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MAJOR ARTICLE

Post-COVID-19 Syndrome 2 Years After the First Wave: The Role of Humoral Response, Vaccination and Reinfection

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Background. The aim of this study was to describe the long-term evolution of post-COVID-19 syndrome over 2 years after the onset of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) in survivors of the first wave.

Methods. This prospective study was based on interviews and investigated post-COVID-19 syndrome 6, 12, and 24 months after the disease onset in all adult in- and outpatients with COVID-19 followed at Udine Hospital (Italy) during the first wave (March–May 2020). Humoral response, vaccination status, and reinfection were assessed.

Results. Overall, 230 patients (53.5% female; mean age 54.7 years) were interviewed 2.3 years (standard deviation = 0.11) after acute onset. Post-COVID-19 syndrome was observed in 36.1% of patients (n = 83) at 2 years. The most common persistent symptoms were fatigue (14.4%), rheumatological (14.4%), and psychiatric symptoms (9.6%). Overall, 55.4% (46 of 83) of long haulers searched for healthcare system support and 21 (45.7%) were visited by a specialist. Female gender (odds ratio [OR] = 2.50, $P = .005$), a proportional increase in the number of symptoms during acute COVID-19 (OR = 1.40, $P = .001$), and the presence of comorbidities (OR = 1.57, $P = .004$) were all independent risk factors for post-COVID-19 syndrome. Vaccination and reinfection had no impact on post-COVID-19 syndrome dynamics. The presence of receptor-binding domain (RBD) SARS-CoV-2 immunoglobulin G (IgG) and non-RBD SARS-CoV-2 IgG titers were not associated with the occurrence of post-COVID-19 syndrome.

Conclusions. Two years after COVID-19, the burden of persistent symptoms remains high among in- and outpatients' population infected during the first wave. Post-COVID-19 dynamic does not seem to be influenced by SARS-CoV-2 immunization status and reinfection.

Keywords. COVID-19; long COVID; post-COVID-19 syndrome; SARS-CoV-2 antibodies.

Coronavirus disease 2019 (COVID-19) has led to an increasing population with a wide range of persistent symptoms after acute infection, referred to as post- or long-COVID-19 syndrome, posing an increasing threat to global health. However, the advancements in the knowledge of this entity are extremely slow due to lack public awareness, precise diagnostic criteria, and resources [1, 2].

The underlying pathophysiology of long-COVID-19 remains unknown and comprehends a complex interaction among the virus persistence, sustained inflammatory, microvascular damage, reactivation of latent viruses, and activation of autoimmune responses [1]. Recovery paths from COVID-19 demand longitudinal clinical and immunological investigation to better understand the post-COVID-19 syndrome dynamics. In a previous description of this cohort at 6 and 12 months, the increased natural humoral immune response was related to the occurrence of post-COVID-19 syndrome [3, 4]. Furthermore, the role of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination and reinfection as possible triggers or modulators that may influence immune response to SARS-CoV-2 and cause variations of post-COVID syndrome is still controversial [1, 3–7]. Thus, the aim of this study was to comprehensively characterize the post-COVID-19 syndrome 2 years after the acute infection. We focused on long-term predictors of post-COVID-19 syndrome, the kinetics of humoral responses, the role of vaccination, and reinfection among survivors of the first wave of SARS-CoV-2 infection.

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METHODS

Study Design, Patients, and Data Collection

The details of this prospective cohort study have been provided previously [3, 4] (Supplementary Tables 1 and 2). In short, the cohort included the following: (1) adult (≥ 18 years) in- and outpatients with COVID-19 during the first wave (March–May 2020) attending the Academic Hospital of Udine (Italy), (2) followed up at 6 (September–November 2020), 12 (March–May 2021) and 24 months (May–November 2022) with a telephonic pilot-tested validated questionnaire on post-COVID-19 syndrome [3, 4, 8], and (3) willingness to participate (Figure 1). Data were collected in a database at the study enrollment and during the follow up. Patients were classified at 24-month follow up compared with 12-month follow up as follows: (1) unaffected when self-reported as asymptomatic at both follow ups; (2) unchanged when symptoms remained the same; (3) worsened when new symptoms emerged or previous symptoms worsened; and (4) improved, when symptoms were reported as recovered or improved.

