model. The colors assigned to the branches are related to their respective lineages and the samples are labeled according to their sampling dates and virus lineages.

mentioned are T19R, T95I, E156G, DEL157/158, L452R, T478K, D614G, Q677H, P681R, D950N, V1104L, and L1265F[6]. Some of these mutations can be related to viral fitness advantages such as enhanced viral entry, pathogenesis, and immune escape (37, 38). Despite that, the number of hospitalizations declined from 6.9% (January to June 2021) to 3.6% (July to December 2021) during the community transmission of the delta variant with the progression of vaccination in the second half of 2021 in Sergipe (39).

The phylogenetic tree revealed that the samples of the delta variant formed a monophyletic clade. In an analysis using the neighbor-joining method with genomes from the delta lineage, a compatible structuring with a monophyletic clade was shown, and the omicron variant emerged from it (40).

In this study, the lineage BA.1.1 was reported as most frequent, followed by BA.1, BA.5.2, and BA.5.2.1. Genomic surveillance detected that in February 2022, the omicron variant was majority; 99.8% of the samples analyzed around the country being positive for the variant (see footnote 5). From January to September, BA.1 (4,253 genomes) and BA.1.1 (2,521 genomes) were also prevalent in infection cases by SARS-CoV-2 in the northeast Brazil (see footnote 5). Since the beginning of the pandemic, the SARS-CoV-2

6 https://outbreak.info/compare-lineages

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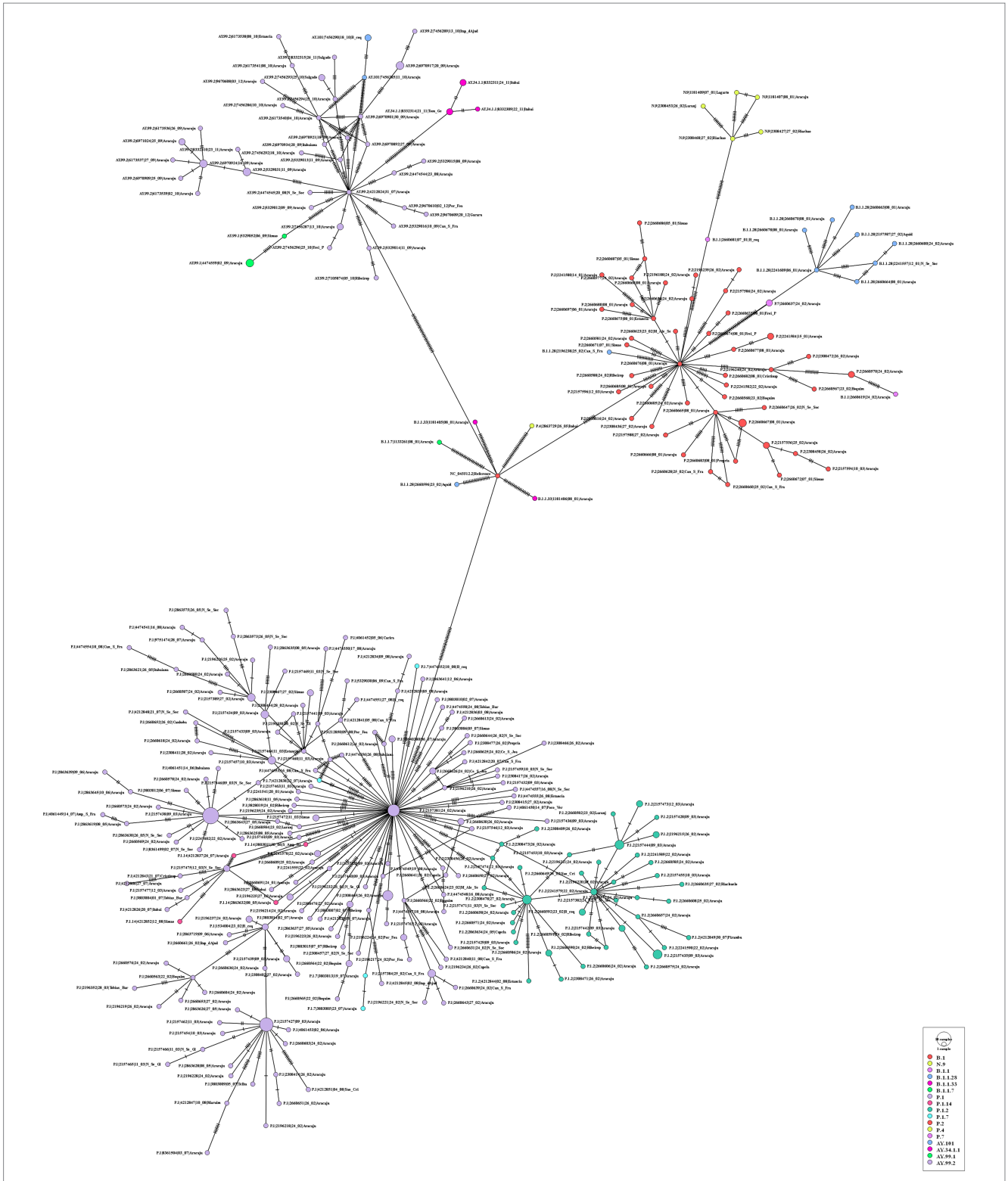

