Connecting European Cohorts to Increase Common and Effective Response to SARS- CoV-2 Pandemic

DELIVERABLE

WP2 D2.6

Final report of 12-month follow up by clinical, immunological, and virological features

UNIVR

ORCHESTRA has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101016167

Project Classification

Project Acronym:	ORCHESTRA
Project Title:	Connecting European Cohorts to Increase Common and Effective Response to SARS- CoV-2 Pandemic
Coordinator:	UNIVR
Grant Agreement Number:	101016167
Funding Scheme:	Horizon 2020
Start:	1st December 2020
Duration:	36 months
Website:	www.orchestra-cohort.eu
Email:	info@orchestra.eu

Document Classification

WP No:	WP2
Deliverable No:	D2.6
Title:	Final report of 12-month follow up by clinical, immunological, and virological features.
Lead Beneficiary:	UNIVR
Other Involved Beneficiaries:	All WP2 members, UMCG
Nature:	Report
Dissemination Level:	Public
Due Delivery Date:	31.08.2022
Submission Date:	23.12.2022
Justification of delay:	To increase sample size and accuracy of results, analyses have been extended from February 2022 (planned) to June 2023 (at least 6-month follow up). This variation increased time for analysis also based on added time needed to complete the shipment of samples and related microbiological and virological analyses.
Status:	Final
Version:	3.0

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History of Changes

Version	**Date**	**Created/Modified by**
1	05.08.22	Elisa Gentilotti, Anna Górska, Lorenzo Maria Canziani, Evelina Tacconelli
2	15.11.22	Elisa Gentilotti, Anna Górska, Lorenzo Maria Canziani, Iris Lopes, Roy Gusinow, Adriana Tami, Samir Kumar Singh, Zeno Pasquini, Evelina Tacconelli
3	23.12.22	Elisa Gentilotti, Lorenzo Maria Canziani, Evelina Tacconelli

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Core content

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Executive summary

The WP2 ORCHESTRA cohort includes outpatients and inpatients, from five countries (France, Italy, Spain, Netherlands, Argentina), with diagnosis of SARS-CoV-2 infection followed at 3-6- and 12-month of diagnosis. The Deliverable 2.3 summarizes the results of the analyses of **clinical, immunological, and virological data collected at 12-month follow up from COVID-19 diagnosis in 1796 individuals**. The statistical analysis was performed using R. Machine learning was used to explore data distribution. Analyses of trend included comparison with 2969 patients data at month-6. Anti-S antibody (Ab) titres were compared by Mann-Whitney U rank test.

According to the WHO definition, 1030 (57%) individuals suffered from long COVID, presenting at least one symptom **at 12-month** from the onset of COVID-19 which could not be explained by an alternative diagnosis. **Fatigue (37%) and dyspnoea (25%)** were the most frequently reported symptoms, followed by memory loss (16%), arthralgia (16%), and myalgia (15%). At univariable analysis, female sex, BMI>30, hospitalisation, and gastrointestinal and/or neurological symptoms at COVID-19 diagnosis were associated with higher risk of persistence of symptoms at 12 months of diagnosis. Vaccination (both pre and post diagnosis of COVID-19), early therapy with monoclonal antibody in high-risk patients for severe COVID-19, and treatment with anticoagulants at the acute phase, appeared to reduce the risk of long COVID. Using machine learning, symptoms were clustered into **4 clinical phenotypes** defined as persistence of specific association of symptoms: **respiratory cluster (RESc: cough and dyspnoea); chronic pain (CPc: arthralgia and myalgia); neurosensorial (NSc: alteration in taste and smell); and chronic fatigue-like (CFSc: fatigue, headache and memory loss,).** Applying a principal component analysis, different patterns of variables associated with each phenotype were identified (p<.001, all comparisons; good accuracy). **Females were at increased risk of the CPc, NSc, and CPc. Risk of RSc was increased by chronic obstructive pulmonary disease. Neurological symptoms at diagnosis were associated with RSc, NSc, and CFSc. Gastrointestinal symptoms at diagnosis were likely associated with CFSc**.

The anti-S Ab response was higher in patients in the RESc (13602 vs 12174 BAU; p=0.05) and **lower in the NSc** presented a lower anti-S Ab response (11307 vs 12436 BAU; p=0.03). The analysis of the varial variant did not show any correlation with the occurrence of long

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COVID being the majority of the VoC identified as Alpha (first wave of COVID-19 pandemic with 12-month follow up).

Early treatment of SARS-CoV-2 infection with monoclonal Ab reduced significantly the risk of all clinical phenotypes while vaccination had highest impact in reducing CFSc ($p < 0.001$).

The clinical assessment of patients included also evaluation of quality of life through the SF-36 questionnaire. **Patients suffering from long COVID had a lower score both in the physical (PCS) and in the mental (MCS) components**. **Being female and suffering from respiratory symptoms were associated with worst results in both components. Having received early therapy with monoclonal antibodies was associated with higher score in PCS and MCS assessment**.

Clinical presentation, immune response, and quality of life, seem to play an important role in the development of different clinical phenotypes of long COVID. Broader view than just a focus on presence of a single symptom is needed for a proper management of long COVID and selection of patients for RCT on treatment and prevention.

Dissemination level: Public.

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Abbreviations

AIC - Akaike Information Criterion

BMI – body mass index

CI – confidence interval

CINECA - Interuniversity Consortium

CINES - National IT Center for Higher Education confidence interval Cis

COVID-19 - COronaVIrus Disease 19

CRF - Clinical research form

GLM - generalized linear model

ICU – Intensive Care Unit

INSERM - Institut National de la Santé et de la Recherche Médicale

LTS – long-term sequelae

MCS - mental component summary

OR – odds ratio

PASC - post-acute sequelae of COVID-19

PCS - physical component summary

QoL – quality of life

REDCap - Research Electronic Data Capture

RCT – randomized clinical trial

RT-PCR - reverse transcriptase–polymerase chain reaction

SAS - Andalusian Health Service

SD – standard deviation

SF-36 - 36-Item Short Form Health Survey questionnaire

UBA - Universidad de Buenos Aires

UMCG - University Medical Center Groningen

UNIBO - University of Bologna

UNIVR – University of Verona

VOCs – variants of concern

WHO – World Health Organization

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Introduction

SARS-CoV-2 pandemic has challenged health care systems worldwide by causing an unprecedented demand for hospital admission and intensive-care assistance during the acute phase of COVID-19. While the presentation of acute COVID-19 and the mechanisms leading to a more severe and rapidly progressing infection have been extensively studied [1], the characteristics and determinants of longer-term sequelae after COVID-19 and their impact on quality of life remain not fully understood. There is evidence in literature of post-COVID-19 syndrome (also referred as "post-acute sequelae of COVID-19 (PASC)", "long COVID-19", or "persistent COVID-19"), although a clear definition of disease and related clinical and laboratory aspects is not yet available [2,3]. According to the WHO definition [4], post-COVID-19 condition occurs in 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. Similarly, the National Institute for Health and Care Excellence (NICE) defines "Post-covid-19 syndrome" as signs and symptoms that develop during or after an infection consistent with COVID-19, present for more than 12 weeks and that are not attributable to alternative diagnoses [5]. Common symptoms include fatigue, shortness of breath and cognitive dysfunction with impact on daily activities. Symptoms may be new onset following initial recovery from an acute COVID-19 episode or persist from the initial illness. Symptoms may also fluctuate or relapse over time. Furthermore, for patients admitted to ICU, potential overlapping with Post-intensive care syndrome (PICS) should also be considered. A summary of systematic reviews and meta-analysis reporting on long COVID symptoms at 12 months after acute infection is available in **table 1** [6-13], showing that fatigue, weakness and general malaise are the most frequently reported symptoms, followed by dyspnoea and cognitive impairment. A literature review of published articles reporting on the impact of long COVID on the quality of life is displayed in **table 2** [14-35]. Overall, patients reporting persistence of symptoms after acute infection experienced also a lower quality of life.

Several hypotheses have been suggested to explain the possible mechanisms leading to the persistence of symptoms weeks after the acute infection, including uncontrolled immune responses, viral direct effects and viral persistence, inflammatory damage, SARS-CoV-2 interactions with host microbiome and virome, and coagulation alterations [36,37]. Finally, the

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characteristics of acute infection in terms of severity, level of assistance needed and oxygen therapy requirement, together with patient-related risk factors, such as sex, age, ethnicity, and presence of comorbidities, may strongly influence the probability of developing long COVID and its severity. For instance, according to a study published in Nature Medicine, which drew on data from 4,182 COVID Symptom Study app users who consistently logged their health, 15% of women experienced symptoms lasting for 28 days or more, compared with 9.5% of men, although this sex difference disappeared among those aged over 70. The greatest difference between men and women was observed among patients aged between 40 and 50, where women had double the risk of developing Long COVID compared to men [38]. In this complex scenario, data from large, international cohorts with the availability of a prolonged follow up after acute infection are needed to better understand the factors involved in the development, persistence and impact of long COVID, to predict its occurrence and severity and, ultimately, to propose a standard evidence-based definition of COVID-19 sequelae.

In this report we present the results of task 2.6: *prospective cohort study for medium and long-term follow up in COVID-19 individuals*, aiming at describing rates and determinants of long COVID in a cohort of patients with previous laboratory-confirmed diagnosis of SARS-CoV-2 infection followed up until 12 months after acute illness. Results hereby summarized include prevalence of long COVID in hospitalized and non-hospitalized individuals, and demographic, clinical, virological and serological determinants of persistence of symptoms. The clinical assessment has been also completed with the analysis of the impact of long COVID on patients' quality of life. Results from other analyses (microbiological, virological and genetic) will be reported to appropriate Deliverables as planned in the description of work.