At the time of the interview, the vaccination status (date, number, and type of vaccine) was also investigated. In Italy, the SARS-CoV-2 vaccination campaign started on December 27, 2020. Vaccines approved were those with Adenovirus Vector (ChAdOx1 nCoV-19 Oxford-AstraZeneca and Ad26.COV2.S Janssen COVID-19 vaccine) and the mRNA (BNT162b2 Pfizer-BioNTech and mRNA-1273 Moderna). Oxford-AstraZeneca COVID-19 vaccine was withdrawn on March 5, 2021 due to suspected related adverse events, and Janssen COVID-19 vaccine was not used for boosters; moreover, the Italian Government adopted the European Digital Pass strategy (from July 2021 to April 2022) [9]. Patients were categorized as vaccinated/hybrid immunity if they had received the vaccine at least 2 weeks before the interview. Patients who received ≥3 shots of SARS-CoV-2 vaccination were considered as fully vaccinated.

In addition, at the time of the interview reinfections were also assessed. Clinical reinfection was defined as a positive SARS-CoV-2 polymerase chain reaction or antigenic test more than 3 months after the onset of the primary infection with or without recurrence of symptoms compatible with COVID-19. Viral sequencing to establish infecting SARS-CoV-2 variants was not routinely performed. On the basis of Italian and Friuli Venezia Giulia sequencing data, the most common variants during the study period were the alfa, beta, delta, and omicron variant [10]. In Supplementary Tables 3 and 4, the clinical definitions are detailed.

Serological Follow-up

Severe acute respiratory syndrome coronavirus 2 antibody titers were performed on a subgroup of patients who participated in a parallel study (CORMOR 3–4) [11] on monthly serological follow up; available serological data at the time of the interview (±2 months) were also recorded in the database. Humoral response was measured with 2 antibody assays: (1) receptor-binding domain (RBD) SARS-CoV-2 immunoglobulin G (IgG), to measure vaccine induced or hybrid humoral response and (2) non-RBD SARS-CoV-2 IgG, to measure natural infection humoral response (Supplementary Table 5).

Patient Consent Statement

The reference Ethics Committee of Friuli Venezia Giulia (CEUR-2020-OS-219 and CEUR-2020-OS-205) approved the study, and all procedures were in accordance with the ethical standards of the Health Care Trust. Informed written consent of all participants was obtained.

Statistical Analysis

Patients were divided into 2 groups (with or without post-COVID-19 syndrome) (Supplementary Table 4) at the time of interview. Absolute values, percentages, mean, and median (standard deviation [SD] or interquartile range [IQR]) were calculated. Categorical variables were compared using the χ^2 test or Fisher's exact test, whereas continuous variables were compared using a Student *t* test or Mann-Whitney *U* test, according to the Shapiro-Wilk test establishing whether data were normally or nonnormally distributed. A multivariable logistic regression was performed to explore variables associated with post-COVID-19 syndrome, estimating the odds ratios ([OR] 95% confidence of interval [CI]). All clinically or microbiologically relevant variables or those that were significant at $P < .05$ in univariable analysis were included, taking into account potential collinearities and adjusting for possible cofounders. Variables included in the multivariable model were the number of comorbidities, the symptoms of acute COVID-19 at the onset, and the setting of care (eg, outpatients, ward, intensive care unit). The model was adjusted for age and gender. Furthermore, in the Supplementary Materials, 2 tables (Supplementary Table *A*, Supplementary Table *B*) comparing people lost and not lost at the follow up and a multivariable model (Supplementary Table *C*) applying stabilized inverse censoring weights were included, to address the bias of the patients lost between the 12- and 24-month follow up. Analyses were performed by STATA 17.

RESULTS

COVID-19 Onset and Post-COVID-19 Syndrome After 2 Years

Overall, during the first wave, 1067 patients were diagnosed with acute COVID-19 in our hospital (Figure 1). Of them, 230 were interviewed with the interview after a mean of 2.3 years (SD = 0.11) after the acute disease, and the prevalence of the post-COVID-19 syndrome was 36.1% (83 of 230; 95%

