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Analysis and clinical determinants of post-COVID-19 syndrome in the Lombardy region: evidence from a longitudinal cohort study

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ABSTRACT

Objective To define macro symptoms of long COVID and to identify predictive factors, with the aim of preventing the development of the long COVID syndrome.

Design A single-centre longitudinal prospective cohort study conducted from May 2020 to October 2022.

Setting The study was conducted at Luigi Sacco University Hospital in Milan (Italy). In May 2020, we activated the ARCOVID (Ambulatorio Rivalutazione COVID) outpatient service for the follow-up of long COVID.

Participants Hospitalised and non-hospitalised patients previously affected by COVID-19 were either referred by specialists or general practitioners or self-referred.

Intervention During the first visit, a set of questions investigated the presence and the duration of 11 symptoms (palpitations, amnesia, headache, anxiety/panic, insomnia, loss of smell, loss of taste, dyspnoea, asthenia, myalgia and telogen effluvium). The follow-up has continued until the present time, by sending email questionnaires every 3 months to monitor symptoms and health-related quality of life.

Primary and secondary outcome measures Measurement of synthetic scores (aggregation of symptoms based on occurrence and duration) that may reveal the presence of long COVID in different clinical macro symptoms. To this end, a mixed supervised and empirical strategy was adopted. Moreover, we aimed to identify predictive factors for post-COVID-19 macro symptoms.

Results In the first and second waves of COVID-19, 575 and 793 patients (respectively) were enrolled. Three different post-COVID-19 macro symptoms (neurological, sensorial and physical) were identified. We found significant associations between post-COVID-19 symptoms and (1) the patients' comorbidities, and (2) the medications used during the COVID-19 acute phase. ACE inhibitors (OR=2.039, 95% CI: 1.095 to 3.892), inhaled steroids (OR=4.08, 95% CI: 1.17 to 19.19) and COVID therapies were associated with increased incidence of the neurological macro symptoms. Age (OR=1.02, 95% CI: 1.01 to 1.04), COVID-19 severity (OR=0.42, 95% CI: 0.21 to 0.82), number of comorbidities (OR=1.22, 95% CI: 1.01 to 1.5), metabolic (OR=2.52, 95% CI: 1.25 to 5.27), pulmonary (OR=1.87, 95% CI: 1.10 to 3.32) and autoimmune diseases (OR=4.57, 95% CI: 1.57 to 19.41) increased the risk of the physical macro symptoms.

Conclusions Being male was the unique protective factor in both waves. Other factors reflected different medical behaviours and the impact of comorbidities. Evidence of the effect of therapies adds valuable information that may drive future medical choices.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The analysis refers to only one hospital in a large metropolitan area.
⇒ The available information refers to a non-probabilistic sample that limits valid inferences for larger populations.
⇒ Our data do not provide information on the treatment of long COVID symptoms.
⇒ Symptoms were recorded as absent/present and severity scales were not reported.

INTRODUCTION

COVID-19 is characterised by a wide spectrum of clinical manifestations ranging from asymptomatic infection to respiratory failure and death[1–3] and may be followed by a postacute condition whose duration is unknown. In October 2021, the WHO[4] presented a clinical definition of post-COVID-19 as 'individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of COVID-19 with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis'.

Several groups have investigated predictors of the post-COVID-19 syndrome, but meta-analyses have shown widespread risks of bias,[5 6] such as a short observation period, an unrepresentative population sample, the use of follow-up methods that were not standardised or reliable, symptom assessment without validated scales, and often telephone

calls or remote clinical technology instead of clinical visits.[7–11]

Although several studies have tried to elucidate the pathogenesis of the post-COVID-19 syndrome, most of the specific mechanisms involved remain unknown.[12] Alongside the increase in the number of COVID-19 cases worldwide, the prevalence of the post-COVID-19 syndrome is also increasing.[13] Understanding the pathogenesis of this syndrome, as well as its duration, is now important in helping patients regain their quality of life.

Another critical issue is to characterise post-COVID-19 in terms of synthetic, clinically relevant, macro-symptoms (MS) and to analyse simultaneously co-occurrences and codurations (correlations) of different symptoms. Our study aimed to define the MS of post-COVID-19 and identify predictive factors, focusing on the first two pandemic waves, in order to understand how to prevent the development of the post-COVID-19 syndrome.

The first COVID-19 wave in Italy (Wave 1) is conventionally dated between 21 February and 31 May 2020, since by the end of May the lockdown effect had sharply reduced the incidence, while the second wave (Wave 2) dates from 1 October 2020 to 31 July 2021.

Wave 1 may have hit harder because of the limited knowledge about the disease and the lack of established therapeutic protocols and specialised equipment. Nonetheless, during Wave 2, a much higher number of infections was observed, again challenging the capabilities of healthcare systems. The therapeutic approach changed considerably between the two waves. Several studies have investigated the different clinical features and mortality of COVID-19 between these waves.[14–16]

MATERIALS AND METHODS

We conducted a single-centre longitudinal prospective cohort study at the Luigi Sacco University Hospital, Milan, Italy. In May 2020, we opened the ARCOVID ('Ambulatorio Rivalutazione COVID') outpatient clinic for the follow-up of COVID-19. Hospitalised and non-hospitalised patients older than 18 years with confirmed COVID-19 (by PCR or anti-N antibody detection) were either referred by specialists or general practitioners or self-referred. After signing a written informed consent, they were enrolled in a clinical and immunological longitudinal study approved by the competent ethical committee ('Comitato Etico Interaziendale' Area 1″, n. 2020/ST/158). During the first visit patients received a standardised clinical examination, blood sampling to detect anti-S1/S2 IgG levels and other parameters according to medical judgement, a 6-minute-walk test and thoracic ultrasound in cases of dyspnoea. Patients were referred to other specialists if necessary. A set of questions investigated the presence of ongoing symptoms or the date of resolution. Eleven symptoms were investigated: palpitations, amnesia, headache, anxiety/panic, insomnia, loss of smell, loss of taste, dyspnoea, asthenia, myalgia and telogen effluvium. The follow-up continued using email questionnaires sent every 3 months to monitor symptoms and health-related quality of life. The few patients who did not have e-mail were contacted by phone to come and answer on plain paper.

