

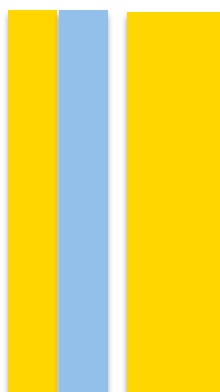
MASTER'S DEGREE
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Prevalence of cognitive impairment after SARS-CoV-2 infection

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Master in Public Health

***Prevalence of cognitive impairment
after SARS-CoV-2 infection***

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This study includes data from the 2013-2015 re-evaluation of the population-based cohort EPIPorto, which has received approval from the Ethics Committee of Hospital São João (CE HSI nº65- 20/10/95).

Both studies, NeurodegCoV-19 and EPIPorto cohorts, were carried out according to the Helsinki Declaration, and all participants completed the informed written consent form. The implementation of these projects was only possible through the cooperation of their participants, whom we thank for their contribution.

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Dedication

Para os meus pais, por sempre acreditarem em mim.

Para o meu marido, pelo apoio constante.

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List of abbreviations

ACE-2 – Angiotensin-converting enzyme 2

BMI – Body mass index

CDC – Centers for Disease Control and Prevention

CI – Confidence interval

COVID-19 – Coronavirus Disease 2019

DAG - Directed Acyclic Diagram

GHD – Homogeneous Diagnoses Groups

HADS – Hospital Anxiety and Depression Scale

MERS-CoV – Middle East respiratory syndrome coronavirus

MoCA – Montreal Cognitive Assessment

aOR – Adjusted odds ratio

PCI – Probable cognitive impairment

PSQI – Pittsburgh Sleep Quality Index

SARS-CoV – Severe acute respiratory syndrome coronavirus

SARS-CoV-2 – Severe acute respiratory syndrome coronavirus 2

SD – Standard deviation

TUG – Timed Up and Go

ULSM – *Unidade Local de Saúde de Matosinhos*

VOC – Variants of Concern

WHO – World Health Organization

Resumo

Antecedentes e objetivo: As queixas cognitivas são sintomas comumente relatados em casos de COVID prolongada, e foi observado déficit no desempenho cognitivo em sobreviventes de COVID-19 até um ano após a infecção, principalmente em doentes hospitalizados. Este estudo tem como objetivo estimar a prevalência do déficit cognitivo em sobreviventes de COVID-19 de dois anos e compará-la com a população em geral.

Métodos: Foram analisados dados da primeira avaliação da coorte NeurodegCoV-19 recolhidos entre o dia 1 de julho de 2022 e 8 de março de 2023. A coorte incluiu 90 indivíduos que foram hospitalizados por SARS-CoV-2 antes da vacinação, entre março de 2020 e fevereiro de 2021 (grupo hospitalizado), e 240 indivíduos que foram infectados, mas não hospitalizados, durante o mesmo período (grupo não hospitalizado). O *Montreal Cognitive Assessment* (MoCA) foi utilizado para avaliar o desempenho cognitivo. Uma pontuação no MoCA abaixo ou igual a 1.5 desvios-padrão (DP) da média das normas específicas para idade e educação foi definido como um provável déficit cognitivo (PDC); abaixo de 2 DP foi considerado PDC severo. Dados da coorte populacional EPIPorto (n=931, avaliada em 2013-2015) foram utilizados para comparação.

Resultados: A prevalência de PDC foi de 43.3% (intervalo de confiança (IC) 95%: 38.3-43.8) no grupo hospitalizado, 43.8% (IC 95%: 40.8-46.8) no grupo não hospitalizado e 30.7% (IC 95%: 29.2-32.2) na coorte EPIPorto. Ajustando para idade, escolaridade, polifarmácia, obesidade, hipertensão, hipercolesterolemia e diabetes, as *odds* de PDC foram maiores tanto no grupo hospitalizado (*odds ratio* ajustadas (aOR), IC 95%: 1.84, 1.17-2.90) quanto no grupo não hospitalizado (aOR, IC 95%: 1.59, 1.17-2.16), em comparação com a coorte EPIPorto. Verificou-se associação entre a ansiedade e o PDC severo (aOR, IC 95%: 1.98, 1.01-3.81), assim como entre a marcha lenta e o PDC e PDC severo (aOR, IC 95%: 1.10, 1.00-1.20; aOR, IC 95%: 1.06, 0.99-1.14 respectivamente).

Conclusão: Este estudo, envolvendo participantes hospitalizados e não hospitalizados, mostra uma elevada prevalência de PDC dois anos após a infecção por COVID-19, em comparação com a população em geral. Realça também a necessidade de identificação precoce de déficit cognitivo pós-COVID-19 em pessoas afetadas no primeiro ano da pandemia, independentemente da gravidade da doença.

Abstract

Background and objective: Cognitive complaints are commonly reported symptoms of long COVID, and lower cognitive performance was reported among COVID-19 survivors, up to one year after infection, mostly in hospitalized cases. This study aims to estimate the prevalence of cognitive impairment in two-year COVID-19 survivors and compare it with the general population.

Methods: Baseline data from the NeurodegCoV-19 cohort collected between July 1, 2022, and March 8, 2023, was analyzed. The cohort included 90 individuals who had been hospitalized for SARS-CoV-2 before vaccination, between March 2020 and February 2021 (hospitalized group), and 240 individuals who had been infected but not hospitalized during the same period (non-hospitalized group). The Montreal Cognitive Assessment (MoCA) was used to assess cognitive performance. A MoCA score below or equal to 1.5 standard deviations (SD) of the mean of age- and education-specific norms was defined as a probable cognitive impairment (PCI); below 2 SD was considered severe PCI. Data from the population-based cohort EPIPorto (n=931, evaluated in 2013–2015) was used for comparison.

Results: The prevalence of PCI was 43.3% (95% confidence interval (95 % CI): 38.3-43.8) in the hospitalized group, 43.8% (95% CI: 40.8-46.8) in the non-hospitalized, and 30.7% (95% CI: 29.2-32.2) in the EPIPorto cohort. Adjusting for age, education, polypharmacy, obesity, hypertension, hypercholesterolemia, and diabetes, the odds of PCI were higher in both the hospitalized (adjusted odds ratio (aOR), 95% CI: 1.84, 1.17-2.90) and the non-hospitalized group (aOR, 95% CI: 1.59, 1.17-2.16) than in EPIPorto. Anxiety was associated with PCI (aOR, 95% CI: 1.98, 1.01-3.81) and slow gait was associated with PCI and severe PCI (aOR, IC 95%: 1.10, 1.00-1.20; aOR, IC 95%: 1.06, 0.99-1.14 respectively).

Conclusion: The study, involving hospitalized and non-hospitalized participants, shows a high prevalence of cognitive impairment two years after COVID-19 infection, compared with the general population. It emphasizes the need for post-COVID-19 cognitive impairment screening in those affected in the first year of the pandemic, regardless of disease severity.

Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

The Coronavirus Disease 2019 (COVID-19), caused by the new coronavirus SARS-CoV-2, was first detected in Wuhan in late December 2019 (1). Since it was declared a global pandemic by the World Health Organization (WHO) in 2020, it has been responsible for more than 690 million cases and 6 million deaths worldwide (2), and has become the most consequential public health crisis since the influenza pandemic in 1918 (3, 4). Disrupting daily life through the introduction of containment measures (use of masks, social distancing, and quarantine), COVID-19 presented an unprecedented challenge to healthcare systems worldwide, with a shortage of protective equipment and intensive care beds and ventilators, ultimately leading to difficulties and weaknesses in patient care (5).

Coronaviruses are common pathogens in humans and animals. Some of them are endemic in humans and usually affect the upper respiratory tract and cause common-cold symptoms (6). In the last two decades, three zoonotic coronaviruses that infect humans have emerged from animal reservoirs: severe acute respiratory syndrome coronavirus (SARS-CoV) (7), which originated in China and was responsible for the 2003 epidemic; Middle East respiratory syndrome coronavirus (MERS-CoV) (8), which currently causes intermittent outbreaks in the Middle East; and SARS-CoV-2, the causative agent of COVID-19 (1). These three viruses can replicate in the lower respiratory tract and cause potentially fatal acute respiratory distress syndrome (6).

SARS-CoV-2 is a single-stranded RNA-enveloped virus that belongs to the beta-coronavirus family alongside MERS-CoV and SARS-CoV (3, 9). SARS-CoV-2 possesses a spike protein, which plays a vital role in the receptor recognition and cell membrane fusion process and is composed of two subunits, S1 and S2. The S1 subunit contains a receptor-binding domain that recognizes and binds to the host receptor angiotensin-converting enzyme 2 (ACE-2), while the S2 subunit mediates viral cell membrane fusion (9). After binding to ACE-2 on the target cell, the spike protein is cleaved at the S2 site. This cleavage activates the S2 subunit trimers to fuse viral and host lipid bilayers, releasing the viral ribonucleoprotein complex into the receptor cell (6, 10). Because ACE-

2 can be found in the endothelial cells of many organs, such as the lung, spleen, skin, heart, kidneys, brain, and intestine (11-13), the spike protein makes the cells possessing ACE-2 main targets of the virus and determines the tropism and virulence of SARS-CoV-2 (3). Common symptoms reported early in the pandemic include fever, cough, and shortness of breath, as well as sore throat, anosmia, dysgeusia, headache, loss of appetite, nausea, malaise, and myalgias (14). Based on clinical symptoms, laboratory and radiographic abnormalities, hemodynamics, and organ function, the severity of COVID-19 can be classified as asymptomatic infection, paucisymptomatic infection, mild illness, moderate illness, severe illness, and critical illness. In more severe cases, acute respiratory failure requiring mechanical ventilation, septic shock, and multiple organ failure may occur (15). A multinational and multicenter observational study involving 43 countries reported that more than 30% of hospitalized patients infected with SARS-CoV-2 developed severe COVID-19 disease with high mortality rates during the first year of the pandemic (16).

SARS-CoV-2, like other RNA viruses, has undergone a rapid genetic evolution, allowing for an improved adaptation to its new human hosts (17), and posing a challenge to disease-control efforts as higher infection rates and lower effectiveness of vaccines to new variants are observed (18). According to the classification of the Centers for Disease Control and Prevention (19), five Variants of Concern (VOC) were identified during this pandemic: Alpha – first described in the United Kingdom in late December 2020; Beta – first reported in South Africa in December 2020; Gamma – first reported in Brazil in early January 2021; Delta – first reported in India in December 2020; Omicron – first reported in South Africa in November 2021 (17, 19). All have spread worldwide and have become the dominant variants in the geographic regions where they were first identified (20, 21). Presently, Omicron is the most widespread VOC in many countries, including Portugal (21, 22).

Figures 1, 2, and 3 illustrate the phylogenetic evolution of the SARS-CoV-2 virus in Portugal (22). Figure 1 shows that since March 2020, when the SARS-CoV-2 virus was first detected in the country, four genetic variations of the virus were frequently detected in the collected genomic samples – clade 19A, 20A, 20B, and 20E. In January

2021, the VOC Alpha became more prevalent and peaked in February. Following Alpha and ancestor forms, the Delta frequency increased from June to December 2021 (Figure 2). In early 2022, Omicron was the most frequently identified variant, which became the dominant lineage of SARS-CoV-2 in Portugal by that time (figure 3).

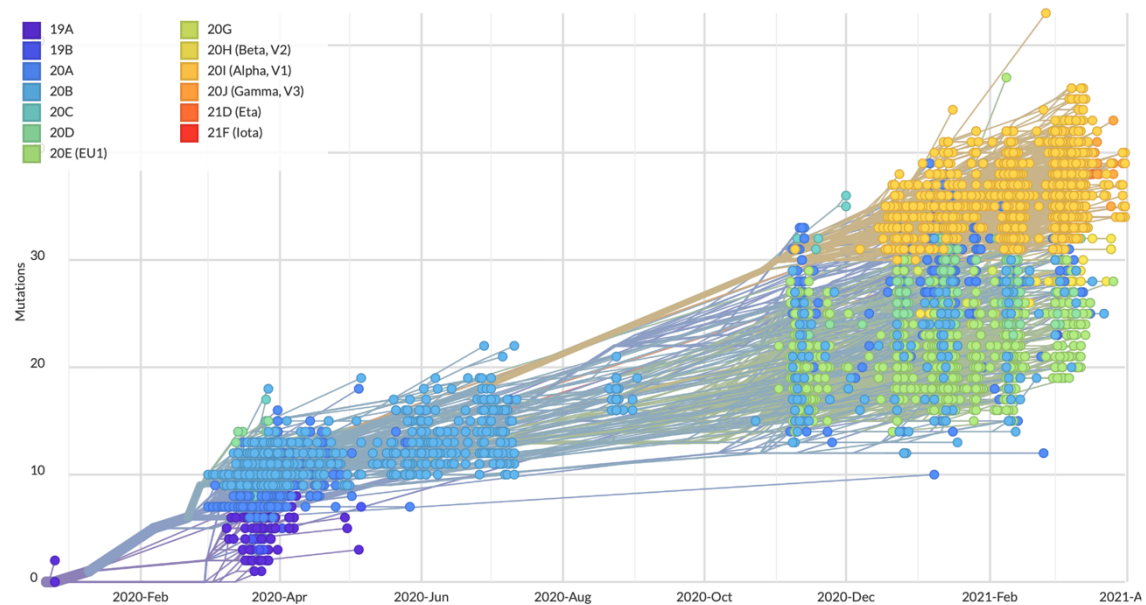


Figure 1. Genomic epidemiology of novel coronavirus in Portugal from March 1, 2020 up to March 3, 2021. [Source: National Institute of Health (INSA). Genetic diversity of the novel coronavirus SARS-CoV-2 (COVID-19) in Portugal Lisbon, Portugal; 2022]

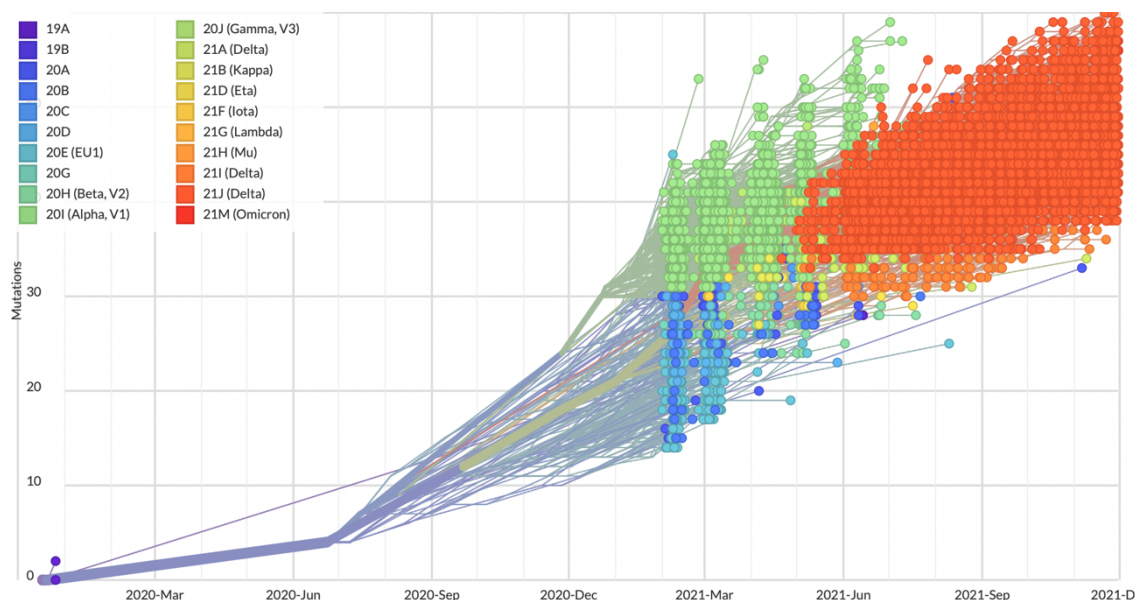


Figure 2. Genomic epidemiology of novel coronavirus in Portugal from March 1, 2020 up to December 31, 2021. [Source: National Institute of Health (INSA). Genetic diversity of the novel coronavirus SARS-CoV-2 (COVID-19) in Portugal Lisbon, Portugal; 2022]