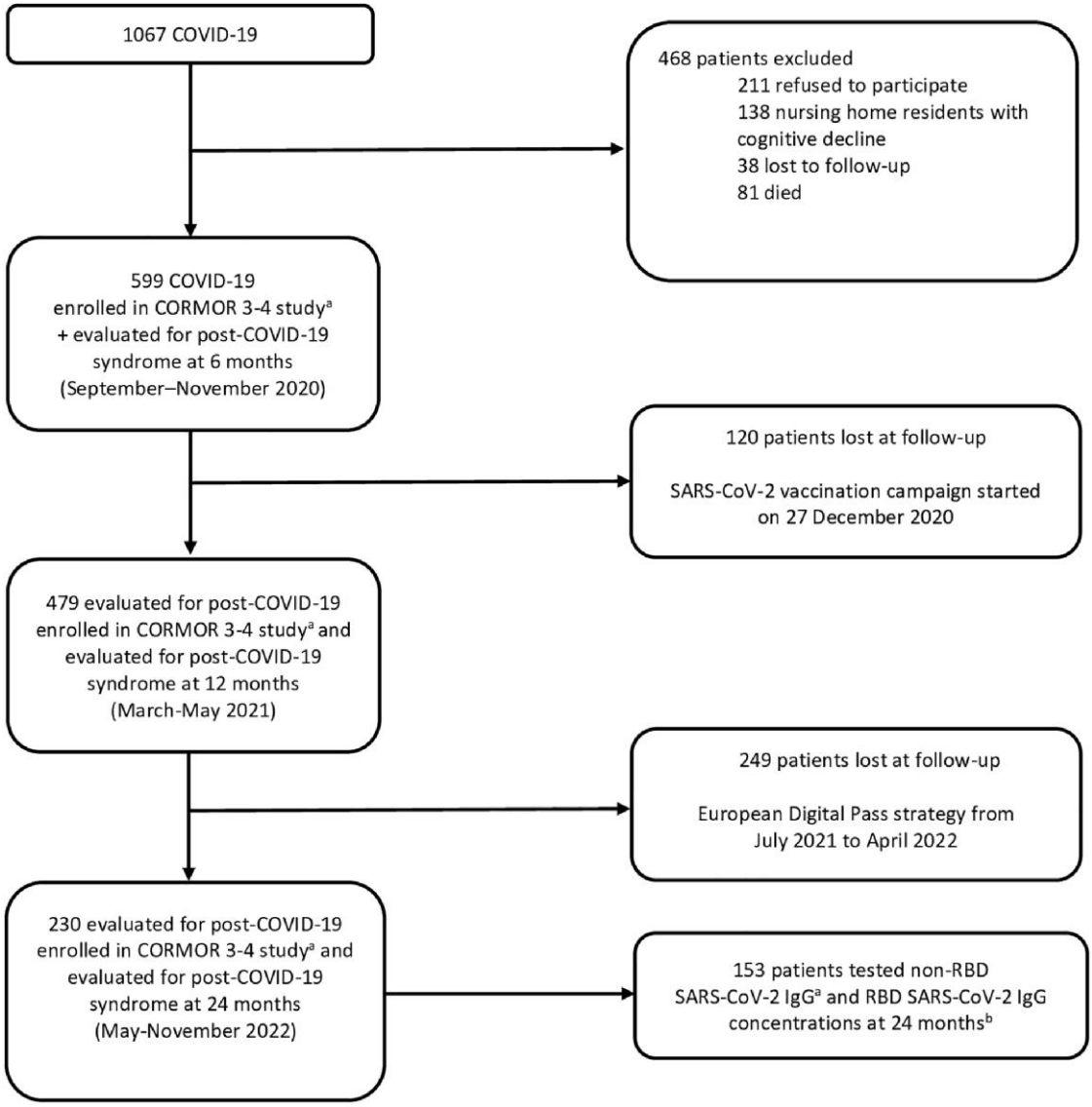

Figure 1. Flow diagram of inpatients and outpatients with coronavirus disease 2019 (COVID-19) included in the post-COVID-19 syndrome study at 6, 12, and 24 months and serological follow-up up to 24 months. [a]Non-receptor binding domain (RBD) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin G (IgG) antibodies (iFlash) concentrations were measured at the serological follow-up visits each month (±5 days) after symptom onset during the first 4 months, and every month up to 12 months (±15 days), and every 3 months up to 24 months. Among the 230 patients, only 153 were evaluated at 24 months. [b]The RBD SARS-CoV-2 IgG antibodies (Roche) at 24 months after the onset of symptom (±60 days). CORMOR, CORonavirus MOnitoRing study.

CI, 29.9–42.7), which was lower than at 6 (40.2%) and 12 months (47.2%) (Table 1, Table 2).

Overall, 35.7% (82 of 230) of patients declared improvement, 19.1% (44 of 230) remained unchanged, and 3.5% (8 of 230) reported worsening of previous symptoms at 2 years compared with 1-year follow up after acute COVID-19.

Among patients with post-COVID-19 syndrome after 2 years, 24.1% (20 of 83) reported at least 1 symptom, 20.5% (17 of 83) reported 2 symptoms, and 55.4% (46 of 83) reported 3 or more symptoms. The most frequently reported persistent symptoms were fatigue (14.4%), rheumatological (14.4%), and psychiatric symptoms (9.6%). Figure 2 summarizes the evolution of specific symptoms at the acute onset and 6- and 12-month follow up. Overall, 46 patients looked for medical evaluation because on persistent symptoms, by accessing the primary care (43.5%, 20 of 46), the emergency department (6.5%, 3 of 46) and specialistic evaluation (50%, 23 of 46).

