NEURAL REGENERATION RESEARCH
www.nrronline.org

The long-COVID-19 in older adults: facts and conjectures

Tino Emanuele Poloni*, Valentina Medici, Antonio Zito, Arenn Faye Carlos

The coronavirus disease-19 (COVID-19) has greatly affected the overall health of the elderly population through direct biological (infection-related) and indirect psychosocial (quarantine- and isolation-related) effects. Because the severe form of COVID-19 most frequently targets this population, the prevalence of long-term sequelae is expected to rise consequentially in people ≥ 65 years old. The prominent neuropsychiatric consequences of COVID-19 and the cognitive frailty seen in older adults can both have a negative impact on their mental health. To explore the behavioral, neurological, and psychosocial consequences of COVID-19, we conducted separate studies on different populations of older adult people residing in Lombardy – the Italian epicenter of the first pandemic wave in spring 2020. In one study, we found that behavioral changes (i.e., delirium) were a frequent symptom of COVID-19, manifesting at disease onset and preceding the typical symptoms in about 1/3 (36.8%) of cases, particularly in patients with neurocognitive disorders (NCD), such as dementia (major-NCD) or mild cognitive impairment (mild-NCD). Delirium was also associated with short-term mortality and potential long-term cognitive sequelae (Poloni et al., 2020). To uncover the neuropathology underlying behavioral changes and their possible effects over time, we compared 9 brains of elderly patients who had died of COVID-19 (with and without dementia) with 6 brains from age-matched non-COVID controls. The main finding was an excessive innate immune response, represented by microglial hyperactivation. Although we observed severe inflammatory changes especially in the brainstem, we did not find neuropathological evidence suggestive of SARS-CoV-2 replication in the brain (Gagliardi et al., 2021; Poloni et al., 2021). In a study evaluating the psychosocial consequences of the lockdown due to the pandemic (Carlos et al., 2021), we observed that those with mild-moderate dementia were unable to cope and adapt to the life changes caused by the restrictions and consequently suffered from depression and cognitive decline. Before COVID, patients with dementia normally engaged in habitual daily activities. The disruption of said routines, the inability to engage in new activities, and the incapability to use modern technologies all triggered psychological distress and some degree of cognitive and motor regression (**Figure 1A**). Although lockdown (the sternest form of quarantine in history) protected them from COVID-19, the social seclusion and the inability to access primary care treatment – aggravated by an unprepared and unequipped primary care health sector – caused further complications (Carlos et al., 2021). Moreover, the general effects of the pandemic in terms of loss of "individual freedom", economic crisis, and mass media conditioning should not be overlooked due to their possible impact on mental health.