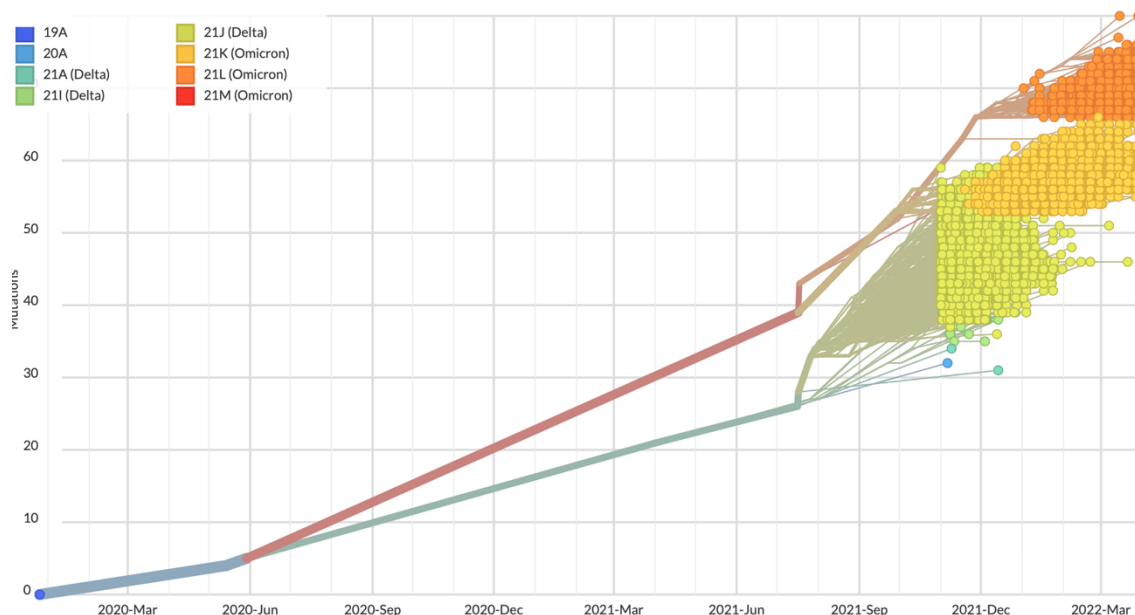


Figure 3. Genomic epidemiology of novel coronavirus in Portugal from March 1, 2020 up to March 31, 2022. [Source: National Institute of Health (INSA). Genetic diversity of the novel coronavirus SARS-CoV-2 (COVID-19) in Portugal. Lisbon, Portugal; 2022]

Figure 4 shows the three peaks in the incidence of new cases from March 2020 to February 2021, representing the first, second, and third waves of COVID-19 in Portugal (23). The highest peak in new cases occurred in early 2022 when the first Omicron cases were detected.

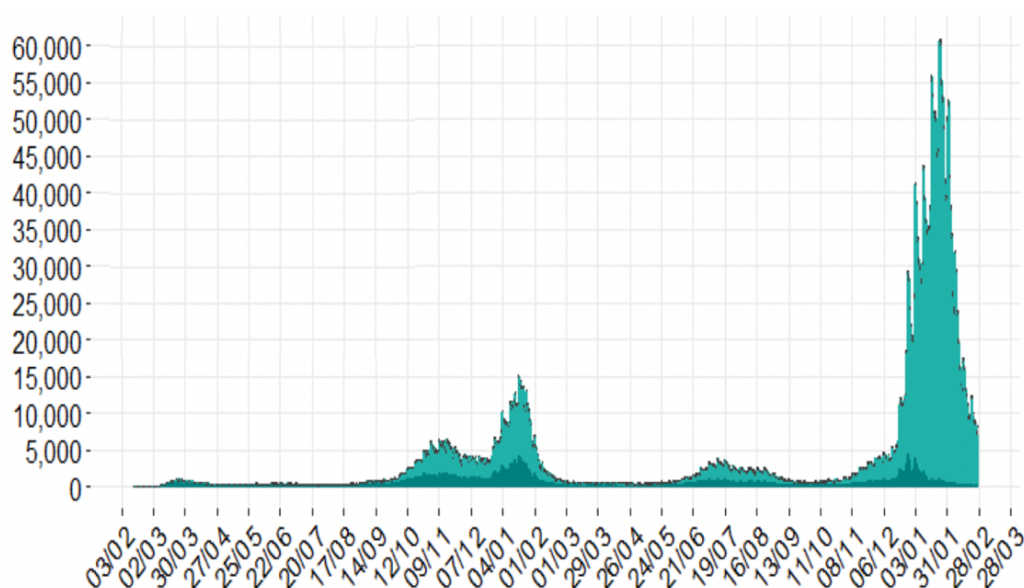


Figure 4. The epidemic curve of SARS-CoV-2 infection cases from March 2020 to February 2022, in Portugal [Source: National Institute of Health (INSA). Covid-19: epidemic curve and transmissibility parameters in Portugal. Lisbon, Portugal; 2022]

According to modeling data, Alpha is estimated to be 43-90% more transmissible than the earlier circulating variants, and Delta is thought to be about 60% more transmissible than Alpha (24, 25). Omicron is described as highly contagious, with recent estimates suggesting that it is roughly 300% more transmissible than Delta (26). However, it is associated with a lower risk of hospitalization and disease severity compared to previous ones (26, 27). Florensa et al. (28) compared the symptoms in the Alpha and Delta periods and found that fever, cough, and vomiting were significantly associated with the Alpha variant. In the Delta period, according to Menni et al. (29), the most reported symptoms among 4990 individuals in the United Kingdom were runny nose (81.6%), headache (77.9%), sneezing (70.7%), sore throat (60.8%), and loss of smell (52.7%). In the same study, authors also noted that during the Omicron period compared to Delta, sore throat was more common (70.5% vs 60.8%; adjusted odds ratio (aOR), 95% confidence interval (CI): 1.55, 1.43–1.69, $p < 0.001$), loss of smell became less common (16.7% vs 52.7%; aOR, 95% CI: 0.17, 0.16–0.19, $p < 0.001$) and fever and persistent cough were less likely to manifest (1.9% vs. 2.6%; aOR, 95% CI: 0.75, 0.57–0.98, $p = 0.03$).

Vaccination is crucial to prevent cases of severe SARS-CoV-2 infection in communities worldwide, as it triggers the immune system to produce neutralizing antibodies against this virus (15, 30, 31). The extraordinary efforts made by clinical researchers worldwide during this pandemic have led to the development of novel vaccines against SARS-CoV-2 at an unprecedented pace (15). In early December 2020, the first COVID-19 vaccine was delivered outside of a clinical trial (32). Next, major vaccination campaigns started to be implemented around the world mainly from December 2020 to mid-2021. Watson et al. (33) estimated that, between December 8, 2020, and December 8, 2021, 14.4 million deaths due to COVID-19 in 185 countries and territories were prevented by vaccination. Recent data shows that 64% of the world's population has a full first vaccination protocol, with Portugal being one of the countries with the highest percentage (87%) (34).

Studies published in the last two years have identified persisting symptoms in COVID-19 survivors in different countries (35), commonly referred to as long COVID. This condition is defined by the WHO as “the continuation or development of new symptoms three

months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least two months with no other explanation” (36). The pooled global prevalence of long COVID after at least 28 days from virus infection was estimated at 43% (95% CI: 39-46) (37), and 45% of COVID-19 survivors experienced unresolved symptoms at four months, regardless of hospitalization status (38). Several systematic reviews that included studies conducted before March 2021 have shown that the most common long COVID symptoms were fatigue, shortness of breath, muscle pain, joint pain, headache, cough, chest pain, altered smell, taste, and diarrhea (39-41). Moreover, Lopez-Leon et al. (40) estimated that 80% of the infected patients with SARS-CoV-2 during the first year of the pandemic developed one or more long-term symptoms up to three months. It seems that Omicron cases are less likely to experience long COVID compared with cases infected with ancestor forms (42, 43), and fatigue seems to be the most common long COVID symptom in all SARS-CoV-2 variants (44, 45). Nevertheless, long COVID is a complex and challenging condition with persistent heterogeneous symptoms and manifestations. It is essential to consider the spectrum of factors and symptoms contributing to long COVID to improve clinical care and help healthcare providers identify populations most at risk for symptoms and persistent consequences (46).

Neurologic manifestations of COVID-19

Neurological symptoms such as altered smell or taste are early symptoms of COVID-19 (47-50). Pooled data from two systematic reviews that included studies from January to September 2020, estimated that the prevalence of smell and taste disturbances in COVID-19 patients ranged from 41.0% to 52.7% and 38.2% to 43.9%, respectively (51, 52). Since Mao et al. (53) reported 214 cases with these symptoms in early 2020, there has been speculation that the virus may target the central nervous system via retrograde olfactory pathways. Currently, evidence shows that acute and long-term neurological manifestations are common in infected individuals (53-56). *In vivo* (human), *in vitro*, and animal models have demonstrated the neuroinvasive potential of SARS-CoV-2, and the following possible mechanisms of neuroinvasion have been described in the literature: trans-synaptic spread (neuron to neuron) (57); binding of the spike protein to glycoproteins on the surface of endothelial cells with subsequent storage in vesicles and transmission across the blood-brain barrier (58); uptake into nonneuronal cells, such as

e.g., sustentacular cells in the olfactory epithelium (typically resulting in anosmia) (59); and infection of leukocytes by coronaviruses, providing a pathway from blood to the brain during periods of severe inflammation and capillary leakage (60).

In addition to smell and taste disturbances, headache, impaired consciousness and cognition, neuropsychiatric manifestations, and ocular symptoms are the most reported neurologic manifestations of SARS-CoV-2 infection (61, 62). The incidence of headaches ranged from 8.0% to 15.4% in hospitalized and non-hospitalized patients in different studies from January-February 2020 (63-66). In a multicenter cohort study, between January and April 2020, the incidence of coma and delirium ranged from 7.5% to 13.9% in patients hospitalized with COVID-19, being these incidences significantly higher in critically ill patients (81.6% and 55.0%, respectively) (67). According to two systematic reviews that included data from January to December 2020, the pooled prevalence of depression ranged from 38% to 45%, anxiety ranged from 38% to 47%, and sleep disorders ranged from 34% to 48% (68, 69). A February 2020 study from China estimated the prevalence of ocular manifestations in hospitalized patients to be 30% (70). Less common neurologic manifestations include stroke, seizures, myopathy, myelopathy, and Guillain-Barré syndrome (61).

Several cohort studies have described the persistence of the neurological manifestations associated with long COVID. In a longitudinal analysis of 1733 COVID-19 patients discharged between January and May 2020 in China, 76% reported at least one of the following symptoms six months after SARS-CoV-2 infection: fatigue or muscle weakness (63%), sleep disturbances (26%), smell or taste disturbance (11% and 7%, respectively), myalgias (2%), and headache (2%) (71). Another cohort study from China of patients who had been hospitalized with COVID-19 between February and April, reported fatigue (30%), myalgias (8%), dizziness (3%), headache (2%), and taste/smell disturbances (1%) in 2433 patients at one-year follow-up (72). In addition, an international online survey of 3762 participants who experienced onset symptoms before June 2020, reported sensorimotor deficits (91%), self-reported cognitive impairment (85%), emotional disturbance (88%), sleep disturbance (79%), headache (77%), memory impairment (73%), and smell/taste disturbance (58%) six months after infection (73). In the same

study, 65% of patients reported that neurologic symptoms persisted beyond six months, with fatigue (80%), post-exertional malaise (73%), and self-reported cognitive impairment (58%) being the most common. In a retrospective cohort study of 273 618 COVID-19 survivors from January to December 2020, anxiety and depression were the most reported complaints (22.8%), occurring most frequently three to six months after infection (74).

A recent cohort study of individuals from Wuhan who survived hospitalization with COVID-19 during January and May 2020, including an age-, sex-, and comorbidity-matched control group of individuals who never had COVID-19, described dynamic health recovery in the two years after symptom onset (75). The proportion of individuals with at least one subsequent symptom decreased significantly from 68% at six months to 55% at two years, with fatigue or muscle weakness being the most reported symptom throughout the follow-up period (52% at six months and 30% at two years, $p<0.0047$). Smell and taste disturbances and sleep difficulties were reported less frequently at two years, whereas the proportion reporting headache and dizziness increased slightly at two years (2% versus 7% and 6% versus 11%, $p<0.0047$, respectively). The proportion of participants reporting anxiety or depression decreased significantly from 23% at six months to 12% at two years ($p<0.0047$).

Cognitive dysfunction after COVID-19

Since Mao et al. (53) pointed out the presence of neurologic manifestations during acute SARS-CoV-2 infection in early 2020, several studies have reported the prevalence of cognitive impairment in COVID-19 survivors. Alemanno et al. (76) assessed cognitive performance from March to June 2020 and found that 80% of COVID-19 survivors were cognitively impaired after hospital discharge. A 6-month retrospective study using medical records of 73 435 patients infected between March and December 2020 showed that after the first 30 days of illness, patients hospitalized with COVID-19 had a higher risk of neurocognitive disorders (diagnoses identified with International Classification Disease-10 codes) than patients hospitalized with seasonal influenza (hazard ratio 95% CI: 1.43, 1.24—1.65), and memory problems were between 1.37 to 8.02 fold higher in COVID-19 affected individuals than in non-infected individuals, with

a gradient of increased risk from non-hospitalized to hospitalized, and to patients admitted in intensive care units (77). Two meta-analyses revealed the occurrence of cognitive impairment at three and six months after diagnosis in approximately 20% of the patients (78, 79). However, because most studies did not establish the presence of cognitive impairment before the COVID-19 diagnosis, it is unclear to what extent the results are related to the SARS-CoV-2 infection. In that respect, the meta-analysis conducted by Crivelli et al. (80) found that patients who recovered from COVID-19 with no prior history of cognitive impairment had lower overall cognition up to seven months post-infection compared with healthy controls. In a study of adults aged 26 to 64 years with confirmed long COVID or in the diagnosis process, 85% had impaired scores in at least one neuropsychological test within a mean elapsed time between infection and evaluation of 508 days (81). A qualitative study (82) explored the cognitive complaints of 50 individuals who experienced mostly mild COVID-19 between February and July 2020 and reported persistent symptoms beyond three weeks after infection. Cognitive domains, namely executive function, attention, memory, and language were described as the most impaired. In the same study, participants also reported difficulties in returning to their previous level of occupational and social activities, opting for reduced hours and adapted roles, often associated with anxiety about potential risks associated with mistakes in cognitively demanding or high-responsibility roles, self-doubt about their abilities, and loss of self-worth. In fact, 86.2% of survivors reported poorer work performance seven months after infection (73), and up to 1/3 of patients with severe COVID-19 had not returned to active work one year after admission (83). Furthermore, various studies suggest that cognitive impairment after SARS-CoV-2 infection seems to be associated with increased anxiety and depression during acute hospitalization/disease and after infection (84-88).