Post-COVID-19 Syndrome and Vaccination

At the time of the interview, only 10 (4.4%) patients were unvaccinated, whereas 220 (95.6%) were fully vaccinated, all with the mRNA vaccine booster. Unvaccinated patients reported similar rates of persistent symptoms at 24 months compared to those vaccinated (30% vs 36.4%, $P = 1.000$), and there was no

Table 1. Post-COVID-19 Syndrome After 2 Years According to Patients' Baseline Characteristics

Characteristics	Post-COVID-19 Syndrome After Two Years: Yes n = 83	No n = 147	*P*
Gender, *n* (%)			**.008**
Female	54 (65.1)	69 (46.9)	
Male	29 (34.9)	78 (53.1)	
Age Group, *n* (%)			.098
18–40	11 (13.2)	33 (22.5)	
41–60	34 (41.0)	65 (44.2)	
> 60	38 (45.8)	49 (33.3)	
Ethnicity, *n/N* (%)			.103
Native Italian	72/79 (91.1)	134/138 (97.1)	
European	7/79 (8.9)	4/138 (2.9)	
Smoking Habit, *n/N* (%)			.962
Nonsmoker	51/82 (62.2)	93/146 (63.7)	
Smoker	10/82 (12.2)	18/146 (12.3)	
Ex-smoker	21/82 (25.6)	35/146 (24.0)	
Alcohol habit, *n/N* (%)			**.011**
Nondrinker	49/82 (59.8)	60/145 (41.4)	
Drinker	32/82 (39.0)	84/145 (57.9)	
Abuser	1/82 (1.2)	1/145 (0.7)	
Work, *n/N* (%)			**.006**
Work in contact with public and HCWs	27/76 (15.8)	57/133 (42.9)	
Work not in contact with public	20/76 (26.3)	45/133 (33.8)	
Retired	12/76 (15.8)	23/133 (17.3)	
Other	17/76 (22.4)	8/133 (6.0)	
Comorbidities, Number, *n* (%)			**<.001**
0	24 (28.9)	83 (56.5)	
1	32 (38.6)	34 (23.1)	
2	12 (14.5)	20 (13.6)	
3	8 (9.6)	8 (5.4)	
≥4	7 (8.4)	2 (1.4)	
Comorbidities, *n/N* (%)			
Hypertension	25/82 (30.5)	22/144 (15.3)	**.007**
Obesity	14/83 (16.9)	15/146 (10.2)	.144
Diabetes	6/83 (7.2)	9/146 (6.2)	.754
Chronic respiratory disease[a]	5/83 (6.0)	3/146 (2.1)	.116
Cardiovascular disease[b]	2/83 (2.4)	2/146 (1.4)	.622
Liver disease	7/83 (8.4)	0/146 (0)	**.001**
Psychiatric disorders[c]	1/83 (1.2)	2/146 (1.4)	1.000
Renal impairment	0 (0)	0 (0)	
Under Chronic Medication, *n* (%)			**.005**
Yes	48/82 (58.5)	57/145 (39.3)	
No	34/82 (41.5)	88/145 (60.7)	

Abbreviations: COVID-19, coronavirus disease 2019; HCW, healthcare workers.

P-value ≤ 0.05 are in bold.

[a]Pulmonary disease: asthma, chronic obstructive pulmonary disease.

[b]Cardiovascular disease: heart failure, ischemic heart disease, tachyarrhythmias, valvular heart disease, venous thromboembolism.

[c]Depression, anxiety.

relationship between the prevalence of post-COVID syndrome and the increasing number of vaccine doses (Table 3). Fully vaccinated status was not associated with worsening of the post-COVID-19 syndrome (Table 3).

Table 2. Post-COVID-19 Syndrome According to the Clinical Presentation and the Onset and the Microbiological Evolution