FIGURE 9
Haplotype network, obtained with PopART software, showing relationships among haplotypes of the SARS-CoV-2 genomes available in 2021. Each circle represents one haplotype. Diameters of the circles correspond to the frequencies of the respective haplotypes. The numbers of dashes display mutational steps (each dash stands for one single nucleotide mutation). Small black circles represent hypothetical (missing) haplotypes.

genome has been rapidly evolving. This is mostly due to the inherent polymerase mistakes and host immune selection factors (32). The omicron variant is the most mutated variant containing more than 60 mutations in its genome. In total, 32 of these mutations lie within the receptor binding domain (RBD) of the spike protein (41, 42). The large number of mutations associated with the spike RBD domain can be related to infectivity rates, high transmission capacity, and rapid dispersal potential (26). Among

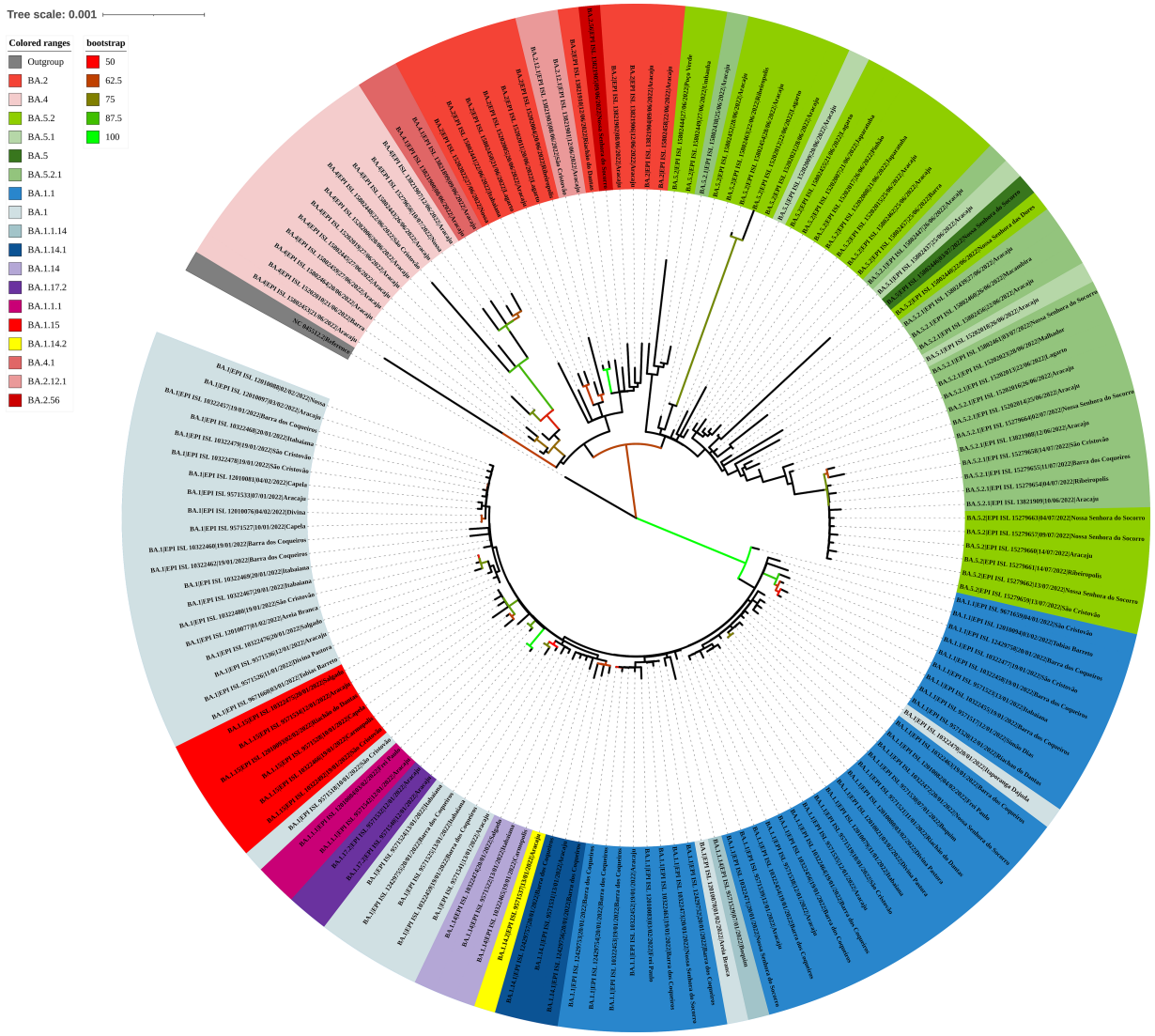

FIGURE 10
Maximum likelihood tree of 150 SARS-CoV-2 whole-genome sequences available at the GISAID database using the TIM + F + I + I + R3 evolutive model. The colors assigned to the branches are related to their respective lineages and the samples are labeled according to their sampling dates and virus lineages.

the mutations identified in the omicron variants, 14 are exclusive and found in all the omicron variants (43).

Our phylogenetic tree for omicron VOC suggests the existence of two main clades, one composed of the sublineages linked to BA.1 and the other associated with BA.2, BA.4, and BA.5 variants. In a study developed by Veneziano et al. (44) with Omicron SARS-CoV-2 genomes in Italy, a group composed of BA.1 and another