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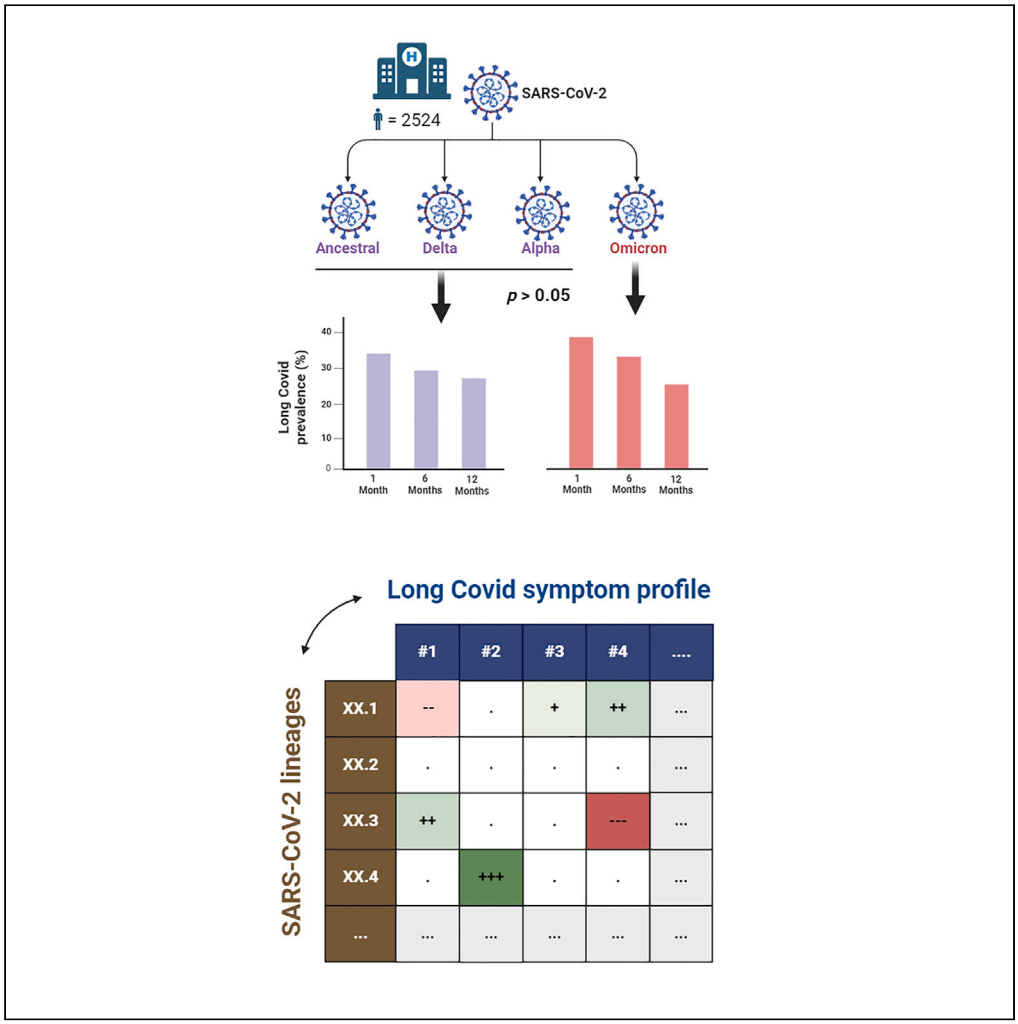

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Highlights

A significant number of Omicron and pre-Omicron patients had symptoms after 1 year

Overall prevalence of long COVID is comparable among Omicron and pre-Omicron variants

Certain SARS-CoV-2 lineages consistently impact severity of post-COVID sequelae

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Long COVID across SARS-CoV-2 variants, lineages, and sublineages

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SUMMARY

This prospective study aimed to determine the prevalence of long COVID in patients hospitalized for SARS-CoV-2 infection from March 2020 to July 2022 and assess the impact of different viral lineages. A total of 2,524 patients were followed up for 12 months, with persistent symptoms reported in 35.2% at one month, decreasing thereafter. Omicron variant patients initially showed higher symptom intensity, but this trend diminished over time. Certain viral lineages, notably Delta lineages AY.126 and AY.43, and Omicron sublineages BA.1.17, BA.2.56, and BA.5.1, consistently correlated with more severe symptoms. Overall, long COVID prevalence and severity were similar across SARS-CoV-2 variants. Specific lineages may influence post-COVID sequelae persistence and severity.

INTRODUCTION

The COVID-19 pandemic has been marked by evolving SARS-CoV-2 variants, lineages, and sublineages, with differential impacts on virus transmissibility, vaccine efficacy, clinical presentation, and outcomes. Soon after it was first identified by the World Health Organization (WHO) in November 2021, Omicron became the predominant variant worldwide, and it remains so today. Unlike previous SARS-CoV-2 variants, Omicron demonstrates an unprecedented number of mutations, resulting in significantly increased transmissibility and spread ability. The Omicron phase has also been characterized by successive sweeps of multiple Omicron sublineages, as opposed to the preceding phases of fewer, independent, and highly divergent lineages.[1,2] Another noteworthy characteristic of the Omicron variant is the reduced risk of severe outcomes, attributed to the decreased intrinsic severity of the variant itself and the protective effect of booster vaccinations.[3]

Moreover, Omicron has also been associated with a reduced incidence of long COVID compared to other variants.[4–7] However, available information about long COVID and its association with different SARS-CoV-2 variants has mostly relied on large-scale, real-world data analyses of electronic health records (EHRs). Despite the recognized advantages of these studies, this methodology also presents significant limitations.[8] Comprehensively capturing all long COVID symptoms, which are often not included in the diagnostic codes of EHR, may be challenging and entails the risk of under-reporting. Variability in the number and type of patient interactions with the healthcare system further exacerbates the problem. Additionally, the usual lack of direct testing for SARS-CoV-2 hampers the determination of the index date from which to commence follow-up and limits the possibility of confidently ascribing a distinct long COVID profile to a particular SARS-CoV-2 variant. Moreover, the substantial proliferation of Omicron sublineages raises the question of whether they might have a differential impact on the severity of persistent symptoms. Another open question is whether the increasing levels of SARS-CoV-2 antibodies over the course of the pandemic exercise a protective role on the risk of long COVID, since in most investigations immune status was inferred exclusively from patients' vaccination history.

The availability of robust and comprehensive clinical and immunovirological data is crucial for evaluating the distinctive characteristics of long COVID according to the SARS-CoV2 variants. Thus, the primary aim of this study was to assess long-term (one year) outcomes associated with the different SARS-CoV-2 variants, lineages, and sublineages. The secondary aim was to compare persistent COVID-19 symptoms in patients with Omicron versus pre-Omicron variants considering the levels of anti-SARS-CoV-2 spike antibodies.