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Table 1. Systematic review and meta-analysis of studies assessing long COVID symptoms at different time-points

Author (ref)	Age	Time-point	Severity	Symptoms	No studies	No subjects	Pooled prevalence %, 95% Confidence Interval
Pinzon RT (6)	>18	<6m	Any	Cognitive disorders: brain fog, difficult thinking, poor attention, memory impairment and other cognitive impairment issues	5	3305	35.4%; 95% CI 2.1-81.7, I^2 99.7%
				Fatigue	9	4546	52.8%; 95% CI 19.9-84.4, I^2 99.8%
				Paresthesia	3	1939	33.3%; 95% CI 2.7-76.6, I^2 99.8%
				Sleep disorder	5	3485	32.9%; 95% CI 6.5-67.4, I^2 99.8%
				Musculoskeletal pain	3	3918	27.8%; 95% CI 12.7-45.9
				Dizziness	5	1783	26.4%; 95% CI 4.6-57.9, I^2 99.9%
				Headache	9	3886	21.3%; 95% CI 3.3-48.9, I^2 99.9%
				Dysosmia	11	2024	17.7%; 95% CI 10.3-26.7, I^2 99.8%
				Dysgeusia	9	1783	16.5%; 95% CI 8.3-27.0, I^2 99.1%
				Movement disorder	2	32	3.6%; 95% CI 2.5-4.9
Lopez-Leon S (7)	0-18	>4m	Any	Mood (sad, tense, angry, depression, anxiety)	5	730	16.5%; 95% CI 7.4–28.2, I^2 97.5%
				Fatigue	16	3015	9.7%; 95% CI 4.5–16.5, I^2 99.1%
				Sleep disorders (e.g., insomnia, hypersomnia, and poor sleep quality)	8	153	8.4%; 95% CI 3.4–15.2, I^2 93.5%
				Headache	13	1875	7.8%; 95% CI 4.0–12.7, I^2 98.5%
				Respiratory symptoms	9	1387	7.6%; 95% CI 2.1–15.8, I^2 99.2%
				Sputum production or nasal congestion	2	11	7.5%; 95% CI 3.8–12.4
				Cognitive symptoms (e.g., less concentration, learning difficulties, confusion, and memory loss)	11	1223	6.3%; 95% CI 4.–58.4, I^2 91.3%
				Loss of appetite	5	747	6.1%; 95% CI 3.9–8.6, I^2 93.5%
				Exercise intolerance	2	8	5.7%; 95% CI 0.0–19.4, I^2 87.8%
				Altered smell (e.g., hyposmia, anosmia, hypersomnia, parosmia, and phantom smell)	10	2048	5.6%; 95% CI 3.1–8.7, I^2 97.1%
	Any	6-12m	Outpatients	Fatigue	5		25-34%

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Nguyen NN (8)				Smell and/or taste disorders	5		3-24%
				Cough	5		2-13%
				Dyspnea	5		13-22%
				Thoracic pain	5		9%
				Arthralgia	5		15%
Alkodaymi MS (9)	Any	6-9m	Any	Sleep disorder	12	24200	29%; 95% CI 15–45, I^2 99.7%
				Depression	6	4377	23%; 95% CI 21–26, I^2 66.3%
				Anxiety	7	240756	23%; 95% CI 13–33, I^2 99.3%
				Difficulty concentrating	4	854	22%; 95% CI 8–40, I^2 96.9%
				Cognitive disorder	5	1987	15%; 95% CI 6–27, I^2 97.6%
				Headache	13	7170	14%; 95% CI 7–23, I^2 99.0%
				Loss of smell	17	6596	15%; 95% CI 10–22, I^2 97.6%
				Loss of taste	16	6505	13%; 95% CI 8–18, I^2 96.9%
				Palpitation	7	4735	14%; 95% CI 8–21, I^2 96.9%
				Effort intolerance	5	850	45%; 95% CI 25–67, I^2 97.4%
				Chest pain	10	4318	12%; 95% CI 8–18, I^2 95.5%
				Cough	21	8737	12%; 95% CI 6–20, I^2 98.0%
				Dyspnoea	13	4384	25%; 95% CI 20–30, I^2 96.8%
				Diarrhoea	8	3318	5%; 95% CI 2–11, I^2 96.4%
				Nausea	8	3419	4%; 95% CI 1–8, I^2 95.2%
				Joint pain	8	5288	23%; 95% CI 15–31, I^2 97.8%
				Myalgia	9	3490	19%; 95% CI 7–35, I^2 99.0%
				Fatigue	19	8191	36 95% CI 27–46, I^2 98.8%
				Hair loss	5	4276	10%; 95% CI 2–22, I^2 99.2%
Ceban F (10)	Any	>12 weeks	Any	Cognitive impairment	43	13232	22%; 95% CI 1728, I^2 98.0%
				Fatigue	68	25268	32%; 95% CI 27-37, I^2 99.1%
Renaud-Charest O (11)	Any	>12 weeks	Any	Depressive symptoms	6	742	11-28%
				Clinically-significant depression and/orsevere depressive symptoms	5	565	3-12%
Michelen M (12)	Any	7m (mean)	Mostly hospitalized	Weakness	1	186	41%; 95% CI 25-59, I^2 96.0%
				General malaise	2	292	33%; 95% CI 15-57, I^2 97.3%

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				Fatigue	17	2207	31%; 95% CI 24-39, I^2 97.9%
				Concentration impairment	2	66	26%; 95% CI 21-32
				Breathlessness	20	1297	25%; 95% CI 18-34, I^2 96.0%
				Reduced quality of life	3	340	37%; 95% CI 18-60, I^2 91.0%
Cares-Marambio K (13)	>18	3 weeks - 3 months	Hospitalized	Fatigue	8	1668	52%; 95% CI 38-66, I^2 97.0%
				Dyspnoea	9	1334	37%; 95% CI 28-48, I^2 93.0%
				Chest pain	5	1066	16%; 95% CI 10-23, I^2 90.0%
				Cough	7	1496	14%; 95% CI 6-24, I^2 96.0%

Table 2 – Studies and systematic reviews assessing quality of life after COVID-19 diagnosis

Author (ref)	**Design**	**QoL test**	**No of patients (studies)**	**Time-point**	**Results**
Tsuzuki S (14)	cross sectional self-report questionnaire survey	EQ-VAS EQ-5D-3L	526	250d	the participants who reported any symptoms showed a lower average value on the EQ-VAS (69.9 vs 82.8, respectively) and on the EQ-5D-3L (0.85 vs 0.96, respectively) than those reporting no symptoms
Malik P (15)	systematic review and meta-analysis	EQ-VAS	4828 (12)	varying (35-151d)	amongst post-acute COVID-19 syndrome patients, the pooled prevalence of poor quality of life (EQ-VAS) was (59%; 95%CI: 42%–75%)
Taboada M (16)	prospective questionnaire 6 months after ICU treatment	EQ-5D-3L EQ-VAS PCFS	91	6 months	ICU survivors showed reduced QoL in 67% of cases. 63% decreased functional status EQ-VAS from 87.6 to 66.36
Huang C (17)	ambidirectional cohort study	EQ-VAS EQ-5D-3L	1733	1y	EQ-VAS 80
Catalan IP (18)	observational cohort study (telephone survey)	SF-36	76	1y	steroids vs. non-steroids physical functioning: comparable median scores mental: lower for non-steroids (76 vs. 86)
Michelen M (19)	systematic review and meta-analysis	Unclear	807 (3)	varying	37% (95% CI 18-60) of patients reported reduced QoL. I2 91%

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Tabacof L (20)	cross-sectional observational study design (self-administered web-based survey)	EQ-5D-5L EQ-VAS	156		EQ-VAS: 64 (6–99)
Poudel AN (21)	systematic review and meta-analysis	SF-36 EQ-5D-5L	(12)		range SF-36 for Long COVID: 60-86 EQ-5D-5L index mean value: 0.61-0.71
McFann K (22)	observational, longitudinal cohort	SF-36	62	0-3m-6m	lower SF-36 (multiple areas) for hospitalized (vs. non-), severity of acute COVID-19; presence of LTS; chronic conditions; overweight
Schouborg LB (23)	retrospective cohort	Functional status scale EQ-5D-5L EQ-VAS	83		EQ-VAS men (80.4; SD 17.8) > women (66.9; SD 23.4)
Kim Y (24)	prospective cohort study	EQ-5D-5L	170	12 months	anxiety/depression 35%; EQ-VAS: LTS+ 80 (range 70-90); LTS- 90 (range 80-95)
Munoz-Corona C (25)	observational, ambispective, longitudinal analytic study	SF-36	141	90d after discharge	ICU heavily correlated with LTS+ SF areas (8) against most common symptoms (13) + ICU
de Sousa KCA (26)	matched case control study	SF-36	80	>12w	LTS+: worse postural balance; worse evaluations in five dimensions of the SF-36 (physical functioning, physical role limitations, bodily pain, general health perceptions, and mental health)
Lloyd-Evans PHI (27)	pre-post study (intervention after acute COVID)	EQ-5D-5L EQ-VAS	110	median 351d (82-457d)	EQ-VAS: pre=48.8 (19.5); post=59.9 (22.1), p<0.01
Kimmig LM (28)	observational cohort study	EQ-VAS	71	up to 1y	EQ-VAS 72.8 (SD ± 17.7); stable over time; not depending on acute severity; not different from same-age population norm (72.9)
Daher A (29)	observational prospective study	EQ-VAS	33	6w after discharge	Acute severity: hospitalization; non-ICU EQ-VAS: 63 (53-80) (median IQR)
Carfì A (30)	observational cohort study	EQ-VAS	143	2m	EQ-VAS 10-point reduction from before Acute infection: 44%
Garrigues E (31)	cohort (phone questionnaire)	EQ-5D-5L EQ-VAS	120	100d	EQ-VAS: 70.3% (21.5) EQ-5D-5L index: 0.86 (0.20) no diff ICU vs. ward

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Moreno-Perez O (32)	prospective cohort study	EQ-VAS	277	8-12w	preinfection: 90 (80-100) post-infection: 83 (70-90)
Weerahandi H (33)	prospective single health system observational cohort study	PROMIS® Global Health-10	161	1m	physical health pre: 54.3, SD 9.3 mental health pre: 54.3, SD 7.8 physical health post: 43.8, SD 9.3 mental health post: 47.3, SD 9.3
Arnold DT (34)	prospective cohort study	SF-36	110	8-12w	reduction in all domains vs. general popul. lower in Severe acute severity
Logue JK (35)	longitudinal prospective cohort	EQ-VAS	177	median 169d	EQ-VAS 10-point reduction from before Acute infection: 30% inpatients > outpatients > asymptomatic > controls

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Methods

Design and objectives of the study

The WP2 is a prospective multinational cohort study enrolling both hospitalized and outpatients with previous laboratory-confirmed diagnosis of SARS-CoV-2 infection followed till 18-months after diagnosis. The Deliverable summarizes results for the 12-month assessment.

Data collection

In WP2 6 prospective cohorts from 5 countries: UBA (Argentina), UNIBO and UNIVR (Italy), SAS (Spain), COVID HOME, UMCG (The Netherlands), and French COVID (France) are included. During the period assessed for this report (7th February 2020 - 30th June 2022), 1796 patients completed the 12-month follow up collecting, according to the protocol, 1285 samples. **Table 3** describes the time points of clinical and laboratory assessment. Hospitalized and non-hospitalized patients aged >14 years old with a laboratory confirmed SARS-CoV-2 infection were enrolled and followed up at 3, 6, 12, and 18-months post-infection at an outpatient clinic setting. Each follow up visit combines clinical and laboratory assessment, including biochemical parameters and serology. One cohort, COVID HOME included only non-hospitalized COVID-19 patients. In this specific cohort participants of all ages are eligible for enrolment if their symptom onset is <5 days and had a positive diagnostic SARS-CoV-2 RT-PCR. Household members of these positive individuals are also enrolled in the cohort. Positive patients are followed weekly at home during their acute disease for at least 3-weeks post-infection, to obtain clinical data, blood samples for laboratory parameters and serological determination; and nasopharyngeal swabs. All the follow up protocols (for prospective cohorts starting before the ORCHESTRA project was financed) were aligned to a common ORCHESTRA cohort protocol (see Milestone7).