Ultimately, this neurologic complication can significantly affect the functional status, daily activities, work performance, and quality of life in both severe and milder cases (89-91), particularly in those infected during the first year of the pandemic. Infections in this period are more likely to be associated with an increased risk of neurological complications because vaccines were essentially unavailable and because *in vitro* and animal models have shown lower neurotropism and neurovirulence of the Delta and

Omicron variants (92). Therefore, it is important to investigate the occurrence and risk factors for this condition in the group of COVID-19 survivors of the first year of the pandemic. Several reviews (79, 80, 93) have indicated that the number of studies examining the impact of COVID-19 on cognitive performance is insufficient to draw adequate conclusions, with most studies presenting small sample sizes, no control group, and underrepresentation of non-hospitalized cases.

Recent evidence suggests that slower gait speed is associated with cognitive impairment and seems to be an early indicator of neurodegenerative disorders such as mild cognitive impairment and dementia (94-97). Gait analysis, including gait speed, is an objective measure of the neuro-motor system and may provide significant information about the pathophysiology of specific diseases such as COVID-19 (98). Therefore, the potential of gait speed assessment for improving the screening of cognitive impairment deserves investigation.

This study aims to:

- 1) Estimate the prevalence of cognitive impairment in two-year COVID-19 survivors infected with the novel coronavirus between March 2020 and February 2021, using the Montreal Cognitive Assessment (MoCA), and compare with the general population.
- 2) Identify associations between cognitive performance (MoCA score and age- and education-based classification for cognitive deficit) and sociodemographic and lifestyle characteristics, medical history, poor sleep quality, anxiety and depression, gait speed, as well as clinical characteristics of SARS-CoV-2 infection, namely the presence of neurological symptoms and the severity of the disease.

By assessing two-year COVID-19 survivors, including hospitalized and non-hospitalized cases, and comparing the cognitive performance with data from the general population, this study will allow the identification of factors that may contribute to long-term cognitive impairment following SARS-CoV-2 infection in those affected by more aggressive variants of the virus.

Methods

The present cross-sectional study is based on the cohort study NeurodegCoV-19 “Neurological Complications after COVID-19”, designed to estimate the occurrence of neurodegenerative complications after COVID-19; and the EPIPorto cohort of the general population. This dissertation analyzed baseline data from the cohort study NeurodegCoV-19 collected between July 1, 2022, and March 8, 2023, and data from the 2013–2015 re-evaluation of the EPIPorto cohort (99).

NeurodegCoV-19 cohort

Citizens of *Matosinhos* represent the study population. The county of Matosinhos is situated in the district of the city of Porto, on the Northern coast of Portugal, the most affected region by the first wave of the COVID-19 pandemic. All citizens of the county (around 175 thousand) have access to public healthcare through the *Unidade Local de Saúde de Matosinhos* (ULSM), which includes and connects all primary healthcare units of the county of *Matosinhos*, its hospital, *Hospital Pedro Hispano*, and continued integrated care units. Participants were retrospectively identified from administrative lists of ULSM as follows: having a first diagnosis of SARS-CoV-2 infection (detection of RNA virus in nasopharyngeal or oropharyngeal swabs, with Real-Time Polymerase Chain Reaction), before any vaccination, during the period from March 2020 (first case diagnosed in Portugal on March 2) to February 2021 [complete vaccination uptake reached 3% and partial vaccination, 6%, on February 28, 2021 (100)]. Infected individuals were grouped according to hospitalization at *Hospital Pedro Hispano* due to infection with SARS-CoV-2 (hospitalized group), or clinical follow-up in the community (non-hospitalized group).

Patients hospitalized between March 2020 and February 2021 due to COVID-19 were identified from the administrative list of all hospitalized patients, in two steps: 1) excluding patients admitted with a diagnosis (description of the Homogeneous Diagnoses Groups—GDH) not compatible with COVID-19 (e.g. “Knee and/or leg procedures other than foot” or “childbirth”); and 2) identification of the remaining patients, by consultation of each medical record to confirm the reason for hospitalization. Nosocomial infections with SARS-CoV-2 were excluded.

Eligibility criteria were checked by consultation of medical records and during recruitment. Participants were aged 18 or older at diagnosis. Those who presented a neurologic condition impairing cognitive performance, consumption of anti-dementia drugs, severe motor, visual or language impairment, and less than one formal year of education and primary school attainment not in Portugal were excluded. Individuals living at senior homes, infected between the two doses of the primary vaccination scheme or within the 14 days following vaccination, or those undergoing clinical or research procedures aiming to prevent cognitive decline were also excluded.

Phone calls to contact potential participants were conducted by trained members of the research team to confirm eligibility, explain objectives and procedures, and schedule an in-presence evaluation at *Hospital Pedro Hispano*.

A structured questionnaire was used in a face-to-face interview to assess sociodemographic data (age, marital status, education, employment status, and occupation), lifestyle (smoking and alcohol consumption), anthropometric characteristics (weight and height), medical history (previous disease diagnoses and current medication), neurologic symptoms during infection, after recover and currently, dates of SARS-CoV-2 infection diagnoses, and vaccination uptake. Polypharmacy was considered as the consumption of more than five medicines, and medicines were classified according to the WHO Anatomical Therapeutic Chemical Classification System (101). Weight and height were reported by participants as the study was originally designed to be conducted remotely and with the shortest possible duration of in presence assessment because of the COVID-19 mitigation measures. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters), and further divided into the categories proposed by the WHO: underweight was defined as BMI $<18.5 \text{ kg/m}^2$; normal weight was defined as BMI between 18.5 kg/m^2 and 24.9 kg/m^2 ; overweight was defined as BMI between 25.0 kg/m^2 and 29.9 kg/m^2 and obesity as BMI $\geq 30.0 \text{ kg/m}^2$ (102). Excessive alcohol consumption was defined, accordingly to the guidelines of *Direção Geral da Saúde*, as the consumption of more than two regular drinks per day for men less than 65 years, and more than one regular drink per day for women and men aged at least 65 years, in the previous six months (103).

Clinical characteristics of SARS-CoV-2 infection were collected through consultation of clinical records by the medical team of the hospital, namely the highest level of care (general ward, intermediate care unit, intensive care unit), and length of hospitalization.

Sleep quality was evaluated using the Pittsburgh Sleep Quality Index (PSQI), which is a 19-item self-rated questionnaire for evaluating subjective sleep quality in general and clinical populations over the previous month (104). It has been used in population-based and clinical studies (105-107), and it is validated for the Portuguese population (104). The PSQI includes seven components – subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction – graded on a score ranging from 0 to 3, and a summary score ranging from 0 to 21, with the highest score indicating worst sleep quality (104). A global PSQI score greater than five indicates major difficulties in, at least, two components or moderate difficulties in more than three (106, 107).

The Hospital Anxiety and Depression Scale (HADS) questionnaire was used for the evaluation of anxiety and/or depressive states. HADS is a validated instrument for the Portuguese population and is formed by 14 questions, seven for each component, that ask the participant to reflect on their mood in the seven previous days (108). Each question has four answer possibilities, graded from 0 to 3, and the total score of each subscale ranges from 0 to 21, being higher scores related to higher states of anxiety and depression (108). Anxiety/depression is considered when the HADS sub-score for anxiety/depression is equal or more than 11 (109).

Gait speed was measured with the Timed “Up and Go” Test (TUG). This is a reliable and valid test for quantifying functional mobility, which gives information about the patient's gait speed, balance, and functional ability (110). Participants are asked to stand up from a chair, walk three meters, turn around, walk back to the chair, and sit down again. One training trial is performed under supervision, then two trials are measured with the chronometer (in seconds), and the results are presented as an average of the two trials.

Cognitive performance was assessed using the validated version of MoCA (version 7.1) for the Portuguese population (111), which is a brief instrument developed for screening

milder forms of cognitive impairment (111, 112). The cognitive domains evaluated are abstraction, attention, executive function, language, memory, orientation, and visuospatial ability. The final score ranges from 0 to 30 points, with higher scores indicating better cognitive performance. A participant was considered to have probable cognitive impairment (PCI) when the score was 1.5 standard deviation (SD) equal to or below the mean of age and education-based group distribution from published normative data according to figure 5 (113). A severe PCI was considered when the score was 2 SD below the mean.

Age (n)	Educational Level (years)				
	Primary (1-4) (6)	Middle (5-9) (31)	High (10-12) (34)	University (>12) (38)	All Education
25-39 <i>SD</i> ²	24.17±2.229 22, 21, 20	27.42±1.893 26, 25, 24	28.50±1.542 27, 26, 25	28.82±1.522 27, 27, 26	28.06±1.992 26, 25, 24
40-49 <i>SD</i> ²	23.50±2.921 20, 19, 17	26.13±2.702 23, 22, 21	26.70±1.750 25, 24, 23	29.07±0.917 28, 28, 27	26.27±2.874 23, 22, 21
50-59 <i>SD</i> ²	23.10±3.267 20, 18, 17	25.04±3.240 22, 20, 19	26.22±2.354 24, 23, 22	27.24±2.420 25, 24, 22	24.71±3.367 21, 20, 18

² MoCA values below 1 SD, 1.5 SDs, and 2 SDs, respectively. *SD*: Standard Deviation

Figure 5. Normative data of the MoCA total scores according to age and educational level [Source: Freitas S, Simões MR, Alves L, Santana I. Montreal Cognitive Assessment (MoCA): normative study for the Portuguese population. *J Clin Exp Neuropsychol*. 2011;33(9):989-96]

EPIPorto cohort

EPIPorto is a population-based closed cohort assembled between 1999 and 2003 in the city of Porto (around 400 000 inhabitants), representative of dwellers aged 18 years or older (n=2485). Random digit dialing of landline telephones was used to select households, and in which one, a permanent resident aged at least 18 years was selected by simple random sampling. The participation rate was 70% (114).

A total of 961 individuals participated in the 2013–2015 re-evaluation of the cohort (99) and 931 were tested with the MoCA. Compared to the originally assembled EPIPorto cohort, participants evaluated in 2013-2015 were younger (mean, SD: 48.8, 11.9 vs. 55.5, 16.9, $p<0.001$) and had higher education (25th percentile, median, 75th percentile: 4, 9, 15 vs. 4, 6, 12, $p<0.001$). The proportion of men was higher in the 2013-2015 re-

evaluation participants, although the difference was not statistically significant (40.5% vs. 36.8% of men, $p=0.069$).

A structured questionnaire was used to collect data on sociodemographic characteristics (age, sex, marital status, and employment situation), medical history (morbidities and medication), and lifestyle characteristics [smoking and alcohol consumption (in the last 12 months)]. Body weight, height, and BMI were collected with participants wearing light clothing and no footwear. Body weight was measured to the nearest 0.1 kg using a digital scale and height was measured to the nearest centimeter in the standing position using a wall stadiometer (115).

Statistical analysis

A total of 330 infected participants of the NeurodegCoV-19 cohort and 931 from the general population (EPIPorto) were considered for analysis. From the NeurodegCoV-19 cohort, 90 participants were part of the hospitalized group and 240 of the non-hospitalized group (median time elapsed between COVID-19 diagnosis and the cognitive test: 675 days). Descriptive statistics of the sample included the mean (SD) and median (25th and 75th percentiles) for continuous variables and absolute and relative frequencies for categorical variables. For inference purposes, comparisons of continuous variables between two or more groups were analyzed with the t-test and one-way analysis of variance or with the Kolmogorov–Smirnov test and the Kruskal-Wallis test, whether the variable was or not normally distributed, respectively. The comparison of categorical variables between groups was analyzed with the Pearson's chi-squared test. PCI prevalence estimates with 95% CI were computed for NeurodegCoV-19 hospitalized and non-hospitalized groups and for the EPIPorto cohort, as well as for the NeurodegCoV-19 groups considering the first, second, and third waves of the first year of the COVID-19 pandemic. Multivariate logistic regression was used to estimate the aOR and corresponding 95% CI of the association between belonging to the hospitalized and non-hospitalized groups of the NeurodegCoV-19 cohort vs. to the EPIPorto cohort with the presence of PCI, and the association of severity and presence of neurological symptoms with PCI, among NeurodegCoV-19 participants. Multiple linear regression analysis was used to explore associations between MoCA cognitive domains, and the time scored in

TUG. Directed Acyclic Diagrams (DAG) were produced to identify variables to be considered as confounders in the mentioned associations (figure A1, A2, A3, appendix). All analyses were performed using STATA v.15 (StataCorp. 2017. Stata Statistical Software: Release 15. StataCorp LLC, College Station, TX, USA). All tests were two-sided, and a $p < 0.05$ was considered significant.

Results

Characteristics of the participants

As presented in Table 1, participants in the hospitalized group were older and less educated than the non-hospitalized group and the EPIPorto cohort. Most of them were men, while in the non-hospitalized group and EPIPorto cohort, most were women.

Table 1. Sociodemographic characteristics of the participants

	NeurodegCo-V19 cohort (n=330)			<i>p</i> value
	Hospitalized group (n=90)	Non-hospitalized group (n=240)	EPIPorto cohort (n=931)	
Age (years), mean (SD)	64.6 (11.5)	53.0 (14.3)	61.9 (12.5)	<0.001*
Age (years), n (%)				<0.001
<50	6 (6.7)	94 (39.2)	147 (15.8)	
50-64	35 (38.9)	90 (37.5)	364 (39.1)	
≥65	49 (54.4)	56 (23.3)	420 (45.1)	
Sex, n (%)				<0.001
Men	59 (65.6)	79 (32.9)	379 (40.7)	
Women	31 (34.4)	161 (67.1)	552 (59.3)	
Education (years), median (25th -75th percentiles)	6 (4-11)	11 (6-15)	10 (4-16)	0.002
Education (years), n (%)				<0.001
1-4	39 (43.3)	50 (20.8)	248 (26.6)	
5-9	20 (22.2)	58 (24.2)	211 (22.7)	
10-12	17 (18.9)	51 (21.3)	149 (16.0)	
>12	14 (15.6)	81 (33.8)	323 (34.7)	
Marital status, n (%)				<0.001
			^b	
Single	5 (5.6)	31 (12.9)	69 (7.4)	
Married	60 (66.7)	145 (60.4)	624 (67.2)	
Non-marital	7 (7.8)	26 (10.8)	38 (4.1)	
Widower	12 (13.3)	13 (5.4)	112 (12.1)	
Divorced	6 (6.7)	25 (10.4)	74 (8.0)	
Separated	0	0	12 (1.3)	
Employment situation, n (%)				<0.001
		^a	^b	
Full-time	27 (30.0)	141 (59.2)	224 (24.2)	
Part-time	1 (1.1)	9 (3.8)	15 (1.6)	
Part-time less than 15 hour per week	1 (1.1)	2 (0.8)	5 (0.5)	
Informal caregiver	0	1 (0.4)	0	

Unemployment	5 (5.6)	21 (8.8)	64 (6.9)
Retired by age	36 (40.0)	37 (15.6)	428 (46.3)
Retired by disablement	8 (8.9)	6 (2.5)	0
Pre-retired	5 (5.6)	8 (3.36)	0
Student	0	3 (1.3)	1 (0.1)
Takes care of the house	2 (2.2)	3 (1.3)	47 (5.1)
Freelancer	2 (2.2)	1 (0.4)	141 (15.2)
Maternity leave	0	1 (0.4)	0
Sick leave	3 (3.3)	5 (2.1)	0

*All groups were statistically significantly different, except when comparing the EPIPorto cohort and the hospitalized group ($p=0.164$). a - number of observations is lower than 240 due to missing data. b - number of observations is lower than 931 due to missing data.