Serum anti-S1/S2 was assessed through a chemiluminescent immunoassay (CLIA) intended for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma (DiaSorin LIAISON S1/S2 IgG) expressed in Arbitrary Units (AU)/mL. On 15 December 2021, it was replaced by another indirect immunoassay (CLIA) for the quantitative determination of antitrimeric spike protein-specific IgG antibodies to SARS-CoV-2 in human serum or plasma samples (DiaSorin Trimeric anti-S IgG), expressed in WHO Binding Arbitrary Units (BAU)/mL. All results were converted into BAU/mL for homogeneity. Serology was performed at 1 month, 3 months and 6 months after clinical onset and every 6 months thereafter. Patients were classified according to the WHO COVID-19 disease severity score into four categories: mild, moderate, severe and critical.[3] Demographic, clinical and acute-phase-related parameters were recorded. In this paper, we monitored the population who contracted COVID-19 between 23 February 2020 and 26 July 2021.

Patient and public involvement

Participants of the study were not involved in setting the research question or the outcome measures, nor were they involved in developing plans for recruitment, design or implementation of the study. No participants were asked for advice on interpreting or writing up of results.

Statistical analysis

First, we compared the characteristics of enrolled patients (pre-COVID-19 characteristics, therapies used during the acute phase of COVID-19) and the duration of symptoms across the waves.

The Shapiro-Wilk test was used to evaluate the normal distribution of quantitative variables, namely the duration of the 11 original symptoms. Descriptive statistics concerning durations and incidence of symptoms are reported as counts, percentages, mean values and SD as well as median values and interquartile ranges (IQR: 25th–75th percentile).

Parametric or non-parametric tests, depending on the Shapiro-Wilk test, were used to compare means of quantitative variables (t-test for independent samples or Mood's median test, respectively) by waves. The z-tests were used to compare the proportions of binary variables. χ^2 statistics were applied to compare distributions of categorical variables across waves.

The primary aim of the analysis was the construction of synthetic scores (aggregation of symptoms based on occurrences and durations) that may reveal the presence of post-COVID-19 in different clinical domains. To this end, a mixed supervised (physician advice about possible MS domains, such as neurological or physical) and empirical strategy was adopted. For the latter, principal

component analysis with different rotation techniques (varimax, oblimin) and Cronbach's alpha analysis were employed to achieve and validate synthetic scores with maximum internal consistency (degree of mean correlation among one synthetic score and its block of symptom variables) and discriminant validity (reducing the risk that a variable that identifies a specific MS correlates with other blocks of variables that measure different MS).

The durations of symptoms for variables that identify the same synthetic score were summed and transformed into a binary variable (primary outcome), assuming a value of 1 in case of a positive sum and identifying the presence of MS of post-COVID-19. Otherwise, the binary variable assumed a value of 0 if the sum equalled 0, meaning the absence of MS. Logistic regression for the occurrence of each MS and wave was performed to assess the effect and significance of demographic and clinical covariates as possible risk factors for the post-COVID-19 syndrome in such domains. Model selection was done using a mixed approach. First, a best subset that minimises the Bayesian information statistics was identified. Second, non-significant variables using p values were removed. A p value of less than 0.1 was considered statistically significant (in such models age and sex were forced in the final models). The same logistic models were fitted separately for each original symptom by wave. Data analysis was performed using the R software.

RESULTS

Data were collected from 575 and 793 patients enrolled in the outpatient service convalescent centre during the first and second COVID-19 waves, respectively.

Table 1 presents the descriptive statistics and differences between waves in terms of pre-COVID-19 characteristics and therapy administered during the acute phase of COVID-19 infection.

In both cohorts, males and females were almost equally distributed. In the second wave patients were older (M=58.1 years in the second vs M=52.5 years in the first). Several case-mix characteristics, such as COVID-19 intensity (36.1% severe and critical intensity vs 28.7%), metabolic diseases (29.9% vs 19%), diabetes (11.2% vs 7.7%), cardiac (38% vs 32%) and total comorbidities (M=2.9 vs 2.2), showed a significantly greater prevalence in the second wave. Pulmonary (10%–11%) diseases were almost equally represented. Large differences emerged among cohorts in terms of therapies during the two waves (table 1, COVID-19 therapies).

Figure 1 and table 2 illustrate the main results and differences between waves in terms of incidence and duration of symptoms, respectively. The mean symptom burden in the second wave was three symptoms, with an IQR ranging from 1 to 5 (compared with an IQR of 0–4 in the first wave).

Asthenia, myalgia and dyspnoea were the main symptoms in both waves (incidence >35%), and they increased by 5%–7% in the second wave. The largest increase was observed in insomnia and amnesia (+12.6% and +8.6%, respectively) shared by a large proportion of patients in the second wave (>30%). Minor symptoms remained stable between the two waves.

Concerning duration, most symptoms remained stable between waves. Only dyspnoea (−20 days on average), asthenia (−45 days), amnesia (−33 days) and insomnia (−8 days) decreased significantly in the second wave.