	Post-COVID-19 Syndrome After 2 Years: Yes n = 83	No n = 147	*P*
Acute COVID-19 Severity[a], *n* (%)			.138
Asymptomatic	2/83 (2.4)	15/146 (10.3)	
Mild	56/83 (67.5)	99/146 (67.8)	
Moderate	16/83 (19.3)	22/146 (15.1)	
Severe	5/83 (6.0)	7/146 (4.8)	
Critical	4/83 (4.8)	3/146 (2.1)	
Symptoms at Onset, Number, *n* (%)			**.002**
0	4 (4.8)	23 (15.7)	
1	11 (13.3)	24 (16.3)	
2	15 (18.1)	34 (23.1)	
3	8 (9.6)	25 (17.0)	
4	18 (21.7)	25 (17.0)	
≥5	27 (32.5)	18 (12.2)	
Symptoms at 6 Months, Number, *n* (%)			**<.001**
0	30 (36.1)	96 (65.3)	
1	24 (28.9)	32 (21.8)	
2	19 (22.9)	9 (6.1)	
3	7 (8.4)	7 (4.8)	
4	2 (2.4)	2 (1.4)	
≥ 5	1 (1.2)	1 (0.7)	
Symptoms at 12 Months, Number, *n* (%)			**<.001**
0	24 (28.9)	92 (65.6)	
1	22 (26.5)	26 (17.7)	
2	16 (19.3)	17 (11.6)	
3	9 (10.8)	7 (4.8)	
4	3 (3.6)	1 (0.7)	
≥ 5	9 (10.8)	4 (2.7)	
Management, *n* (%)			.060
Outpatients	52 (62.7)	112 (76.2)	
Inpatients			
Ward[b]	24 (28.9)	30 (20.4)	
ICU	7 (8.4)	5 (3.4)	
Length of in-hospital stay, days, median (IQR)	7 (4–10)	6 (3–8)	.283
Viral shedding, days, median (IQR)	21 (14.5–28)	18 (12–24)	**.014**
Vaccinated, *n* (%)	80 (96.4)	140 (95.2)	1.000
Reinfection, *n* (%)	15 (18.1%)	23 (15.7%)	.634
Number of vaccine's doses, median (IQR)	3 (3–3)	3 (3–3)	.959
Non-RBD SARS-CoV-2 IgG[c,d], median (IQR)	9.6 (3.7–28.1)	7.6 (3–45.7)	.753
Positive non-RBD SARS-CoV-2 IgG[b], *n/N* (%)	29/65 (44.6)	41/111 (36.9)	.315
RBD SARS-CoV-2 IgG[b], *n/N* (%)			.405
<0.9 U/mL	0/62 (0)	0/91 (0)	
0.9–2500 U/mL	1/62 (1.6)	0/91 (0)	
>2500 U/mL	61/62 (98.4)	91/91 (100)	

Abbreviations: ICU, intensive care unit; IgG, immunoglobulin G; IQR, interquartile range; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

P-value ≤ 0.05 are in bold.

[a]Asymptomatic; mild (without pneumonia); moderate (with pneumonia); severe (with severe pneumonia); critical including acute respiratory distress syndrome, sepsis, and/or septic shock.

[b]Infectious Disease or Pneumology Department.

[c]At the time of the telephone interview.

[d]Increase in unit in anti-SARS-CoV-2 IgG.

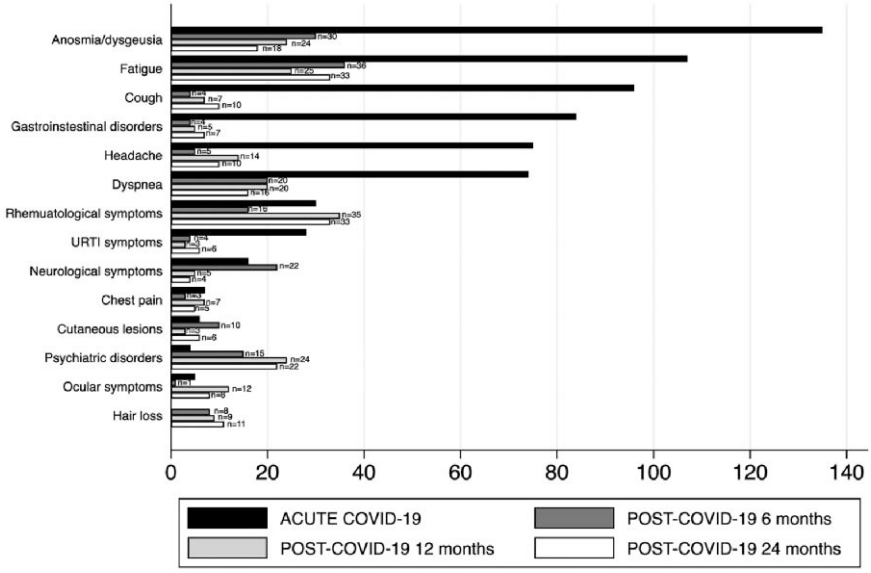

Figure 2. Acute- and post-coronavirus disease 2019 (COVID-19)-related symptoms at 6, 12 and 24 months referred to the population that completed the 24-month follow up (n = 230). URTI, upper respiratory tract infection.

Post-COVID-19 Syndrome and Reinfection

At the time of the evaluation, reinfection had occurred in 16.5% (38 of 230) of patients. Cases of reinfection occurred at a median of 701 days after acute onset of the disease. All patients experienced mild or asymptomatic reinfection. No significant difference in post-COVID-19 syndrome incidence at 24 months emerged in reinfected patients compared to non-reinfected patients (39.5% vs 35.4%, $P = .634$), and reinfection was not associated with the worsening of post-COVID-19 syndrome (Table 2).