RESULTS

Baseline characteristics, clinical status upon admission and short-term outcomes

Between March 1, 2020 and July 31, 2022, a total of 2,524 patients were hospitalized in our center due to SARS-CoV-2 infection (Figure S1), resulting in 172,768 and 1,269 patient-years of cumulative cohort follow-up at 1, 6, and 12 months, respectively. Baseline

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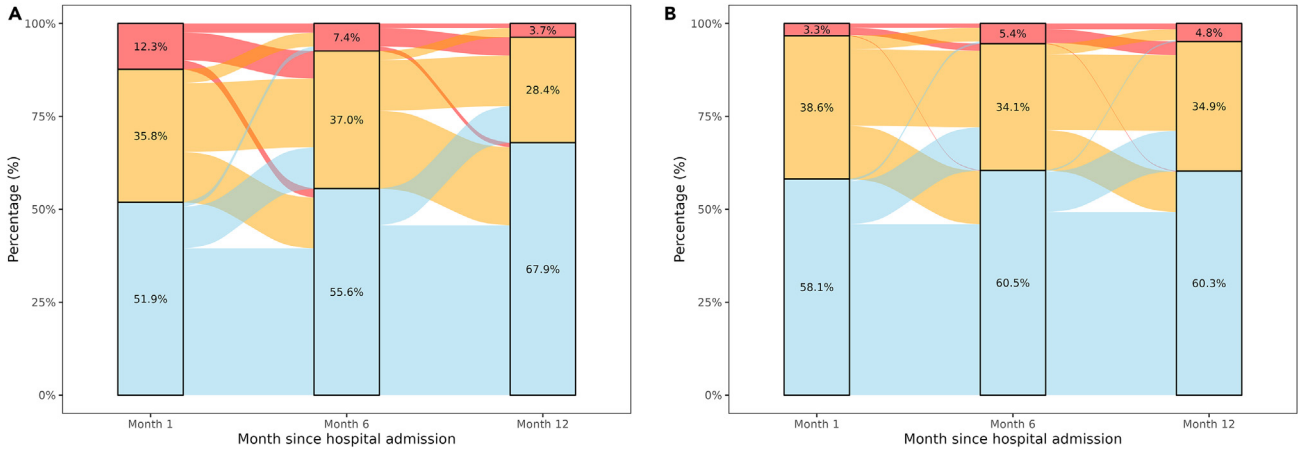

Figure 1. Sankey diagrams illustrating trajectories in composite scores of symptom intensity categories across study visits

(A) Participants infected with any pre-Omicron variant of SARS-CoV-2; (B) participants infected with the Omicron variant. Composite score was computed by standardizing the sum of all survey items, and deemed present if it was equal to or exceeded 2 SD population scores. The symptom intensity categories are defined based on the distance from the standardized mean score for that visit: in blue, below the mean; in orange, between the mean and two standard deviations; and in red, above two standard deviations. On the x axis are the three visits (Months 1, 6, and 12) following hospital admission.

characteristics are shown in Table S2. The SARS-CoV-2 variant was confirmed by viral genome sequencing (n = 393) or multiplex PCR (n = 661) in 1,054 participants (41.8%). Altogether, 552 (22%) cases were Omicron, 1954 (77%) pre-Omicron variants (n = 1224 Ancestral, n = 511 Alpha, and n = 219 Delta) and in 18 (<1%) cases SARS-CoV-2 variant could not be determined. These accounted for 2506 patients with known SARS-CoV-2 variant, who were included in all analyses. Hospitalized patients infected with the Omicron variant were older, were more likely to have comorbidities, and were more likely to be vaccinated. These patients also exhibited a less severe clinical presentation on admission, characterized by lower WHO severity score, lower frequency of pneumonia, and higher peripheral arterial oxygen saturation/inspired fraction of oxygen ratios (SpO2/FiO2) compared to patients infected with other variants (Table S2). The levels of SARS-CoV-2 antibodies against the spike and the nucleocapsid were significantly higher and lower, respectively, in patients with Omicron, consistent with a population with higher vaccination rates and fewer previous SARS-CoV-2 infections. The median (IQR) antibody levels in unvaccinated subjects upon admission were not different between the unvaccinated Omicron-infected group (n = 101) and those infected with pre-Omicron variants and unvaccinated (n = 1833) (5.75 (4.8, 29.0) vs. 8.5 (4.68, 68.8); p = 0.389).

Long-term COVID-19 symptoms

Symptom questionnaires were available for 1,749 (69%) participants for at least one of the follow-up points (1, 6, and/or 12 months following hospital admission; Figure S2). The prevalence of persistent symptoms at one month varied according to the definitions applied. The prevalence of patients reporting long COVID-19 symptom/s of any intensity in at least two symptom domains was 35.2%, and of severe intensity, 6.4%. For strictly defined long COVID and severe long COVID, prevalence was 28.6% and 2.2%, respectively Figures S3 and S4 for comparisons between Omicron and individual pre-Omicron variants). As shown in Table S3, at the one-month visit, patients infected with the Omicron variant displayed a higher proportion of severe (>2 SDs) symptoms across most domains as well as in the composite score (14.4% in Omicron vs. 2.4% in pre-Omicron variants; p = 0.001). Table S3 also shows the quantitative analysis of symptom scores and composite scores using adjusted linear models that included the levels of anti-spike IgG antibodies among covariates. Patients infected with Omicron exhibited higher scores in general, respiratory, and gastrointestinal symptoms, and in the composite score with global symptom aggregates. Similar results were observed when analyses were performed with the matched patient sample using propensity scores and when analyses were repeated in patients with viral variants confirmed through sequencing (data not shown).

To assess the evolution of post-COVID symptoms while controlling for patient response variability, we chose a subgroup of 598 individuals with available symptom surveys for all visits and examined their standardized scores. Most patients within a specific symptom intensity category—and particularly those with Omicron—either remained stable or experienced a decrease in symptom intensity. However, there were also minor shifts of patients to higher intensity categories over time in both Omicron and pre-Omicron groups. A small percentage of patients consistently remained in the high-intensity symptom category; at the initial follow-up visit, this was more frequent in the Omicron group (Figure 1). By symptom category, the proportion of patients exhibiting symptoms of an intensity of 2 or more SDs of the standardized population scores was usually below 20% in all domains at the 1-month follow-up visit, and was more frequent among patients infected with Omicron (Figure 2). There was a downward trend in the presence of symptoms to the 12-month follow-up visit, when the proportion of patients with Omicron vs. pre-Omicron variants was similar for most symptoms and