Three MSs were found (comprising 67% of the total variance of the 11 symptoms): the neurological–psychological ('Neuro-MS'; including palpitations, amnesia, headache, anxiety/panic and insomnia), the sensorial ('Sens-MS'; including loss of smell and taste) and the physical ('Phys-MS'; including dyspnoea, asthenia and myalgia). The correlation structure between symptoms and MSs found by the varimax-rotated principal component analysis is presented in online supplemental table S1.

Hair loss was eliminated according to medical advice for the low health impact.

Each MS explains a large quota (70% Neuro-MS, 87% Sens-MS and 75% Phys-MS) of the variance of its block of variables. Cronbach alpha's confirmed that no alternative structures (eg, by reassigning a symptom to another or new MS) would assure higher internal consistency of each block. The adopted rotation criterium prevents a single symptom from being associated with different MS. In addition, results were validated and confirmed by clinicians.

Depending on the presence of each of the three MS (at least 1 day of duration for original symptoms associated with each MS), the patients were classified into eight groups per wave, as illustrated in figure 2.

Significant differences emerge among distributions (p value <0.0001), particularly due to differences in the proportion of patients without MS (decreasing over time, from 38% to 24%) and of the simultaneous presence of Neuro-MS and Phys-MS (increasing from 23% to 34%) among waves.

The effect and significance of demographic and clinical covariates, as meaningful risk factors for post-COVID-19 occurrence in different domains and emerging from logistic regression, are illustrated in table 3 for Neuro-MA and Phys-MS and in table 2 for Sens-MS. Age and gender were forced in the final models.

Overall, the structure of significant factors (retained at least at the 0.1 level of significance) varied among MS and for each over the two waves (table 3). For both waves, males were less exposed to Neuro-MS, independent of age and COVID-19 intensity (p value=0.322). Statin and calcium antagonists resulted in protective factors in the first wave, whereas ACE inhibitors and inhaled steroids were associated with increased incidence. Other risk factors were therapies administered during the COVID-19 infection phase, such as hydroxychloroquine (HCQ) (Wave 1) and steroids and antibiotics (Wave 2). In the second wave, patients with pre-existing diseases, such as diabetes and immunologic disease, were at lower risk.

Table 1 Enrolled patients, pre-COVID-19 characteristics and therapies during acute phase of COVID-19: descriptive and differences by waves

		Levels	Wave 1 (n=575)	Wave 2 (n=793)	P value
Personal info	Sex	Male	297 (51.7%)	385 (48.5%)	0.634
		Female	278 (48.3%)	408 (51.5%)	
	WHO scale (COVID-19 intensity)	Mild	139 (24.2%)	209 (26.4%)	0.004
		Moderate	266 (46.3%)	295 (37.2%)	
		Severe	57 (9.9%)	94 (11.9%)	
		Critical	108 (18.8%)	192 (24.2%)	
	Age	Mean (SD)	52.5 (15.9)	58.1 (15.0)	<0.001
		Median (IQR)	54.0 (43.0–62.0)	58.0 (49.0–69.0)	
	BMI	Mean (SD)	25.9 (11.9)	26.3 (4.5)	0.483
		Median (IQR)	24.8 (22.4–27.3)	26.0 (23.2–28.1)	
Therapies pre-COVID-19	DOAC, VKA	0	554 (96.3%)	756 (95.3%)	
		1	11 (1.9%)	33 (4.2%)	0.028
	Antiaggregant	0	524 (91.1%)	679 (85.6%)	
		1	41 (7.1%)	111 (14.0%)	<0.001
	Hypolipidaemic statin	0	506 (88.0%)	662 (83.5%)	
		1	59 (10.3%)	128 (16.1%)	0.004
	Hypoglycaemic	0	528 (91.8%)	712 (89.8%)	
		1	37 (6.4%)	76 (9.6%)	0.041
	Antihypertensive	0	411 (71.5%)	516 (65.1%)	
		1	154 (26.8%)	274 (34.6%)	0.007
	Calcium antagonists	0	524 (91.1%)	702 (88.5%)	
		1	41 (7.1%)	88 (11.1%)	0.019
	Beta blockers	0	502 (87.3%)	662 (83.5%)	
		1	62 (10.8%)	128 (16.1%)	0.009
	ACE inhibitors (sartani)	0	454 (79.0%)	587 (74.0%)	
		1	111 (19.3%)	203 (25.6%)	0.012
	Diuretics	0	528 (91.8%)	699 (88.1%)	
		1	37 (6.4%)	91 (11.5%)	0.004
	Immune steroids	0	552 (96.0%)	783 (98.7%)	
		1	13 (2.3%)	6 (0.8%)	0.019
	Antiretrovirals	0	551 (95.8%)	753 (95.0%)	
		1	14 (2.4%)	36 (4.5%)	0.052
	Neuropsycoactive	0	524 (91.1%)	701 (88.4%)	
		1	41 (7.1%)	89 (11.2%)	0.016
	Steroids inhalators	0	557 (96.9%)	768 (96.9%)	
		1	18 (3.1%)	25 (3.1%)	0.955
Comorbidity pre-COVID-19	Pulmonary	0	517 (89.9%)	703 (88.7%)	
		1	58 (10.1%)	90 (11.3%)	0.513
	Kidney	0	548 (95.3%)	751 (94.7%)	
		1	27 (4.7%)	42 (5.3%)	0.691
	Neoplasm	0	554 (96.3%)	774 (97.6%)	
		1	21 (3.7%)	19 (2.4%)	0.176