Post-COVID-19 Syndrome and Antibody Response

Approximately 153 patients underwent a serological test at the time of the interview (±2 months), and 23 patients who seroreverted (negative non-RBD IgG at previous serological control) were analyzed. Approximately 70 of the 176 patients (39.8%) maintained non-RBD SARS-CoV-2 IgG induced by natural infection after 2 years, and, overall, the median non-RBD SARS-CoV-2 IgG titer was 8.6 (IQR, 3.2–31.9). The presence of non-RBD IgG was not associated with the occurrence of post-COVID-19 syndrome (OR = 1.38; 95% CI, .74–2.56; $P = .316$), and the median non-RBD SARS-CoV-2 IgG was similar in long haulers compared with patients without symptoms (9.6 [IQR, 3.7–28.1] vs 7.6 [IQR, 3–45.7], $P = .753$). All 153 patients (100%) maintained positive RBD SARS-CoV-2 IgG induced by natural infection or hybrid immunity after 2 years.

Post-COVID-19 Syndrome-Associated Factors

Risk factors for post-COVID-19 syndrome were evaluated for the study population (n = 230) and the subgroup with the serological follow-up available at the time of the interview (n = 153). Table 4 summarized the results of univariate and multivariate logistic regression analysis. Female gender (OR = 2.50; 95% CI, 1.32–4.76; $P = .005$), a proportional increase in the number of symptoms at the onset of COVID-19 (OR = 1.40; 95% CI, 1.16–1.70; $P = .001$), and the presence of comorbidities (OR = 1.57; 95% CI, 1.15–2.13; $P = .004$) were all independent risk factors for post-COVID-19 syndrome at 2-year follow ups.

DISCUSSION

Our study offers a comprehensive dynamic overview of the trajectory after acute COVID-19 and highlights that the overall burden of post-COVID-19 syndrome disease is still high (36.1%) even 2 years after the onset in both hospitalized and nonhospitalized patients. Female sex, the number of symptoms at the acute onset, and the number of chronic comorbidities are all independently associated with persistent symptom, whereas vaccination and reinfection do not have an impact on risk and on the dynamics of the negative outcomes in long-term, postacute infection.

The exact prevalence (range, 10%–70%) of post-COVID-19 is unknown due to the (1) definitions available, (2) differences in the study population, (3) duration of the follow-up, (4) type of variants causing acute disease, (5) vaccination status, and (6) medications used for COVID-19 [1, 12–16]. Furthermore, the trajectory of post-COVID-19 syndrome is not completely understood, because few studies have reported that health is affected by the COVID-19 more than 2 years after its acute onset, mainly among hospitalized patients and/or regarding specific subsets of health

Table 3. Post-COVID-19 Symptoms Evolution at 24 Months Compared With Post-COVID-19 Symptoms at 12 Months Stratified According to the Vaccination and Reinfection Status

Post-COVID-19 Symptoms Evolution[a]	<3 Vaccine Doses n = 44 (22.8%)	≥3 Vaccine Doses n = 149 (77.2%)	*P* Value .645	Reinfected n = 38 (16.5%)	Not Reinfected n = 192 (83.5%)	*P* Value .618	Reinfected + <3 Vaccine Doses n = 11 (32.4%)	Reinfected ≥3 Vaccine Doses n = 23 (67.6%)	*P* Value .364
Unaffected	19 (43.2)	61 (40.9)		13 (34.2)	83 (43.2)		3 (27.3)	9 (39.2)	
Unchanged	9 (20.4)	28 (18.8)		7 (18.4)	37 (19.3)		4 (36.4)	3 (13.0)	
Worsened	2 (4.6)	3 (2.0)		1 (2.6)	7 (3.6)		0 (0)	0 (0)	
Improved	14 (31.8)	57 (38.3)		17 (44.8)	65 (33.9)		4 (36.4)	11 (47.8)	

Abbreviations: COVID-19, coronavirus disease 2019.

[a]Information on the number of vaccine doses is missing for 37 patients.

domains (neurological, psychiatric symptoms, fatigue, dyspnea) [1, 2, 12–14, 16–20]. The strengths of our study reside in the extensive assessment from asymptomatic through critical life-threatening disease experienced during the first wave caused by the original strain with 6-, 12-, and 24-month longitudinal assessment and the whole evaluation of factors linked to the post-COVID-19 syndrome, including vaccination, reinfection, and immunological humoral response.