Nasal swab was performed to define variant of concern (VOC) at baseline and repeated at 3-month only in case of positive sampling after 30 day of infection diagnosis. Antibodies response was assessed at each follow up visit at ORCHESTRA central laboratory (Antwerp) or at local laboratories applying the same protocol (details in Deliverable 6.1).

The data collection was carried out using a dedicated structured electronic case report form (eCRF) developed in REDCap (Research Electronic Data Capture) and hosted at Consorzio Interuniversitario (CINECA). The variables underwent a process of homogenization across the different cohorts and standardization according to the protocol. Since French COVID and COVID-HOME started before the ORCHESTRA project was financed, data from

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these two cohorts went through a post-data collection harmonization process under the supervision of the Charité – Universitätsmedizin Berlin and transformation conducted by the Centre Informatique National de l'Enseignement Superieur (CINES).

Data collected in WP2 ORCHESTRA cohort at baseline include date of symptom onset, date of positive diagnostic SARS-CoV-2 test, demographic characteristics, comorbidities, clinical presentation, treatment during the acute infection, hospitalization, admission to intensive care and post-acute infection complications classified as respiratory (pneumothorax, pleural effusion, etc.), cardiac (congestive heart failure, pericarditis, arrhythmia, etc.), embolic (pulmonary embolism, deep vein thrombosis, disseminated intravascular coagulation, etc.), neurological (meningitis, encephalitis, stroke, etc.), renal (acute renal failure), and gastrointestinal complications (pancreatitis, haemorrhage, acute liver dysfunction, etc.). Presence of symptoms are recorded at each assessment. A symptom was considered to be associated to COVID-19 if newly diagnosed after SARS-CoV-2 acute infection (or if a significant worsening in terms of severity and/or presentation of the symptom was registered after COVID-19. Dates of start and end of symptoms were collected in order to be able to derive the duration of the symptoms. Occurrence of new medical events, vital signs and physical examination, laboratory parameters and vaccination status were also collected at each time point.

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Table 3. [1]Day 0: first positive SARS-CoV-2 PCR test. Follow up of 3, 6, 12, and 18 months start from Day 0. [2]Reassessed only if outside the normal ranges at the previous assessment or if clinically indicated. [3]At least one of the three timepoints (month 3, month 6, month 12) is required to perform metagenomic analyses. [4]At least one of the three timepoints (month 3, month 6, month 12) is required.

	D0[1]	3 months[1] ±1 month	6 months[1] ±1 month	12 months[1] ±1 month	18 months[1] ±2 month	
NP swab	x	x[2,3]	x[2,3]	x[2,3]	x[2]	Viral variant and metagenomic sequencing
2 x 2 mL serum tube (serum)	x	x	x	x	x	Immune - serology and type I IFNs autoantbodies
4 mL EDTA blood tube (plasma)	x	x[4]	x[4]	x[4]	x[2]	Immune - cytokine and chemokine
1 (if possible 2) 9 mL heparin tube (PBMCs)	x	x[4]	x[4]	x[4]	x[2]	Immune - cellular
2 x 2 mL EDTA tube (whole blood)	x	x	x	x	x	Genetic and epigenetic analyses
Stool sample (faeces or rectal swab)	x		x		x	Metagenomic sequencing

Level I	Light blue	**Assessments of Level I are mandatory**
Level II	Pink	**Customized according to the feasibility of each cohort**

<u>Data quality assessment</u>

The data are collected with the common eCRF in REDCap, harmonized according to the SNOMED-CT system, and in line with the pre-existing REDCap used by INSERM. In addition, CINECA runs quality checks based on the semantic value of the variables and reported to the cohorts to be corrected. Finally, mistakes found at the analysis step are corrected systematically by communicating with the corresponding cohorts.

<u>Data imputation</u>

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Data imputation was performed only in case of data that could be derived by known information, such as availability of the vaccination or treatments. E.g. we considered all patients infected before the onset of the vaccination campaigns within each country as not vaccinated. No other, especially algorithmic, imputation was done.

Data analysis

Prevalence of long COVID was assessed using WHO case definition. Symptoms were analysed individually and divided per organ/system involved: general symptoms (fever, fatigue, myalgia, arthralgia, headache, conjunctivitis, lymphadenopathy, anorexia, skin rash, haemorrhage); upper and lower respiratory symptoms (cough, dyspnoea, sore throat, nasal congestion, rhinorrhoea, chest pain, chest retraction, wheezing); gastrointestinal symptoms (abdominal pain, diarrhoea, nausea, vomiting); neurological and neurosensorial symptoms (ageusia, anosmia, syncopal episodes, confusion, memory loss, aphasia, anomia, seizures, inability to walk). Distribution of symptoms among patients with long COVID was analysed using machine learning and principal component analysis (PCA). Sensitivity analysis included: demographic and epidemiological data, hospitalization, ICU admission, clinical presentation during acute infection, treatment received including early and late treatment, vaccination status, VoC, anti-S antibody titer.

Virological and immunological analyses

Viral variant and immunological analysis were performed at central laboratory (Antwerp) or at local laboratories using the same protocols (details in Deliverable 6.1). Serological results reported in arbitrary units were converted into BAU from Roche Elecsys Anti-SARS-CoV-2 assay in AU/ml and UI/ml were multiplied by 1.0288 to convert to BAU, those from the Alignity_Abbott in AU/mL were multiplied by 0.142 and from MSD assay was converted from AU/mL by multiplying by 0.00901. The distributions of the titers were compared between the groups by Mann-Whitney U rank test. Immunological response was classified as non-reactive (<5.58 BAU/mL), inconclusive (5.58-<45 BAU/mL), positive-low (45-<205 BAU/mL), positive-mild (205-<817 BAU/mL), and positive-high (>817 BAU/mL) according to WHO criteria. Thus, negative antibody response was defined as an anti-rapid binding domain (RBD) titer < 45 BAU/mL (including non-reactive and inconclusive results).

Statistical analyses

Means and standard deviations (SD) were calculated for continuous variables, and frequency tables and respective percentages were calculated for categorical variables. For the univariable analysis, crude odds ratios (OR) with 95% confidence intervals (95% CI) of the

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categorical variables were shown with corresponding *p*-values, where a *p*-value less than 0.05 was deemed as statically significant. For continuous variables, a comparison of medians using a Mann-Whitney U test or Kruskal-Wallis test for independent samples was made. Analyses of trend were performed analysing also cohort data at month-6. To determine risk factors for the primary endpoint, their associations with the continuous, discrete, and categorical covariates (including subgroups) as well as interaction terms were assessed using methods from single- and multi-variable risk factor analysis. Specifically, logistic regression models were used by considering a generalized linear model (GLM) with log-odds linking function (i.e., Bernoulli distribution) was used, assuming information to be missing at random. The identified important risk factors (as defined based on *p*-value and odds ratio) of the univariable analysis were selected to be included in the logistic regression models. Model selection is done by evaluating the AIC (Akaike Information Criterion) of models that use all possible combinations (subsets) of risk factors deemed significant. The model with the lowest AIC score was then selected; age and sex were considered as additional risk factors. The statistical analysis was performed using R.

Patients' quality of life was assessed through the SF-36 questionnaire at 6- and 12-months after acute infection. The questionnaire is composed of 36 items, which are categorised into 8 scales (dimensions): physical functioning, role limitations due to physical health, pain, general health, vitality (energy/fatigue), social functioning, role limitations due to emotional problems, and mental health. Each of the items may have from 2 to 6 levels of answers. For each scale, the items are (re)codified, transformed, and aggregated into a scale ranging from 0 to 100. In case of missing information, if the respondent has answered at least 50% of the items within the scale, the scale average is imputed into the missing items. Otherwise (i.e., more than 50% missing information within the scale), the scale is not computed. Once the score for each of the 8 scales was computed, these were aggregated into two main components: the physical component summary (PCS) and the mental component summary (MCS) based on population-representative weights computed via PCA.

Results

<u>Population characteristics</u>

Since February 2020 until 30th of June 2022, 1796 patients completed 12-month follow up and were included in the analysis (see **table 4**). The majority of patients were male (1016, 57%)

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and aged between 41 and 60 years (774, 43%) and 61 and 80 years (689, 38%). Cardiovascular diseases were the most frequently reported underlying clinical conditions (710, 40%), followed by chronic respiratory diseases (297, 17%), and diabetes (154, 9%). Smokers or former smokers accounted for 546 (30%) individuals, while BMI>30 was observed in 138 (8%). At least one dose of SARS-CoV-2 vaccination was administered in 283 (16%) subjects before SARS-CoV-2 acute infection and in 1081 (60%) after COVID-19 within the 12-month follow up. Acute hospitalisation for COVID-19 occurred in 1267 (71%) patients. Of these, 419 (33%) patients required ICU admission. Early treatment for COVID-19 with monoclonal antibodies (available in Europe from March 2021) was prescribed in 123 (7%) patients at high risk of complications (age >65 years old and presence of at least one comorbidity among the following: BMI ≥30, chronic kidney disease, diabetes, HIV, cardiovascular diseases, chronic respiratory diseases, chronic liver disease, and neurological disorders), while 661 (43%) patients received steroids, and 929 (60%) received oxygen therapy. SARS-CoV-2 variants were identified in the ORCHESTRA central laboratory and local laboratories in 230 (13%) patients.

The cohorts did not differ in terms of age distribution and past medical history with exception for UBA and COVID-HOME (outpatients setting), that enrolled substantially younger and healthier patients (comorbidities detected only in 6% of the cohorts' participants). The distribution of gender was homogeneous across the cohorts except for UBA that enrolled a higher proportion of male individuals.

A comparison of male and female population showed differences in the age distribution with a higher proportion of male individuals in the 61-80 age group ($p<0.001$) and presenting cardiovascular diseases (especially coronary heart diseases, $p<0.001$), diabetes ($p=0.001$) and renal diseases ($p<0.001$). Auto-inflammatory diseases were more frequent among women ($p=0.008$). Hospitalization and ICU admission rates were higher in men compared to women ($p<0.001$), while a higher proportion of females reported more than 8 symptoms during the acute infection ($p<0.001$), with more constitutional symptoms ($p=0.044$), and gastrointestinal ($p<0.001$) and neurological ($p<0.001$) involvement. Post-COVID acute complications, such as cardiac, renal and embolic acute events, were more often observed in men compared to women. Compared to outpatients, individuals admitted to hospital were more often male, older, with pre-existing medical conditions and a lower rate of vaccination before acute infection ($p<0.001$).