Table 2 displays the clinical characteristics and lifestyle of the three groups. The hospitalized group consumed more medicines and presented the highest proportion of psycholeptics and psychoanaleptics consumption. This group also presented the highest proportion of hypertension (67.8%), hypercholesterolemia (65.6%), diabetes (30.0%), overweight (46.7%), and obesity (35.6%). Similar proportions of never smokers were observed in the three groups, but current smokers were more frequent among EPIPorto participants, as well as those exceeding the recommended threshold for excessive alcohol consumption.

Table 2. Clinical and lifestyle characteristics of the participants

	NeurodegCoV19 cohort (n=330)			p value
	Hospitalized group (n=90)	Non-hospitalized group (n=240)	EPIPorto cohort (n=931)	
Number of medications, n (%) ^a				<0.001
0	7 (7.8)	71 (29.6)	201 (21.6)	
1-2	21 (23.3)	70 (29.2)	291 (31.3)	
3-5	26 (28.9)	64 (26.7)	275 (29.5)	
>5	36 (40.0)	35 (14.6)	164 (17.6)	
Consumption of psycholeptics, n (%) ^{a b}	21 (23.3)	34 (14.2)	203 (21.8)	0.026
Consumption of psychoanaleptics, n (%) ^{a b}	28 (31.1)	50 (20.8)	161 (17.3)	0.004
Co-morbidities, n (%) ^c			^g	
Hypertension	61 (67.8)	88 (36.7)	360 (38.8)	<0.001
Hypercholesterolemia	59 (65.6)	82 (34.2)	418 (45.0)	<0.001
Diabetes	27 (30.0)	34 (14.2)	111 (11.9)	<0.001
BMI, n (%) ^d		^f	^g	<0.001
Underweight	1 (1.1)	3 (1.3)	9 (1.0)	

Normal	15 (16.7)	72 (30.1)	291 (31.4)	
Overweight	42 (46.7)	107 (44.8)	407 (43.9)	
Obesity	32 (35.6)	57 (23.58)	221 (23.8)	
Smoking status, n (%)^a			^g	0.008
Never smoker	50 (55.6)	132 (55.0)	508 (54.8)	
Former smoker	37 (41.1)	74 (30.8)	267 (28.8)	
Current smoker	3 (3.3)	34 (14.2)	152 (16.4)	
Excessive alcohol consumption, n (%)^{a e}	15 (16.7)	21 (8.8)	246 (26.4)	<0.001

a - data reported by participants. b - medicines were classified according to the WHO Anatomical Therapeutic Chemical Classification System (101). c - co-morbidities reported by participants and retrieved from medical files. d – data reported by participants (NeurodegCoV-19) and measured using a digital scale and a stadiometer (EPIPorto); underweight defined as BMI <18.5 kg/m²; normal weight defined as BMI between 18.5 kg/m² and 24.9 kg/m²; overweight defined as BMI between 25.0 kg/m² and 29.9 kg/m² and obesity as BMI ≥30.0 kg/m² (102). e – excessive alcohol consumption defined as the consumption of more than two regular drinks for men with less than 65 years and more than one regular drink for women and men over 65 years in the previous six to 12 months (103). f - number of observations is lower than 240 due to missing data. g - number of observations is lower than 931 due to missing data.

PCI in the three groups of participants

PCI proportion was approximately the same in both NeurodegCoV-19 groups two years after the infection (≈43%) and 24.4% and 19.2% of those hospitalized or not, had severe PCI, respectively (figure 6). These proportions were statistically significantly higher than in EPIPorto cohort (p<0.001 in both comparisons).

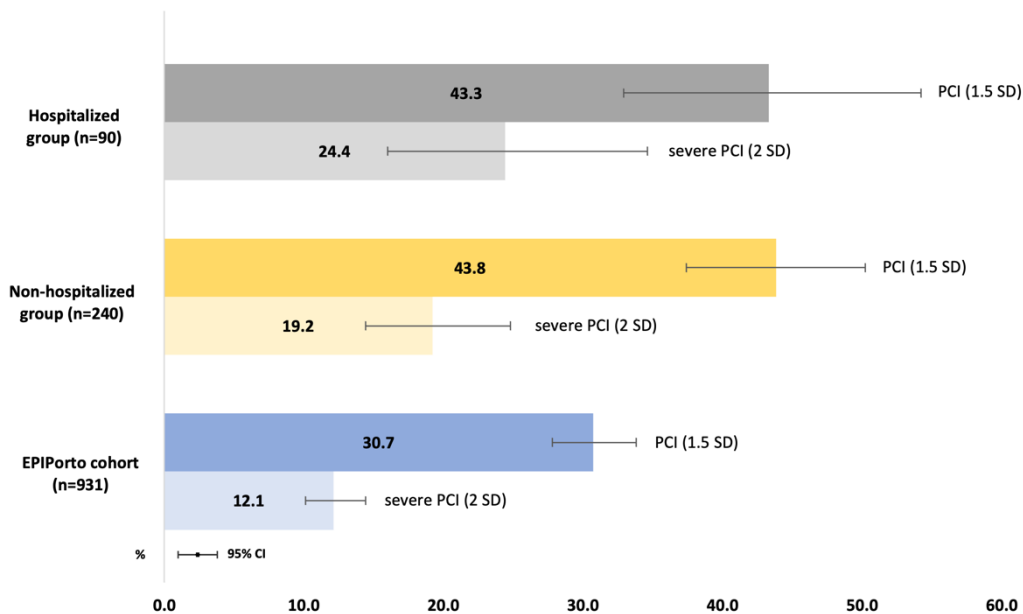


Figure 6. Distribution of PCI and severe PCI and corresponding 95% confidence interval (95% CI) in NeurodegCoV-19 groups (hospitalized and non-hospitalized) and in EPIPorto cohort

After adjusting for age, education, polypharmacy, obesity, hypertension, hypercholesterolemia, and diabetes, hospitalized individuals due to SARS-CoV-2 infection presented a 84% increase in the odds of having PCI compared to the general population (aOR, 95% CI: 1.84, 1.17-2.90, $p=0.003$). Those not hospitalized had a 59% increase (aOR, 95% CI: 1.59, 1.17-2.16, $p=0.008$). The association increased for those over 55 years of age, doubling the odds of PCI (aOR, 95% CI: 2.11, 1.39-3.23, $p=0.001$; aOR, 95% CI: 2.23, 1.34-3.70, $p=0.002$, for non-hospitalized and hospitalized COVID-19 survivors vs. EPIPorto, respectively). Using a MoCA cut-off of 2 SD for defining severe PCI showed a similar association (aOR, 95% CI: 2.20, 1.26-3.84, $p=0.006$; aOR, 95% CI: 2.57, 1.39-4.76, $p=0.003$, for non-hospitalized and hospitalized COVID-19 survivors vs. EPIPorto, respectively).

Neurological symptoms in the NeurodegCoV-19 cohort

Table 3 describes the characteristics of SARS-CoV-2 infection among participants in the NeurodegCoV-19 cohort. Most hospitalizations occurred in the general ward setting (55.6%), and in both groups, the mean number of neurological symptoms during infection and at two years post-infection was similar. Fatigue, myalgia, hypogeusia/dysgeusia, and hyposmia/anosmia were the most common symptoms during infection in both groups and, two years after the infection, fatigue remained the most common symptom, followed by headache and vision changes. Difficulty staying awake, difficulty staying asleep, and waking up too early were statistically significantly higher in the hospitalized group ($p=0.004$, $p<0.001$, $p=0.046$, respectively). Memory loss was reported by approximately 50% of participants from both groups and at least one neurological symptom was reported in 91.1% of the hospitalized group and in 77.9% of the non-hospitalized group two years after the infection. Results from PSQI and HADS showed slightly higher proportions of poor sleep, anxiety, and depression in the hospitalized group with over half of participants classified as having poor sleep. Hospitalized individuals were statistically significantly slower in performing TUG than the non-hospitalized ($p<0.001$).

Table 3. Characteristics of SARS-CoV-2 infection and proportion of poor sleep (PSQI), anxiety and depression (HADS) and gait speed (TUG) in NeurodegCoV19 participants

	Hospitalized group (n=90)	Non-hospitalized group (n=240)	p value
Level of care during hospitalization, n (%) ^a			
General ward	50 (55.6)	n/a	n/a
Intermediate care unit	16 (17.8)	n/a	n/a
Intensive care unit	24 (26.7)	n/a	n/a
Post-infection hospitalization episode due to COVID-19 complications, n (%) ^b	8 (9.0)	2 (0.8)	<0.001
Asymptomatic cases, n (%) ^b	1 (1.1)	19 (7.9)	<0.001
Number of neurological symptoms during SARS-CoV-2 infection, mean (SD) ^b	3.4 (2.1)	3.4 (2.2)	0.891
COVID-19 symptoms during SARS-CoV-2 infection, n (%) ^b			
	^e	^f	
Headaches	34 (38.2)	134 (60.1)	<0.001
Hyposmia/Anosmia	35 (39.3)	137 (61.4)	<0.001
Hypogeusia/Dysgeusia	41 (46.1)	136 (61.0)	0.016
Fatigue	63 (70.8)	154 (69.4)	0.806
Myalgia	52 (58.4)	147 (65.9)	0.214
Vision changes	9 (10.1)	37 (16.6)	0.145
Disorientation	23 (5.8)	29 (13.0)	0.006
Delirium	14 (15.7)	13 (5.8)	0.005
Changes in gait or balance	32 (36.0)	42 (18.8)	0.001
Number of neurological symptoms at two years post-infection, mean (SD) ^b	3.5 (3.5)	4.3 (3.1)	0.073
At least one neurological symptom at two years post-infection, n (%) ^b	82 (91.1)	187 (77.9)	0.006
COVID-19 symptoms and complications at two years post-infection, n (%) ^b			
	^e	^f	
Headache	17 (18.9)	70 (29.0)	0.062
Hyposmia/Anosmia	8 (8.9)	30 (12.4)	0.372
Hypogeusia/Dysgeusia	5 (5.6)	28 (11.6)	0.103
Fatigue	45 (50.0)	101 (41.7)	0.177
Myalgia	17 (18.9)	42 (17.4)	0.757
Vision changes	21 (23.3)	53 (21.9)	0.780
Disorientation	12 (13.3)	18 (7.5)	0.098
Delirium	0	1 (0.4)	0.541
Changes in gait or balance	21 (23.3)	33 (13.6)	0.033
Sleep complaints:			
Excessive sleepiness during the day	19 (21.1)	50 (20.7)	0.928
Difficulty falling asleep	35 (38.9)	56 (23.1)	0.004
Difficulty staying asleep	46 (51.1)	63 (26.0)	<0.001
Waking up too early	26 (28.9)	45 (18.8)	0.046
Cognitive complaints:			
Memory loss	52 (57.8)	128 (52.9)	0.427

Slowing down of thinking	36 (40.0)	75 (31.0)	0.122
Distraction	25 (27.8)	69 (28.5)	0.895
PSQI poor sleep, n (%)^{b c}	57 (64.8)	115 (57.4)	0.308
HADS anxiety, n (%)^{b d}	18 (20.2)	47 (19.8)	0.937
HADS depression, n (%)^{b d}	12 (13.5)	23 (9.7)	0.326
TUG Gait speed, mean (SD, seconds)	12.9 (7.0)	9.7 (2.3)	<0.001

n/a: non-applicable. a – level of care was retrieved from medical files. b – data reported by participants. c – poor sleep was considered when the total score was $\geq$ five in the PSQI (104). d - anxiety/depression was considered when the HADS sub-score for anxiety/depression was $\geq$ 11 (108). e - number of observations is lower than 240 due to missing data. f - number of observations is lower than 90 due to missing data.

PCI over the three waves

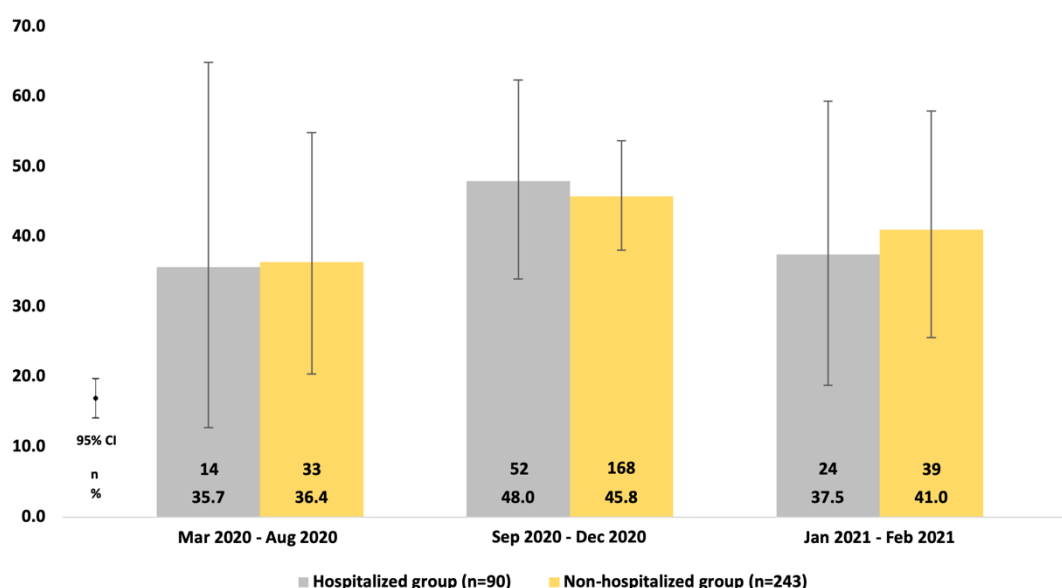


Figure 7. Distribution of PCI and corresponding 95% confidence interval (95% CI) in NeurodegCoV-19 cohort groups considering the first, second, and third wave of the first year of the pandemic

Figure 7 displays the distribution of PCI in the first year of the pandemic. Overall, PCI was observed in 36.2%, 46.1% and 39.1% of participants from the first, second, and third wave, respectively, with no statistically significant differences between waves ($p=0.321$). No statistically significant differences were observed between groups overall ($p=0.946$) and in any of the waves ($p=0.776$), nor between waves in any of the groups ($p=0.563$).