Post-COVID-19 still has a significant impact on patients' everyday life after 2 years. Among long haulers, approximately 55.4% still experienced 3 or more symptoms, with a high prevalence of fatigue, rheumatological, and psychiatric symptoms. However, only half of patients experiencing post-COVID-19 syndrome looked for medical support and 43.5% were visited by a general practitioner. These findings suggest that there is a lack of public awareness and that healthcare systems should design primary healthcare strategies capable of implementing tailored interventions according to the symptoms experienced by patients.

The mechanism of long-term consequences of COVID-19 is likely to be multifaceted and heterogeneous. Post-COVID-19 disproportionately affected women in our cohort, in line with evidence already available [12–14]. Previous studies indicated that the syndrome is associated with the presence of increased number of comorbidities, most often hypertension, obesity, diabetes mellitus, and liver and pulmonary disease, as observed in our cohort [12–14]. Whether the constellation of persistent symptoms experienced by patients represents a new syndrome unique to COVID-19 or there is overlapping with other comorbidities has not been established to date. The high burden of chronic diseases emerged in our study should raise the awareness to manage this entity with a multidisciplinary specialistic approach to respond holistically to the complex clinical needs of these patients. A higher number of symptoms during the acute phase of COVID-19 was also associated with higher risk for post-COVID-19 in our cohort, as previously observed [3, 21].

Severe acute respiratory syndrome coronavirus 2 vaccination has been observed to reduce the risk of post-COVID-19 syndrome. However, there is limited and conflicting evidence on the impact of vaccination on post-COVID-19 symptoms in previously infected patients [7, 14]. These variable results are probably due to the high heterogeneity across studies and the presence of potential confounders, including some protective behaviors and missing data [22]. In our study, we found that vaccination was not associated with the presence of post-COVID-19 symptoms, independently of the number of doses. Moreover, fully vaccinated patients did not experience worsening of post-COVID symptoms. Altogether, our results suggest that SARS-CoV-2 vaccination is not contraindicated in individuals with a history of acute COVID-19 and long haulers should be recommended to undertake vaccination. More robust comparative observational studies and are needed to clearly establish the effectiveness of vaccine on post-COVID-19 syndrome.

Reinfections are progressively common. The impact of reinfection on patients recovered from a first infection with or without pre-existing post-COVID-19 is crucial to understand this entity and to update future policies. Existing literature suggests that repeated infections (second and third infection) may also raise the risk of long COVID-19 compared with patients infected only once [5]. In contrast, we did not find any association between reinfection, risk for post-COVID, and dynamics of long-term effects of COVID-19.

The pathophysiology underlying post-COVID-19 remains unclear, but a common element of most proposed etiologies is the potential role of excess humoral activation. Previous studies showed that people with long COVID-19 continued to have elevated antibody levels due to natural infection and/or vaccination, with variable antispike antibody levels but increased nucleocapsid antibodies [23–25]. In our previous study on this cohort at 6- and 12-month follow up, we found a significant association between SARS-CoV-2 IgG induced by natural infection and post-COVID-19 syndrome [3, 4]. In contrast, this relationship is not confirmed after 2 years, likely due to the expected decline of natural humoral immunity leading to different convalescent immune states in association with post-COVID-19 and the possible role of vaccination resetting immune responses [11].

Table 4. Univariable and Multivariable Analysis of Risk Factors Associated With Post-COVID-19 Syndrome at 24 Months