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Assessment of Long COVID

Clinical presentation

During the acute infection, the predominant symptoms were fever (78%), fatigue (68%), cough (65%), dyspnoea (57%) and myalgia (40%). Headache was reported in 32% of the cohort, while ageusia and anosmia (including also disgeusia and disosmia) were observed in 31% and 29%, respectively. **According to the WHO definition, long COVID was diagnosed in 1660 (56%) out of 2969 patients at 6-month assessment and in 1030 (57%) out of 1796 patients 12-months after acute infection, ranging from 39% to 68% of the overall population among cohorts**. At 6- and 12-month assessment, post-COVID fatigue and dyspnoea were the most frequently reported symptoms, followed by memory loss, arthralgia and myalgia (15%). Percentage of people still reporting anosmia and ageusia after 12-months follow up was 9% and 8%, respectively. **Figure 1 and 2** display the distribution of symptoms, classified per organ and system, at SARS-CoV-2 diagnosis and at 6- and 12-month follow up.

The analysis of blood test parameters showed that several inflammatory markers' levels were increased during the acute infection among patients developing long COVID after 12 months (red) compared with individuals without long-term sequelae (blue). In particular, **significantly higher levels of AST ($p \leq 0.05$), PCT ($p \leq 0.01$) and CRP ($p \leq 0.001$) were detected in the long COVID group during the acute phase**, while fibrinogen levels were also found to be higher in the long COVID group, although not statistically significant (**Figure 3**).

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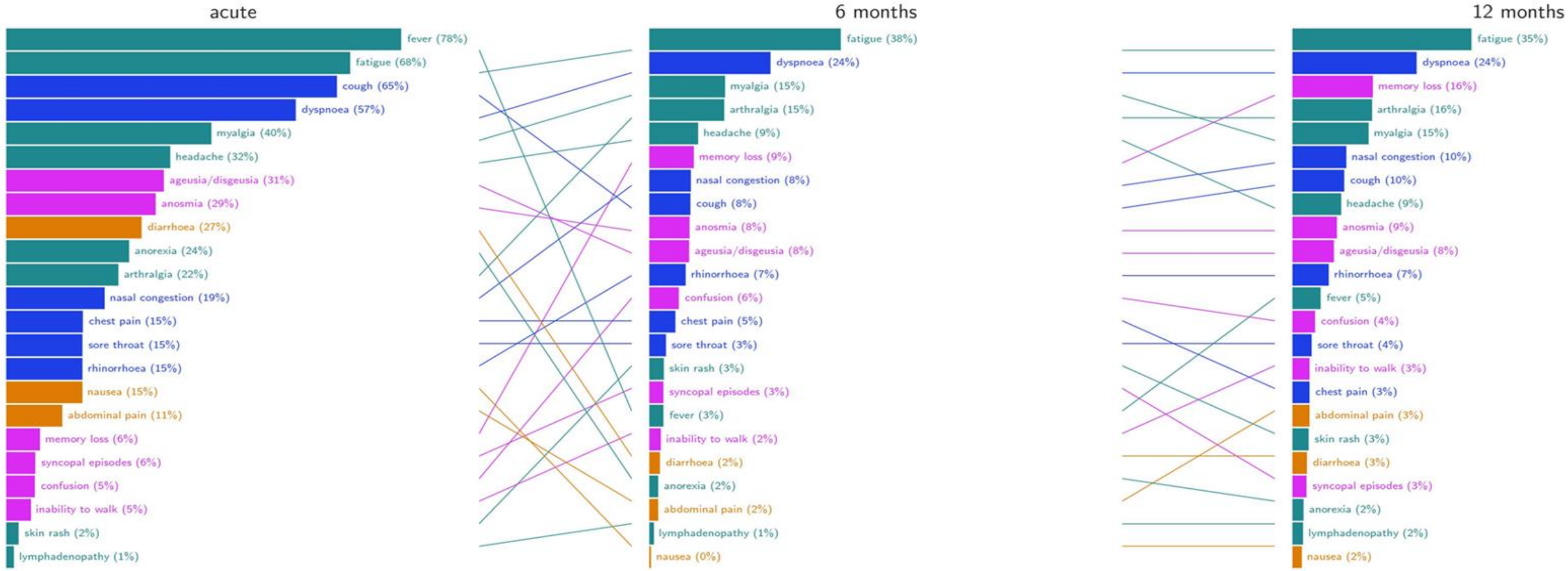

Figure 1. Percentages of symptoms reported during the acute infection, at 6-month follow up assessment and at 12-months follow up assessment. The lines connect symptoms between the bar-charts to underline the changes in the ranking of the most frequent symptoms, Symptoms are classified according with organ/system involved as follows: green: general symptoms (fever, fatigue, myalgia, arthralgia, headache, conjunctivitis, lymphadenopathy, anorexia, skin rash, haemorrhage); blue: upper and lower respiratory symptoms (cough, dyspnoea, sore throat, nasal congestion, rhinorrhoea, chest pain, chest retraction, wheezing); orange: gastrointestinal symptoms (abdominal pain, diarrhoea, nausea, vomiting); Pink: neurological and neurosensorial symptoms (ageusia, anosmia, syncopal episodes, confusion, memory loss, aphasia, anomia, seizures, inability to walk).

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Figure 2. Heat-map presents the relative frequency of symptoms across the population subgroups, according to the demographic features and comorbidities during acute infection and 6- and 12-month follow up

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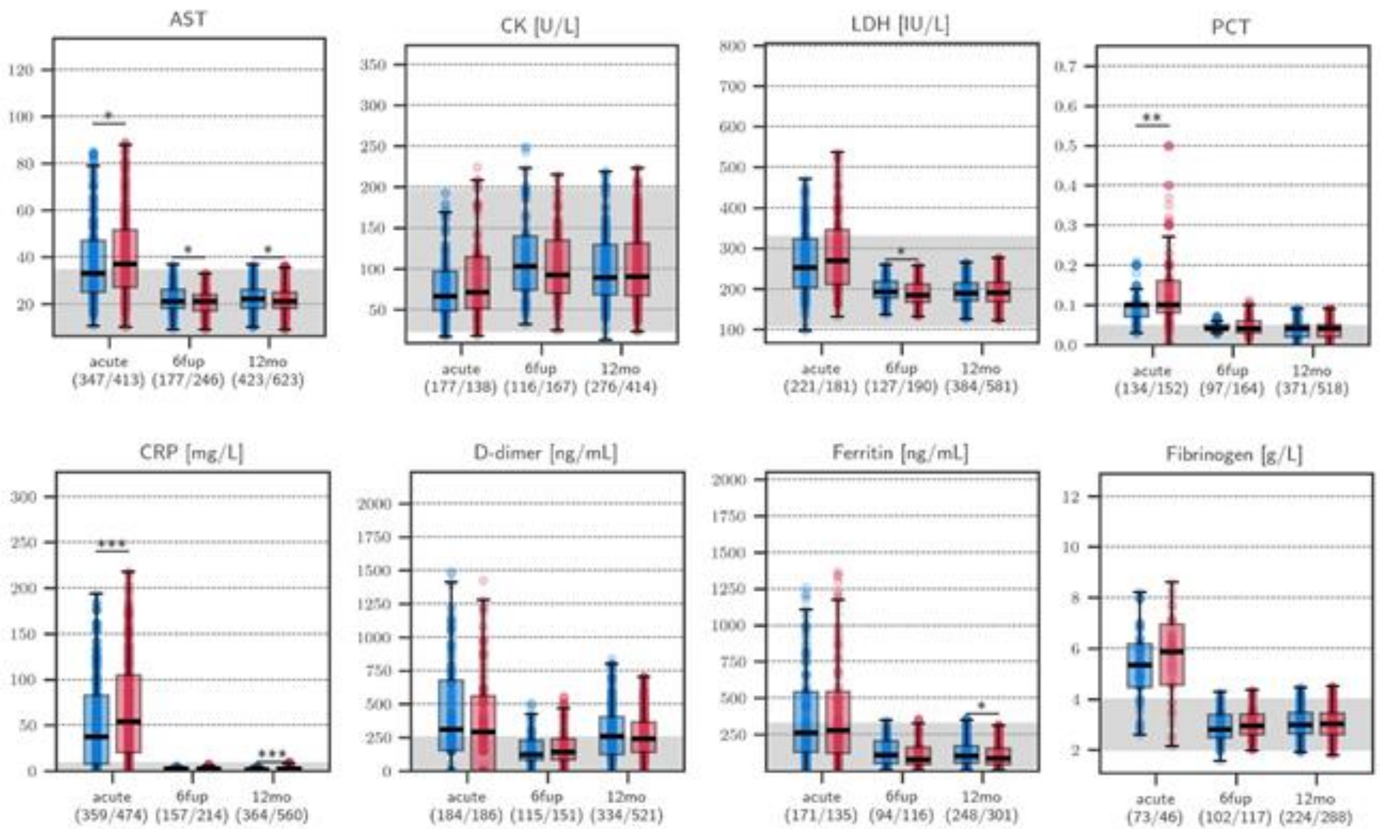

Figure3. Blood test comparison between patients with (red) and without (blue) long COVID disaggregated per time-point (acute infection, 6- and 12-months assessment). ***≤0.001,**≤0.010, *≤0.050.

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Quality of life

The quality of life was assessed through the SF-36 questionnaire in a subpopulation of 1273 patients, 586 (46%) of whom being females. Overall, **patients with long COVID syndrome (yellow) presented a lower score both in the physical (PCS) and in the mental (MCS) component** (Figure 4) compared with patients without long COVID syndrome (control group, grey).

