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Cognitive impact of COVID-19: looking beyond the short term

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Abstract

COVID-19 is primarily a respiratory disease but up to two thirds of hospitalised patients show evidence of central nervous system (CNS) damage, predominantly ischaemic, in some cases haemorrhagic and occasionally encephalitic. It is unclear how much of the ischaemic damage is mediated by direct or inflammatory effects of virus on the CNS vasculature and how much is secondary to extracranial cardiorespiratory disease. Limited data suggest that the causative SARS-CoV-2 virus may enter the CNS via the nasal mucosa and olfactory fibres, or by haematogenous spread, and is capable of infecting endothelial cells, pericytes and probably neurons. Extracranially, SARS-CoV-2 targets endothelial cells and pericytes, causing endothelial cell dysfunction, vascular leakage and immune activation, sometimes leading to disseminated intravascular coagulation. It remains to be confirmed whether endothelial cells and pericytes in the cerebral vasculature are similarly targeted. Several aspects of COVID-19 are likely to impact on cognition. Cerebral white matter is particularly vulnerable to ischaemic damage in COVID-19 and is also critically important for cognitive function. There is accumulating evidence that cerebral hypoperfusion accelerates amyloid-β (Aβ) accumulation and is linked to tau and TDP-43 pathology, and by inducing phosphorylation of α-synuclein at serine-129, ischaemia may also increase the risk of development of Lewy body disease. Current therapies for COVID-19 are understandably focused on supporting respiratory function, preventing thrombosis and reducing immune activation. Since angiotensin-converting enzyme (ACE)-2 is a receptor for SARS-CoV-2, and ACE inhibitors and angiotensin receptor blockers are predicted to increase ACE-2 expression, it was initially feared that their use might exacerbate COVID-19. Recent meta-analyses have instead suggested that these medications are protective. This is perhaps because SARS-CoV-2 entry may deplete ACE-2, tipping the balance towards angiotensin II-ACE-1-mediated classical RAS activation: exacerbating hypoperfusion and promoting inflammation. It may be relevant that *APOE* ε4 individuals, who seem to be at increased risk of COVID-19, also have lowest ACE-2 activity. COVID-19 is likely to leave an unexpected legacy of long-term neurological complications in a significant number of survivors. Cognitive follow-up of COVID-19 patients will be important, especially in patients who develop cerebrovascular and neurological complications during the acute illness.

Keywords: COVID-19, SARS-CoV-2, Stroke, White matter ischaemia, Angiotensin-converting enzyme-2, Angiotensin-converting enzyme inhibitors, Angiotensin receptor blockers, Cognitive impairment, Dementia

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Background

COVID-19, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is primarily a respiratory disease but has the capacity to damage other organs including the brain. Similarly to the severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MER) viruses [1–3], SARS-CoV-2 targets the brain (reviewed [4]) and a growing number of case reports and cohort studies indicate significant neurological disturbance in COVID-19 patients (reviewed [5]). Central nervous system (CNS) involvement including non-specific encephalopathy (headache, confusion, and disorientation) was first documented in 53/214 (25%) hospitalised patients in Wuhan, China [6]. More recent studies in Europe have reported higher rates of CNS involvement: 69% of 58 hospitalised patients in a French Study [7], and 31% of 125 cases with altered mental state, including psychosis and neurocognitive changes, in a recent UK survey [8]. A recent report described a 'dysexecutive syndrome consisting of inattention, disorientation, or poorly organised movements in response to command' in 33% of 43 patients discharged from hospital [7]. Moreover, neuroradiological evidence of microstructural damage and disruption of functional brain integrity at 3-month follow-up in recovered COVID-19 patients [9] indicates potential long-term neurological consequences in severely affected COVID-19 patients (reviewed [10]). Acute cerebrovascular disease (CVD), typically presenting as ischaemic stroke but occasionally as intracerebral haemorrhage (ICH), has emerged as an important clinical feature in COVID-19 (reviewed in [5]). There have also been multiple case reports of encephalitis with brain-stem involvement (reviewed in [5]). CNS involvement with neurological presentation is more frequent in older and more severely ill COVID-19 patients [6]. Based on the minimum prevalence of neurological complications in SARS and MERS, Ellul et al. [5] estimated that of the reported 4.8 million COVID-19 cases at the time, 1805–9671 had developed CNS complications.