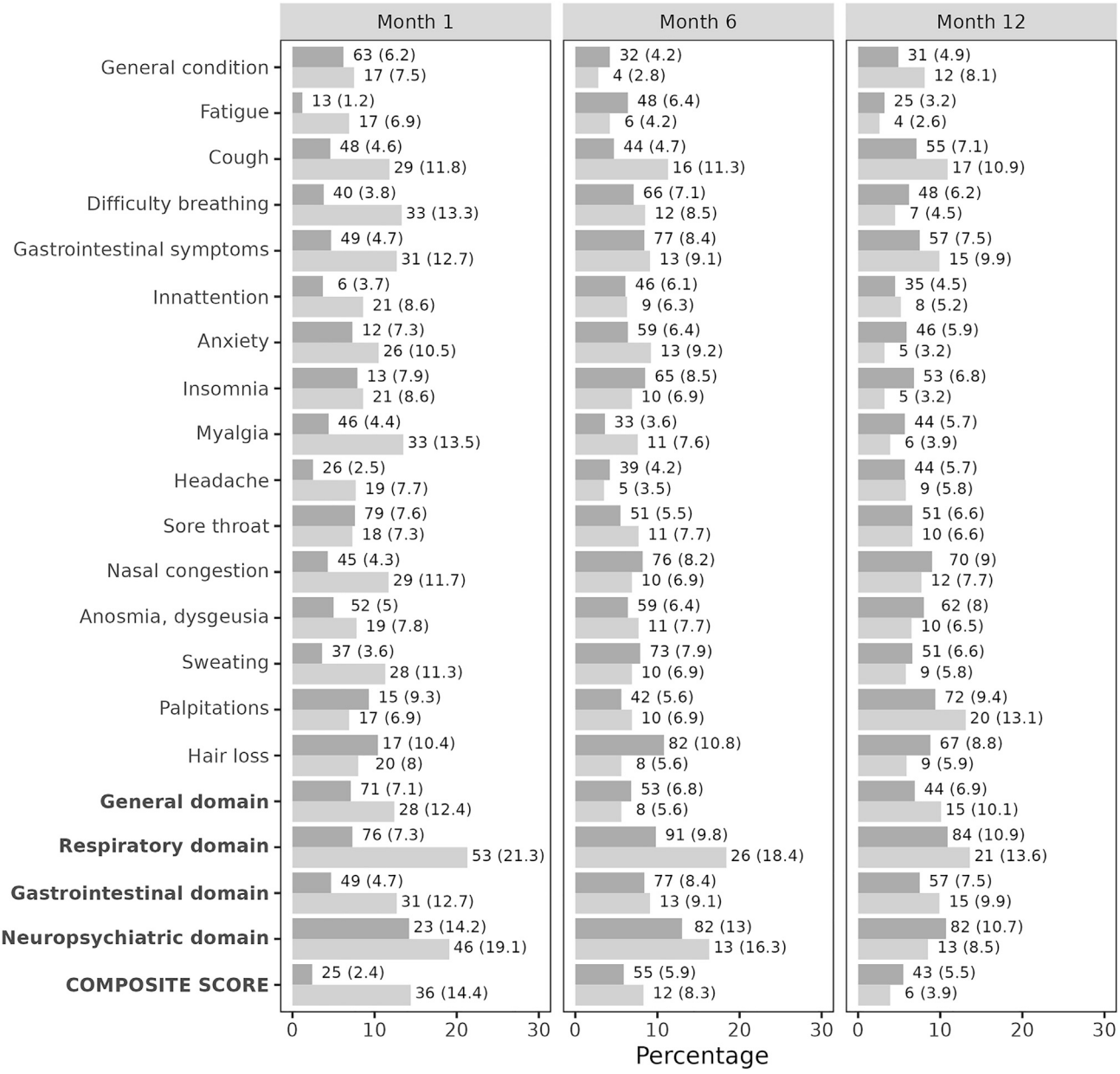

Figure 2. Proportion of patients with persistent symptoms with an intensity equal or higher than 2 standard deviations across the study visits

Light gray bars represent subjects with Omicron variant infection, and dark gray with pre-Omicron variants. Domains are met if any of the symptoms comprising the domain (General domain: general condition and fatigue; Respiratory domain: cough and difficulty breathing; Digestive domain: gastrointestinal symptoms; Neuropsychiatric domain: inattention, anxiety and insomnia) are above 2 SD. The composite score was computed by standardizing the sum of all survey items, and deemed present if it was equal to or exceeded 2 SD population scores.

symptom domains. The proportion of patients with a severe composite score (above 2 population SDs) remained stable throughout the study ($p > 0.05$), although patients with the Omicron variant experienced a greater decrease from the initial 1-month visit. When the analyses were repeated, including all subjects in the study with at least one survey available, the results followed a similar trend (Figure S5).

The quantitative analysis of symptom scores over the entire study period and across different visits using adjusted linear regression models showed that the higher scores observed at 1 month in patients infected with the Omicron variant tended to attenuate at the 6-month visit, and differences dissipated at the 12-month visit (Table 1). Similar results were observed for the comparison of questionnaire scores between viral variants in the propensity-matched analyses (Table 1) and when analyses were repeated in patients with viral variants confirmed by sequencing

Table 1. Comparison of questionnaire scores between Omicron pre-Omicron SARS-CoV-2 variants across assessment time points

	All study period				1 - Month visit				6 - Month visit				12 - Month visit			
	Propensity-matched cohort		All study participants		Propensity-matched cohort		All study participants		Propensity-matched cohort		All study participants		Propensity-matched cohort		All study participants	
	EE (SE)	P	EE (SE)	P	EE (SE)	P	EE (SE)	P	EE (SE)	P	EE (SE)	P	EE (SE)	P	EE (SE)	P
General																
General condition	−0.03 (0.15)	0.86	0.01 (0.11)	0.93	−0.06 (0.22)	0.78	0.01 (0.14)	0.94	−0.11 (0.19)	0.57	−0.04 (0.14)	0.78	0.13 (0.22)	0.54	0.06 (0.14)	0.66
Fatigue	0.08 (0.14)	0.58	0.16 (0.11)	0.14	0.15 (0.19)	0.43	0.18 (0.13)	0.18	0.22 (0.19)	0.25	0.28 (0.14)	0.03	−0.13 (0.19)	0.51	0.02 (0.13)	0.88
Respiratory																
Cough	0.14 (0.16)	0.40	0.21 (0.10)	0.04	0.24 (0.21)	0.27	0.28 (0.13)	0.03	0.06 (0.21)	0.78	0.24 (0.13)	0.09	0.08 (0.19)	0.70	0.08 (0.13)	0.56
Difficulty breathing	0.04 (0.16)	0.78	0.01 (0.11)	0.97	0.25 (0.21)	0.23	0.10 (0.14)	0.46	0.06 (0.19)	0.76	0.06 (0.13)	0.64	−0.18 (0.19)	0.36	−0.16 (0.14)	0.24
Gastrointestinal	0.35 (0.14)	0.02	0.15 (0.10)	0.14	0.62 (0.21)	0.01	0.36 (0.14)	0.01	0.35 (0.17)	0.04	0.13 (0.14)	0.34	0.09 (0.19)	0.61	−0.04 (0.14)	0.77
Neuropsychiatric																
Inattention	0.18 (0.15)	0.24	0.12 (0.11)	0.27	0.66 (0.36)	0.06	0.30 (0.17)	0.08	0.18 (0.18)	0.33	0.16 (0.13)	0.23	0.09 (0.19)	0.59	−0.01 (0.13)	0.98
Insomnia	0.21 (0.15)	0.17	0.04 (0.11)	0.73	0.15 (0.33)	0.66	−0.25 (0.18)	0.17	0.25 (0.19)	0.19	0.19 (0.14)	0.17	0.19 (0.17)	0.27	−0.02 (0.13)	0.88
Anxiety	0.25 (0.16)	0.11	0.12 (0.11)	0.27	0.37 (0.35)	0.29	0.03 (0.18)	0.88	0.26 (0.20)	0.20	0.27 (0.14)	0.05	0.18 (0.18)	0.30	0.01 (0.13)	0.96
Other symptoms																
Myalgia	0.01 (0.15)	0.92	−0.02 (0.11)	0.87	0.14 (0.19)	0.48	−0.01 (0.14)	0.92	0.03 (0.18)	0.85	0.03 (0.13)	0.85	−0.14 (0.19)	0.47	−0.07 (0.13)	0.62
Sweating	0.11 (0.15)	0.47	0.05 (0.10)	0.62	0.43 (0.21)	0.04	0.31 (0.14)	0.02	0.08 (0.18)	0.66	0.06 (0.13)	0.69	−0.19 (0.16)	0.23	−0.21 (0.13)	0.11
Headache	0.09 (0.16)	0.58	0.16 (0.11)	0.15	0.43 (0.19)	0.03	0.30 (0.14)	0.02	0.07 (0.20)	0.74	0.15 (0.14)	0.27	−0.22 (0.20)	0.29	0.03 (0.13)	0.80
Sore throat	0.02 (0.16)	0.91	0.09 (0.10)	0.82	0.09 (0.22)	0.67	0.05 (0.14)	0.70	0.26 (0.21)	0.21	0.21 (0.14)	0.12	−0.32 (0.20)	0.12	−0.21 (0.13)	0.11
Nasal congestion	0.12 (0.13)	0.39	0.06 (0.11)	0.54	0.45 (0.21)	0.03	0.30 (0.14)	0.03	0.23 (0.16)	0.17	0.14 (0.13)	0.31	−0.32 (0.17)	0.07	−0.25 (0.14)	0.06
Anosmia, dysgeusia	−0.02 (0.16)	0.90	0.03 (0.11)	0.77	−0.09 (0.20)	0.62	−0.06 (0.14)	0.65	0.06 (0.21)	0.77	0.17 (0.14)	0.22	−0.03 (0.19)	0.86	−0.02 (0.14)	0.87
Palpitations	0.16 (0.16)	0.31	0.12 (0.11)	0.25	0.23 (0.36)	0.53	0.10 (0.18)	0.58	0.42 (0.21)	0.05	0.42 (0.13)	0.01	−0.09 (0.18)	0.59	−0.17 (0.14)	0.22
Hair loss	−0.10 (0.16)	0.52	−0.03 (0.11)	0.78	0.04 (0.34)	0.91	−0.03 (0.18)	0.88	−0.04 (0.17)	0.80	−0.04 (0.14)	0.76	−0.16 (0.20)	0.44	−0.04 (0.13)	0.76
Composite score	0.14 (0.16)	0.39	0.12 (0.11)	0.29	0.37 (0.21)	0.08	0.23 (0.14)	0.09	0.19 (0.19)	0.32	0.21 (0.13)	0.13	−0.13 (0.19)	0.52	−0.07 (0.13)	0.59