by BA.2, BA.4, and BA.5 lineages was identified. Six mutations have been identified in all the omicron variants, excluding omicron BA.1: Del24-26, V213G, T376A, S371F, D405N, and R408S (43). These mutations may have contributed to the structure of the clades of the phylogenetic tree and also in the haplotype network, as observed in other organisms (45, 46). Notably, our study presents a limitation due to the fact that in some months of 2020 and 2022, there were no records of SARS-CoV-2 genomes in the GISAID database. However, there is agreement between our genomic surveillance results and those observed in other states of Brazil.

Finally, the increase in the average number of SARS-CoV-2 lineages during the studied periods is related to the average number of infections in both unvaccinated and vaccinated individuals. Tarkowski et al. (47) demonstrated that vaccinated individuals presented higher levels of IgG against viral proteins of spike protein-1 (S1) and receptor-binding domain (RBD), which resulted in a better immune response to B.1 and P.1 variants although immune activation is less noticeable in response to the B.1.617.2 variant. A similar study revealed differences in the efficiency of humoral activity in vaccinated individuals against B.1.617.1, B.1.617.2, B.1.351, and P.1 lineages due to mutations in the spike protein (S) (48). Unvaccinated individuals are intrinsically associated with daily cases and deaths. Martins-Filho et al. (39) studied the dynamics of hospitalizations and the predominance of delta and omicron variants in the Northeast of Brazil and found that during the circulation of the delta variant (July to December 2021), the majority of deaths occurred in people who