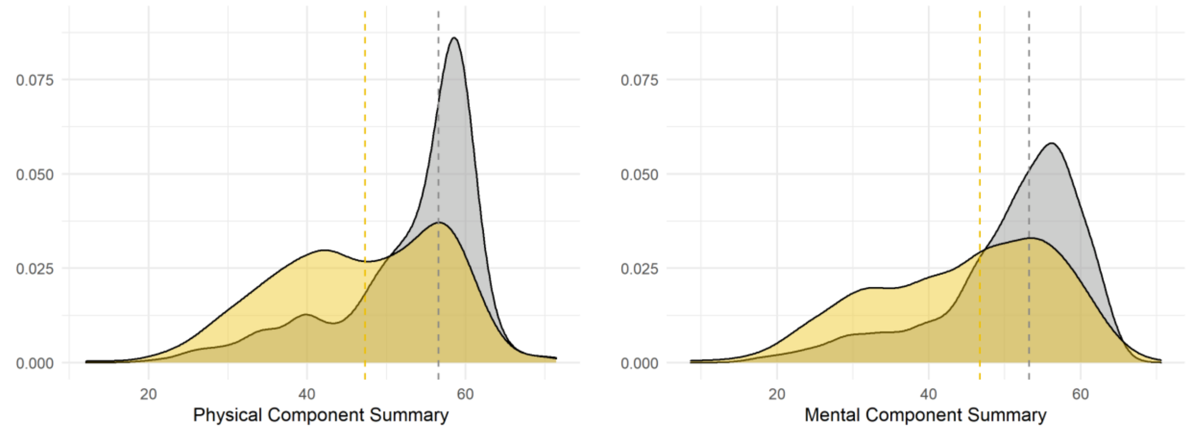

Figure 4. Density plot of PCS and MCS scores in the 12 months follow up cohort. Yellow: patients with long COVID; grey: control group. The dashed lines depict group medians.

Immunological features

Out of 549 samples with anti-S titer available, 2 samples (0.4%) resulted non-reactive or inconclusive, 6 (1%) positive-low, 14 (2.6%) positive-mild, and 527 (96%) positive-high.

Virological features

SARS-CoV-2 variants were identified in the ORCHESTRA central laboratory and local laboratories in 230 (13%) patients, being the majority of strains Alpha (B.1.1.7) variants (125, 54%) and 49 (21%) Delta (B.1.351).

<u>Univariable analysis of determinants of long COVID and impact on quality of life</u>

Demographic, clinical and epidemiological features

Female patients had a higher risk of persistence of symptoms and of having a low SF-36 score after 12 months compared to men (p<0.001). No differences were found in the diagnosis of long COVID according to age groups, while an increasing trend with age was observed for the

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SF-36<50 score. Obesity and chronic respiratory diseases were associated with a higher risk of developing long COVID (p=0.036 and p=0.016, respectively). Being infected with Alpha variant was associated with a higher risk of persistence of symptoms at 12-months assessment (p=0.045). Being vaccinated for SARS-CoV-2 with one or more doses before the acute infection and before 12-month assessment had a protective role against the occurrence of long COVID (p<0.001 and p=0.002, respectively). Hospitalization increased the risk of long COVID (p=0.002), especially in patients transferred to ICU (p<0.001). Regarding the therapy received during the acute infection, univariable analysis of data from this prospective cohort show that monoclonal antibodies (bamlanivimab, bamlanivimab/etesevimab, casirivimab/imdevimab) reduced the risk of developing long COVID (p<0.001). As per the clinical presentation during the acute infection, data did not show any association between number of symptoms reported at the time of acute illness and the risk of persistence of complains after one year, although an increasing trend was observed. Patients suffering from neurological or gastrointestinal symptoms during acute infection had an increased risk of long COVID (p<0.001 and p=0.003, respectively).

SF-36 PCS cut-off of 50 was identified to define a poor QoL, based on the distribution of patients not reporting symptoms at 12-months assessment below 25th percentile. The analysis was available on 527 patients with complete SF-36 questionnaire, showing an overall worst performance among female (p<0.001), patients aged<41 years old and with the majority of individuals with pre-existing medical conditions (see **table 4**). Anti-SARS-CoV-2 vaccination and early treatment for COVID were associated with a better SF-36 PCS score (p<0.001 and p=0.011, respectively), while patients hospitalized, with respiratory symptoms during the acute infection and renal post-acute COVID complication presented a lower score (p<0.001).

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Table 4. Univariable analysis of variables associated with the presence of at least one symptom at 12-month follow up assessment and with a SF-36 PCS score<50. [a]Analysis performed on samples available at baseline; [b] 20A, 20B, 20E (EU1); [c]monoclonal antibodies administered during the study period: bamlanivimab, bamlanivimab/etesevimab, casirivimab/imdevimab; [d]fever, fatigue, myalgia, arthralgia, headache, conjunctivitis, lymphadenopathy, anorexia, skin rash, haemorrhage.

Variable	Total	Long COVID	%	Crude OR	95% CI		*p*-value	Total	SF-36 PCS<50	%	Crude OR	95% CI		p-value
					lower	upper						lower	upper	
Demographic and clinical characteristics														
Sex														
Male	1016	536	52.8	1				646	254	39.3	1			
Female	779	493	63.3	1.543	1.276	1.869	**<0.001**	547	272	49.7	1.526	1.212	1.922	**<0.001**
Age groups (years old)														
15-30	98	50	51.0	1				67	15	22.4	1			
31-40	150	83	55.3	1.188	0.712	1.985	0.509	104	29	27.9	1.333	0.656	2.797	0.431
41-60	774	456	58.9	1.376	0.901	2.101	0.139	553	235	42.5	2.540	1.427	4.790	**0.001**
61-80	689	403	58.5	1.352	0.883	2.070	0.165	426	214	50.2	3.467	1.934	6.576	**<0.001**
>80	83	38	45.8	0.812	0.450	1.461	0.487	43	34	79.1	12.571	5.115	33.889	**<0.001**
Smoking status														
Non-smoker	1040	600	57.7	1				685	299	43.6	1			
Former smoker	434	248	57.1	0.978	0.780	1.227	0.845	272	128	47.1	1.147	0.865	1.521	0.340
Smoker	112	70	62.5	1.220	0.819	1.838	0.330	80	33	41.3	0.908	0.563	1.449	0.687
Body Mass Index														
<30	263	133	50.6	1				146	75	51.4	1			
≥30	138	85	61.6	1.565	1.030	2.391	**0.036**	73	42	57.5	1.280	0.727	2.271	0.394
Cardiovascular disease														
No	1012	569	56.2	1				714	261	36.6	1			

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Yes	710	410	57.7	1.064	0.876	1.292	0.531	434	238	54.8	2.106	1.653	2.687	**<0.001**
Cardiovascular disease														
None	1012	569	56.2	1				714	261	36.6	1			
Congestive heart failure	9	5	55.6	0.966	0.245	4.095	0.960	7	6	85.7	9.305	1.523	241.61	**0.013**
Coronary heart disease	175	108	61.7	1.254	0.904	1.750	0.176	94	57	60.6	2.667	1.721	4.178	**<0.001**
Hypertension	468	272	58.1	1.080	0.866	1.349	0.495	293	157	53.6	2.002	1.520	2.640	**<0.001**
Other	58	25	43.1	0.591	0.343	1.007	0.053	40	18	45.0	1.422	0.737	2.706	0.289
Diabetes														
No	1545	883	57.2	1				1051	434	41.3	1			
Yes	154	77	50.0	0.750	0.538	1.046	0.090	88	54	61.4	2.253	1.447	3.553	**<0.001**
Chronic respiratory disease														
No	1485	833	56.1	1				998	406	40.7	1			
Yes	297	189	63.6	1.369	1.060	1.776	**0.016**	185	116	62.7	2.447	1.775	3.397	**<0.001**
Renal disease														
No	1673	959	57.3	1				1121	483	43.1	1			
Yes	90	56	62.2	1.224	0.794	1.914	0.363	48	35	72.9	3.526	1.886	7.014	**<0.001**
Liver disease														
No	1721	986	57.3	1				1146	502	43.8	1			
Yes	52	33	63.5	1.290	0.733	2.335	0.381	29	19	65.5	2.417	1.131	5.502	**0.022**
HIV infection														
No	1015	615	60.6	1				629	316	50.2	1			
Yes	38	14	36.8	0.382	0.189	0.740	**0.004**	27	10	37.0	0.587	0.253	1.290	0.187
Active cancer														
No	1675	951	56.8	1				1122	478	42.6	1			
Yes	121	79	65.3	1.429	0.976	2.122	0.067	72	49	68.1	2.858	1.734	4.845	**<0.001**
Transplant														

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No	1764	1011	57.3	1				1172	510	43.5	1			
Yes	32	19	59.4	1.084	0.534	2.274	0.825	22	17	77.3	4.312	1.678	13.452	**0.002**
Autoimmune diseases														
No	909	575	63.3	1				539	291	54.0	1			
Yes	95	62	65.3	1.089	0.703	1.715	0.706	60	45	75.0	2.536	1.408	4.823	**0.002**
Ongoing immuno-suppressive therapy														
No	87	37	42.5	1				58	23	39.7	1			
Yes	50	22	44.0	1.062	0.522	2.151	0.868	35	22	62.9	2.538	1.075	6.199	**0.033**
Vaccination														
≥1 dose before acute infection														
No	1492	919	61.6	1				978	460	47.0	1			
Yes	283	104	36.7	0.363	0.278	0.471	**<0.001**	202	65	32.2	0.535	0.386	0.735	**<0.001**
Number of doses before acute infection														
0	960	549	57.2	1				700	277	39.6	1			
1	66	19	28.8	0.304	0.172	0.519	**<0.001**	46	16	34.8	0.818	0.426	1.514	0.529
≥2	48	9	18.8	0.175	0.078	0.351	**<0.001**	14	5	35.7	0.860	0.255	2.561	0.792
No	384	241	62.8	1				239	132	55.2	1			
Yes	1081	581	53.7	0.690	0.542	0.875	**0.002**	764	300	39.3	0.525	0.391	0.703	**<0.001**
Number of doses														
0	7	2	28.6	1				6	2	33.3	1			
1	481	265	55.1	2.932	0.597	22.953	0.191	337	141	41.8	1.388	0.252	11.353	0.715
≥2	571	301	52.7	2.666	0.544	20.867	0.234	417	155	37.2	1.142	0.207	9.324	0.883
Acute infection features														
Variants of concern[a]														

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Alpha (B.1.1.7)	125	53	42.4	1				91	24	26.4	1			
Other variants[b]	105	31	29.5	0.571	0.327	0.987	**0.045**	54	17	31.5	1.282	0.603	2.694	0.514
First wave														
No	944	465	50.7	1				649	229	35.3	1			
Yes	852	301	64.7	1.776	1.470	2.149	**<0.001**	545	298	54.7	2.211	1.752	2.794	**<0.001**
Hospitalization														
No admission	529	264	49.9	1				373	92	24.7	1			
Admission to non-intensive ward	848	496	58.5	1.414	1.137	1.760	**0.002**	580	302	52.1	3.311	2.494	4.425	**<0.001**
Admission to ICU	419	270	64.4	1.817	1.398	2.367	**<0.001**	241	133	55.2	3.750	2.658	5.320	**<0.001**
Acute infection treatment														
Steroid therapy														
No	868	500	57.6	1				560	242	43.2	1			
Yes	661	387	58.5	1.039	0.847	1.277	0.712	439	209	47.6	1.194	0.929	1.535	0.167
Anticoagulant therapy														
No	522	270	51.7	1				347	119	34.3	1			
Yes	608	338	55.6	1.168	0.924	1.478	0.194	397	194	48.9	1.829	1.361	2.464	**<0.001**
Immunomodulator therapy														
No	1399	807	57.7	1				906	404	44.6	1			
Yes	85	55	64.7	1.341	0.854	2.146	0.204	64	32	50.0	1.242	0.745	2.072	0.404
Monoclonal antibodies therapy[c]														
No	1524	945	62.0	1				1027	484	47.1	1			
Yes	123	24	19.5	0.149	0.092	0.232	**<0.001**	49	14	28.6	0.452	0.232	0.835	**0.011**
Acute infection clinical presentation														
Number of symptoms														
0	47	27	57.4					31	16	51.6	1			
1	77	38	49.4	0.725	0.345	1.507	0.389	43	26	60.5	1.425	0.555	3.689	0.461