Continued

Table 1 Continued

		Levels	Wave 1 (n=575)	Wave 2 (n=793)	P value
	Cardiac	0	378 (65.7%)	479 (60.4%)	
		1	187 (32.5%)	303 (38.2%)	0.033
	Metabolic	0	456 (79.3%)	552 (69.6%)	
		1	109 (19.0%)	237 (29.9%)	<0.001
	Diabetes	0	521 (90.6%)	701 (88.4%)	
		1	44 (7.7%)	89 (11.2%)	0.040
	Immunological disease	0	544 (94.6%)	744 (93.9%)	
		1	30 (5.2%)	48 (6.1%)	0.619
	Autoimmune disease	0	552 (96.0%)	758 (95.6%)	
		1	23 (4.0%)	33 (4.2%)	0.723
	Total comorbidities	Mean (SD)	2.2 (1.5)	2.9 (2.0)	
		Median (IQR)	2.0 (1.0–2.0)	2.0 (1.0–4.0)	<0.001
COVID-19 therapies		**Levels**	**Wave 1 (n=575)**	**Wave 2 (n=793)**	**P value**
O_2 therapy	None		329 (57.2%)	368 (46.4%)	<0.001
	Cannula nose		68 (11.8%)	116 (14.6%)	
	Venturi mask		61 (10.6%)	112 (14.1%)	
	Reservoir mask		15 (2.6%)	12 (1.5%)	
	Not Invasive ventilation		63 (11.0%)	150 (18.9%)	
	Orotracheal		26 (4.5%)	25 (3.2%)	
O_2 intensity	None		325 (56.5%)	367 (46.3%)	<0.001
	≤2 L/min		45 (7.8%)	66 (8.3%)	
	6–2 L/min		29 (5.0%)	58 (7.3%)	
	>6 L/min		155 (27.0%)	287 (36.2%)	
Steroids	No		497 (86.4%)	271 (34.2%)	
	Yes		62 (10.8%)	519 (65.4%)	<0.001
Remdesevir	No		518 (90.1%)	663 (83.6%)	
	Yes		39 (6.8%)	127 (16.0%)	<0.001
Antibiotic	No		478 (65.7%)	727 (66.6%)	
	Yes		197 (34.3%)	266 (33.4%)	0.497
HCQ	No		340 (59.1%)	785 (99.0%)	
	Yes		217 (37.7%)	5 (0.6%)	<0.001
Inhibitors protease HIV	None		417 (72.5%)	789 (99.5%)	<0.001
	Lopinavir		134 (23.3%)	0 (0%)	
	Lopinavir and darunavir		4 (0.7%)	0 (0%)	
Tocilizumab	No		524 (91.1%)	788 (99.4%)	
	Yes		32 (5.6%)	2 (0.3%)	<0.001
Heparin	No		411 (71.5%)	287 (36.2%)	
	Yes		146 (25.4%)	502 (63.3%)	<0.001

Note: therapies as DMARDs and anticancer were not presented (less than 10 patients in each wave). For binary therapy, p value refers to z-tests on equality of percentages among waves (two-sided). For categorical therapies, p value refers to χ^2 tests on equality of distributions among waves (one sided). Missing data for each variable were excluded from the analysis. BMI, body mass index; DMARDs, disease-modifying antirheumatic drugs; DOAC, direct oral anticoagulants; VKA, vitamin K antagonists.

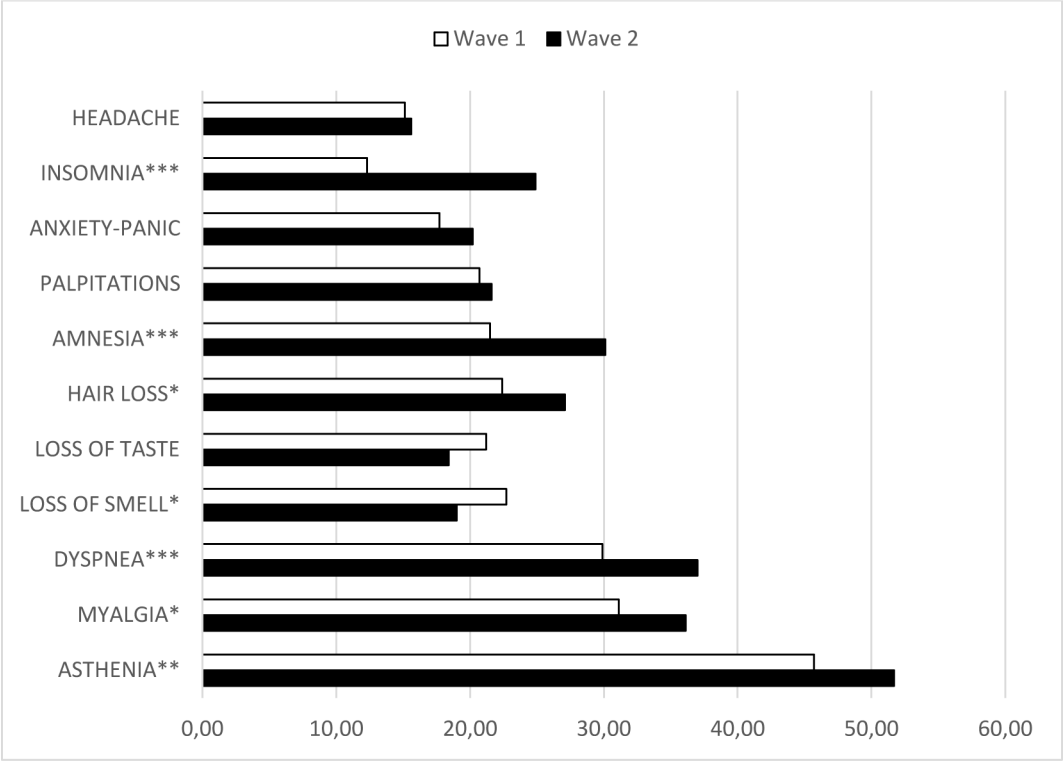

Figure 1 Incidence (%) of symptoms by COVID-19 wave and differences by waves. Note: incidence is calculated as number of new cases suffering from a symptom as a proportion of the patients in ARCOVID (in each wave) as population at risk. Missing values were excluded. Asterisks refer to significance of incidence differences over waves: *p value <0.1, **p value <0.05, ***p value <0.01. ARCOVID, Ambulatorio Rivalutazione COVID.