EE: adjusted estimated effect for the difference between Omicron vs. non-Omicron (reference category) variants in the linear regression model (N = 598 participants); SE: standard error.

or multiplex PCR (data not shown). A global symptom aggregation including the entire study period was constructed for each domain (Table 1); no differences in the individual scores or in the composite scores were observed between Omicron and pre-Omicron variants in the adjusted linear models, which accounted for the within-individual correlation.

The analysis of persistent symptoms according to SARS-CoV-2 viral clades included 131 patients. Following cluster analysis, participants were divided into two groups, one (n = 101) showing significantly more severe symptoms than the remaining group with mild symptoms (n = 30) across various domains (Table S4). When these groupings were overlaid with survey symptoms, expressed in two dimensions after principal component analysis, there was still minimal overlap between them, suggesting the existence of two groups of lineages that differ in the expression of persistent symptoms (Figures 3 and S6 for individual domains). Throughout the follow-up visits, we consistently observed the presence of these two distinct groups of clades, each displaying enduring associations with either severe or mild symptoms at 1, 6, and 12 months. Notably, Delta lineages such as AY.126 and AY.43, along with the Omicron sublineages BA.1.17, BA.2.56 and BA.5.1, were consistently allocated within the most severe symptom category, while lineages B.1.1.7 (Alpha), AY.47 (Delta), and the Omicron sublineage BA.1.15.1 fell within the milder symptom group (Tables 2 and S6 for individual domains).

DISCUSSION

This study took place throughout the first three years of the pandemic and followed a cohort of patients hospitalized with COVID-19, who were closely investigated and monitored following a predefined protocol. Persistent symptom scores were standardized to ensure accuracy of analyses. The results revealed no overall significant differences in long-term outcomes between the SARS-CoV-2 variants. Unlike previous studies, the prevalence of persistent symptoms one month after admission was higher within certain domains among patients infected with the Omicron variant. However, this prevalence gradually decreased and converged to rates comparable with the pre-Omicron cases at one year. Certain lineages and sublineages exhibited consistent profiles in the severity of COVID-19 sequelae. Patients infected with the Delta lineages AY.126 and AY.43, and Omicron sublineages BA.1.17, BA.2.56, and BA.5.1, consistently experienced more severe persistent symptoms throughout the 12-month follow-up period, while patients infected with the Omicron sublineage BA.1.15.1 exhibited persistently milder symptoms.

Establishing a clear definition for long-term, persistent symptoms following the acute phase of COVID-19 has posed a challenge for researchers, as exemplified by the significant variability in the estimated prevalence of long COVID, ranging from 10% to 30%.[9–13] The main problem is the lack of a well-delineated, consensus-based definition for this complex condition, which has resulted in the use of a wide range of variables, formats, and measurement methodologies across different studies. This diversity represents an obstacle when attempting to compare prevalence estimates between studies. A distinguishing aspect of our investigation is the standardization of post-COVID symptoms, yielding precise and remarkably consistent data, enabling us to compare the scores of dissimilar variables measured at different time points. Data standardization in studies of long COVID would enable the comparison of prevalence estimates across different studies. Additionally, we performed sensitivity analyses using different definitions, some more inclusive and others more stringent, for the symptoms that were most specific to long COVID.[14,15] The results across all analyses were similar in terms of the frequency of COVID-19 sequelae in people infected with Omicron and pre-Omicron variants.

The comparable occurrence of long-lasting symptoms in our cohort of Omicron-infected inpatients represents a novel finding. The greater frequency of post-COVID syndrome with pre-Omicron variants had been associated with the higher intensity and number of symptoms during the acute infection caused by these variants.[6,16–18] Of note, the available data on Omicron-induced post-COVID syndrome are derived exclusively from non-hospitalized patient cohorts. To our knowledge, this is the first study addressing the long-term outcomes after infection with the SARS-CoV-2 Omicron variant in patients requiring admission. Furthermore, in our cohort, patients hospitalized with Omicron exhibited a differential profile, characterized by older age and greater comorbidity burden, despite having milder disease severity. Additionally, these Omicron infected patients had higher vaccination rates and a lower proportion of previous infections, which could contribute to the decreased severity in this group of patients, as observed in other studies.[19] Cumulative evidence suggests that development of post-COVID syndrome is more frequent in people with pre-existing comorbidities, which might be involved in the pathogenesis of the syndrome.[20,21] The increased comorbidity burden likely accounts for the greater initial frequency of COVID-19 sequelae in patients infected with Omicron, even after adjusting for this and other distinctive factors compared to other variants. Although the presence and severity of post-COVID symptoms at 6 and 12 months following hospital admission tended to converge with those of patients infected with pre-Omicron variants. While the differences found during the initial months could indeed be attributed to the distinctive features of the variants, we cannot rule out some residual impact of the underlying comorbidities on the enduring persistence of COVID-19 sequelae in patients infected with Omicron, which was probably not completely controlled for in the adjustments we made. Notably, a relevant proportion of patients infected with both Omicron and pre-Omicron variants continued to experience symptoms throughout the entire one-year follow-up period, albeit with fluctuations in symptom intensity. A subset of these patients consistently presented severe symptoms. This is in line with the reported occurrence of persistent health impairment even up to 24 months after infection in a Swiss cohort[22] and other studies showing a slow recovery,[23,24] which may point to development of chronic sequelae. Fluctuations in symptom intensity and prolonged duration, observed in our subjects, have been linked to the persistence of the SARS-CoV-2 virus.[25] This persistence triggers immune dysregulation, heightened release of inflammatory cytokines, and abnormal endothelial damage, culminating in chronic inflammation, vascular injury, hypercoagulability, microthrombosis, and multiorgan symptoms.