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2	150	76	50.7	0.763	0.389	1.478	0.424	92	55	59.8	1.390	0.605	3.187	0.435
3-5	750	406	54.1	0.876	0.476	1.588	0.664	487	200	41.1	0.654	0.311	1.367	0.257
6-8	488	303	62.1	1.215	0.653	2.227	0.533	329	142	43.2	0.713	0.336	1.505	0.373
>9	197	128	65.0	1.374	0.710	2.629	0.342	149	64	43.0	0.708	0.321	1.551	0.386
General symptoms[d]														
No	45	23	51.1	1				27	14	51.9	1			
Yes	1673	957	57.2	1.279	0.701	2.327	0.420	1116	483	43.3	0.709	0.324	1.542	0.383
Respiratory symptoms														
No	148	74	50.0	1				103	29	28.2	1			
Yes	1498	870	58.1	1.385	0.987	1.945	0.060	1001	449	44.9	2.068	1.335	3.283	**0.001**
Gastrointestinal symptoms														
No	1017	551	54.2	1				684	296	43.3	1			
Yes	662	407	61.5	1.349	1.106	1.648	**0.003**	436	196	45.0	1.070	0.840	1.363	0.581
Neurological symptoms														
No	285	103	36.1	1				211	74	35.1	1			
Yes	779	477	61.2	2.787	2.107	3.702	**<0.001**	543	217	40.0	1.231	0.886	1.720	0.216
Acute infection complications														
Pulmonary complications														
No	1606	939	58.5	1				1051	480	45.7	1			
Yes	47	30	63.8	1.248	0.689	2.338	0.470	28	17	60.7	1.828	0.852	4.091	0.122
Cardiac complications														
No	1558	908	58.3	1				1024	466	45.5	1			
Yes	97	62	63.9	1.265	0.830	1.958	0.276	57	33	57.9	1.643	0.959	2.854	0.071
Embolic complications														
No	1562	909	58.2	1				1027	470	45.8	1			
Yes	90	60	66.7	1.433	0.920	2.276	0.112	52	28	53.8	1.381	0.788	2.438	0.259

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Neurological complications														
No	1644	962	58.5	1				1074	496	46.2	1			
Yes	9	7	77.8	2.354	0.550	17.473	0.266	6	3	50.0	1.165	0.199	6.808	0.858
Renal complications														
No	1552	903	58.2	1				1018	456	44.8	1			
Yes	100	64	64.0	1.275	0.841	1.961	0.254	61	43	70.5	2.927	1.689	5.281	**<0.001**
Gastrointestinal complications														
No	1528	898	58.8	1				1000	460	46.0	1			
Yes	127	71	55.9	0.889	0.618	1.285	0.529	81	39	48.1	1.090	0.690	1.719	0.710

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Distribution and correlation among symptoms

Using machine learning, symptoms were clustered into **4 clinical phenotypes** defined as persistence of specific association of symptoms (**figures 5 and 6**): **respiratory cluster (RESc: cough and dyspnoea); chronic pain (CPc: arthralgia and myalgia); neurosensorial (NSc: alteration in taste and smell); and chronic fatigue-like (CFSc: fatigue, headache and memory loss).**

Figure 5. Example of two cluster distribution of symptoms reported at month-12.

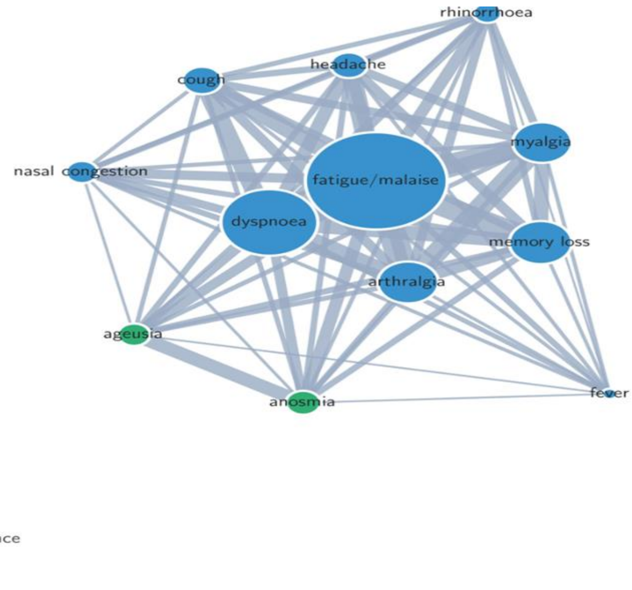
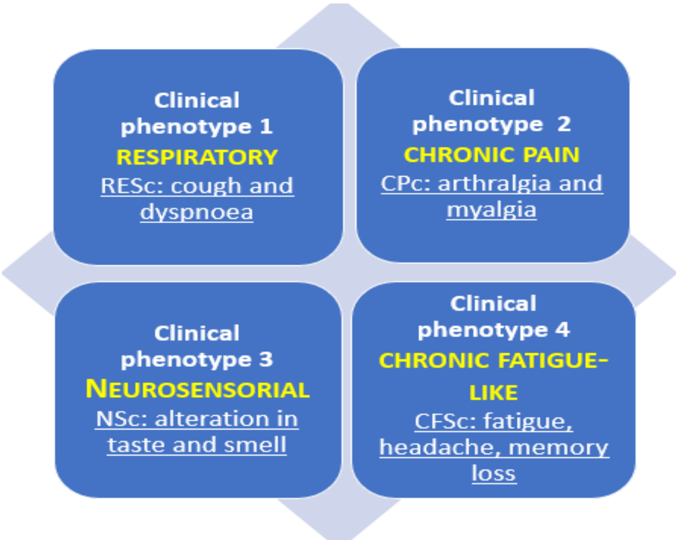


Figure 6. Clusters of long COVID symptoms identified in 1796 patients.



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Serological assessment

In the 6-month cohort, no differences were observed between people experiencing long COVID and those without any persisting complain (9273 BAU +/- 6543 *vs* 7900 BAU +/- 6322; p=0.140), while at 12-month post-acute infection a lower mean of anti-S antibodies was observed in people with persisting symptoms (12144 BAU +/- 5594 *vs* 13030 BAU +/- 4984; p=0.050). When considering long COVID per clusters of symptoms, the anti-S response was higher in patients in RESc (13602 BAU +/- 4296 *vs* 12174 BAU +/- 5632; p=0.050), while patients in NSc presented a lower anti-S response (11307 BAU +/- 5786 *vs* 12436 BAU +/- 5519; p=0.030). **Figure 7** shows the differences in anti-S response between patients with and without long COVID and SF-36 PCS<50 both at 6- and at 12-month follow up. **Figure 8** reports differences in anti-S response according to the four clinical clusters of long COVID.

Figure 7. Differences in anti-S response between patients with (red) and without (blue) long COVID and SF-36 PCS<50 both at 6- and at 12-month follow up. Anti-S titer is provided in BAU. The numbers denote the number of samples used for each of the boxes.

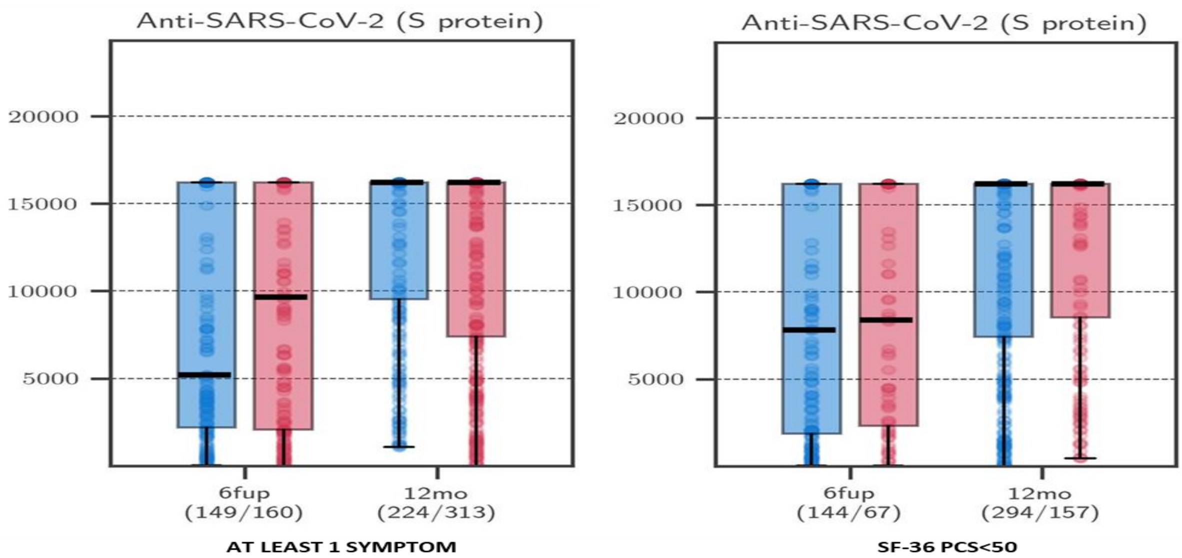

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Figure 8. Differences in anti-S response between patients with (red) and without (blue) the four clusters of symptoms: respiratory, chronic pain, neurosensorial, and chronic fatigue-like symptoms, both at 6- and at 12-month follow up. Anti-S titer is provided in BAU. The numbers denote the number of samples used for each of the boxes. *** <= 0.001, ** <= 0.010, * <= 0.050.