Males were less prone than females to the risk of developing Phys-MS, whereas age and COVID-19 severity were predictive of Phys-MS (P value <0.001, both from likelihood ratio tests) in Wave 1. Critically ill patients with COVID-19 were more likely to suffer from Phys-MS than moderately (OR=2.36, 95% CI: 1.21 to 4.69) or mildly ill patients (OR=2.33, 95% CI: 1.27 to 4.36). A higher number of comorbidities (OR=1.23, 95% CI: 1.01 to 1.50), particularly metabolic diseases (OR=2.52, 95% CI 1.25 to 5.27), as well as beta blockers and inhaled steroids increased the risk of Phys-MS, whereas the use of an anticoagulant was protective. In the second wave, patients with pulmonary and autoimmune diseases were strongly predisposed to Phys-MS.

Moreover, similar to Neuro-MS, therapies administered during the first wave, such as HCQ, and during the second wave, such as steroids and remdesivir, were significantly associated with increased risk of post-COVID-19.

Regarding results about Sens-MS (online supplemental table S2), having kidney disease or undergoing tocilizumab/ACE inhibitors was protective in Wave 1, whereas being male and young was protective in Wave 2.

Online supplemental table S3 shows the main results (significance and direction of the sign of each covariate) related to the estimated occurrence of each original symptom.

DISCUSSION

The landscape of post-COVID-19 analyses

A recent meta-analysis of post-COVID-19 symptoms has included clinical trials whose mean or median observation period was at least 1 year.[17] Evaluable studies were 18. Thirteen included only hospitalised patients. Most cohorts were small and some concerned a single aspect of post-COVID-19.[18 19] The selection identified three large cohorts (>1000 patients), limited to hospitalised patients. Two studies employed a telephone interview 1 year after discharge.[18–20] One large cohort included 1272 hospitalised patients assessed with the modified Medical Research Council scale for dyspnoea. Patients were evaluated with questionnaires about symptoms and underwent medical examination.[21] One year after acute COVID-19 infection, symptom persistence ranged from 12% (insomnia) to 28% (fatigue/weakness). Female sex was predictive of sequelae at 1 year, while age was not. COVID-19 severity was associated with post-COVID-19 in some studies but not in others, in one study only with muscle fatigue.[22]

One study showed a correlation with the duration of mechanical ventilation,[19] another[23] associated corticosteroid therapy during COVID-19 with a lower incidence of headache, dysphagia, chest pain and depression, while a further study[21] correlated steroid therapy with increased fatigue and muscle weakness.

Our analysis aimed to understand which elements of the acute disease could positively or negatively influence

Table 2 Number of symptoms, incidence and duration (in days) of each symptom: descriptive and differences by first two waves of COVID-19

	Wave 1 (n=575)	Wave 2 (n=793)	P value
Number of symptoms			
Mean (SD)	2.6 (2.9)	3.0 (2.7)	0.008
Median (IQR)	2.0 (0.0, 4.0)	3.0 (1.0, 5.0)	
Dyspnoea			
No. (%)	172 (29.9 %)	294 (37.0 %)	0.007
Mean (SD)	62.6 (131.9)	42.8 (72.8)	
Median (IQR)	0.0 (0.0, 31.0)	0.0 (0.0, 70.0)	0.006
Hair loss			
No. (%)	129 (22.4 %)	215 (27.1 %)	0.057
Mean (SD)	46.5 (112.9)	33.4 (65.9)	
Median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 45.0)	0.049
Asthenia			
No. (%)	263 (45.7 %)	410 (51.7 %)	0.034
Mean (SD)	114.8 (172.5)	69.8 (88.6)	
Median (IQR)	0.0 (0.0, 185.5)	21.0 (0.0, 127.0)	0.029
Myalgia			
No. (%)	179 (31.1 %)	287 (36.1 %)	0.059
Mean (SD)	89.3 (166.1)	48.9 (82.2)	
Median (IQR)	0.0 (0.0, 73.0)	0.0 (0.0, 81.0)	0.051
Palpitations			
No. (%)	119 (20.7 %)	172 (21.6 %)	0.707
Mean (SD)	54.9 (131.8)	27.5 (63.3)	
Median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.623
Loss of smell			
No. (%)	131 (22.7 %)	151 (19.0 %)	0.105
Mean (SD)	46.8 (119.7)	22.1 (59.4)	
Median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.091
Loss of taste			
No. (%)	122 (21.2 %)	146 (18.4 %)	0.222
Mean (SD)	38.4 (107.8)	21.5 (61.1)	
Median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.169
Amnesia			
No. (%)	124 (21.5 %)	238 (30.1 %)	<0.001
Mean (SD)	75.4 (159.8)	42.5 (77.5)	
Median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 67.0)	<0.001
Headache			
No. (%)	87 (15.13 %)	124 (15.6 %)	0.857
Mean (SD)	38.5 (114.7)	19.5 (58.3)	
Median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.818
Anxiety/panic			
No. (%)	102 (17.7 %)	160 (20.2 %)	0.289
Mean (SD)	48.0 (126.5)	27.0 (64.4)	
Median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.258
Insomnia			
No. (%)	71 (12.35 %)	198 (24.9 %)	<0.001