We observed a distinct contribution of SARS-CoV-2 lineages and sublineages to long COVID. Delta AY.126 and AY.43 lineages were associated with consistently higher scores in post-COVID symptoms throughout the study period, whereas the Alpha B.1.1.7 lineage and Delta

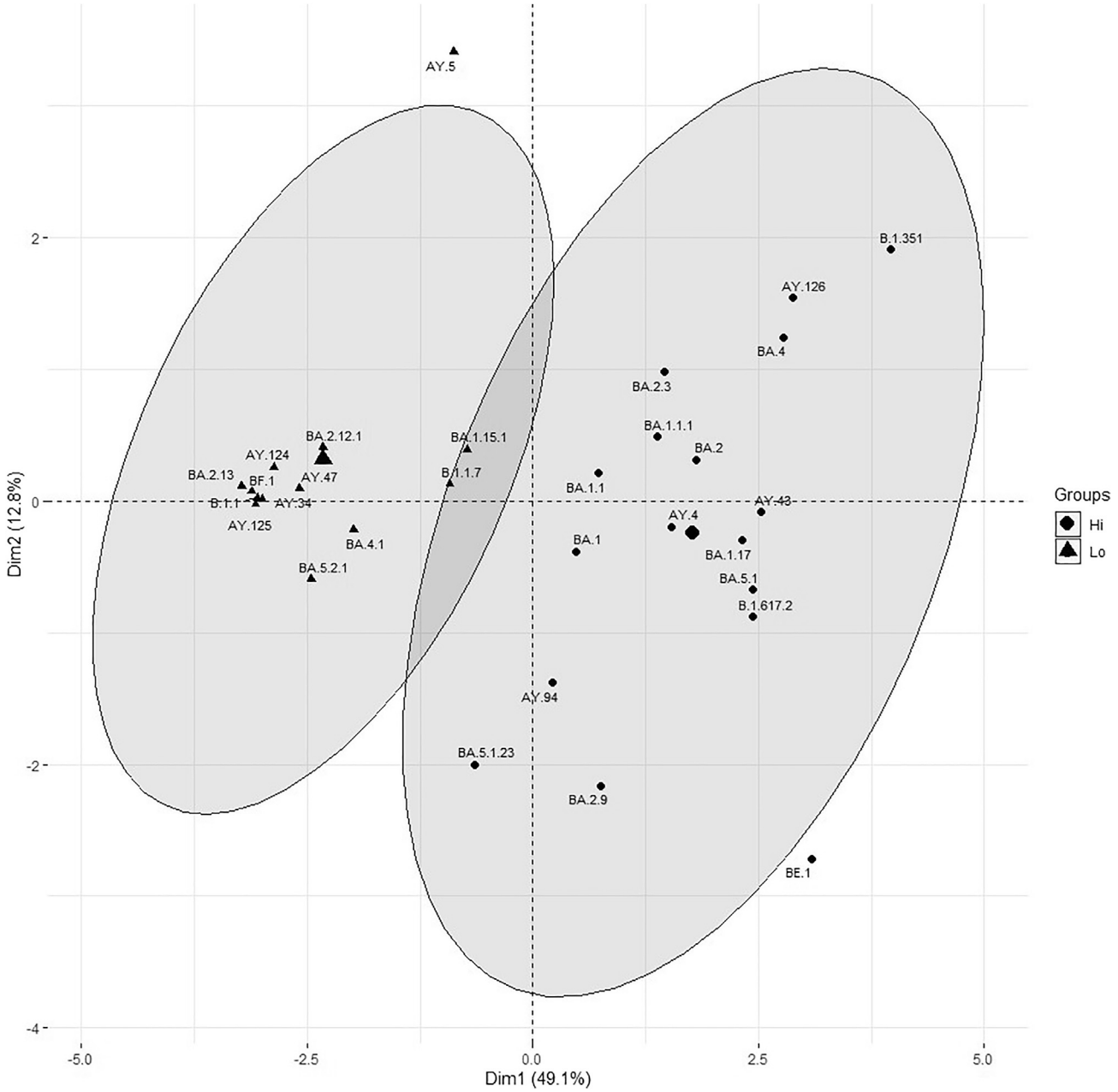

Figure 3. Two dimensions principal component scatterplot of symptoms questionnaires displaying the two groups defined in viral clade cluster analysis

Two clusters were defined by cluster analysis of COVID-19 questionnaire scores: Hi for "Highscorers", Lo for "Lowscorers". Dim1, Dimension 1 (Dimension 1 primarily comprises the subsequent variables (loading coefficient): general condition (0.32), myalgia (0.30), inattention (0.30), sweating (0.28), anxiety (0.28) and palpitations (0.28)); Dim2, Dimension 2 (Dimension 2 primarily comprises the subsequent variables (loading coefficient): cough (−0.59), nasal congestion (−0.45) and gastrointestinal symptoms (0.35).

AY.47 were repeatedly associated with mild-intensity symptoms. For the Omicron variant, different sublineages showed differential impacts on the post-COVID sequelae, with persistently high symptom scores observed with some clades and consistently low scores with others. Accordingly, certain lineages might be associated with a higher capacity to evade selective host immune responses or with higher viral persistence in particular organs.[1,26,27] To our knowledge, this is a novel finding that may help explain the chronicity and severity of COVID-19 sequelae. Although the numbers were low, our findings generate new knowledge about long COVID and take us toward a deeper understanding of its etiology. Future research through larger studies is warranted to confirm our results, which may also help explain discrepancies between studies examining long-term outcomes of SARS-CoV-2 infection. The potential existence of a differential effect in persistent

Table 2. Cluster classification of symptom scores by viral lineages and study visits

		Visit		
	All visits	1st month	6th month	12th month
Samples no., Total (Hi, Lo)	131 (101, 30)	126 (95, 31)	80 (49, 31)	78 (31, 47)
SARS-CoV-2 lineages				
AY.126	**Hi**	**Hi**	**Hi**	**Hi**
AY.43	**Hi**	**Hi**	**Hi**	**Hi**
BA.1.17	**Hi**	**Hi**	**Hi**	**Hi**
BA.2.56	**Hi**	**Hi**	**Hi**	**Hi**
BA.5.1	**Hi**	**Hi**	**Hi**	**Hi**
B.1.351	Hi	–	Hi	Hi
AY.133	Hi	Hi	Hi	–
AY.94	Hi	Hi	Hi	–
BA.1.17.2	Hi	Hi	–	Hi
BA.2.3	Hi	Hi	Hi	–
AY.4	Hi	Hi	Hi	Lo
BA.1.1.1	Hi	Hi	Hi	Lo
BA.2	Hi	Hi	Hi	Lo
AY.42	Hi	Hi	–	–
AY.5.4	Hi	Hi	–	–
BA.4	Hi	Hi	–	–
BA.5.2	Hi	Hi	–	–
BE.1	Hi	Hi	–	–
B.1.617.2	Lo	Lo	Hi	Hi
BA.1.1	Hi	Hi	Lo	Lo
BA.2.9	Hi	Hi	Lo	–
BA.4.1	Hi	Hi	Lo	–
BA.5.1.23	Hi	Hi	Lo	–
BA.1	Lo	Lo	Hi	Hi
AY.34	Lo	Lo	Lo	Hi
AY.100	Lo	Lo	–	–
AY.124	Lo	Lo	–	–
AY.5	Lo	Lo	–	–
BA.5.2.1	Lo	Lo	–	–
B.1.1	Lo	–	Lo	–
BA.2.12.1	Lo	–	Lo	–
AY.125	Lo	Lo	Lo	–
BF.1	Lo	Lo	Lo	–
B.1.1.7	**Lo**	**Lo**	**Lo**	**Lo**
AY.47	**Lo**	**Lo**	**Lo**	**Lo**
BA.1.15.1	**Lo**	**Lo**	**Lo**	**Lo**

Clusters were defined by cluster analysis of COVID questionnaires scores. Hi, "highscorers"; Lo, "lowscorers".

symptoms based on SARS-CoV-2 lineage could significantly imply forthcoming changes in the future prevalence, intensity, and clinical profile, depending on the virus's future trajectory. If confirmed, the lineages/sublineages associated with severe long-term symptoms should be given preferential consideration in the development of forthcoming SARS-CoV-2 vaccines or in COVID-19 therapy.