Factors associated with PCI in the NeurodegCoV-19 cohort

Table 4 presents the associations between participants' and infection characteristics with PCI. PCI was associated with age ($p=0.018$), with anxiety (HADS) considering the MoCA cut-off of 2 SD for defining severe PCI ($p=0.028$), and with slow gait speed (TUG), regardless of the MoCA cut-off. No association was found between the level of care

during hospitalization, the number of neurological symptoms during infection and two years post-infection, with PCI.

Table 4. Associations between sociodemographic, medical, lifestyle, and SARS-CoV-2 infection characteristics, as well as poor sleep (PSQI), anxiety and depression (HADS) and gait speed (TUG) with PCI considering 1.5 standard deviations (SD) or 2 SD of published normative data from the MoCA in NeurodegCoV-19 participants (n=330)

	PCI (cut-off 1.5 SD)	<i>p</i> value	Severe PCI (cut-off 2 SD)	<i>p</i> value
Age (years), n (%)		0.018		0.062
<50	54 (37.5)		27 (39.7)	
50-64	44 (30.6)		18 (26.5)	
≥65	46 (31.9)		23 (33.8)	
Sex, n (%)		0.343		0.501
Men	56 (38.9)		26 (38.2)	
Women	88 (67.1)		42 (61.8)	
Education (years), n (%)		0.158		0.260
1-4	39 (27.1)		22 (32.4)	
5-9	41 (28.5)		19 (27.9)	
10-12	23 (16.0)		9 (13.2)	
>12	41 (28.5)		18 (26.5)	
Marital status, n (%)		0.529		0.535
Single	13 (9.0)		5 (7.4)	
Married or Non-marital	108 (75.0)		52 (76.5)	
Widower, Divorced or Separated	23 (16.0)		11 (16.2)	
Professionally active, n (%)	73 (52.1)	0.112	31 (47.7)	0.085
Smoking status, n (%)		0.334		0.115
Never smoker	86 (59.7)		45 (66.2)	
Former smoker	44 (30.6)		18 (26.5)	
Current smoker	14 (9.7)		5 (7.4)	
Excessive alcohol consumption, n (%)	17 (11.8)	0.646	5 (7.4)	0.291
Obesity, n (%)	42 (29.4)	0.406	22 (32.4)	0.269
Number of co-morbidities, mean (SD)	4.0 (2.9)	0.801	4.2 (2.6)	0.472
Hypertension, n (%)	65 (45.1)	0.997	37 (54.4)	0.085
Hypercholesterolemia, n (%)	59 (51.0)	0.571	32 (47.1)	0.418
Diabetes, n (%)	24 (16.7)	0.454	13 (19.1)	0.880
Heart disease, n (%)	24 (22.0)	0.899	11 (22.9)	0.924
Osteoarticular disease, n (%)	55 (38.2)	0.360	29 (42.6)	0.164
Obstructive sleep apnea, n (%)	79 (54.9)	0.169	37 (54.4)	0.378
Consumption of psycholeptics, n (%)	20 (13.9)	0.243	10 (14.7)	0.626
Consumption of psychoanaleptics, n (%)	34 (23.6)	0.992	14 (20.6)	0.507

Level of care during hospitalization, n (%)		0.901		0.262
Followed in community	105 (72.9)		46 (67.6)	
General ward	20 (13.9)		9 (13.2)	
Intermediate care unit	8 (5.6)		5 (7.4)	
Intensive care unit	11 (7.6)		8 (11.8)	
Length of hospitalization $\geq$ 11 days, n (%)	22 (56.4)	0.609	12 (54.5)	0.896
Number of cognitive complains at two years post-infection, mean (SD)	1.4 (1.3)	0.144	1.4 (1.4)	0.208
Number of neurological symptoms during infection, mean (SD)	3.5 (2.3)	0.475	3.6 (2.5)	0.319
Number of neurological symptoms at two years post-infection, mean (SD)	4.0 (3.5)	0.241	4.2 (3.5)	0.217
PSQI poor sleep, n (%)	71 (56.3)	0.219	35 (54.4)	0.592
HADS anxiety, n (%)	31 (22.0)	0.419	20 (29.4)	0.028
HADS depression, n (%)	18 (12.5)	0.360	9 (13.2)	0.454
TUG Gait speed (seconds), mean (SD)	11.2 (5.7)	0.026	11.7 (7.2)	0.016

Among NeurodegCoV-19 participants, those with anxiety (HADS classification) were statistically significantly more likely to have severe PCI considering a MoCA cut-off of 2 SD (aOR, 95% CI: 1.98, 1.01-3.81, $p=0.044$), and those between 50 and 64 years old were less likely to have PCI regardless of the MoCA cut-off (aOR, 95% CI: 0.46, 0.27-0.79, $p=0.005$ for PCI; aOR, 95% CI: 0.45, 0.23-0.89, $p=0.014$ for severe PCI) (Table 5). For each additional second in the TUG, PCI odds increased by 10% (95% CI: 1 – 20, $p=0.028$) when considering the cut-off of 1.5 SD of MoCA (table 5). Furthermore, specific-cognitive domains of MoCA, namely abstraction, attention, executive functions, language, and visuospatial ability were negatively associated with the time scored in the TUG (adjusted $\beta=-0.030$, $p=0.011$; $\beta=-0.082$, $p<0.001$; $\beta=-0.070$, $p<0.001$; $\beta=-0.046$, $p=0.005$, $\beta=-0.038$, $p=0.009$, respectively) (table A1, appendix).

Table 5. Adjusted odds ratio (aOR) and corresponding 95% confidence interval (95% CI) of the associations between age, patient-reported anxiety (HADS) and gait speed (TUG) with PCI, considering 1.5 standard deviations (SD) or 2 SD of published normative data from the MoCA in NeurodegCoV-19 participants ($n=330$)

	PCI (cut-off 1.5 SD)	Severe PCI (cut-off 2 SD)
	aOR (95% CI)	aOR (95% CI)
Age (ref <50)		
50-64	0.46 (0.27 – 0.79)	0.45 (0.23 - 0.89)
≥ 65	0.66 (0.38 – 1.15)	0.76 (0.40 – 1.44)

HADS anxiety (ref. no)				
Yes	1.16 (0.66 - 2.11)	b	1.98 (1.01 - 3.81)	b
TUG Gait speed ^a	1.10 (1.01 - 1.20)	c	1.06 (0.99 - 1.14)	c

a- number of observations is lower than 330 due to missing data. b - adjusted for age and sex. c – adjusted for age, hypoacusis, hypertension, professionally active, fatigue, myalgia, changes in gait or balance, vision changes, disorientation, headache, and depression. DAG was used to identify the confounders to adjust for in the logistic model (figure A2 and A3, appendix)

Discussion

The present study examined the prevalence of PCI evaluated with MoCA in 330 participants two years following a SARS-CoV-2 infection. Nearly 2/5 of two-year COVID-19 survivors had PCI, and 1/4 and 1/5 of those hospitalized or not, respectively, had severe PCI, being these proportions higher than in the general population. Fatigue was the most common symptom, and over 50% experienced memory loss two years after infection. Those with anxiety and slower walking were more likely to present PCI.

Fatigue, myalgia, and changes in gait are usually categorized as musculoskeletal symptoms, and although they are often attributed to indirect effects resulting from inflammatory or immune responses, they can also be attributed to direct damage of the virus on the endothelial cells of the brain or peripheral nerves (116), and so result from neurological damage. Previous studies examining the presence of long COVID neurological symptoms, two years after acute SARS-CoV-2 infection, found that fatigue and cognitive complaints were among the most commonly reported symptoms (117-119), which was also observed in this study. Consistent with Fernández-de-las-Penãs et al. (117), no differences in the aforementioned symptoms were observed between the hospitalized and non-hospitalized groups two years after infection. Furthermore, there were no differences between groups for the majority of symptoms included in this analysis, except for gait or balance changes and sleep complaints. The similar proportion observed in this study of participants from both groups with symptoms two years after the acute infection suggests that the neurological symptoms of long COVID may be independent of being hospitalized or not at the time.

Most studies in the literature addressing the prevalence of cognitive impairment in COVID-19 survivors have focused on cases hospitalized during the acute phase of infection (80). In this study, the prevalence of PCI was approximately the same in both the hospitalized and non-hospitalized groups. Consistent with our results, three studies also described a higher prevalence of cognitive impairment in COVID-19 survivors compared with a control group using the MoCA (120-122). However, comparing these results is challenging due to differences in cut-offs for impaired performance, the time between infection and examination, and the definition of the clinical severity of COVID-

19. The use of a control group is crucial when outcome definitions diverge, yet the different periods in which NeurodegCoV-19 and EPIPorto evaluations occurred, as well as the different investigation teams involved, may not allow for a more precise comparison. Furthermore, the EPIPorto cohort was assembled as a representative sample of the population of the city of Porto in 1999–2003, and considering the attrition suffered until the re-evaluation in 2013–2015, it is relevant to note that it is likely that PCI might be more frequent in the general population since those evaluated in 2013–2015 were younger and more educated than the original assembled cohort. Still, the MoCA was first used in the 2013–2015 re-evaluation of EPIPorto.

No association between PCI and the level of care of the infected participants was found, suggesting that the severity of the disease considering the level of care during hospitalization (general ward, intermediate care, or intensive care) or not being hospitalized due to SARS-CoV-2 may not be related with long-term cognitive impairment. Given the previous knowledge on cognitive deficits from post-intensive care unit syndrome (123, 124), a worse cognitive outcome in more severe cases of COVID-19 was expected. However, current evidence on the association between the severity of COVID-19 and cognitive impairment is not consistent, with studies indicating a relationship (91, 125–127) and others not (128–130). The heterogeneous classification of disease severity found in the literature (levels of care, oxygen therapy/ventilation requirement, or WHO disease severity score) makes the comparison challenging, with most studies focusing on hospitalized cases, with no control group and not being specifically designed to investigate this association.

According to our results, two-year COVID-19 survivors with anxiety (measured with HADS) presented higher odds for more severe cognitive impairment (corresponding to a MoCA cut-off of 2 SD). Anxiety, as well as depression, are commonly reported alongside long COVID, and it is known that it can lead to cognitive dysfunction (131–133). Previous analyses in COVID-19 survivors are in accordance with anxiety being a predictor of PCI, however, these studies had the maximum follow-up of 4 months (120, 134, 135). The present study shows that this association was observed two years after infection by SARS-CoV-2.

Older age is considered to increase the likelihood of cognitive impairment (136). A relationship between age and PCI was also observed in the present study, in the EPIPorto and NeurodegCoV-19 cohorts, although the youngest group (<50 years) was more likely to have PCI than the age group 50-64 years. This unexpected result in the youngest age category highlights the importance of a comparison cohort and further confirmation of PCI cases with a comprehensive battery of neuropsychological tests.

In the present study, those who were hospitalized had a statistically significant slower gait than those who were not, and those with slower gait were more likely to have PCI two years after infection after adjusting for potential confounders, and regardless of MoCA cut-off. In addition, the majority of specific cognitive domains of MoCA were negatively associated with time achieved at TUG, except for two domains. To the best of our knowledge, no studies to date have analyzed the association between gait speed and cognitive impairment in COVID-19 survivors. Gait is a complex functional movement, involving the integration of multiple systems including motor, perceptual, and cognitive processes, which, in the case of failure or dysfunction in these systems can lead to gait disturbances, including slowing gait (137). Slow gait speed has been associated with neurodegenerative disorders such as mild cognitive impairment and dementia in elderly populations (94-97). Two case studies of COVID-19 survivors identified disruptions in the neural system-related gait functions after the disease (138, 139), and Helms et al. (55) reported that neurological damages that could occur after COVID-19 may affect gait by impairing cerebellar coordination. Overall, the ability to ambulate underlies important functional actions necessary for the individual's independence, and so further investigation of this outcome is needed through longitudinal studies with a long follow-up period. The results of the present study suggest the potential contribution of gait speed for cognitive impairment screening after SARS-CoV-2 infection, particularly in individuals hospitalized due to the infection.

To the best of our knowledge, this study is the first to estimate and explore the prevalence of cognitive impairment in two-year COVID-19 survivors, including hospitalized and non-hospitalized cases with a representation of cases from the first three waves of the pandemic, and compare it with the general population.

However, participants had retrospectively reported their symptoms during acute SARS-CoV-2 infection, which may be affected by recall bias, particularly among those who were hospitalized. Because potential participants with more health impairments due to COVID-19 may be more willing to participate in the study than those without any health impairments after COVID-19, selection bias cannot be ruled out in the NeurodegCoV-19 cohort, nor can a possible overestimation of the prevalence of cognitive impairment. As mentioned above, due to attrition in the EPIPorto cohort since the cohort was assembled, selection bias also cannot be ruled out in the comparison cohort. Furthermore, this is a cross-sectional study which does not allow us to conclude on the effect of certain variables on cognitive performance, namely anxiety that could also be a consequence of experiencing cognitive complaints. Still, the use of two different age- and education-based classifications for cognitive deficit from MoCA's published normative data (referring to the presence of PCI or severe PCI) provided well-grounded estimations of the prevalence of cognitive impairment in this population. Nevertheless, the NeurodegCoV-19 cohort is a two-year prospective longitudinal study designed to include sex- and age-matched controls for the hospitalized and non-hospitalized groups. By assembling the control groups and collecting one- and two-year follow-up data, it will be possible to mitigate the aforementioned limitations to clarify the increased risk and causes for cognitive impairment in those affected by COVID-19 in the first year of the pandemic.

Conclusion

The present study consisting of participants that were hospitalized due to SARS-CoV-2 infection and participants followed in the community, highlights the high frequency of cognitive impairment two years after COVID-19 infection, as well as the need to consider post-COVID-19 cognitive impairment screening in the large group of people affected in the first year of the pandemic, regardless of the severity of the disease. Future studies should include longer follow-up periods, neuropsychological examinations, and further investigation of the potential role of speed gait in the screening for cognitive impairment in COVID-19 survivors. Such results may help develop preventive interventions for cognitive dysfunction in this population.