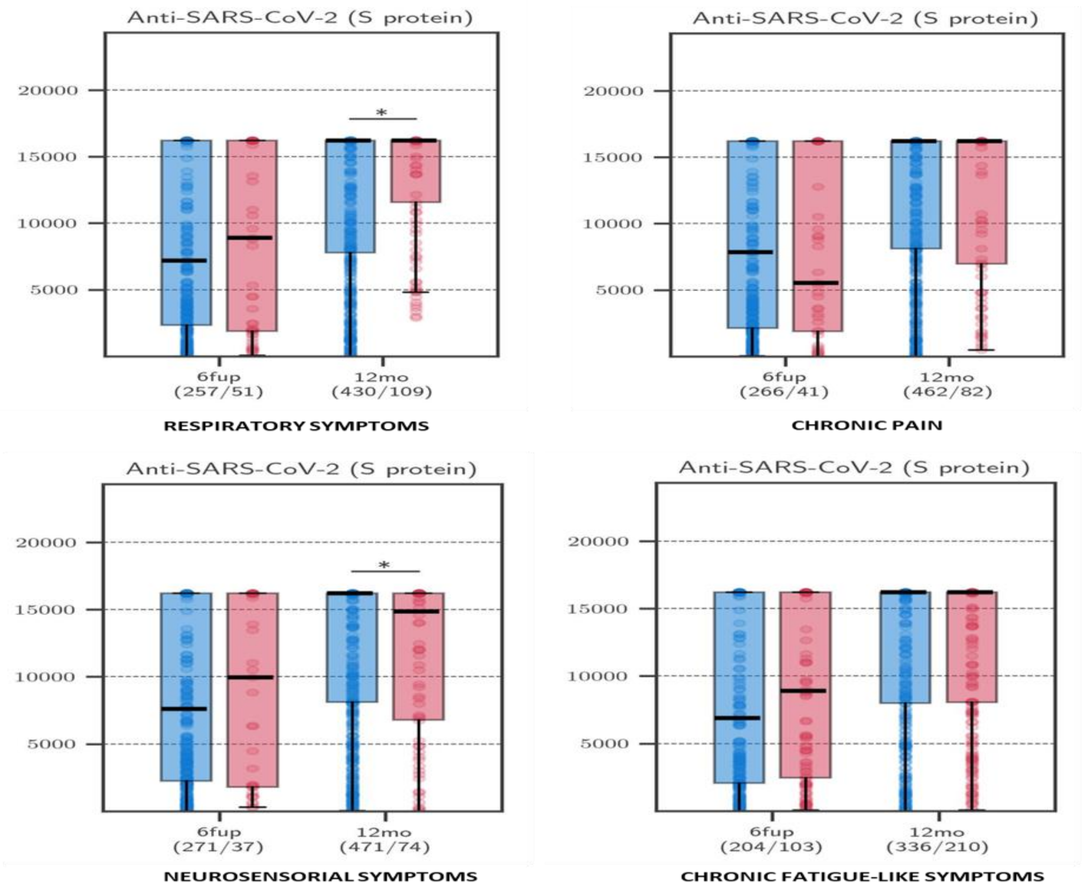

Figure 9 and 10 show the trend of the immunological response with regards to the time from the last dose of anti-SARS-CoV-2 vaccination was administered. No differences were found in the overall titer of anti-S antibodies according to the time from the last dose of vaccine administration and the presence of at least one symptom at 12-month assessment. The analysis of antibody trend with respect to the SF-36 PCS<50 showed that at 4-month after last dose of vaccine, patients experiencing a lower SF-36 PCS score presented a lower anti-S titer (SF-36 PCS<50 group: 8846 BAU +/- 5929 *vs* SF-36 PCS>50 group: 12895 BAU +/- 4242; p=0.010). No differences were found regarding the four symptoms clusters.

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Figure 9. Differences in anti-S response between patients with (red) and without (blue) long COVID and SF-36 PCS<50 both at 6- and at 12-month follow up, according with time since last dose of anti-SARS-CoV-2 vaccine was received. Anti-S titer is provided in BAU. The numbers denote the number of samples used for each of the boxes. *** <= 0.001, ** <= 0.010, * <= 0.050.

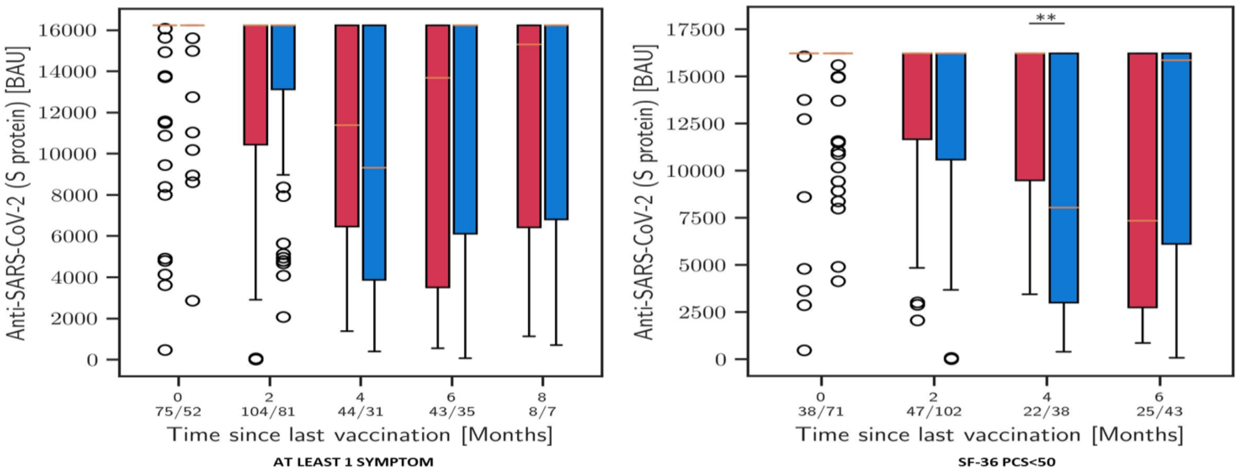
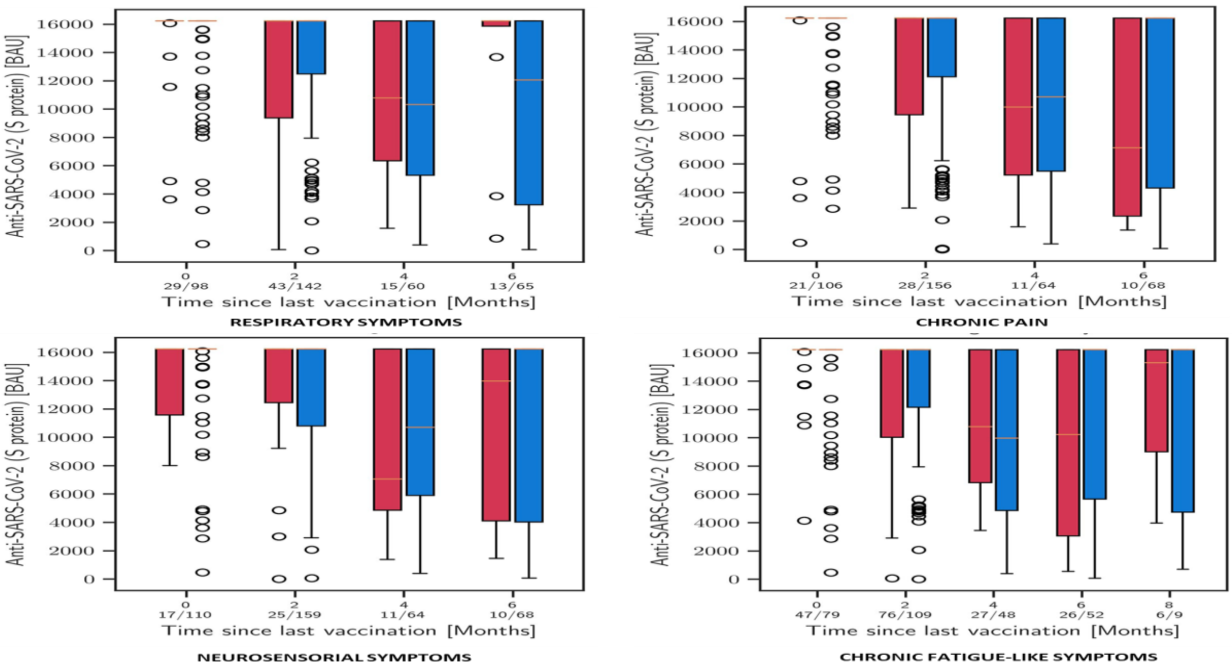


Figure 10. Differences in anti-S response between patients with (red) and without (blue) the four clusters of symptoms: respiratory, chronic pain, neurosensorial, and chronic fatigue-like symptoms, both at 6- and at 12-month follow up, according with time since last dose of anti-SARS-CoV-2 vaccine was received. Anti-S titer is provided in BAU. The numbers denote the number of samples used for each of the boxes.



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Quality of life

A univariable analysis of the association of demographic, epidemiological and clinical characteristics with both PCS and MCS score was computed, showing that for the **PCS,** age >65 years, and male sex were associated with an overall higher score (p<0.001 and p=0.006, respectively). Absence of comorbidities (cardiovascular diseases, chronic respiratory diseases, diabetes, renal impairment, active cancer, transplant, auto-inflammatory diseases) was also associated with a higher performance. Patients vaccinated before acute infection scored better compared with those not vaccinated (55.68 vs 51.11, p<0.001). Overall, when considering persistent symptoms per category, only patients not reporting respiratory symptoms showed to have a better outcome at SF-36 (p<0.001). Not requiring hospital admission or transfer to ICU was also associated with a higher score (p<0.001). Interestingly, patients receiving monoclonal antibodies presented a better score (57.52 vs 50.92, p=0.001). When considering the **mental component (MCS),** male sex confirmed to be associated with a higher score (p<0.001), together with BMI<30 (p=0.028). The presence of comorbidities did not show a relevant association, while gastrointestinal (p=0.004) and neurological (p=0.0031) symptoms during acute infection had a negative impact on the mental component score. Higher scores were found among patients vaccinated before acute infection (p=0.001). Monoclonal antibodies were associated with higher score also for the mental component.

Determinants of long COVID: multivariable analysis

A logistic regression was performed with variables that showed statistically significant association (p<0.05) with the outcomes in univariable analysis (**table 5**). Estimates and accuracy of the models were computed for long COVID defined as presence of at least 1 symptom at 12-month assessment, quality of life measured through SF-36 PCS with a cut off of 50, and the four clusters of long COVID symptoms (**figure 6**).

Neurological symptoms during the acute infection and being female were independently associated with development of long COVID (p<0.001), while receiving early treatment for COVID with monoclonal antibodies was inversely correlated with the outcome (p<0.001). Variables independently associated with a poor quality of life were: being female (p<0.001), older age (p=0.001), hospital admission (p<0.001), pre-existing chronic respiratory diseases (p<0.001), diabetes (p=0.043), respiratory symptoms during the acute infection (p=0.039), and renal post-COVID complication (p=0.029).