Human coronaviruses are known to target the CNS and cause damage by direct neurotoxicity or activation of the host immune response [1]. The propensity of SARS-CoV-2 to cause cerebral vascular injury greatly increases the risk of chronic brain damage, not only because of the cumulative destructive effect of multifocal cerebral ischaemia or haemorrhage, but potentially also through chronic post-infective complications of CVD, including endothelial and blood-brain barrier (BBB) dysfunction and upregulation of pro-inflammatory cytokines within the brain [11]. Long-term cognitive decline and neurodegeneration, with associated hippocampal atrophy [12], were previously reported to complicate systemic inflammation associated with severe sepsis [13, 14]. Acute respiratory distress syndrome (ARDS), a common clinical presentation in COVID-19 patients, is also associated with cognitive decline and neurodegeneration [15, 16]. Long-term follow-up of COVID-19 patients that includes detailed cognitive assessment will be important, to determine the extent and prevalence of long-term neurological and psychiatric consequences of COVID-19 [17], especially in patients who develop cerebrovascular and neurological complications during the acute illness.

In this review, we discuss the pathophysiological processes and risk factors shared by COVID-19 and dementia, focussing particularly on the role of cerebrovascular disease and the involvement of the renin-angiotensin system (RAS) (Table 1). We consider whether SARS-CoV-2 infection may increase the risk of later developing dementia, particularly in people with underlying cerebrovascular disease and high-risk co-morbidities, such as diabetes and hypertension.

Cerebral vascular disease (CVD) is common in severe COVID-19

Unlike in SARS and MERS, COVID-19 patients are at substantial risk of developing acute CVD. Studies to date indicate that CVD has affected 2–6% of hospitalised patients with COVID-19 (reviewed in [5]). In a Spanish cohort, 23 of 1683 patients (1.4%) developed CVD, with cerebral ischaemia accounting for 74% and ICH for 23% of the 23 cases [18]. Amongst COVID-19 patients with