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Appendix

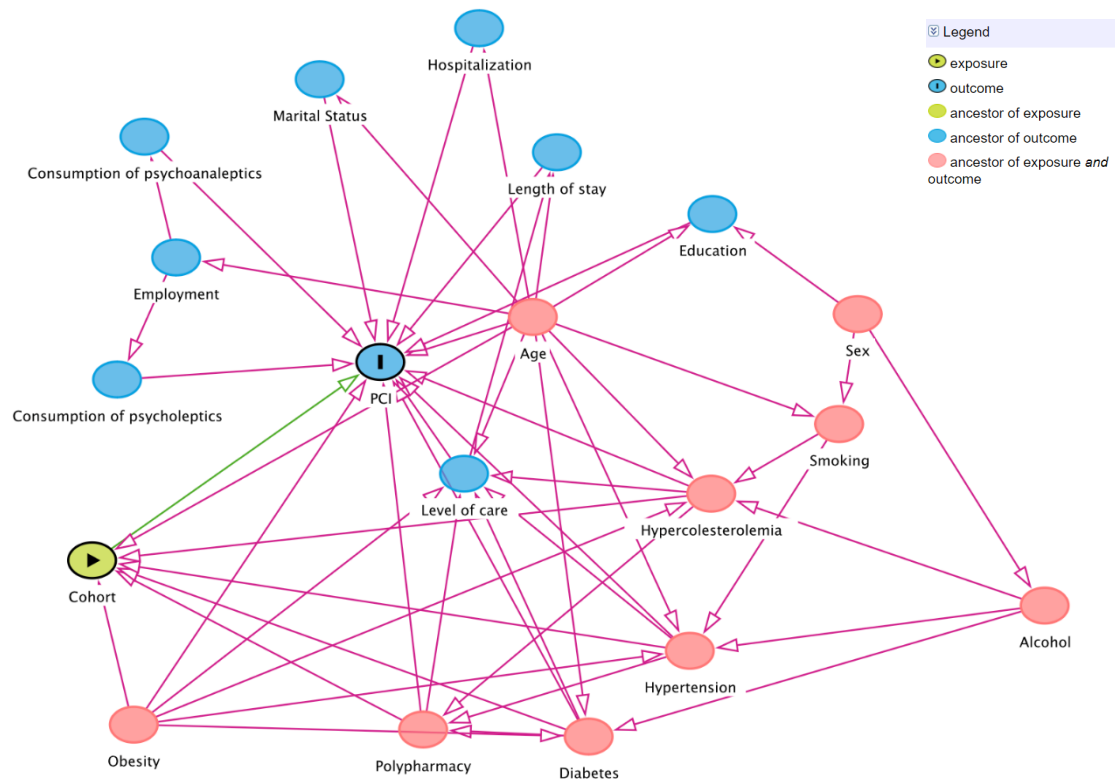


Figure A1. DAG to identify possible confounders in the association between belonging to NeurodegCoV-19 and PCI

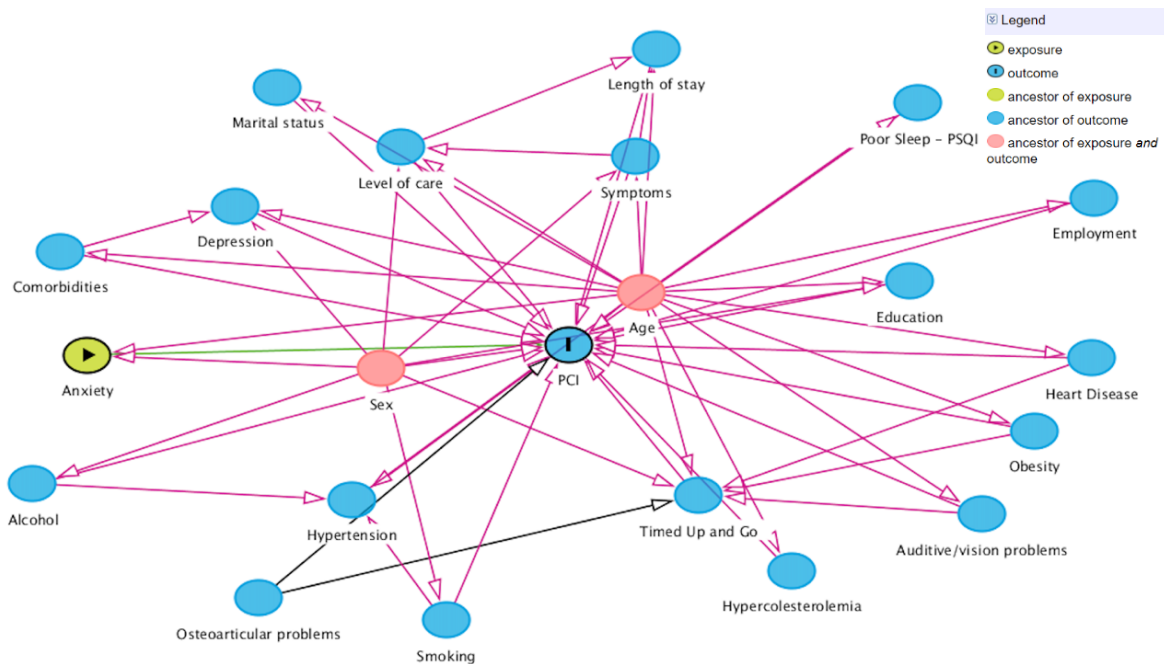


Figure A2. DAG to identify possible confounders in the association between anxiety (HADS) and PCI

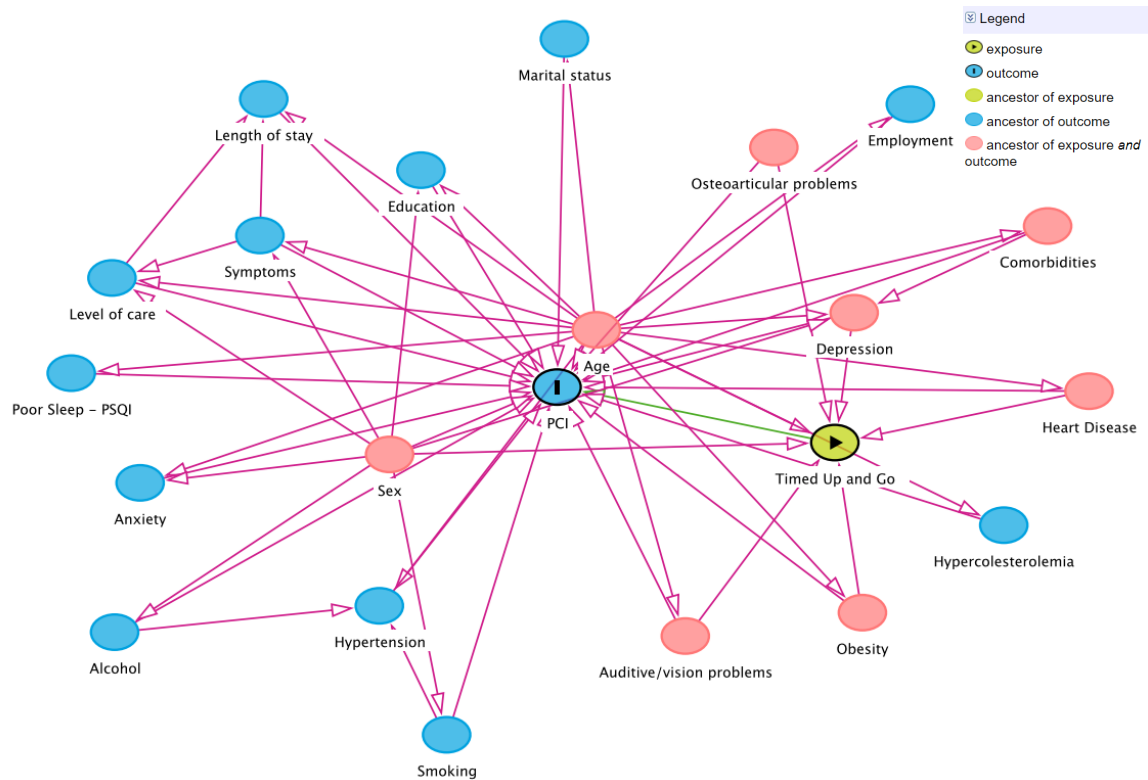


Figure A3. DAG to identify possible confounders in the association between gait speed (TUG) and PCI

Table A1. Regression coefficient (β) and corresponding 95% confidence interval (95% CI) from multiple linear regression model on the association between MoCA cognitive domains score and score of TUG (seconds) in NeurodegCoV-19 participants (n=330)*

	β (SE)	95% CI	p value
Abstraction^a	-0.030 (0.012)	-0.053, -0.007	0.011
Attention^b	-0.082 (0.018)	-0.118, -0.048	<0.001
Executive functions^c	-0.070 (0.019)	-0.107, -0.033	<0.001
Language^d	-0.046 (0.016)	-0.079, -0.014	0.005
Memory (delayed recall)^e	0.002 (0.023)	-0.043, 0.048	0.924
Orientation^f	0.009 (0.005)	-0.001, 0.019	0.078
Visuospatial ability^g	-0.038 (0.014)	-0.066, -0.009	0.009

*Number of observations is lower than 330 due to missing data. SE: Standard error. Model included covariates: age, hypoacusis, hypertension, professionally active, fatigue, myalgia, changes in gait or balance, vision changes, disorientation, headache, and depression. DAG was used to identify the confounders to adjust for in the logistic model (figure A3, appendix). a – $R^2=0.0186$, adjusted $R^2=0.147$. b – $R^2=0.176$, adjusted $R^2=0.137$. c – $R^2=0.287$, adjusted $R^2=0.253$. d – $R^2=0.223$, adjusted $R^2=0.186$. e – $R^2=0.171$, adjusted $R^2=0.132$. f – $R^2=0.108$, adjusted $R^2=0.066$. g – $R^2=0.228$, adjusted $R^2=0.191$.

BMJ Open Long-term neurological complications in COVID-19 survivors: study protocol of a prospective cohort study (NeurodegCoV-19)

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ABSTRACT

Background Evidence suggests an association between SARS-CoV-2 infection and worse performance on cognitive tests, and a higher risk of Parkinson's disease (PD) and dementia up to 6 and 12 months after infection, respectively. Longer follow-ups with comparison groups are needed to clarify the potentially increased risk of neurodegenerative diseases in COVID-19 survivors, namely those infected before mass vaccination.

Methods A prospective study started in July 2022 with four cohorts of 150 individuals each, defined according to SARS-CoV-2 infection and hospitalisation status between March 2020 and February 2021: cohort 1—hospitalised due to SARS-CoV-2 infection; cohort 2—hospitalised, COVID-19-free; cohort 3—infected, not hospitalised; cohort 4—not infected, not hospitalised. Cohort 2 will be matched to cohort 1 according to age, sex, level of hospitalisation care and length of stay; cohort 4 will be age-matched and sex-matched to cohort 3. Baseline, 1-year and 2-year follow-up evaluations will include: cognitive performance assessed with the Montreal Cognitive Assessment (MoCA) and neuropsychological tests; the assessment of prodromal markers of PD with Rapid Eye Movement Sleep Behaviour Disorder single-question Screen and self-reported olfactory and gustative alterations; screening of PD with the 9-item PD screening questionnaire; gait evaluation with Timed Up&Go test. Suspected cases of cognitive impairment and PD will undergo a clinical evaluation by a neurologist. Frequency measures of neurological complications, prodromal markers and diagnoses of dementia and PD, will be presented. The occurrence of cognitive decline—the difference between baseline and 1-year MoCA scores below 1.5 SD below the mean of the distribution of the variation—will be compared between cohorts 1 and 2, and cohorts 3 and 4 with OR estimated using multivariate logistic regression.

Ethics and dissemination This study received ethics approval from the Ethics Committees of the health units Unidade Local de Saúde de Matosinhos and Centro Hospitalar de Entre Douro e Vouga, and informed consent is signed for participating. Results will be disseminated among the scientific community and the public.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ Prodromal markers of Parkinson's disease and dementia, as well as diagnoses of these diseases, will be evaluated with screening tests, neuropsychological tests and a clinical evaluation by a neurologist.
- ⇒ In addition to assessing episodic cognitive impairment 2–3 years after SARS-CoV-2 infection, we will evaluate cognitive performance over 2 years and compare individuals who had mild/severe SARS-CoV-2 infections with the general population or a group of hospitalised patients.
- ⇒ Data on cognitive performance will only be obtained after SARS-CoV-2 infection, limiting the assessment of causality between SARS-CoV-2 infection and cognitive impairment.
- ⇒ Sample size, calculated to detect an association between SARS-CoV-2 infection and cognitive decline defined based on the variation in Montreal Cognitive Assessment score over 1 year, may not be large enough to identify a significant association between COVID-19 and dementia or Parkinson's disease, requiring a longer follow-up and/or external collaborations.
- ⇒ Misclassification of the exposure in the comparison groups and the possibility of infection after vaccination may lead to a possibly weaker association than if comparison groups had been completely COVID-19 free over all the study period.

INTRODUCTION

COVID-19 mortality is still high in some countries and the response to the pandemic is not uniform around the world. Mass vaccination, booster vaccine doses offered to the most vulnerable population subgroups, access to adequate treatment of COVID-19, and less aggressive variants have allowed many countries to face new cases of SARS-CoV-2 infection with a lower load on the health system.¹ However, the long-term health consequences

of SARS-CoV-2 or long COVID may represent an important health problem. Long COVID is defined by the WHO as the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation.² It may affect 45% of hospitalised and non-hospitalised SARS-CoV-2 infected individuals within 4 months postinfection,³ and its worldwide prevalence at 120 days from infection, was estimated at 0.49% (higher prevalence among hospitalised patients: 0.54% vs 0.34%), accounting for nearly 38 million cases.⁴ Fatigue and cognitive complaints are the most frequent symptoms of long COVID⁴ and were associated with difficulties in returning to full-time employment.⁵

Although COVID-19 is a respiratory disease, neurological manifestations during the acute phase of SARS-CoV-2 infection are common.⁶ A few cases of parkinsonism developing between 5 and 32 days after initial symptoms of SARS-CoV-2 infection were described, suggesting parkinsonism as a consequence of mild and severe COVID-19 infection.^{7–11} In a large retrospective study (January–December 2020), COVID-19 was also associated with an increased risk of neurological conditions during the 6 months following infection, namely, parkinsonism (increased risk in 45%) and dementia (increased risk in 71%), when compared with matched controls of individuals infected with other respiratory tract infections, although the incidence of parkinsonism and dementia were low: 0.11% and 0.67%, respectively, at 6 months after infection with SARS-CoV-2.¹² A four-point decrease in the Montreal Cognitive Assessment (MoCA) was observed in 21% and 2% of seropositive symptomatic and seronegative asymptomatic individuals for SARS-CoV-2 infection, respectively, from a prepandemic to a postpandemic evaluation (253.4 person-years of follow-up),¹³ and a cumulative incidence of cognitive impairment (assessed with a telephone interview for cognitive status) of 12.45% was reported 12 months after hospital discharge in a large study in Wuhan, China.¹⁴ Neurological symptoms after infection have been corroborated with brain microstructural changes on imaging exams, namely in the central olfactory system, 3 months after recovery from COVID-19, with no differences according to COVID-19 severity.¹⁵ Indeed, there is evidence from in vivo (humans), in vitro and animal studies, that the novel COVID-19 has the potential to be neuroinvasive.¹⁶

Early detection of neurodegenerative complications during prodromal phases of disease is essential for implementing disease-modifying interventions. Impaired olfaction and rapid eye movement (REM) sleep behaviour disorder (RBD) are prodromal markers with the highest sensitivity and specificity, respectively, to predict Parkinson's disease (PD).^{17,18} Non-dementia cognitive impairment and low gait speed can be used to predict dementia.^{19–21} Moreover, cognitive decline over 1 year, that is, a deterioration in cognitive performance over this period, may help in identifying individuals who have normal cognitive performance but who will

develop a long-term declining trajectory of cognitive performance.²² Therefore, this study protocol describes the ongoing prospective cohort study NeurodegCoV-19 which aims to:

1. Quantify the association between SARS-CoV-2 infection and 1-year cognitive decline, comparing individuals infected with SARS-CoV-2 virus (hospitalised patients or cases with clinical follow-up in the community) and a control group (hospitalised patients or the general population).
2. Describe trajectories of cognitive performance over 2 years.
3. Estimate frequency measures of the occurrence and persistence of long-term neurological symptoms and diagnoses, and prodromal markers of PD, in COVID-19 survivors.
4. Explore the associations between SARS-CoV-2 infection and prodromal markers of PD.