In addition to the viral variant, vaccination represents another significant factor involved in the development of post-COVID-19 syndrome in the Omicron era. Accordingly, higher levels of neutralizing antibodies against Omicron were observed in individuals who had recovered

from the infection and were vaccinated, compared to those who were unvaccinated[28] and data from the pre-Omicron waves suggest that COVID-19 vaccines may confer protective effects against long COVID.[29] However, the effect of vaccination on the long-term outcomes after infection with Omicron in non-hospitalized patients has yielded inconsistent results, ranging from reduced prevalence of COVID-19 sequelae in vaccinated patients with cancer[5] to null protective effects in a cohort of health care workers.[6] In our study, the availability of antibody titers as opposed to vaccination history as an adjustment covariate may help elucidate the true protective impact of the immune response against the virus versus the pathogenicity of the clade on long-term outcomes. Likewise, it may have also contributed to the novel findings compared to previous studies.

A strength of our study lies in the representativeness of the emerging SARS-CoV-2 variants in this cohort of hospitalized patients throughout the entire course of the pandemic. Patients were closely and comprehensively monitored, and symptom scores were standardized, which contributed to mitigating the impact of individual variations and ensuring the consistency of data and the comparability between the different variants and clades to support the results. We measured COVID-19–related sequelae longitudinally, through prospective and protocol-driven evaluation of participants starting from the time of SARS-CoV-2 infection, which shed light on how problems evolve in people who survive COVID-19. This approach also minimized the variability in the definition of long COVID, which could pose challenges in multicenter studies. Additionally, the identification of the SARS-CoV-2 lineages and sublineages through sequencing, as well as the measurement of antibody levels to assess the true protective effect of immunization, further enhanced the robustness of our research. Our findings may contribute to the understanding of long-COVID dynamics by acknowledging the potential for prolonged symptom persistence with contemporary variants, as well as the differential impacts of existing and emerging clades. This may aid in the management and mitigation of the impact of the COVID-19 pandemic.

In conclusion, the overall prevalence and severity of long COVID is comparable among the SARS-CoV-2 variants. Certain SARS-CoV-2 lineages and Omicron sublineages may have distinctive and consistent effects on the severity and persistence of post-COVID sequelae.

Limitations of the study

Our findings should be considered in light of several limitations. First, participants were recruited from a single hospital. We relied on self-reported symptoms and comparisons with baseline health status. While this captures participants' experiences of their own sequelae, it also introduces the risk of misclassification, as perceptions may evolve over time, and some participants' symptoms may be due to a health condition unrelated to COVID-19. Our findings could also be attributed to the inherent selection bias toward both more severe and longer-lasting clinical conditions in patients who completed all questionnaires from 1 month to 12 months, which may contribute to an overestimate of the frequency and severity of persistent symptoms. Consequently, our findings may reflect a skewed representation of the patient population, primarily comprising those with more persistent and severe symptoms. The SARS-CoV-2 lineage/sublineage was not available in all samples, and certain lineages and sublineages were underrepresented. Having a control group would have been valuable given the nonspecific nature of long COVID symptoms.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109536.

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AUTHOR CONTRIBUTIONS

S.P.: Investigation, writing—original draft preparation and editing. M.F., A.d.R., and A.G.: Investigation, writing – review and editing. C.L. and J.A.G.: Data curation, software, and formal analysis, writing – review and editing. J.G.: Writing—review and editing. F.G. and M.M.: Conceptualization, methodology, writing—original draft preparation, reviewing and editing, and supervision. All authors contributed to the article and approved the submitted version.

DECLARATION OF INTERESTS

None to declare.