Continued

Table 2 Continued

	Wave 1 (n=575)	Wave 2 (n=793)	P value
Mean (SD)	39.8 (124.9)	31.5 (67.5)	
Median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	<0.001

Note: significance of differences of durations was based on parametric (mean: t-test) or non-parametric (median: Mood's test) tests depending on results of normality (Shapiro-Wilk) test. P values of tests on the rows 'No.' refer to z-test on equality of percentages among waves. All tests are two-sided. No. indicates the number of patients. Missing data for each variable were excluded from the analysis.

the postacute phase. Dividing the main post-COVID-19 symptoms into three macro areas allowed us to analyse the impact of a changing affected population (older and more severely affected patients in the second wave) and therapeutic approaches used during the acute phase (steroids and low-weight heparin vs antiretrovirals and HCQ) in the post-COVID-19 syndrome.

Strengths and limitations

A strength of this paper is the new information it provides about the incidence and duration of post-COVID-19 symptoms in Wave 1 and Wave 2.

We clarify how therapies administered during the acute phase of illness changed during waves, identifying COVID-19 treatments as well as pre-existing diseases and therapies that significantly affect the probability of post-COVID-19 symptoms. We hypothesise that treatments in the acute phase of the illness may influence the incidence of long-term symptoms.

However, this paper also has several limitations.

First, the analysis covered only one hospital in a large metropolitan area.

Second, this analysis is not a whole population survey nor limited to hospitalised patients, rather it is an open clinic to which any subject with COVID-19 can be referred or can self-refer. Therefore, the available information refers to a non-probabilistic sample that limits valid inferences for larger populations. For this reason, we limit our study to the incidence of post-COVID-19 as a primary outcome and avoid the analysis of duration that requires more sophisticated methodologies (inference

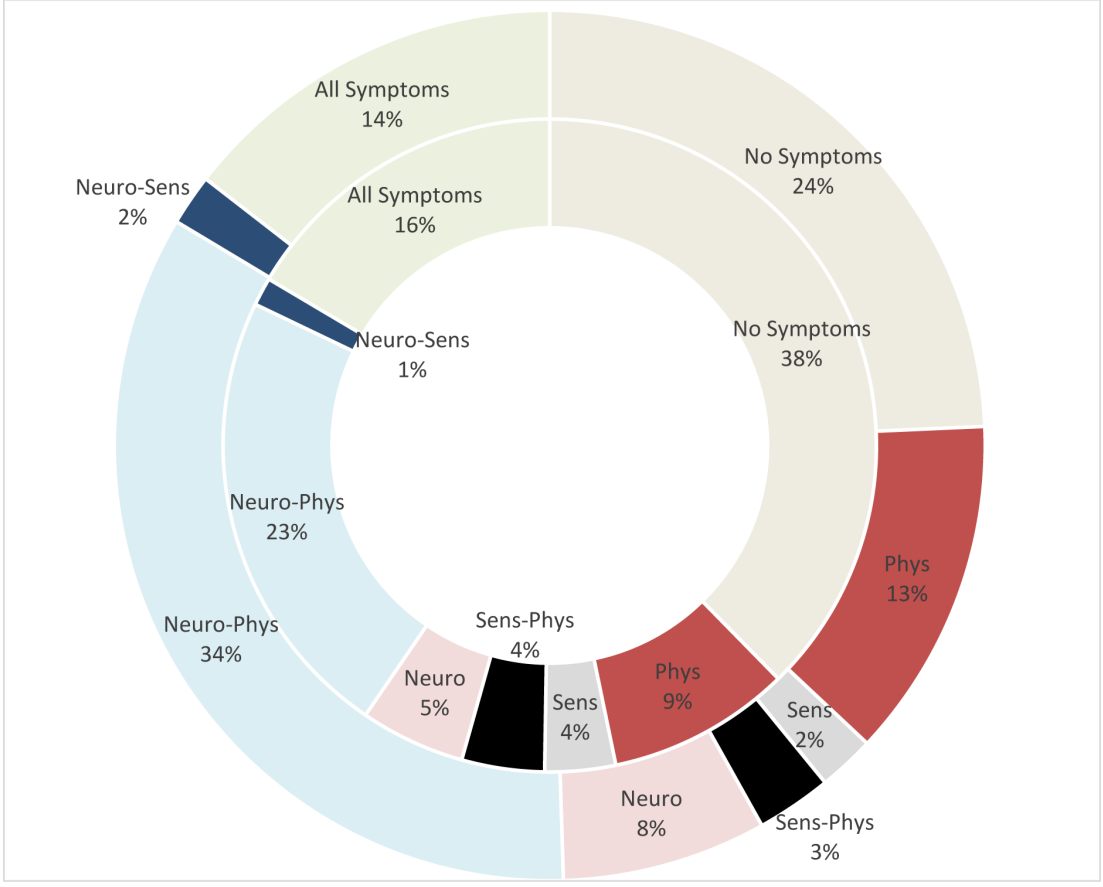

Figure 2 Profile of macro-symptoms by wave (internal ring wave 1; external ring wave 2). Note: profiles indicate the eight possible groups indicating the presence or absence of each of three macro-symptoms: Physical (Phys), Neurological (Neuro) and Sensory (Sens). Missing values were excluded.