In this study, the reference period to define SARS-CoV-2 infection/COVID-19 hospitalisation was the first year of the pandemic. Infections in this period are more likely to be associated with an increased risk of neurological complications because vaccines were essentially unavailable and because in vitro and animal models have shown the lower neurotropism and neurovirulence of the Delta and Omicron BA.1 variants.²³ Recruitment began on 1 July 2022 and is expected to end in July 2023. Therefore, the baseline evaluation is being performed 2–3 years after SARS-CoV-2 infection, and the 2-year follow-up is expected to be complete in July 2025.

METHODS AND ANALYSIS

Setting

This prospective cohort study is based on the population of Matosinhos, one of the counties of the Porto Metropolitan Area, Northern Littoral of Portugal, the region with the highest number of COVID-19 cases during the first months of the pandemic (326 378 cases until 28 February 2021).²⁴ Nearly all dwellers in Portugal are enrolled in public primary healthcare units, either as regular users, sporadic users or whether they have never used this service, and receive a unique identification number in the National Health System. Those enrolled at these units of Matosinhos (176 299 inhabitants enrolled in 2020, and 177 715 in 2021) were the source population for this study. The local health unit of Matosinhos, Unidade Local de Saúde de Matosinhos (ULSM), integrates primary and continuous palliative care units and its hospital, Hospital Pedro Hispano, under a single entity and the same management. The clinical pathology laboratory of ULSM performed all COVID-19 diagnostic tests prescribed by family doctors and public health services of the ULSM, except when results could not be obtained in 24 hours due to an overload of testing; private laboratories collaborated in the latter cases.

Additionally, the hospital unit Centro Hospitalar de Entre Douro e Vouga (CHEDV) in Santa Maria da Feira,

another county of the Porto Metropolitan Area, which includes three hospitals—Hospital de São Sebastião, E.P.E. (in Santa Maria da Feira), Hospital Distrital de São João da Madeira, and Hospital São Miguel (in Oliveira de Azeméis)—also participated for the selection of hospitalised participants. CHEDV does not include primary healthcare nor continuous palliative care units.

Study design

This is a prospective study with a baseline assessment 2–3 years after SARS-CoV-2 infection and follow-up evaluations 1 and 2 years later. Baseline assessments started on 1 July 2022, and are expected to end on July 2023. Participants were retrospectively identified from administrative lists of ULSM and CHEDV, and according to the exposure definition, as follows: having a first diagnosis of SARS-CoV-2 infection (detection of RNA virus in nasopharyngeal or oropharyngeal swabs, with Real-Time PCR), before any vaccination, during the period from March 2020 (first case diagnosed in Portugal on 2 March) to February 2021 (complete vaccination uptake reached 3% and partial vaccination, 6%, on 28 February 2021).²⁵ Exposed individuals are grouped according to hospitalisation at Hospital Pedro Hispano or at one of the three hospitals of CHEDV due to infection with COVID-19—cohort 1, or clinical follow-up in the community—cohort 3. The unexposed group is defined as having no diagnosis of SARS-CoV-2 infection in the same period, nor after if unvaccinated, and with at least the primary scheme of vaccination, to avoid the inclusion of controls who could get infected during the follow-up period without being vaccinated. Unexposed individuals were also grouped according to having been hospitalised at Hospital Pedro Hispano or at CHEDV during the same period—cohort 2, or not—cohort 4.

According to instructions from the Ministry of Health, from 27 October 2020, all patients had to be tested for SARS-CoV-2 infection before hospital admission.²⁶ Therefore, efforts were initially made to select participants infected and/or hospitalised after this date to ensure the non-infection status of the hospitalised comparison group. However, due to a low number of eligible participants for cohort 1, we extended the period to March 2020.

Misclassification regarding the non-exposed comparison groups cannot be excluded as the seronegativity for SARS-CoV-2 infection of the comparison groups could not be confirmed due to the retrospective identification of the participants. Although the National Serological Survey reported a low seroprevalence (2.9%) in May–June 2020,²⁷ it was higher in February–March 2021 (13.5% due to natural infection).²⁸

Participants

Eligible participants are 18 or older at the COVID-19 diagnosis (similar age at baseline for the comparison groups). General and cohort-specific eligibility criteria are checked by consultation of medical records and

complemented with information from the participant during recruitment.

SARS-CoV-2-infected patients

Participants with a positive test for SARS-CoV-2 infection between March 2020 and February 2021 were retrospectively identified from the ULSM and CHEDV databases, which, in the case of ULSM, includes tests sampled at the ULSM (96.7%), at temporary emergency units outside the hospital (0.3%), and the drive-in community centre (3.1%), whereas all tests from CHEDV were sampled at the hospitals.

Individuals infected with SARS-CoV-2 were included into one of two groups: cohort 1: individuals hospitalised at Hospital Pedro Hispano or at CHEDV due to COVID-19 infection, and cohort 3, individuals with clinical follow-up of COVID-19 in the community (asymptomatic or not). Individuals for cohort 1 were identified in two steps: (1) exclusion, from the list of hospitalisation episodes at Hospital Pedro Hispano or at CHEDV, of those with a description of Homogeneous Diagnostic Groups surely not compatible with virus infection (eg, childbirth); (2) consultation of medical records of hospitalisation episodes not excluded in the previous step, to identify if hospital admission was due to COVID-19, the highest level of care (general ward, intermediate care, intensive care unit, ICU) and length of stay (figure 1). All individuals identified were contacted for recruitment.

Individuals for cohort 3 were those on the ULSM list of positive tests for SARS-CoV-2 infection and not on the ULSM list of hospitalised patients (criterion confirmed by the participant) (figure 1). Three pools of individuals were selected from the pandemic's first, second and third waves (earliest 30 diagnosis dates in 2020; all cases from 27 October 2020 to 3 November 2020, and the latest 30 diagnosis dates in February 2021).

Unexposed comparison groups

A comparison group of hospitalised patients (cohort 2), with no positive test for SARS-CoV-2 infection between March 2020 and February 2021, never tested positive afterwards or infected after complete primary vaccination, was identified from the list of hospitalised patients between March 2020 and February 2021 who were not on the list of positive tests for COVID-19 for this period (figure 1). Individuals for cohort 2 will be matched with individuals from cohort 1 regarding the hospital (Hospital Pedro Hispano or CHEDV), age (18–24, 25–34, 35–44, 45–54, 55–64, 65–74 and ≥75 years), sex, level of hospitalisation care (general ward, intermediate care, ICU), number of days of hospitalisation (1, 2–7, 8–15, 16–30 and >30 days), and month of hospital admission.

For comparison with cohort 3, cohort 4 will include (baseline) age-matched and sex-matched individuals randomly selected from the pool of individuals enrolled at ULSM, who were not on the list of a positive diagnostic test for COVID-19 between March 2020 and February

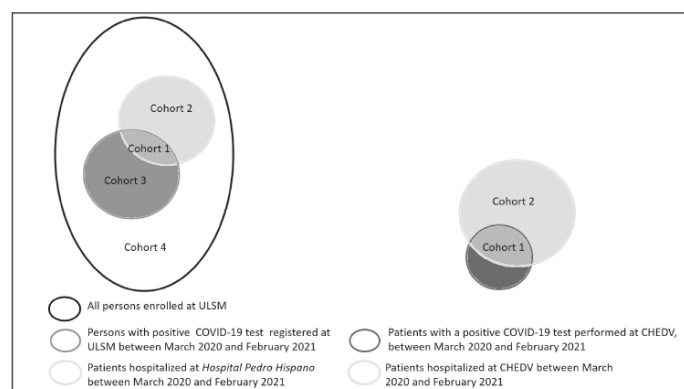


Figure 1 Identification of potential participants from the lists of COVID-19 test results and hospitalisation episodes during the period from March 2020 to February 2021, and from the list of individuals enrolled at primary healthcare units of Unidade Local de Saúde de Matosinhos (ULSM). Cohort includes individuals with a positive diagnosis test for COVID-19 registered at ULSM and hospitalised at hospital Pedro Hispano and those with a positive test performed at CHEDV and hospitalised at one of the three hospitals of CHEDV. Cohort 2 includes individuals with no positive diagnosis test for COVID-19 and hospitalised at hospital Pedro Hispano and at CHEDV. Cohort 3 includes individuals with a positive diagnosis test for COVID-19 registered at ULSM and with clinical follow-up in the community. Cohort 4 includes individuals enrolled at ULSM with no positive diagnosis test for COVID-19 and without any hospitalisation episode between March 2020 and February 2021. Areas are not proportional to the number of cases. CHEDV, Centro Hospitalar de Entre Douro e Vouga.

2021, nor on the list of hospitalised patients in the same period (figure 1).

Exclusion criteria

We will exclude: (1) individuals diagnosed with a condition impairing cognitive function or enrolled in treatments for cognitive decline (consumption of Memantine, Rivastigmine or Donepezil; participation in psychotherapeutic interventions for clinical or research purposes), or under clinical neuropsychological assessment, or with a history of substance abuse (registered in the medical file or spontaneously reported by the participant); (2) individuals with less than 1 year of formal education, and primary education (grades 1–4) not in the Portuguese education system (criteria for the use of cognitive tests) and (3) individuals with a motor, visual or auditory problem incompatible with the performance of the cognitive tests.

Individuals living at senior homes are excluded, as well as those infected between the two doses of the primary vaccination scheme or within the 14 days following vaccination.

The report from the participant of an infection with SARS-CoV-2 virus during the period from March 2020 to February 2021 that could not be confirmed with a registry of a positive result at the RT-PCR test, or a hospitalisation episode in a private hospital reported by the participant during this period, will lead to the exclusion of the participant.

Recruitment

Phone calls to contact the participants are performed during the day (morning, afternoon and evening) and with repeated attempts, in case of failure. A trained member of the research team conducts a structured conversation aiming to confirm the eligibility of participants, explain the objectives and procedures of the study, and schedule the in-person evaluation at Hospital Pedro Hispano or Hospital São Sebastião. Reasons for not participating (impossible to contact, exclusion criteria or refusals) are registered for all potential participants identified for the cohorts 1–4.

Baseline and follow-up evaluations

Participants are evaluated in person, at Hospital Pedro Hispano or Hospital São Sebastião, at baseline, that is, approximately 2–3 years after the index date, which corresponds to the date of the first positive test for SARS-CoV-2 infection, for cohort 3, the date of hospital admission due to acute COVID-19, for cohort 1, and the date of hospital admission due to other reasons, for cohort 2. No index date was attributed to participants of cohort 4 as they were neither infected nor hospitalised during the period March 2020–February 2021.

Follow-up evaluations after 1 and 2 years will be similar to the baseline assessment.

Instruments

Table 1 presents the instruments administered to all participants of the four cohorts.

Table 2 Description of the battery of instruments used for the neuropsychological evaluation of the participants with probable cognitive impairment*

Instrument	Description	Cognitive domains/function	Score
SMC ^{54 55}	A 10-item scale regarding subjective memory complaints.	Subjective memory.	Range: 0–21 Higher scores reflect maximal memory complaints.
Verbal Fluency Test ^{56 57}	Verbal fluency tests consist of four trials, one of semantic fluency and three of phonemic fluency, of 1 min each. The participants are asked to generate the name of as many species of animals as possible in the semantic fluency test, and as many words as possible beginning with a specific letter in the phonemic fluency test, within 1 min.	Non-motor processing speed, language production and executive functions	The total trial score corresponds to the number of words correctly produced within 1 min. The total test score corresponds to the sum of the three trials. Higher scores correspond to better performance.
TMT ^{58 59}	Part A: the participants are asked to draw lines to connect 25 randomly scattered numbered circles in ascending order. They should do so as quickly as possible. Part B: the participants are asked to draw lines to connect circles in numeric and alphabetic order, alternating between numbers and letters, progressively up to no 13. They should do so as quickly as possible.	Part A: attention; visual scanning; and speed of eye-hand coordination and information processing. Part B: working memory and executive functions; particularly, the ability to switch between sets of stimuli.	Direct measures of performance: time (seconds) to complete part A and part B and performance errors during part A and part B. Derived scores: difference score (B–A), ratio score (B/A), proportion score (B–A/A), sum score (A+B), and multiplication score (A×B/100). Lower raw scores and higher adjusted scores correspond to better performance.
WMS-III ^{60 61}	Scale composed of 17 subtests that assess different memory functions using verbal and visual stimuli. Useful in the evaluation of various clinical conditions, including neurodegenerative diseases such as Alzheimer's disease. Subtests used: Logical Memory I, II; Visual reproduction I, II; Digit Span.	Verbal and visual memories, working memory.	Range: Immediate recall and delayed recall: 0–50; Auditory recognition; digit span: 0–30; Visual reproduction: 0–104 Higher scores correspond to better performance.
WAIS-III ^{62 63}	Scale composed of 14 subtests that assess the verbal component and the perceptual-motor component of intelligence in adults and older adolescents. Subtests used: Digit-Symbol-Coding; Symbol Search; Information.	Attention/concentration, executive function (sequencing), motor function; processing speed.	The number of correct symbols within the allowed time (120 s) is measured.
Stroop Test ^{64 65}	The Stroop Test evaluates the interference effects between the two cerebral hemispheres. Consist in three trials: (1) word reading; (2) colours naming and (3) identifying the colours of words, without considering the meaning of the word.	Executive functions (inhibitory control), selective attention.	Scores for each trial indicate the number of correct responses. An interference score can be generated that quantifies the participant's ability to inhibit the inappropriate response of reading the colour name as opposed to the colour of the ink used to print the colour name in the third trial.
Token Test-short form ⁶⁶	A test designed to assess the comprehension of commands that vary in degree of linguistic difficulty. The participant is presented with tokens of different geometric shapes, sizes and colours, and is required to perform certain acts with the tokens, such as point to tokens, touch them, pick them up, and place one token on top of another.	Attention and vigilance; verbal functions.	Range: 0–36

Continued

Table 1 Description of the instruments used for the evaluation of all participants

Instrument	Description	Domains/subscales	Score
MoCA ²⁹	Paper and pencil cognitive screening test for evaluating mild cognitive impairment.	Attention and concentration, executive functions, memory, language, visuoconstructional skills, calculations, orientation.	Range: 0–30 Higher scores represent better cognitive performance.
18-point CDT ^{52 53}	The CDT is an assessment tool where participants are asked to draw a big circle, place the clock numbers and then indicate the time as '10 past 11'. An 18-point system will be used to score the test.	Visuospatial, executive function.	Range: 0–18 Scoring system with three main components: (A) assessment of circle integrity (two points); (B) number placement and sequencing (six points); and (C) placement and size of the hands (six points). Additionally, there are two points for representation of the clock's centre and two points for general gestalt.
Brain on Track ^{33 34}	Computerised and self-administered test that allows longitudinal monitoring of cognitive performance.	Attention, memory, executive functions, language, calculation, constructive ability, visuospatial processing.	Range: virtually unlimited (maximum number of correct answers in a fixed time). Higher scores represent better cognitive performance. Scores falling below an expected performance threshold for each age/education group represent a pattern of decline in individual performance.
HADS ⁴⁵	Scale with 14 questions designed to assess anxiety and depression. Patients should answer considering the previous week.	Depression, anxiety.	Range (for each subscale): 0–21 Scores greater than or equal to 11 represent a case of anxiety or depression, as applicable.
PSQI ⁴⁴	Quality of sleep index with 18 questions about the sleeping habits of the patients during the previous month.	Subjective sleep quality, sleep latency, duration of sleep, habitual sleep efficiency, sleep disorders, use of medications for sleep, daytime dysfunction.	Range: 0–21 Scores greater than 5 indicate poor sleep quality.
REM Sleep Behaviour Disorder Single-Question Screen ³⁵	Single screening yes/no question about the classic dream-enactment behaviour in the REM behaviour disorder.	REM sleep behaviour disorder.	A positive answer suggests a presence of REM behaviour Disorder.
STOP-Bang questionnaire ⁴⁶	Screening tool with eight yes or no questions related to the clinical features of sleep apnoea.	Obstructive sleep apnoea (OSA).	Range: 0–8 Each positive answer sum 1 to the final score, with higher scores representing more risk of OSA. Scores equal or greater than three indicate OSA.
9-item PD screening questionnaire ³⁸	Screening tool with nine yes or no questions	PD	Range: 0–9 Higher scores indicate higher likelihood of Parkinson's Disease: unlikely if scores are 0–1 ; possible if scores are 2–4; and probable if scores are ≥5.
Timed "Up and Go" test ⁴⁰	Test to evaluate the ability of patients to perform sequential locomotor tasks that incorporate walking and turning.	Mobility, gait and balance (ability to stand up from the chair, walking characteristics, presence of 'freezing' walking, posture).	5-point Likert scale (normal, discreet, mild, moderate and severe) and global gait diagnosis: normal or altered, with parkinsonism and/or osteoarticular and/or vascular and/or others features.