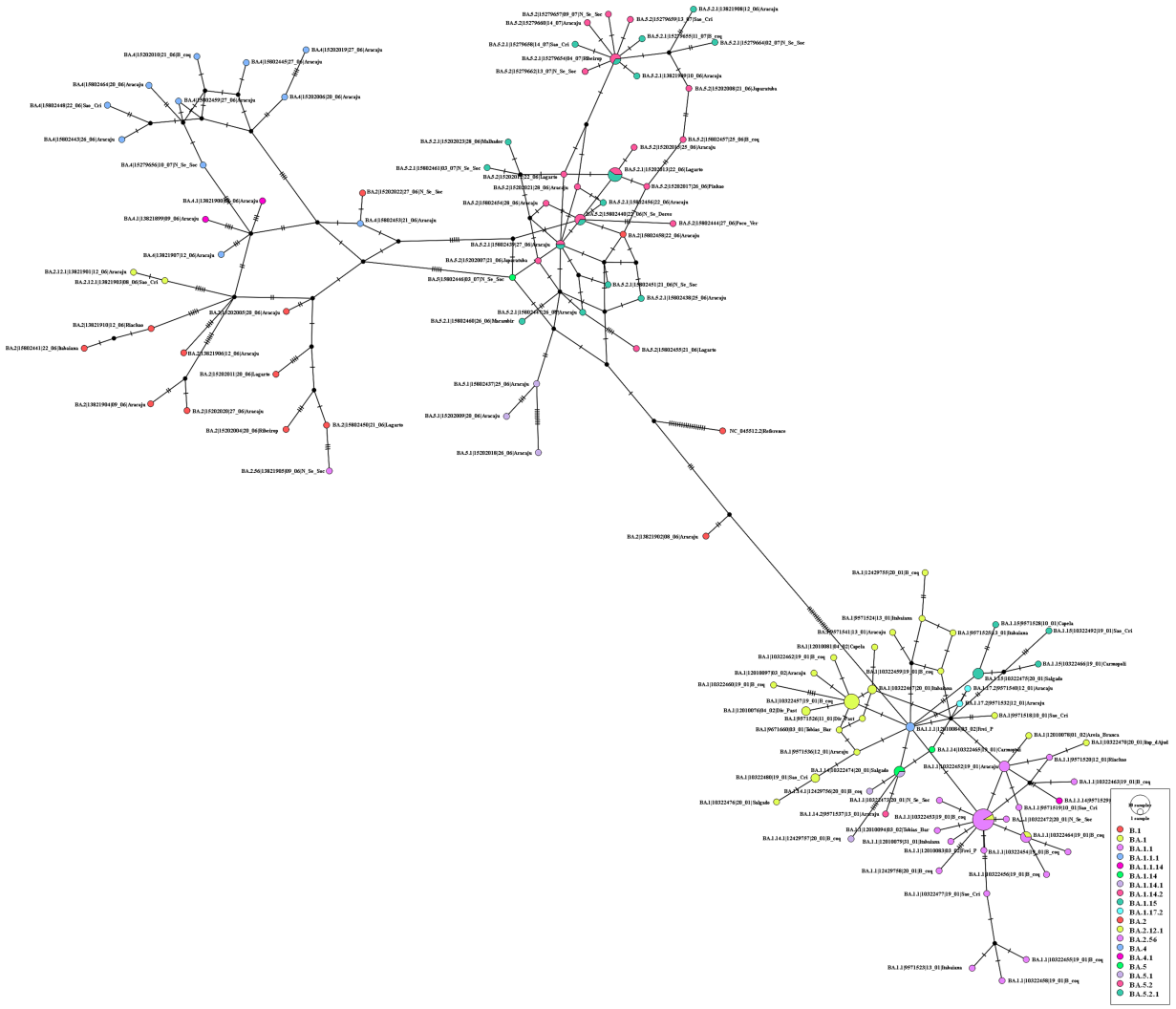

FIGURE 11
Haplotype network, obtained with PopART software, showing relationships among haplotypes of the SARS-CoV-2 genomes available in 2022. Each circle represents one haplotype. Diameters of the circles correspond to the frequencies of the respective haplotypes. The numbers of dashes display mutational steps (each dash stands for one single nucleotide mutation). Small black circles represent hypothetical (missing) haplotypes.

were not vaccinated or who had not completed the vaccination schedule. Furthermore, in 2020, vaccines were scarce, with high hospitalization rates (46% of the population with active infection) (39).

Conclusion

Our data suggest a correlation between the increase in the mean number of variant lineage strains and the mean number of infections in unvaccinated and vaccinated individuals. It is important to note that 3 years after the beginning of the SARS-CoV-2 pandemic, and despite the availability of several vaccines for 2 years, the restrictive measures to contain SARS-CoV-2 spreading were met with several challenges among most countries. In particular, the recent variants are generating new outbreaks of infection, even in countries where the level of vaccinations is high. However, it becomes necessary for continuous monitoring of the most predominant SARS-CoV-2 lineages as well as their specific dynamic and processes of evolution. Therefore, this knowledge gain and continual analysis of variant lineages is imperative for epidemiologists to define public health measures, perform adequate diagnostic tests, and strategically employ vaccines (49).