Table 3 Significant covariates and OR that predict the occurrence of Neuro macro-symptom (top) and Phys macro-symptom (bottom) by first two waves of COVID-19

	Wave 1				Wave 2			
	OR	95% CI		P value	OR	95% CI		P value
Neuro macro-symptom								
(intercept)	1.138	0.538	2.405		2.552	1.324	4.978	
Male vs female	**0.392**	**0.256**	**0.595**	**<0.001**	**0.311**	**0.221**	**0.434**	**<0.001**
Age	1.003	0.988	1.017	0.723	0.991	0.980	1.003	0.149
Hypolipidaemic statin	**0.297**	**0.140**	**0.608**	**0.001**	**1.551**	**0.961**	**2.532**	**0.072**
Calcium antagonists	**0.453**	**0.179**	**1.123**	**0.087**				
Beta blockers	1.931	0.914	4.281	0.085				
ACE inhibitors (sartani)	2.039	1.095	3.892	0.025				
Steroids inhalators	4.083	1.175	19.192	0.026				
Kidney disease	**0.301**	**0.072**	**1.097**	**0.069**				
Hydroxychloroquine	*2.548*	*1.644*	*3.997*	*<0.001*				
Neuro-psychoactive					1.642	0.965	2.866	0.068
Diabetes					**0.572**	**0.331**	**0.985**	**0.044**
Immunologic disease					**0.269**	**0.127**	**0.536**	**<0.001**
Steroids					*1.729*	*1.169*	*2.569*	*0.006*
Remdesivir					*1.500*	*0.951*	*2.393*	*0.082*
Antibiotics					*1.392*	*0.980*	*1.985*	*0.065*
McFadden R^2	0.12				0.09			
Phys macro-symptom								
(intercept)	1.209	0.444	3.323		1.19	0.632	2.247	
Age	1.022	1.006	1.038	0.005	1.005	0.994	1.016	0.397
Male vs female	**0.600**	**0.384**	**0.931**	**0.022**	**0.455**	**0.327**	**0.632**	**<0.001**
WHO scale: mild vs critical	**0.429**	**0.229**	**0.787**	**0.007**				
WHO scale: moderate vs critical	**0.422**	**0.213**	**0.822**	**0.012**				
WHO scale: severe vs critical	0.957	0.395	2.400	0.922				
Number of comorbidity	1.227	1.010	1.495	0.039				
Metabolic disease	2.516	1.247	5.270	0.010				
Antiaggregant	**0.286**	**0.121**	**0.667**	**0.004**				
Beta blockers	2.642	1.141	6.711	0.022				
Steroids inhalators	5.591	1.376	37.95	0.014				
Hydroxychloroquine	*1.612*	*0.958*	*2.720*	*0.072*				
Pulmonary disease					1.876	1.103	3.324	0.020
Autoimmune disease					4.570	1.571	19.416	0.003
Steroids					*1.761*	*1.225*	*2.539*	*0.002*
Remdesivir					*1.636*	*1.023*	*2.676*	*0.040*
Mc Fadden R^2	0.10				0.11			

Note: protective factors in bold; rows in italics refers to therapies during the acute phase of COVID-19, white background to pre-COVID-19 therapies and pre-existing diseases. Missing data were excluded from the analysis.

for sample selected sample). Further research is in progress to complete this figure.

Third, the database did not provide information on the treatment of post-COVID-19 symptoms, which may have affected their duration, and this is a possible confounder.

Fourth, symptoms were recorded as absent/present and severity scales were not reported. This enables us only to define the disappearance of a symptom and not its increase or reduction in intensity.

Patient referrals to our clinic changed slightly between the two waves. Asymptomatic patients in the cohort were relatives of symptomatic subjects (mainly in Wave 1) and subjects willing to measure their anti-S response for evaluating vaccine effectiveness (in Wave

2). During the second wave, a larger number of hospitalised patients was referred by physicians. This may explain the increase in post-COVID-19 symptoms described in figure 1. We do not have a clear explanation for why taste and smell loss appeared to diminish in the second wave.

The study has confirmed some risk factors, but also reduced the importance of others and revealed unexpected novelties. Male sex appears to be protective across all MSs in both waves, whereas age was not predictive. Mild and moderate COVID-19 were protective against Phys-MS, but only in the first wave.

The use of statins and calcium antagonists seemed to be protective against the risk of developing Neuro-MS during the first wave. This can be related to a possible cardiovascular component in the development of neurological post-COVID-19 symptoms. These drugs seem not to have had an impact during the second wave, probably because heparin was introduced in the management of COVID-19 acute disease as a cardiovascular protector. HCQ in the first wave and steroids in the second wave could have played an important role in promoting these symptoms. Steroids may have impacted patients' abilities to fully control the virus, preventing the cytokine storm, while the role of HCQ is more difficult to explain, as it does not directly act on the immune system and seems to have little effect on the virus.

Therapies showed significantly more use of cardioprotective drugs at baseline, reflecting the overall older age of second-wave patients. Data reflected changes in the therapeutic approach to hospitalised and home-care patients, in particular the increased use of steroids, heparin and remdesivir as well as the sharp reduction in use of HCQ, antiretroviral and tocilizumab, according to the international recommendations.[24]

However, examination of the drugs used in the acute phase from a post-COVID-19 perspective is important: for example, it shows that steroids should be used as life-saving therapy only in case of pneumonia. The role of diabetes as a protective factor in the second wave could be related to the high mortality rate in this population caused by COVID-19 pneumonia[25] which may have selected a more 'fit' population. Subjects with diabetes may have been less exposed to steroids given their impact on glucose levels. Last, the protective effect of nephropathy may be related to the higher threshold of tolerance of adverse events (ie, a polypathological population).