Given these premises, it is not surprising that many elderly patients continue to have symptoms after the acute phase of COVID-19. Despite having eradicated the infection, many of them do not appear restored to health and instead experience persistent disturbances of the neuropsychiatric type, such as fatigue, anosmia, sleep disorders, anxiety, depression, and cognitive dysfunction (e.g., difficulties in concentration, problem-solving and spatial planning – the so-called "brain fog"). The extent of cognitive dysfunction is directly proportional to the severity of the respiratory distress (although cognitive deficits can also be observed in non-hospitalized patients). Neuropsychiatric symptoms are currently considered part of a possible early chronic phase of COVID-19 (Hampshire et al., 2021). While a universal definition is still lacking, the American Centers for Disease Control and Prevention has defined this post-COVID condition, also called "long-COVID", as a syndrome that extends beyond four weeks after the initial infection. The pathophysiology of these persistent symptoms is clearly multifactorial, but whether they are related to incomplete viral eradication or indirect factors (e.g., prolonged inflammation, lung fibrosis, sequelae of neurological complication, or psychosocial distress) remains controversial. SARS-CoV-2 is a virus that clearly produces an acute disease but there is currently no evidence suggesting that it can directly infect neurons and persist inside the central nervous system (CNS). Moreover, the expression of angiotensin-converting enzyme-2 and transmembrane protease serine 2, the main factors allowing the virus to enter cells, is generally low in the human brain (Iadecola et al., 2020). Late neurological manifestations related to previous coronavirus outbreaks (SARS and MERS) and clear evidence of neuroinvasiveness of the previous SARS-CoV-1 are not reported in the literature. A pathological study on SARS-CoV-1 by Ding et al. (2004) did reveal viral localization in some neurons, but their topographical distribution was not described and their appearance was rather similar to that observed by us in a few brainstem neurons. Nonetheless, several authors have proposed theories about a possible neurotropism of SARS-CoV-2 and a possible direct invasion of the brain by the virus, which could remain within the CNS producing long-term damage, as is the case with other viral infections (Yachou et al., 2020). These assumptions have generated fear among the recovered and recovering COVID-19 patients, producing negative psychological conditioning even in subjects who have had mild forms of the disease. Our data suggest that SARS-CoV-2 slightly penetrates the CNS but does not actively replicate within it. The detection of viral proteins is very rare and limited to the lower brainstem; they probably consist of virions or virions fragments, which migrated from the respiratory and pharyngeal mucosa through the lower cranial nerves (Poloni et al., 2021). Additionally, despite being present, the viral genome in the brain is detectable in minimal quantities and only by using a very sensitive method like digital droplet PCR, suggesting an origin from the blood (Gagliardi et al., 2021). Hence, SARS-CoV-2 cannot cause direct injuries to the neurons and consequently viral encephalitis. Indeed, all the COVID-19 patients we studied showed a clear predominance of the monocyte-microglia component (non-specific innate immunity) with scant lymphocytes and no evidence of lymphocytic clonal activation (specific adaptative immunity) within the CNS (Poloni et al., 2021). These features are quite different from those of viral or autoimmune encephalitis, in which frequent viral inclusions or many lymphocytes are detectable, in association with a direct damage of brain tissues. These facts strongly suggest that SARS-CoV-2 is not a neurotropic virus and, importantly, there is no evidence proving its persistence within the CNS after the acute infection. Therefore, we believe that the long-term neurological prognosis will be favorable in most COVID-19 patients, with the exception of those who had severe complications like the elderly patients who presented with delirium. Like other severe systemic infections or conditions requiring intensive care, COVID-19 may have detrimental effects on the nervous system, including long-term consequences, that are indirect and mostly non-specific to SARS-CoV-2. These include: rare post-infectious immune-mediated diseases (such as autoimmune encephalitis with ADEM-like features and Guillain-Barré syndrome),

reported as a single case or small series and involving all age groups (Reichard et al., 2020; Zito et al., 2020); delirium caused by innate immunity hyperactivation (i.e., abnormal microglial activation in the brain) that is particularly frequent and detrimental to the elderly (Poloni et al., 2021); and the vascular and neurological complications due to hypoxia, protracted intensive care, immobilization, and isolation. Also, even those who did not get infected with COVID-19 eventually suffered from the effects of social deprivation, which led to a decline in cognition and functional abilities especially in the elderly with NCD (**Figure 1A**) (Carlos et al., 2021).