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When considering the four clusters of long COVID, female gender was strongly associated to all the clusters except respiratory symptoms, while early treatment of SARS-CoV-2 infection was independently associated with a lower risk of developing any of the four clusters of long COVID. Corticosteroid administration was a protective factor for the development of neurosensorial symptoms, while presence of neurological symptoms during the acute infection increased the risk of developing the neurosensorial cluster of symptoms (p<0.001). Vaccination before 12-month follow up was a protective factor against the development of the chronic pain cluster of symptoms (p=0.035). Neurological and gastroenterological symptoms, together with oxygen therapy requirement, were independently associated with persisting chronic fatigue-like symptoms, while respiratory and neurological symptoms during the acute infection and oxygen need were associated with the respiratory cluster. Overall, accuracy estimates for the cluster analysis were better compared with long COVID defined as presence of at least one symptom, reaching 86% balanced accuracy for the neurosensorial cluster and 75% for the chronic pain (**figure 11**).

Table 5. Multivariable analysis of variables associated with the presence of at least one symptom and SF-36 PCS<50 at 12-month follow up assessment. LB: lower bound; UB: upper bound. Red: variable directly associated to the outcome; blue: variable inversely associated to the outcome.

Variable	At least 1 symptom				SF-36 PCS<50			
	OR	95% CI		p-value	OR	95% CI		p-value
		LB	UB			LB	UB	
Female sex	1.811	1.337	2.461	**<0.001**	3.083	2.220	4.318	**<0.001**
Age	1.007	0.996	1.019	0.197	1.022	1.009	1.036	**0.001**
Neurological symptoms	2.163	1.559	3.009	**<0.001**				
Hospital Admission	0.616	0.373	1.015	0.058	2.355	1.488	3.754	**<0.001**
Monoclonal antibody therapy	0.191	0.105	0.333	**<0.001**	0.645	0.285	1.398	0.276
Oxygen therapy	1.536	0.961	2.459	0.073				
First Wave					1.384	0.963	1.995	0.080
Chronic respiratory disease					2.385	1.588	3.614	**<0.001**
Diabetes					1.830	1.025	3.315	**0.043**
Cardiovascular disease					1.339	0.946	1.894	0.099
Transplant patient					2.173	0.693	7.759	0.199
Respiratory symptoms					1.824	1.042	3.281	**0.039**
Renal Events					2.328	1.108	5.119	**0.029**
Anti-viral Agent					0.822	0.551	1.225	0.336

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Figure 11. ROC curves for the outcomes: at least one symptom (A) and SF-36 PCS<50 (B).

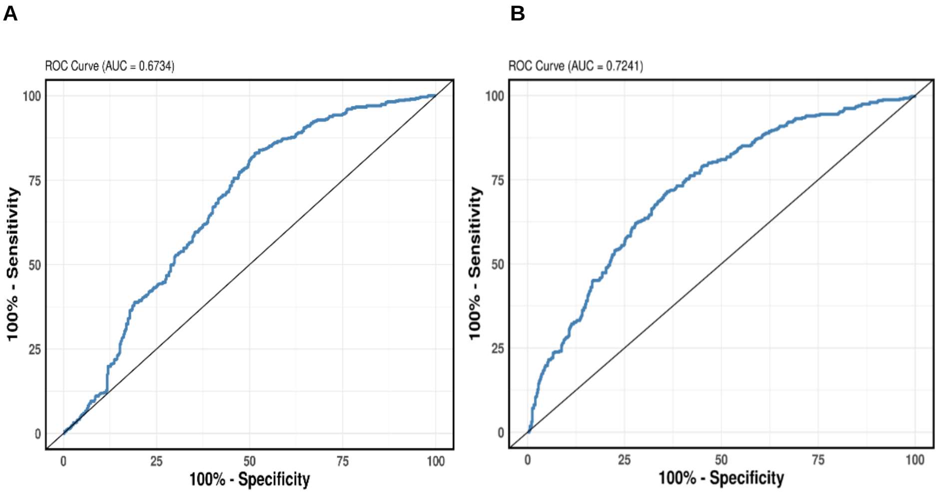

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Table 6. Multivariable analysis of variables associated with the presence of clusters of long COVID at 12-month follow up. LB: lower bound; UB: upper bound. GI: gastrointestinal. ICU: intensive care unit. Red: variable directly associated to the outcome; blue: variable inversely associated to the outcome.

Variable	Respiratory symptoms				Neurosensorial symptoms				Chronic pain				Chronic fatigue-like			
	OR	95% CI		p-value	OR	95% CI		p-value	OR	95% CI		p-value	OR	95% CI		p-value
		LB	UB			LB	UB			LB	UB			LB	UB	
Female sex	1.190	0.834	1.700	0.338	2.248	1.346	3.848	**0.002**	1.941	1.249	3.052	**0.004**	2.144	1.529	3.019	**0.000**
Age	0.997	0.983	1.011	0.703	1.000	0.982	1.019	0.978	1.007	0.991	1.024	0.374	1.005	0.993	1.017	0.455
First Wave					0.380	0.182	0.754	**0.007**	0.653	0.333	1.212	0.195				
Chronic respiratory disease	1.657	1.051	2.590	**0.028**												
Symptoms													7.135	1.419	129.9	0.059
Neurological	1.713	1.137	2.629	**0.012**	40.76	8.845	724.2	**0.000**	1.514	0.932	2.521	0.101	1.608	1.109	2.346	**0.013**
Respiratory	4.438	2.031	11.68	**0.001**												
Gatrointestinal													1.484	1.062	2.074	**0.021**
Vaccination					1.314	0.690	2.633	0.421	0.547	0.315	0.971	**0.035**	0.698	0.466	1.047	0.082
Monoclonal antibody therapy	0.194	0.057	0.496	**0.002**	0.159	0.025	0.557	**0.014**	0.159	0.038	0.455	**0.003**	0.320	0.160	0.600	**0.001**
Oxygen therapy	1.684	1.106	2.579	**0.016**									1.494	1.039	2.154	**0.031**
Corticosteroid					0.412	0.237	0.704	**0.001**								
Anticoagulant									1.191	0.753	1.892	0.456				
ICU admission	1.221	0.763	1.941	0.402												

ROC by outcome: respiratory cluster 0. 69; neurosensorial cluster 0. 80; chronic pain cluster 0. 66; chronic fatigue-like cluster 0. 70.

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Major conclusions

- Long COVID (WHO definition) has been detected in 57% of the population enrolled in the ORCHESTRA prospective cohort including 1796 hospitalised and not hospitalised patients at month 12 of SARS-CoV-2 diagnosis;
- Fatigue, dyspnoea, memory loss, arthralgia, and myalgia are the most frequently reported symptoms;
- Analysis of distribution of type of symptoms suggests that long COVID includes 4 different clusters of symptoms: respiratory (persistence of at least cough and dyspnoea); chronic pain (arthralgia and myalgia); neurosensorial (alteration in taste and smell); and chronic fatigue-like (fatigue, headache and memory loss).
- Long COVID has a clear impact on quality of life assessed prospectively through the SF-36 scores. Being female was associated with persistence of at least one symptom at 12-month follow up and lower SF-36 scores both in the physical and mental components.
- Logistic regression identified different patterns of variables associated with each cluster. Females had higher probability of developing long COVID (specifically neurosensorial, chronic pain and chronic fatigue-like condition); risk of respiratory cluster was increased by chronic obstructive pulmonary disease; neurological symptoms at diagnosis were associated with respiratory, neurosensorial, chronic fatigue-like condition; gastrointestinal symptoms at diagnosis increased the risk of chronic fatigue-like condition.
- Early treatment for COVID-19 with monoclonal antibodies appeared to reduce the risk of all long COVID clusters.
- Corticosteroid treatment was associated to a lower occurrence of neurosensorial impairment.
- Vaccination (3 dosages) decreased the risk to develop chronic pain cluster;

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- Patients suffering from long COVID had a lower mean of anti-S antibodies at 12-month post-acute infection; lower level were detected in those within the neurosensorial cluster.
- Long COVID cannot be identified only by the presence of a single symptoms. The distribution in clusters of symptoms seem to suggest **possible different pathogenetic mechanisms for at least 4 clinical presentations of long COVID**.
- The **early identification of patients at risk for long COVID identified through the ORCHESTRA clusters' risks can play a pivotal role in driving selection of patients for RCT** on preventive measures and early treatment, increasing the accuracy of results and facilitating the rapid achievement of appropriate sample size.
- Consistently with previous reports, this cohort showed a **higher proportion of long COVID-19 in females**. Women elicit a stronger humoral and cellular immune response compared to men, also associated to increased CD4:CD8 ratio, more rapid rejection of allograft, reduced incidence and regression of cancer, and higher occurrence of auto-immune disorders. Sex hormones and genetic factors have been proposed as underlying mechanisms for these gender differences and could also explain female prevalence of long COVID in adults. Future analyses in ORCHESTRA and other cohorts should specifically focus **to understand the role of biological sex in the persistence of symptoms after COVID.**
- The **effect monoclonal antibody early therapy suggests a role for early therapy in reducing long COVID and** encourage a stronger investment on R&D for specific new treatments but also in including post treatment follow up for new therapies released for SARS-CoV-2 treatments.
- The effect of vaccination on long COVID, confirmed in other cohorts, needs analyses of larger sample size underlying the importance of data homogeneity among countries' cohorts to be included in the preparedeness plans.

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Scientific dissemination

Coordinating Partner	Title	Status	Journal / Conference
UNIVR	Determinants of persistence of symptoms and impact on physical and mental wellbeing in Long COVID: a prospective cohort study	Published	Journal of Infection 2022
INSERM	Post-acute COVID-19 symptoms are highly prevalent 12 months after hospitalisation-analyses stratified by gender from a large prospective cohort	Published	Clinical Microbiology and infection 2022
FCRM	Assessment of neutralizing antibody responses after natural SARS-CoV-2 infection and vaccination in Congolese individuals	Published	BMC Infectious diseases 2022
UNIVR	Omicron Neutralizing and Anti-SARS-CoV-2 S-RBD Antibodies in Naïve and Convalescent Populations After Homologous and Heterologous Boosting With an mRNA Vaccine	Submitted to journal	
UMCG	The COVID HOME study research protocol: Prospective cohort study of non-hospitalised COVID-19 patients	Published	PLOS ONE 2022
UNIVR	Clinical phenotypes and cluster of symptoms predicting long COVID the European ORCHESTRA cohort experience	Submitted	ECCMID 2023
UNIVR	Impact of long COVID on quality of life: a functional assessment of post-COVID long term sequelae in the ORCHESTRA cohort	Submitted	ECCMID 2023
Antwerp	Cytokine dynamics in the acute and post-acute phase of hospitalized COVID-19 patients and their link to disease severity, mortality and Long Covid	Submitted	ECCMID 2023

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ORCHESTRA has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101016167

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