Table 1 Pathophysiological processes contributing to increased risk of chronic neurological disease, including dementia, in COVID-19 patients
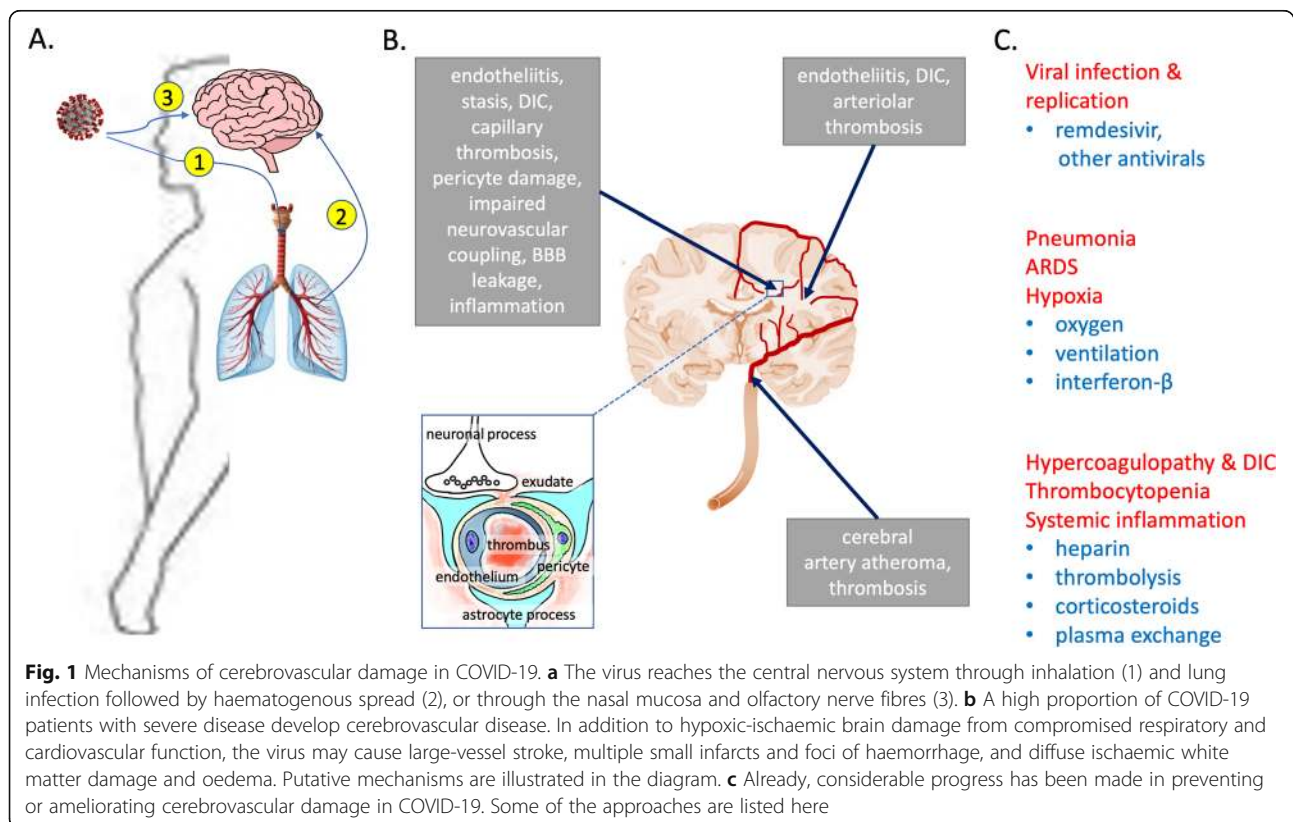
	References
1. Hypoxia and cerebral hypoperfusion secondary to cardiorespiratory disease	[25, 26]
- Hypoxic-ischaemic brain injury, diffuse white matter damage	
2. Coagulopathy, with thrombotic occlusion of cerebral blood vessels	[22]
- Cerebral artery thrombosis, disseminated intravascular coagulation	
3. Cerebral microvascular damage and dysfunction	[23, 24]
- Endotheliitis, pericyte damage, BBB leakiness, neurovascular dysfunction, impaired autoregulation, impaired vascular/para-vascular drainage	
4. Dysregulation of renin-angiotensin system	[125–127, 160, 161]
- Loss of regulatory RAS and overactivity of classical RAS signalling	
5. SARS-CoV-2 encephalitis / post-infective encephalitis (rare)	[27, 28, 38], reviewed in [5]
- CNS viral neuroinvasion via olfactory nerve fibres or vasculature/post-infective immune injury to CNS	

neurological complications, the reported incidence of CVD is much higher. Acute CVD was diagnosed in 77% of 56 patients admitted to a neurology ward in Italy [19]. In a recent UK-wide survey of 153 COVID-19 cases with neurological and/or psychiatric disturbance, most patients (62% of 125), for whom a full clinical dataset was available, had had a cerebrovascular event, compared to 31% with encephalopathy; of those with CVD, 74% had presented with ischaemic stroke, 12% with ICH, and 1% with CNS vasculitis [8]. A common theme across most of these studies is the predominance of CVD in older patients in association with more severe disease, and in those with co-morbidities, including hypertension, diabetes and underlying cerebrovascular disease [20]. However, large-vessel stroke has also been reported in younger adults with COVID-19 [21].