The main purpose of this Perspective is to give an interpretation of the long-COVID phenomenon in the elderly based on the data that emerged from our studies. The neuropathological assessment of our series includes COVID-19 cases with a clinical course not influenced by prolonged intensive care. In fact, none of our patients had been subjected to orotracheal intubation or mechanical ventilation. Arguably, this is a possible reason why neuropathological analysis revealed no macroscopic vascular lesions, which appear to correlate more with protracted intensive care than COVID-19 per se. From our study, the two neuropathological hallmarks are: 1- diffuse cortical edema due to extreme hypoxia with cortical swelling, spongiosis, and severe neuronal rarefaction (**Figure 1Ba**); and 2 - increased microglial activation forming CD68-positive ameboid cells, which then aggregated into nodules (**Figure 1Bc–e**). Nevertheless, when comparing the frontal cortex of people with COVID-19 to that of matched controls, we found that cortical microglial boosting was not only related to COVID-19 but also to pre-existing neurodegeneration. In those with both dementia and COVID-19, the distribution of the inflammatory nodules closely paralleled that of amyloid plaques and was probably enhanced by an infection-induced cytokine release (**Figure 1Bb** and **c**). This phenomenon was particularly prominent in the hippocampus. Indeed, the hippocampi of people with COVID-19 and dementia who suffered from hyperactive delirium showed significantly higher microglial activation (**Figure 1Bd**). Regardless of dementia, ameboid CD68-positive microglial nodules were more abundant in the brainstem of all our COVID-19 cases (**Figure 1Be**), while scant lymphocytic infiltration within a few nodules (**Figure 1Bf**) and very limited traces of SARS-CoV-2 antigens were detected (**Figure 1Bg**). Contrary to what occurs in the lungs, proteins from the virus localizing within the brain were exceedingly rare and were only observed in the lower brainstem with no associated evidence of viral encephalitis (**Figure 1Bg**). The microglial hyperactivation is probably due to an extreme cytokine outpouring ("cytokine storm") or entry of viral antigens into the nervous system (Poloni et al., 2021). It is intriguing to hypothesize specific reasons behind the observed topography. Microglial activation might be induced by the presence of viral antigens in the lower brainstem and by the degenerative burden in the hippocampus. The brainstem is involved in the regulation of alertness and vegetative states while the hippocampus is a central hub in the limbic system involved in memory, emotions, and behavior. The nodules in the brainstem and hippocampus probably represent the neuropathological basis of the neurological manifestations frequently observed in older adults affected by COVID-19. Indeed, aside from the patients who suffered the consequences of prolonged intensive care and the few who had post-infectious immune-mediated complications, which should be considered separately, the most frequent clinical condition we observed was what we referred to as the "COVID-19 encephalopathic syndrome". As observed by us, "COVID-19 encephalopathic syndrome" is characterized by acute changes in behavior (hyperactive delirium with agitation and psychosis or hypoactive delirium with severe psychomotor slowing down), and by autonomic changes (hypotension, hypersomnia, lack of respiratory hypoxic drive) (Poloni et al., 2020). All these symptoms are consistent with the topography of the inflammatory changes in the brain (Poloni et al., 2021). From a biochemical standpoint, a transcriptome analysis of frontal cortex in patients with severe COVID-19 showed a downregulation of the hypoxia inducing factor system, a regulator of oxygen in cells, associated with increased hemoglobin subunits (HBB, A1 and A2) and long non-coding RNA CTB-36O1.7 that is probably involved in microglial modulation (Gagliardi et al., 2021). Another study confirmed the pivotal role of microglia and the persistent CNS inflammation after the acute phase of the disease by showing transcriptional changes of genes involved in several microglial biological functions, including activation, migration, and phagocytic induction (Fullard et al., 2021). These results are consistent and support our interpretation of the two main mechanisms of neurological damage: extreme cortical hypoxia and inflammatory changes.

In summary, we observed a boosting of the innate immunity and a concurrent dampening of the adaptive immune system

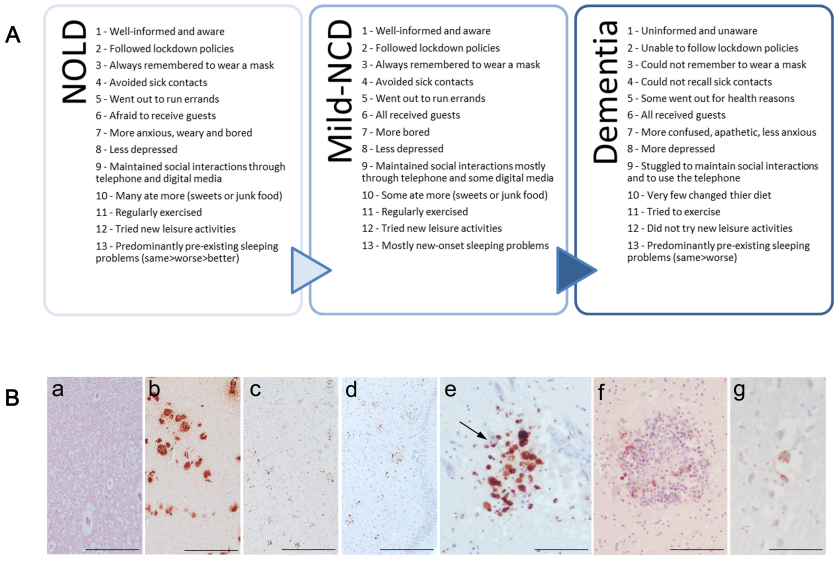

Figure 1 | **Psychosocial and neuropathological impact of the SARS-CoV-2 pandemic.**
(A) Psycho-social and behavioral profiles during a lockdown of 204 elderly subjects grouped based on their cognitive state. Overall, NOLD and mild-NCD subjects tolerated lockdown well, subjects with dementia did not. Dementia: only subjects with mild to moderate dementia were included. NOLD: Cognitively normal old; Mild-NCD: mild neurocognitive disorder or mild cognitive impairment (MCI). Reprinted with permission from Carlos et al. (2021). (B) Main microscopic neuropathological pictures in patients who died from COVID-19. (a) Diffuse cortical edema due to extreme hypoxia with cortical swelling, spongiosis, and severe neuronal rarefaction (hematoxylin & eosin staining); (b) amyloid-β plaques in the frontal cortex (4G8 antibodies); (c) microglial nodules in the frontal cortex showing a distribution similar to that of amyloid-β plaques (CD68 antibodies, specific for microglia); (d) microglial nodules in the hippocampus (CD68 antibodies); (e) typical picture of a microglial nodule in the brainstem showing ameboid cells with some features of neuronophagia (arrow) (CD68 antibodies); (f) one of the rare nodules containing some lymphocytes (CD20 antibodies, specific for B-lymphocytes); (g) one of the extremely rare and small clusters of SARS-Cov-2 positive neurons, which were detected only in the lower brainstem (SARS-CoV-2 nucleocapsid antibodies). Scale bars: 300 µm in a–d; 62 µm in e; 76 µm in f; 53 µm in g. Adapted from Poloni et al. (2021).