Phys-MS was shown to be prevented by chronic anticoagulant consumption during the first wave. This role seems not to be relevant in the second wave when heparin became the gold standard of COVID-19 therapy. Chronic uptake of inhaled corticosteroids was negatively associated with Phys-MS incidence. This observation may reflect a higher risk of developing Phys-MS among people suffering from pulmonary disease (ie, COPD or pulmonary fibrosis). People with autoimmune diseases have a risk of developing Phys-MS at a rate that is 4.5-fold higher than others after Wave 2. A clear explanation is lacking but it may be related to a worsening of the baseline condition and time to recovery.

Lastly, Sens-MS was not sensitive to the modified therapeutic approach in the acute phase. The apparent protection by nephropathy in first-wave patients may be due to an already damaged sensorial profile, as described in the literature.[26] Other correlations of this macro-system involved few patients and only approached a significant p value of 0.05, so we are unable to offer a confident explanation.

To conclude, the incidence of post-COVID between the two alpha waves dropped dramatically, likely due to the different therapeutic approach. Our clinic has registered 575 post-COVID cases over 88 968 positive swabs in Lombardy (0.65%) in the first wave and 793 over 840 978 (0.09%) in the second wave.[27] The only protective factor emerging among all macro areas in both waves was the male sex. Other factors reflect the different therapeutic approach (HCQ, steroids or heparin) as well as the impact of comorbidities. Considering the effect of therapies such as HCQ from a post-COVID-19 perspective adds important information that may help drive medical choices in the future: for example, considering not only the evidence of their efficacy in the acute infection but also the possibility of leading to chronic or long-lasting symptoms. In addition, the use of steroids in patients with mild to moderate COVID-19 may increase the risk of Neuro-MS and Phys-MS. This experimental and innovative health service can be useful for effectively monitoring post-COVID-19 symptoms.

As the pattern of post-COVID-19 continues to evolve with the appearance of new variants, we will update the present observations to include the delta and omicron waves.

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Online only supplement

eTable 1: Correlations among Macro-symptoms scores (Varimax-rotated principal components) and original symptoms

	Neuro-MS	Sens-MS	Phys-MS
PALPITATIONS	**0.73**	0.23	0.55
AMNESIA	**0.80**	0.25	0.56
CEPHALALGIA	**0.66**	0.23	0.46
ANXIETY-PANIC	**0.73**	0.21	0.48
INSOMNIA	**0.67**	0.24	0.49
LOSS OF SMELL	0.28	**0.95**	0.31
LOSS OF TASTE	0.32	**0.92**	0.29
DYSPNEA	0.56	0.22	**0.74**
ASTHENIA	0.64	0.29	**0.92**
MYALGIA	0.61	0.30	**0.88**

eTable 2: Risk of Sensorial Macro-symptom incidence: Significant covariates and odds ratio (OR)

	Wave 1				Wave 2			
	OR	95% IC		P-value	OR	95% IC		P-value
(Intercept)	0.278	0.135	0.560		1.812	0.902	3.651	
Age	1.010	0.998	1.024	0.109	**0.976**	**0.964**	**0.988**	**<0.001**
Male	**0.693**	**0.469**	**1.021**	**0.064**	**0.392**	**0.267**	**0.569**	**<0.001**
Kidney disease	**0.095**	**0.005**	**0.466**	**0.001**				
Neoplasm	**0.282**	**0.044**	**1.020**	**0.054**				
Tocilizumab	**0.366**	**0.106**	**0.970**	**0.043**				
Immunologic disease					**0.484**	**0.179**	**1.102**	**0.087**
ACE-inhibitors (Sartani)					**0.619**	**0.367**	**1.011**	**0.056**
McFadden R^2	0.11				0.07			

Note: protective factors for symptom occurrence in bold.

eTable 3: Significant factors for probability of symptom incidence*, by symptom and wave

		Dyspnea		Asthenia		Myalgia		Palpitations		Amnesia		Cephalalgia		Anxiety-Panic		Insomnia		Loss Smell		Loss Taste	
		I wave	II wave	I wave	II wave	I wave	II wave	I wave	II wave	I wave	II wave	I wave	II wave	I wave	II wave	I wave	II wave	I wave	II wave	I wave	II wave
WHO scale	Moderate vs Mild	+++	+++						+	+											
WHO scale	Severe vs Mild	++	++				+				++										
WHO scale	Critical vs Mild	+++	+							+											
Age				+++					□				□ □								
Female			++		+++	++	+++	+++	+++	++	+++	+	+++	+	+++	++	+++	++	++	++	++
BMI			+							+	++		++			+					
ACE-inhibitor															□ □ □				□ □		□
Diuretics				□																	
Ca Antagonist												□ □		□ □							
Hypolipid. Statin								□ □		□ □											
Beta Blockers		++		++				+				+				+					
Steroids Inhalat.		++		++	++			+++				++		++							
Antiaggregant		□ □		□ □																	
Pulmonary dis.			++																		
Cardiac dis.															+						
Metabolic dis.				+																	
Kidney dis.								□		□								□		□	
Neoplasm																					
Autoimmune dis.			+++			++															
Immunologic dis.																			□ □		□
No. Comorbidity		++			+												□				
O2_therapy	Cannula vs None		□																		
O2_therapy	Venturi Mask vs None								□												
O2_therapy	Reservoir Mask vs None				+																
O2_therapy	Not Invasive Ventil. vs None					++								+							
O2_therapy	Orotracheal vs None					+															
Steroid			++		++				+						++						
Remdesivir			++	++											++						

Antibiotic														++		+				
Heparin Tocilizumab				+					+								+++	□ □		□	

* + indicates significant risk factor for symptom incidence, □ significant protective factor for symptom incidence. The number of + and □ indicates significant levels: +++, p<0.001; ++, p<0.05; +, p<0.1). Grey areas divide variables of three macro-symptoms (vertically) and three blocks of variables (horizontally)