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REFERENCES

1. Markov, P.V., Ghafari, M., Beer, M., Lythgoe, K., Simmonds, P., Stilianakis, N.I., and Katzourakis, A. (2023). The evolution of SARS-CoV-2. Nat. Rev. Microbiol. *21*, 361–379.
2. Xu, A., Hong, B., Lou, F., Wang, S., Li, W., Shafqat, A., An, X., Zhao, Y., Song, L., Tong, Y., and Fan, H. (2022). Sub-lineages of the SARS-CoV-2 Omicron variants: Characteristics and prevention. MedComm *3*, e172.
3. Nyberg, T., Ferguson, N.M., Nash, S.G., Webster, H.H., Flaxman, S., Andrews, N., Hinsley, W., Bernal, J.L., Kall, M., Bhatt, S., et al. (2022). Comparative analysis of the risks of hospitalisation and death associated with SARS-CoV-2 omicron (B.1.1.529) and delta (B.1.617.2) variants in England: a cohort study. Lancet *399*, 1303–1312.
4. Antonelli, M., Pujol, J.C., Spector, T.D., Ourselin, S., and Steves, C.J. (2022). Risk of long COVID associated with delta versus omicron variants of SARS-CoV-2. Lancet *399*, 2263–2264.
5. Cortellini, A., Tabernero, J., Mukherjee, U., Salazar, R., Sureda, A., Maluquer, C., Ferrante, D., Bower, M., Sharkey, R., Mirallas, O., et al. (2023). SARS-CoV-2 omicron (B.1.1.529)-related COVID-19 sequelae in vaccinated and unvaccinated patients with cancer: results from the OnCovid registry. Lancet Oncol. *24*, 335–346.
6. Kahlert, C.R., Strahm, C., Güsewell, S., Cusini, A., Brucher, A., Goppel, S., Möller, E., Möller, J.C., Ortner, M., Ruetti, M., et al. (2023). Post-Acute Sequelae After Severe Acute Respiratory Syndrome Coronavirus 2 Infection by Viral Variant and Vaccination Status: A Multicenter Cross-Sectional Study. Clin. Infect. Dis. *77*, 194–202.
7. Willan, J., Agarwal, G., and Bienz, N. (2023). Mortality and burden of post-COVID-19 syndrome have reduced with time across SARS-CoV-2 variants in haematology patients. Br. J. Haematol. *201*, 640–644.
8. Zhang, H.G., Honerlaw, J.P., Maripuri, M., Samayamuthu, M.J., Beaulieu-Jones, B.R., Baig, H.S., L'Yi, S., Ho, Y.L., Morris, M., Panickan, V.A., et al. (2023). Potential pitfalls in the use of real-world data for studying long COVID. Nat. Med. *29*, 1040–1043. https://doi.org/10.1038/s41591-023-02274-y.
9. Arjun, M.C., Singh, A.K., Pal, D., Das, K., Alekhya, G., Venkateshan, M., Mishra, B., Patro, B.K., Mohapatra, P.R., and Subba, S.H. (2022). Characteristics and predictors of Long COVID among diagnosed cases of COVID-19. PLoS One *17*, e0278825.
10. Havervall, S., Rosell, A., Phillipson, M., Mangsbo, S.M., Nilsson, P., Hober, S., and Thålin, C. (2021). Symptoms and functional impairment assessed 8 months after mild COVID-19 among health care workers. JAMA *325*, 2015–2016.
11. Nalbandian, A., Sehgal, K., Gupta, A., Madhavan, M.V., McGroder, C., Stevens, J.S., Cook, J.R., Nordvig, A.S., Shalev, D., Sehrawat, T.S., et al. (2021). Post-acute COVID-19 syndrome. Nat. Med. *27*, 601–615.
12. Whitaker, M., Elliott, J., Chadeau-Hyam, M., Riley, S., Darzi, A., Cooke, G., Ward, H., and Elliott, P. (2022). Persistent COVID-19 symptoms in a community study of 606,434 people in England. Nat. Commun. *13*, 1957.
13. Davis, H.E., Assaf, G.S., McCorkell, L., Wei, H., Low, R.J., Re'em, Y., Redfield, S., Austin, J.P., and Akrami, A. (2021). Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. EClinicalMedicine *38*, 101019.
14. Thaweethai, T., Jolley, S.E., Karlson, E.W., Levitan, E.B., Levy, B., McComsey, G.A., McCorkell, L., Nadkarni, G.N., Parthasarathy, S., Singh, U., et al. (2023). Development of a Definition of Postacute Sequelae of SARS-CoV-2 Infection. JAMA *329*, 1934–1946.
15. Office for National Statistics (ONS) (2023). ONS website, statistical bulletin, Prevalence of ongoing symptoms following coronavirus (COVID-19) infection in the UK: 30 March 2023. https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/prevalenceofongoingsymptomsfollowingcoronaviruscovid19infectionintheuk/previousReleases.
16. Strahm, C., Seneghini, M., Güsewell, S., Egger, T., Leal-Neto, O., Brucher, A., Lemmenmeier, E., Meier Kleeb, D., Möller, J.C., Rieder, P., et al. (2022). Symptoms compatible with long-COVID in healthcare workers with and without SARS-CoV-2 infection—results of a prospective multicenter cohort. Clin. Infect. Dis. *75*, e1011–e1019.
17. Bahmer, T., Borzikowsky, C., Lieb, W., Horn, A., Krist, L., Fricke, J., Scheibenbogen, C., Rabe, K.F., Maetzler, W., Maetzler, C., et al. (2022). Severity, predictors and clinical correlates of post-COVID syndrome (PCS) in Germany: a prospective, multi-centre, population-based cohort study. EClinicalMedicine *51*, 101549.
18. Hernández-Aceituno, A., García-Hernández, A., and Larumbe-Zabala, E. (2023). COVID-19 long-term sequelae: Omicron versus Alpha and Delta variants. Infect. Dis. Now *53*, 104688.
19. Bowe, B., Xie, Y., and Al-Aly, Z. (2022). Acute and postacute sequelae associated with SARS-CoV-2 reinfection. Nat. Med. *28*, 2398–2405.
20. Russell, C.D., Lone, N.I., and Baillie, J.K. (2023). Comorbidities, multimorbidity and COVID-19. Nat. Med. *29*, 334–343.
21. El Moussaoui, R., Opmeer, B.C., de Borgie, C.A.J.M., Nieuwkerk, P., Bossuyt, P.M.M., Speelman, P., and Prins, J.M. (2006). Long-term symptom recovery and health-related quality of life in patients with mild-to-moderate-severe community-acquired pneumonia. Chest *130*, 1165–1172.
22. Ballouz, T., Menges, D., Anagnostopoulos, A., Domenghino, A., Aschmann, H.E., Frei, A., Fehr, J.S., and Puhan, M.A. (2023). Recovery and symptom trajectories up to two years after SARS-CoV-2 infection: population-based, longitudinal cohort study. BMJ *381*, e074425.
23. Tran, V.-T., Porcher, R., Pane, I., and Ravaud, P. (2022). Course of post covid-19 disease symptoms over time in the ComPaRe long covid prospective e-cohort. Nat. Commun. *13*, 1812.
24. Peter, R.S., Nieters, A., Kräusslich, H.G., Brockmann, S.O., Göpel, S., Kindle, G., Merle, U., Steinacker, J.M., Rothenbacher, D., and Kern, W.V.; EPILOC Phase 1 Study Group

(2022). Post-acute sequelae of covid-19 six to 12 months after infection: population based study. BMJ *379*, e071050.

25. Yang, C., Zhao, H., Espín, E., and Tebbutt, S.J. (2023). Association of SARS-CoV-2 infection and persistence with long COVID. Lancet Respir. Med. *11*, 504–506.
26. Yang, C., Zhao, H., Shannon, C.P., and Tebbutt, S.J. (2022). Omicron variants of SARS-CoV-2 and long COVID. Front. Immunol. *13*, 1061686.
27. Davis, H.E., McCorkell, L., Vogel, J.M., and Topol, E.J. (2023). Long COVID: major findings, mechanisms and recommendations. Nat. Rev. Microbiol. *21*, 133–146.
28. Sullivan, D.J., Franchini, M., Joyner, M.J., Casadevall, A., and Focosi, D. (2022). Analysis of anti-SARS-CoV-2 Omicron-neutralizing antibody titers in different vaccinated and unvaccinated convalescent plasma sources. Nat. Commun. *13*, 6478.
29. Byambasuren, O., Stehlik, P., Clark, J., Alcorn, K., and Glasziou, P. (2023). Effect of covid-19 vaccination on long covid: systematic review. BMJ Med. *2*, e000385.
30. Guillén, L., Padilla, S., Fernández, M., Agulló, V., García, J.A., Telenti, G., García-Abellán, J., Botella, Á., Gutiérrez, F., and Masiá, M. (2020). Preemptive interleukin-6 blockade in patients with COVID-19. Sci. Rep. *10*, 16826.
31. COVID-19 Rapid Guideline: Managing the Long-Term Effects of COVID-19 (2020 (London: National Institute for Health and Care Excellence (NICE)).
32. Masiá, M., Fernández-González, M., García, J.A., Padilla, S., García-Abellán, J., Botella, Á., Mascarell, P., Agulló, V., and Gutiérrez, F. (2022). Robust long-term immunity to SARS-CoV-2 in patients recovered from severe COVID-19 after interleukin-6 blockade. EBioMedicine *82*, 104153.
33. Websites: Liaison SARS-CoV-2 Trimerics IgG assay brochure. https://artemisialab.it/wp-content/uploads/2021/03/liaisonr_sars-cov-2_trimerics_igg_assay_m0870004408_lr_digital.pdf.
34. Fernández-González, M., Agulló, V., García, J.A., Padilla, S., García-Abellán, J., de la Rica, A., Mascarell, P., Masiá, M., and Gutiérrez, F. (2023). T-cell immunity against SARS-CoV-2 measured by an interferon-γ release assay is strongly associated with patient outcomes in vaccinated persons hospitalized with Delta or Omicron variants. J. Infect. Dis. *7*, jiad260.
35. García-Abellán, J., Padilla, S., Fernández-González, M., García, J.A., Agulló, V., Andreo, M., Ruiz, S., Galiana, A., Gutiérrez, F., and Masiá, M. (2021). Antibody Response to SARS-CoV-2 is Associated with Long-term Clinical Outcome in Patients with COVID-19: a Longitudinal Study. J. Clin. Immunol. *41*, 1490–1501.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Plasma and nasopharyngeal frozen samples.	Infectious Diseases Unit and Microbiology Service Biobank. Hospital General Universitario de Elche, Spain.	Local Biobank.
Critical commercial assays		
Detection of Nucleocapsid IgG: SARS-CoV-2 IgG (Alinity i)	Abbott Laboratories	Cat# 06R9022
Detection of Spike IgG: Liaison SARS-CoV-2 IgG Trimeric IgG assay	Diasorin	Cat# 311510
Sequencing: Ligation Sequencing Kit	MinION (Oxford Nanopore Tech)	SQK-LSK109
Deposited data		
De-identified patient data	Mendeley Data	https://doi.org/10.17632/whgfwf35j5.1
SARS-CoV-2 genome sequences	hCoV-19 data GISAID	https://gisaid.org/
R code	Mendeley Data	https://doi.org/10.17632/rvv6r2nrh5.2