of the brain. This was represented by a large number of microglial nodules and the small number of lymphocytes. This phenomenon is probably enhanced by the occurrence of "immunological senescence" (reduced adaptive immunity with decreased specific immune responses) and "inflammaging" (excessive inflammatory activation in aging) in the elderly. At the same time, elderly patients are affected by both cerebrovascular comorbidities, which reduce oxygen supply, and neurodegenerative changes, which recruit inflammatory cells and "prime" microglial cells. Hypoxia and inflammation may accelerate neurodegeneration, which in turn may cause long-term cognitive worsening, especially in those who suffered from delirium during the acute phase (Davis et al., 2017). In light of our recent investigations and previous studies, we can conclude that long-COVID in elderly patients is not the result of a direct invasion of the brain by SARS-CoV-2, but instead derives from a complex interaction between the biological and the psychosocial factors described above. A proper long-COVID definition should only include the consequences of established complications of SARS-CoV-2 infection. Otherwise, it remains a confusing clinical entity with possible misinterpretations, especially regarding the neuropsychiatric symptoms. Longitudinal studies will be necessary to verify the progress of patients through long-term observation, considering that the recovery from COVID-19 requires termination of both viral infection and its associated inflammatory processes which can take > 4 weeks in severe cases. Notwithstanding, some of the biological and psychosocial detrimental effects are at least partially reversible, as well as the associated mental and motor decline. Through a deeper understanding of these phenomena, we could improve the management of the long-COVID patients. First, policies strongly encouraging primary prevention through extensive vaccination of those over 65 (also with eventual booster shots) should be implemented. Following the acute phase of the disease, rehabilitative interventions, such as physical therapy, cognitive training, and psychological support, should be provided promptly to restore previous functional performances. Looking to the future, appropriate clinical and public health management should not be delayed in order to prevent the long-term neuropsychiatric complications related to COVID-19 and to attend to the needs of long-COVID patients.

We are very grateful to Prof. Mauro Ceroni (Department of Brain and Behavioral Disorders, University of Pavia & IRCCS Mondino Foundation, Pavia, Italy) and Dr. Antonio Guaita (Department of Neurology and Neuropathology; Abbiategrasso Brain Bank; Golgi-Cenci Foundation; Abbiategrasso; Milan, Italy) for their valuable contributions.

Our work was partially funded by 'Fondo di Beneficenza' Intesa Sanpaolo (Italy). Project code: B/2020/0045 (to TEP).

The data concerning the clinical-neuropathological correlations were presented as an oral communication at the AD/PD congress - Barcelona 2021.

Tino Emanuele Poloni*, Valentina Medici, Antonio Zito, Arenn Faye Carlos

Department of Neurology-Neuropathology and Abbiategrasso Brain Bank, Golgi-Cenci Foundation & Department of Rehabilitation, ASP Golgi-Redaelli - Abbiategrasso, Milan, Italy (Poloni TE)
Department of Neurology-Neuropathology and Abbiategrasso Brain Bank, Golgi-Cenci Foundation-Abbiategrasso, Milan, Italy (Medici V, Carlos AF)
Department of Brain and Behavioral Disorders, University of Pavia & IRCCS Mondino Foundation, Pavia, Italy (Zito A)

***Correspondence to:** Tino Emanuele Poloni, MD, e.poloni@golgicenci.it or tepoloni@gmail.com. https://orcid.org/https://orcid.org/0000-0002-8463-6879 (Tino Emanuele Poloni)