Despite the number of positive cases of COVID-19 in Sergipe, these did not have minimum values to be submitted for genetic sequencing; and we were unable to establish a stratified correlation between the number of lineages and the severity of COVID-19 cases in both vaccinated and unvaccinated individuals. This correlation could have demonstrated how lineage variability impacts the severity of infections.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

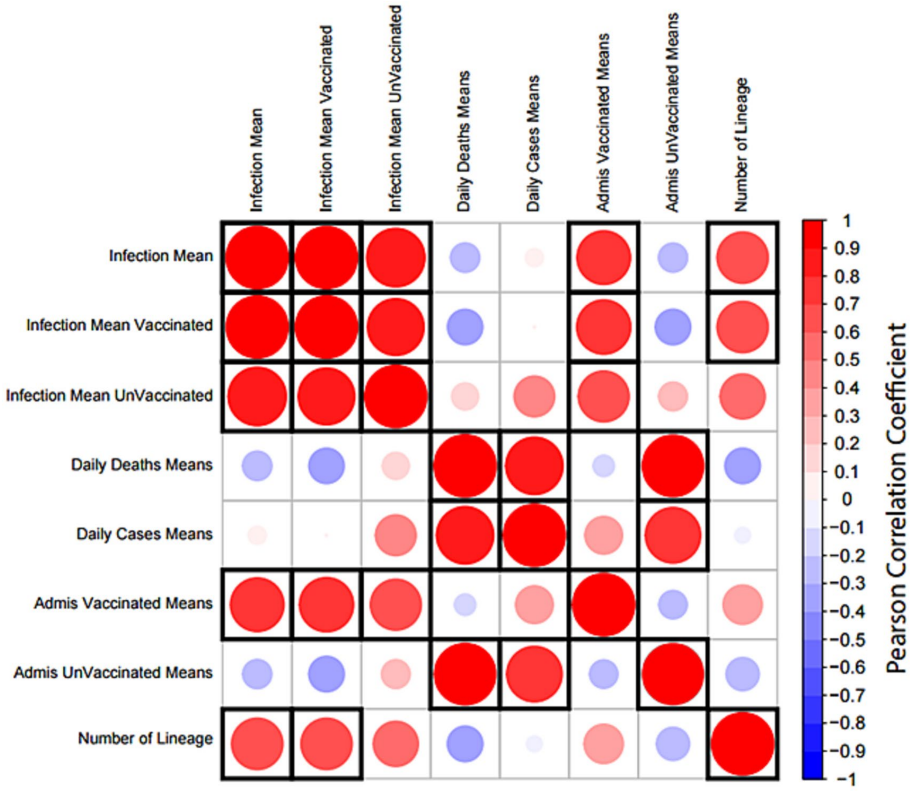

FIGURE 12
Correlation matrix analysis of epidemiological data and SARS-CoV-19 lineages. The distribution means of lineage and epidemiological data per month were obtained from a cross-country COVID-19 database. All variables were tested for correlation using the Pearson correlation test. The size and color of each circle indicate the strength of the correlation coefficient; red indicates a positive correlation and blue indicates a negative correlation. A significant correlation (p-value <0.05) is indicated by a black frame. The matrix was created using the corrplot package in R.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

MF and VQ: conceptualization. MF, KF, LS, FN, and JR: investigation. MF, KF, and ER: methodology. MF and KF: formal analysis. MF: project administration. MF and TM: writing – original draft. MF, TM, ML, MA, SS, KF, JR, LS, and CS: writing – review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1222152/full#supplementary-material

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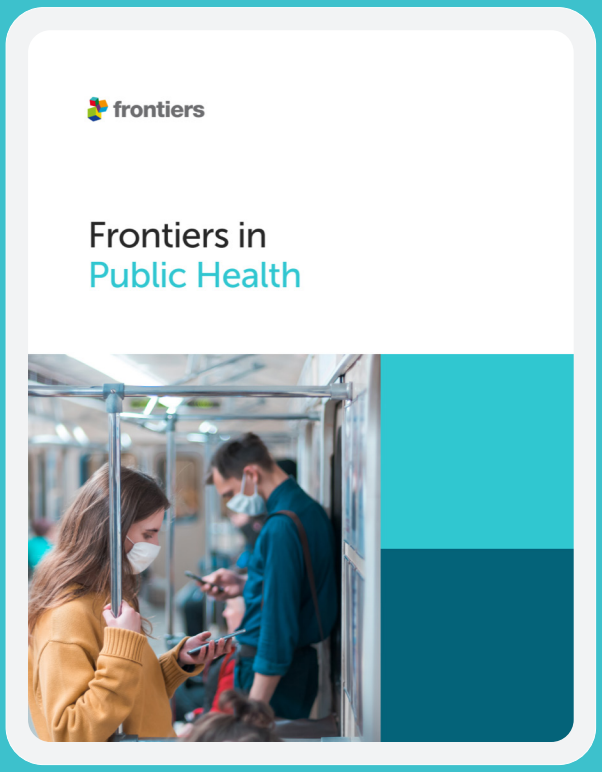

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