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Mar Masiá (marmasiac@gmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- De-identified patient data have been deposited at Mendeley Data public repository. Accession numbers are listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.
- Original R code has been deposited at Mendeley Data Repository and is publicly available as of the date of publication. DOI are listed in the key resources table.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study participants

This prospective longitudinal study took place at the Hospital General Universitario de Elche (Spain) and included all cases of COVID-19 confirmed by real-time PCR (RT-PCR) from nasopharyngeal swab samples and admitted from 1 March 2020 to 31 July 2022. During the COVID-19 pandemic, hospitalized patients with COVID-19 were managed according to a local protocol that outlined specific procedures for diagnosis and treatment.[30] Patients older than 18 years of age of both sexes were included. Long COVID was defined as having new or ongoing symptoms four weeks or more after the start of acute COVID-19, in line with the National Institute for Health and Care Excellence guidelines.[31] Prospective information on the occurrence of SARS-CoV-2 reinfections not requiring hospital admission was not available during follow-up. The study was approved by the institutional ethics committee as part of the COVID-19 Elx/Spain project. Written informed consent for participation was not required for this study, in accordance with national legislation and institutional requirements.

METHOD DETAILS

COVID symptoms questionnaire

Follow-up assessments were performed using a self-administered questionnaire (Supplementary material) at the specified time points. Specifically, patients rated the presence and severity of 16 symptoms across four domains: general, respiratory, abdominal, and neuropsychiatric

symptoms, using a 0–10-point visual analog scale (VAS). On initial analysis, the prevalence and severity of persistent symptoms were assessed based on these scores, considering various definitions of long COVID-19 symptoms—some more stringent than others. Patients were classified as having long COVID-19 symptoms if they reported at least one symptom in any of the domains. If one of these symptoms was rated with a score of 7 points or higher, they were classified as having severe long COVID-19 symptoms. The criterion of fatigue (its reported presence or a severity of 7 or more on the VAS) was used to define strict long COVID and strict severe long COVID, respectively.

We also conducted an analysis using standardized scores. With the aim of mitigating the impact of individual variations in patient responses and enabling cross-temporal comparison, Z-scores were computed for each symptom score and overall questionnaire scores. For each symptom item, we calculated Z-scores by subtracting the item's mean from the observed score and dividing the result by the item's standard deviation. This process was performed on a per-item basis and using surveys from the same assessment time point. As for the overall questionnaire scores, the *Z* score for each participant's total score was computed by subtracting the mean total score and dividing by the standard deviation of total scores. The resulting distribution of scores exhibited a mean of 0 points (standard deviation [SD] 1). Standardized symptom scores or overall questionnaire scores that surpassed 2 SDs from the population scores at that time point were defined as severe COVID symptoms; from 0 to 2 SDs, as symptoms of mild intensity; and below 0, as an absence of significant symptoms.

SARS-CoV-2 antibody levels

To assess humoral immunity against SARS-CoV-2 at the time of hospital admission, plasma levels of anti-SARS-CoV-2 spike IgG were determined, as detailed elsewhere.[32] The antibody levels determined by this technique have been correlated with neutralizing capacity against various Omicron variants.[33] Following discharge, structured follow-up was carried out at 1, 6, and 12 months.

SARS-CoV-2 viral genome sequencing

Variants of SARS-CoV-2 were identified through viral genome sequencing or multiplex PCR on archived nasopharyngeal samples obtained from hospital COVID-19 admissions, as previously described.[34] In patients with no available sample, the variant was assumed to be the prevalent one circulating in our health department during the period when admission occurred. In weeks where there was no predominant variant, the variant variable was considered missing. In the subset of patients with available spike SARS-CoV-2 RNA gene sequencing, the corresponding viral clade was determined using the Nextclade tool.[35]

QUANTIFICATION AND STATISTICAL ANALYSIS

Unless otherwise stated, the analysis was carried out on the whole study cohort, including patients in whom the SARS-CoV-2 variant was ascribed based on circulating infections as well as those in whom it was confirmed through sequencing or multiplex PCR. The Wilcoxon test or Student's *t* test was used to compare groups according to continuous variables, and the χ^2 test or Fisher's exact test according to categorical variables. Regression models were developed that controlled for potential confounders. In particular, all adjusted analyses included the levels of anti-SARS-CoV-2 spike IgG in order to account for the potential impact of vaccine-induced, prior infection-induced, or hybrid immunity against SARS-CoV-2. Additionally, gender, age, Charlson comorbidity index, the WHO COVID-19 severity score, which are recognized predictors of clinical outcomes, and any clinically relevant variables that were significant in the univariate analysis were also included.

To analyze standardized questionnaire scores and identify potential differences in persistent symptoms among viral variants, different sensitivity analyses were carried out. Firstly, the proportions of patients with severe symptoms at each visit were compared across variants according to standardized scores. Secondly, linear regression models of standardized symptom scores and composite scores were constructed, adjusting for gender, age, levels of anti-spike SARS-CoV-2 IgG immunoglobulin at hospital admission, and the SARS-CoV-2 viral variant (Omicron vs. pre-Omicron). Thirdly, to mitigate selection biases when comparing potentially dissimilar populations (patients infected with the Omicron versus other variants) that are non-contemporaneous and possess distinct comorbidity profiles, the regression analysis was replicated, incorporating infected patients with the Omicron variant matched through propensity scores to a similar group of those infected with other variants. The covariates for propensity matching included gender, age, Charlson comorbidity index, and the WHO COVID-19 severity score. In Table S5, the balance between the groups is depicted after matching through propensity. Finally, the analyses were repeated, considering only patients in whom the variant was confirmed through sequencing.

In the subgroup of participants with available viral sequence data and surveys across all study visits, we conducted an exploratory cluster analysis to investigate potential associations between viral clades and symptom profiles. The objective of this analysis was to cluster distinct viral clades based on reported symptom patterns. The Ward linkage method was employed for clustering, utilizing the Euclidean distance as the similarity metric. Subsequently, a principal component analysis (PCA) was executed to reduce the dimensionality of the symptom questionnaire scores. After the PCA, we evaluated the coherence of the previously derived cluster groupings with respect to viral clades, utilizing visual scrutiny of viral clades through PCA to validate the robustness of these patterns. This analysis has been conducted in two ways: firstly, incorporating all symptoms from the questionnaire, and secondly, including only the symptoms from each individual domain separately. Statistical analyses were performed using R software (R-Core Team 2020, R-4.1.2.1). We used case-wise deletion, without data imputation, because missingness rates were small for all variables (Table S1).