Date of submission: August 26, 2021
Date of decision: November 22, 2021
Date of acceptance: December 11, 2021
Date of web publication: April 29, 2022

https://doi.org/10.4103/1673-5374.339483
How to cite this article: *Poloni TE, Medici V, Zito A, Carlos AF (2022) The long-COVID-19 in older adults: facts and conjectures. Neural Regen Res 17(12):2679-2681.*
Availability of data and materials: *All data generated or analyzed during this study are included in this published article and its supplementary information files.*

Open peer reviewer: *Zuleide Maria Ignácio, Federal University of the Southern Frontier, Brazil.*
Additional file: *Open peer review report 1.*

References

Carlos AF, Poloni TE, Caridi M, Pozzolini M, Vaccaro R, Rolandi E, Cirrincione A, Pettinato L, Vitali SF, Tronconi L, Ceroni M, Guaita A (2021) Life during COVID-19 lockdown in Italy: the influence of cognitive state on psychosocial, behavioral and lifestyle profiles of older adults. Aging Ment Health 15:1-10.

Davis DHJ, Muniz-Terrera G, Keage HAD, Stephan BCM, Fleming J, Ince PG, Matthews FE, Cunningham C, Ely EW, MacLullich AMJ, Brayne C, Members for the ECS in E (EClipSE) C (2017) Association of delirium with cognitive decline in late life: a neuropathologic study of 3 population-based cohort studies. JAMA Psychiatry 74:244-251.

Ding Y, He L, Zhang Q, Huang Z, Che X, Hou J, Wang H, Shen H, Qiu L, Li Z, Geng J, Cai J, Han H, Li X, Kang W, Weng D, Liang P, Jiang S (2004) Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. J Pathol 203:622-630.

Fullard JF, Lee HC, Voloudakis G, Suo S, Javidfar B, Shao Z, Peter C, Zhang W, Jiang S, Corvelo A, Wargnier H, Woodoff-Leith E, Purohit DP, Ahuja S, Tsankova NM, Jette N, Hoffman GE, Akbarian S, Fowkes M, Crary JF, et al. (2021) Single-nucleus transcriptome analysis of human brain immune response in patients with severe COVID-19. Genome Med 13:118.

Gagliardi S, Poloni ET, Pandini C, Garofalo M, Dragoni F, Medici V, Davin A, Visonà SD, Moretti M, Sproviero D, Pansarasa O, Guaita A, Ceroni M, Tronconi L, Cereda C (2021) Detection of SARS-CoV-2 genome and whole transcriptome sequencing in frontal cortex of COVID-19 patients. Brain Behav Immun 97:13-21.

Hampshire A, Trender W, Chamberlain SR, Jolly AE, Grant JE, Patrick F, Mazibuko N, Williams SCR, Barnby JM, Hellyer P, Mehta MA (2021) Cognitive deficits in people who have recovered from COVID-19. EClinicalMedicine 39:101044.

Iadecola C, Anrather J, Kamel H (2020) Effects of COVID-19 on the nervous system. Cell 183:16-27.

Poloni TE, Carlos AF, Cairati M, Cutaia C, Medici V, Marelli E, Ferrari D, Galli A, Bognetti P, Davin A, Cirrincione A, Ceretti A, Cereda C, Ceroni M, Tronconi L, Vitali S, Guaita A (2020) Prevalence and prognostic value of Delirium as the initial presentation of COVID-19 in the elderly with dementia: an Italian retrospective study. EClinicalMedicine 26:100490.

Poloni TE, Medici V, Moretti M, Visonà SD, Cirrincione A, Carlos AF, Davin A, Gagliardi S, Pansarasa O, Cereda C, Tronconi L, Guaita A, Ceroni M (2021) COVID-19-related neuropathology and microglial activation in elderly with and without dementia. Brain Pathol 31:e12997.

Reichard RR, Kashani KB, Boire NA, Constantopoulos E, Guo Y, Lucchinetti CF (2020) Neuropathology of COVID-19: a spectrum of vascular and acute disseminated encephalomyelitis (ADEM)-like pathology. Acta Neuropathol 140:1-6.

Yachou Y, El Idrissi A, Belapasov V, Ait Benali S (2020) Neuroinvasion, neurotropic, and neuroinflammatory events of SARS-CoV-2: understanding the neurological manifestations in COVID-19 patients. Neurol Sci 41:2657-2669.

Zito A, Alfonsi E, Franciotta D, Todisco M, Gastaldi M, Cotta Ramusino M, Ceroni M, Costa A (2020) COVID-19 and Guillain-Barré syndrome: a case report and review of literature. Front Neurol 11:909.

P-Reviewer: Ignácio ZM; C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y