Risk Factors	OR	Univariable 95% CI	*P* Value	OR	Multivariable 95% CI	*P* Value
Gender						
Female	2.10	1.21–3.67	**.009**	2.50	1.32–4.76	**.005**
Age Group						
41–60 vs 18–40	1.57	0.71–3.49	.269	1.04	0.44–2.47	.921
>60 vs 18–40	2.33	1.04–5.19	**.039**	1.37	0.52–3.58	.524
>60 vs 41–60	1.48	0.82–2.68	.193	1.31	0.64–2.69	.464
Ethnicity						
European vs Native Italian	3.26	0.92–11.50	.067	…	…	
Smoking Habits						
Smoker vs nonsmoker	1.01	0.44–2.36	.976	…	…	
Ex-smoker vs nonsmoker	1.09	0.58–2.07	0.783	…	…	
Ex-smoker vs smoker	1.08	0.42–2.77	.873	…	…	
Alcohol Habits						
Drinker vs nondrinker	0.47	0.27–0.81	**.007**	…	…	
Alcohol use disorder + vs nondrinker	1.22	0.07–20.08	.887	…	…	
Alcohol use disorder r + vs drinker	2.63	0.16–43.23	.500	…	…	
Comorbidities, number	1.60	1.24–2.05	**<.001**	1.57	1.15–2.13	**.004**
Chronic medication	2.18	1.26–3.78	**.006**	…	…	
Work						
Work in contact with public *vs* not in contact with public	1.07	0.53–2.14	.858	…	…	
Retired vs work in contact with public	1.10	0.48–2.54	.821	…	…	
Retired vs work not in contact with public	1.17	0.49–2.81	.719	…	…	
Retired vs other	0.25	0.08–0.73	**.012**	…	…	
Other vs work in contact with public	4.49	1.72–11.68	**.002**	…	…	
Other vs work not in contact with public	4.78	1.77–12.89	**.002**	…	…	
Severity of Acute COVID-19[a]						
Mild vs asymptomatic	4.24	0.94–19.23	.061	…	…	
Moderate vs asymptomatic	5.45	1.09–27.28	**.039**	…	…	
Moderate vs mild	1.29	0.62–2.65	.495	…	…	
Severe vs asymptomatic	5.36	0.83–34.73	.078	…	…	
Severe vs mild	1.26	0.38–4.17	.702	…	…	
Severe vs moderate	0.98	0.26–3.66	.979	…	…	
Critical vs asymptomatic	10	1.22–81.81	**.032**	…	…	
Critical vs mild	2.36	0.51–10.91	.273	…	…	
Critical vs moderate	1.83	0.36–9.35	.466	…	…	
Critical vs severe	1.87	0.28–12.31	.517	…	…	
Symptoms of acute COVID-19 at the onset, number	1.40	1.18–1.67	**<.001**	1.40	1.16–1.70	**.001**
Management						
Ward vs outpatients	1.72	0.92–3.23	.090	1.28	0.61–2.70	.513
ICU vs outpatients	3.02	0.91–9.95	.070	3.24	0.84–12.58	.089
ICU vs ward	1.75	0.49–6.21	.387	2.53	0.63–10.16	.191
Hospitalization, days	1.04	0.98–1.12	.243	…	…	
Viral shedding, days	1.05	1.02–1.08	**.003**	…	…	
Non-RBD SARS-CoV-2 IgG[c,b]	0.99	0.98–1.01	.312	…	…	
Positive non-RBD SARS-CoV-2 IgG[c]	1.38	0.74–2.56	.316	…	…	
Reinfection	1.19	0.58–2.43	.634	…	…	
Number of doses of vaccine	1.03	0.72–1.47	.891	…	…	

Abbreviations: CI, confidence interval; COVID-19, coronavirus disease 2019; Ct, cycle threshold; ICU, intensive care unit; IgG, immunoglobulin G; OR, odds ratio; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

P-value ≤ 0.05 are in bold.

[a]Asymptomatic; mild (without pneumonia); moderate (with pneumonia); severe (with severe pneumonia); critical including acute respiratory distress syndrome, sepsis and/or septic shock.

[b]Increase in unit in anti-SARS-CoV-2 IgG.

[c]At the interview.

Our study has several limitations including the monocentric design, the significant loss of patients at clinical and serological follow ups, the absence of a control population, the low statistical power to assess the relationship between vaccination and/or reinfection and post-COVID-19 syndrome, and the absence of international homogeneous assessment protocols.

However, our longitudinal study offers a perspective regarding the dynamic trajectory at 6, 12, and 24 months after acute COVID-19 with the use of a standardized research protocol and consistent results supporting the strictness of our methodology [3, 4]. In addition, our results on post-COVID-19 are related to the original Wuhan strain of the virus; thus, their relevance to infections with other variants is unknown. Moreover, during the first wave, patients received COVID-19 treatments without high-quality evidence supporting their effectiveness and with several concerns for potential toxicities that may have influenced long-term outcomes; however, medications administered were not recorded in the database of our cohort. Furthermore, reinfections may be underestimated and may have resulted in misclassification of exposure. Also, SARS-CoV-2 variants may have dissimilar acute and long-term effects, but we did not routinely do viral sequencing to distinguish infecting SARS-CoV-2 variants in reinfected patient. Finally, humoral immunity evaluation may be assay dependent.

CONCLUSIONS

In conclusion, a prospective, 2-year follow up of a large cohort of recovered COVID-19 patients after the first wave with diverse severities and managed in different settings suggested that 36.1% of them still experience post-COVID-19 syndrome, without accessing healthcare settings, thus substantially self-managing the condition. Severe acute respiratory syndrome coronavirus 2 vaccination subsequent to primary infection and reinfections may not impact the trajectory of long-COVID-19 in previously infected patients. Internationally coordinated, multidisciplinary research informing tailored healthcare programs is warranted to improve our understanding of the pathogenesis and management of this new medical challenge that still seems to be neglected by the health services but may lead to an emerging global crisis.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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