The pathophysiology of CVD in COVID-19 has yet to be fully determined (Fig. 1). Inflammation-induced disseminated intravascular coagulation (DIC), often complicated by pulmonary embolism, has been documented in a high proportion of patients with neurovascular complications and is likely to be a major contributor to most acute CVD events in COVID-19 [22], especially in younger healthy adults. A recent review [23] highlighted a number of pathways involving localised endothelial cell dysfunction, vascular leakage and unregulated immune activation

that contribute to DIC formation in ARDS in COVID-19 patients. Activation of the kallikrein-bradykinin system leading to reduced blood flow, upregulation of adhesion molecules that mediate leucocyte recruitment, activation of platelets and neutrophils, and increased inflammation and immune surveillance all potentially contribute to vascular damage (and pulmonary injury) in COVID-19 patients. SARS-CoV-2 has also been shown to target and infect endothelial cells in vascular beds in multiple tissues [24], but it remains to be confirmed whether endothelial cells in the cerebral vasculature are similarly targeted.

Most studies to date have implicated vascular dysfunction and ischaemic damage in the major neurological complications associated with COVID-19. Post-mortem neuropathological examination in 18 COVID-19 cases showed all to have acute hypoxic-ischaemic brain injury affecting the cerebrum and cerebellum, with rare foci of perivascular inflammation in two of the brains but no convincing evidence of virus within the CNS [25]. MR imaging of autopsy brains from deceased COVID-19 patients within 24 h of death revealed white matter changes including foci of haemorrhage in two cases and evidence of posterior reversible encephalopathy syndrome in another [26]. Plasma markers of neuronal and astrocyte injury (neurofilament light chain protein and glial fibrillary acidic protein) were elevated in COVID-19

Fig. 1 Mechanisms of cerebrovascular damage in COVID-19. **a** The virus reaches the central nervous system through inhalation (1) and lung infection followed by haematogenous spread (2), or through the nasal mucosa and olfactory nerve fibres (3). **b** A high proportion of COVID-19 patients with severe disease develop cerebrovascular disease. In addition to hypoxic-ischaemic brain damage from compromised respiratory and cardiovascular function, the virus may cause large-vessel stroke, multiple small infarcts and foci of haemorrhage, and diffuse ischaemic white matter damage and oedema. Putative mechanisms are illustrated in the diagram. **c** Already, considerable progress has been made in preventing or ameliorating cerebrovascular damage in COVID-19. Some of the approaches are listed here

patients and associated with disease severity [27]. The authors concluded that further studies were needed to assess the relationship of brain damage to ischaemic and inflammatory processes. The key unanswered question is how much damage is mediated by direct effects of virus on the CNS parenchyma or vasculature (damage that would be expected to persist after the virus is cleared from the CNS), how much is indirect CNS vascular injury mediated by immune activation, and how much is hypoxic-ischaemic damage secondary to the extracranial effects of the virus on the respiratory and cardiovascular systems.

SARS-CoV-2 infects human brain

Both SARS-CoV-2 antigen and RNA have been detected within brain tissue in human post-mortem studies, the antigen mainly within the medulla and lower cranial nerves [28]. SARS-CoV-2 was detected in cerebrospinal fluid from a patient with viral encephalitis [29] and was observed at autopsy in neural and capillary endothelial cells in COVID-19 brain tissue [30]. These observations need to be confirmed in further studies, particularly given the high Ct values used for the PCR detection of viral RNA and the difficulty of electron microscopic interpretation of virus-like particles. Although retrograde axonal transport via the olfactory bulb, associated with anosmia, is a potential route for neuroinvasion, it is likely that the cerebral vasculature plays a more important role in entry of the virus into the CNS. ACE-2, the principal SARS-CoV-2 receptor, is highly expressed by endothelial cells [31] and pericytes throughout the body [32] and analysis of publicly available databases indicates that ACE-2 is also expressed in the brain [33].