CDT, Clock Drawing Test; HADS, Hospital Anxiety and Depression Scale; MoCA, Montreal Cognitive Assessment; PD, Parkinson's disease; PSQI, Pittsburgh Sleep Quality Index; REM, rapid eye movement.

Structured questionnaire

Trained interviewers conduct face-to-face interviews using a structured questionnaire to collect baseline data on: (1) sociodemographic characteristics: age, years of education, employment status, occupation; (2) lifestyles: alcohol and tobacco consumption; (3) previous medical diagnoses and current medication intake; (4) neurological symptoms during the first infection and following recovery (cohorts 1 and 3, and individuals from the comparison groups who got infected after vaccination), and current symptoms (all participants); (5) dates of SARS-CoV-2

infection diagnoses (reinfections for cohorts 1 and 3, and infection episodes after complete vaccination, for cohorts 2 and 4 and (6) data on vaccination uptake.

Evaluation of cognitive performance and identification of cognitive impairment: MoCA and brain on track, neuropsychological evaluation and clinical evaluation by a neurologist

Cognitive performance of all participants is evaluated with the MoCA, V.7.1,²⁹ translated, adapted and validated in the Portuguese population.³⁰ MoCA is a screening

instrument for mild cognitive impairment, with good psychometric characteristics and high sensitivity.³¹ It assesses visuoconstructive ability, language, attention, concentration and working memory, executive functions, delayed memory and orientation. Scores range 0–30, with higher values corresponding to better cognitive performance. Interviewers received training and certification from the MoCA Training and Certification Module at www.mocacognition.com, and the Manual of the Portuguese V.7.1 was discussed within the research team to clarify administration procedures and the rating process.

Two outcome measures will be used: cognitive decline and probable cognitive impairment. We will define the former based on the distribution of the MoCA scores variation over time (score at 1 year minus score at baseline) in the comparator non-exposed group (either cohort 2 or cohort 4), as being a variation in MoCA scores below 1.5 SD below the mean. For defining probable cognitive impairment, we use age-specific and education-specific norms³¹ and the cut-off point of 1.5 SD below the mean.

Participants with probable cognitive impairment are invited for a neuropsychological assessment, to be scheduled as soon as possible, to confirm cognitive deficits. The neuropsychological evaluation includes neurocognitive tests validated for the Portuguese population, assessing verbal and visual memory, working memory, information processing speed, executive functions and language. It also includes the assessment of subjective memory complaints, emotional functioning, functional disability, independent living skills and premorbid intelligence (table 2).

Based on the number of tests used for evaluating each cognitive domain, cut-off points (below 1 SD, 1.5 SD or 2 SD below the mean of age-norms) are applied to identify cognitive deficit³² (table 3). Participant with cognitive deficit in at least one cognitive domain are considered to have cognitive impairment. These are referred to a clinical consultation with a neurologist of the research team at the hospital, for clinical follow-up according to the best clinical practices in use and a structured protocol of classification of the probable aetiology of the cognitive impairment. Brain imaging and laboratory tests will complement the diagnosis whenever necessary.

Additionally, cognitive performance is evaluated with the web-based remote monitoring test Brain on Track. This instrument showed good internal consistency, discriminative ability and reliability.^{33 34} It assesses executive functions, short and delayed memories, attention, calculation, visuoconstructive ability and language. A complete session of tests of Brain on Track takes 24 min and includes 11 exercises with fixed duration. The participant is asked to answer to as many items as possible in each exercise, as the score is the number of correct answers. Based on this score and using a predictive model adjusted for age (to be published), a score of risk for cognitive impairment is computed. Participants with a high score of risk will be invited for the neuropsychological assessment.

Eligibility criteria to use Brain on Track are: at least 3 years of education, access to a computer at home with internet and sufficient digital literacy to log into the website, or with help from another person but with autonomy for using the mouse and to do the exercises independently.

All participants eligible to use Brain on Track are invited for remote monitoring. The first and second sessions of tests are scheduled with a 1-week interval, and the following sessions every 3 months thereafter.

Identification of prodromal markers and screening for PD

RBD Single-Question screen

We use the RBD Single-Question Screen³⁵ to identify idiopathic RBD, which is an important risk factor for PD.³⁶ RBD may be considered a prodromal marker of PD, dementia with Lewy bodies and multiple system atrophy, and about 75% of those suffering from RBD develop PD or a parkinsonism within about 10 years.³⁷ This rapid test has high sensitivity, specificity and reliability.³⁵

The nine-item PD screening questionnaire

We use the nine-item PD screening questionnaire³⁸ which has a sensitivity and a specificity ranging 61%–92% and 29%–92%, respectively, to identify possible cases of PD in community settings.³⁹ The test includes nine questions to be answered with yes or no. The sum of positive answers is the final score of the test which defines the likelihood of having PD: 0–1 (unlikely), 2–4 (possible) and ≥ 5 (probable).³⁹

Participants screening positive for possible parkinsonism are formally evaluated by a neurologist of the research team, following usual clinical practices.

Timed 'Up and Go' test and gait assessment

We use the Timed 'Up and Go' Test (TUG)⁴⁰ to measure the ability of participants to perform sequential locomotor tasks that incorporate walking and turning. This test was designed to measure mobility in elderly people and could be a useful tool for quantifying locomotor performance in people with PD. Patients are required to stand up from a chair, walk three metres, turn around, walk back to the chair and sit down again. One training trial is performed under instructions of a trained member of the research team and then two trials are videotaped to allow a trained neurologist of the research team to later evaluate gait and balance. The time, in seconds, necessary for the participant to perform the test is recorded. The two videotaped trials of the TUG test are evaluated using a structured report comprising: (1) four gait-related features (ability to stand up from a chair, walking characteristics, presence of 'freezing' walking, posture); (2) the rating of arms swing, asymmetry of shoulder lift, and tremor at rest on gait; (3) and a global gait diagnosis ('normal' or 'altered', with parkinsonism and/or osteoarticular and/or vascular and/or others features).

Table 2 Continued

Instrument	Description	Cognitive domains/function	Score
TeLPI ⁶⁷	Designed to assess premorbid intelligence, TeLPI is a Portuguese irregular word reading test. The participants are asked to read 46 irregular words, infrequent Portuguese words.	Premorbid IQ: full scale IQ, Verbal IQ, performance IQ	Range: number of errors (maximum of 46) and years of education are inserted in three linear equations to calculate the three types of IQ
BDI-II ^{68 69}	A 21-question measure assessing the presence of depressive symptoms experienced by the participant within the past week.	Emotional functioning.	Range: 0–63 A cut-off score indicative of mild depressive symptoms is greater than 10 and for severe depressive symptoms is greater than 30.
Barthel ADL Index ^{70 71}	An index to measure functional disability, focused on bodily oriented personal care.	Functional domains: feeding, incontinence, transferring, toileting, dressing, bathing.	Range: 0–100 Lower scores reflect increased disability.
IADL ^{72 73}	An eight-item scale used to assess independent living skills which include more complex activities (ie, 'instrumental activities of daily living') necessary for functioning in community settings.	Functional domains: using the telephone, shopping, food preparation, housekeeping, laundry, transport, medication, finances.	Range: 0–8 Higher scores reflect high function, independence.

*Participants with a MoCA score below age- and education-specific values based on norms and the cut-off point of 1.5 SD below the mean. Barthel ADL Index, Barthel Activities of Daily Living Index; BDI-II, Beck Depression Inventory – Second Edition; IADL, instrumental ADL; MoCA, Montreal Cognitive Assessment; SDMT, Symbol and Digit Modalities Test; SMC, Subjective Memory Complaints scale; TeLPI, Irregular Word Reading Test; TMT, Trail Making Test; WAIS-III, Wechsler Adult Intelligence Scale – Third Edition; WMS-III, Wechsler Memory Scale – Third Edition.

Assessment of anxiety, depression, sleep quality and obstructive sleep apnoea

Levels of anxiety and depression symptoms are measured using the Hospital Anxiety and Depression Scale (HADS)⁴¹ validated in the Portuguese population.⁴² This self-administered questionnaire includes seven items assessing anxiety symptoms and seven items for depression symptoms, resulting in the HADS-A and HADS-D subscales, respectively, rated with a four-point Likert

scale (0–3). Scores of 11 or higher in the subscale are indicative of clinically significant symptoms.⁴² HADS was designed to be used in patients with physical illness, and for this reason, the questionnaire does not include somatic symptoms, namely insomnia, loss of appetite and fatigue, to avoid overlapping of symptoms due to depression and due to physical conditions. Several studies have shown its suitability in both clinical and community settings.⁴³

Table 3 Criteria used for the classification of cognitive impairment, considering the number of tests administered to assess each cognitive domain

Cognitive domain	Test	Criteria for impairment
Verbal memory	WMS III-Logical memory I and II	2 scores <1.5 SD or 1 score <2 SD
Visual memory	WMS III-Visual reproduction I and II	2 scores <1.5 SD or 1 score <2 SD
Attention/information processing speed	WAIS III-Digit-Symbol-Coding and Symbol search Trail making test, part A Stroop test-word reading	At least 3 scores <1 SD or 2 scores <1.5 SD
Executive functions	Stroop test (colour naming and word colour naming) Trail making test, part B and B-A Phonemic fluency-letters M, R and P Phonemic fluency-categories of animals WMS III-digit span	At least three scores <1.5 SD or 2 scores <2 SD
Language	Token test-short-form	Score <2 SD

WAIS III, Wechsler Adult Intelligence Scale Third Edition; WMS III, Wechsler Memory Scale Third Edition.

collected, namely clinical data from their electronic health record, questionnaires, cognitive tests and a video; expected individual benefits and risks (minimal); procedures to respect their right to confidentiality, their right and how to manifest this right, to abandon the study and withdraw their consent at any time and that this will not interfere with their medical care, and their right to access their data and have their data corrected and erased. After this explanation, patients clearly manifesting that they do not understand the information will not be considered eligible. Participants will have time to decide. Participation will be assumed after a participant signs the informed consent. No direct or indirect incentives for participation will be given. This is an observational study and no risk is expected. Participants with neurological complications of COVID-19 will be followed as are non-participants of the study, according to clinical practice implemented at the health institution. Therefore, participants will not be submitted to additional risk.

The findings of this project will be submitted for publication in international peer-reviewed journals, and proposed for presentation in relevant national and international conferences, which will allow for the dissemination of the main findings across the medical community. Press releases through mass media will also be issued to promote the dissemination of information relevant to the general population and policy-makers. Furthermore, the project will contribute to the training of researchers through the production of masters' dissertations and doctoral theses.

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Abstract: Cognitive impairment after SARS-CoV-2 infection: preliminary results of the NeurodegCoV-19 project (*XLI Reunión Anual de la SEE e XVIII Congresso da APE Porto 2023*)

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Introduction: Lower cognitive performance was reported among individuals infected with SARS-CoV-2 virus, up to one year after infection, mostly in hospitalized cases. The present analysis aims to estimate the prevalence of cognitive impairment about two years after infection with SARS-CoV-2, of individuals who had not received any dose of vaccination against COVID-19 before the infection.

Methods: Participants having a first diagnosis of SARS-CoV-2 infection (detection of RNA virus in nasopharyngeal or oropharyngeal swabs, with Real-Time Polymerase Chain Reaction), were retrospectively identified from administrative lists of *Unidade Local de Saúde de Matosinhos*. Baseline data was collected from July 2022 to February 2023. The NeurodegCoV-19 cohort includes 91 individuals who had been hospitalized due to COVID-19, between March 2020 and February 2021 (hospitalized group), and 243 individuals who had been infected but not hospitalized during the same period (non-hospitalized group) (median time elapsed between COVID-19 diagnosis and the cognitive test: 698 and 673.5 days, respectively). Sociodemographic data and current medication were collected during a structured interview. Medicine consumption (none, 1-2, 3-5, and >5 medicines) was considered as a proxy of general health. The Montreal Cognitive Assessment (MoCA) was used to assess cognitive performance. A MoCA score below 1.5 standard deviations of the mean of age- and education-specific norms was defined as a probable cognitive impairment (PCI). Data from the population-based cohort EPIPorto (n=929, evaluated in 2013–2015) was used for comparison. Odds ratio (OR) and corresponding 95% confidence interval (95%CI) adjusted for age, sex, education, and medication were computed using multivariate logistic regression.

Results: The prevalence of PCI was 33.0% (95%CI: 23.5–43.6) in hospitalized, 31.3% (95%CI: 25.6–37.5) in non-hospitalized group, and 18.7% (95%CI: 16.3–21.4) in EPIPorto. Adjusting for age, sex, education and medication, the odds of PCI were higher in both the hospitalized (OR=2.01, 95%CI: 1.29, 3.17) and the non-hospitalized group (OR=1.58, 95%CI: 1.17, 2.16) than in EPIPorto. A stronger association was observed for hospitalized group versus cohort EPIPorto than for non-hospitalized group versus cohort EPIPorto (p for trend<0.001).

Conclusion: Preliminary results show that PCI is common about two years after SARS-CoV-2 infection in individuals who had not received any dose of vaccination against COVID-19 before the infection. Both hospitalized and non-hospitalized individuals infected with SARS-CoV-2 virus are more likely to present PCI, when compared with the general population.

Keywords: COVID-19, SARS-CoV-2, probable cognitive impairment

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