Despite apparent low levels of ACE-2 mRNA within the brain [34–37], SARS-Cov-2 infects induced pluripotent stem cell-derived human neural stem and progenitor cells, neurospheres, and cortical neurons with brain organoids (all of which express ACE-2) [38–41]. These data suggest that the mRNA levels do not necessary reflect ACE-2 protein or enzyme activity within the brain, although we would point out that some of the information (e.g. [38–41]) has been published only on preprint servers; peer review may lead to modification of the conclusions. We and others have detected ACE-2 immunohistochemically within the cerebral vasculature in human post-mortem brains [38, 42] and a pre-published study by the Betsholtz lab indicates that ACE-2 is also enriched in brain pericytes [43]. In addition to ACE-2, other docking receptors for SARS-CoV-2 have been identified, including basigin (BSG, CD147) (preprint [44]) and neuropilin (NRP1) (preprint [45]), and these are highly expressed in endothelial cells and pericytes [46]. These receptors may have an important role to play, alongside or separately to ACE-2, in viral entry and disease pathogenesis (reviewed [46]).

Activation of the cerebral endothelium in a range of disease states, including AD, is associated with increased expression of integrins and selectins that are responsible for the attachment, tethering and passage of immune cells across the BBB. This leads to infiltration of brain tissue by immune cells, including neutrophils, monocytes, and lymphocytes, contributing to the pathogenesis of disease (reviewed [47]). In view of the activation of endothelium and inflammatory cell infiltration in lung and other tissues in COVID-19, it is conceivable that activation of the cerebral endothelium and infiltration by immune cells also contributes to neurological disturbance in many patients, although this too remains to be determined.

Pericytes are mural cells located within the basement membrane of microvessels [48] and communicate with endothelial cells to maintain integrity of the BBB [49] and regulate essential vascular functions: blood flow [50] and neurovascular coupling, endothelial transcytosis [51], and angiogenesis [52]. Transcriptomic analysis of murine heart [32, 53] and brain [54–56] indicates that pericytes express high levels of ACE-2 and are therefore likely targets of SARS-CoV-2. Lung biopsies from 4 hospitalised Covid-19 patients revealed a dramatic reduction in pericyte coverage of alveolar capillaries in addition to thickening of the capillary wall [57]. Pericyte degeneration and the consequent disruption of endothelial signalling and homeostasis are likely to be important contributors to vascular instability in COVID-19 [32]. In pericyte-deficient mice (Pdgfrb^ret/ret^), levels of von Willebrand factor, which promotes platelet aggregation and coagulation, are elevated, suggesting that pericyte loss contributes to the pro-angiogenic response in COVID-19 patients [43]. These studies implicate pericyte dysfunction as a mediator of pathophysiology in COVID-19. It is not yet known whether pericytes within the brain degenerate or become dysfunctional in COVID-19 patients with neurological manifestations.

A recent study has provided further evidence of the neuroinvasive potential of SARS-CoV-2 [38]. The authors demonstrated ACE-2-dependent infection of nerve cells within human brain organoids, and hypoxia-like metabolic changes and damage in neighbouring uninfected cells. Expression of humanised ACE-2 within the brain of mice experimentally infected with SARS-CoV-2 caused vascular remodelling throughout the cortex and greatly increased their mortality. The authors examined brain tissue from three COVID-19 patients and reported that SARS-CoV-2 spike protein could be detected immunohistochemically within the walls of small vessels cortical adjacent to microinfarcts. They also reported spike protein immunopositivity in some cortical neurons. However, these findings need to be confirmed.

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The types of cerebral ischaemic damage seen in COVID-19 are major contributors to cognitive decline and dementia

Pre-existing dementia ranks as one of the most significant risk factors, or co-morbidities, in COVID-19. Retrospective assessment of UK health records in the UK OpenSAFELY platform indicated a hazard ratio of 2.16 (fully adjusted model) in association with pre-existing dementia/stroke [58]. An odds ratio of 3.07 was associated with dementia in a UK biobank community study [59]. The reasons for the increased risk and mortality in patients with pre-existing dementia have been well reviewed [60]. What is perhaps not as widely appreciated is that the types of brain damage seen in COVID-19 are themselves major contributors to cognitive decline and dementia.

Ischaemic brain damage is the defining pathological process in vascular dementia (VaD), and stroke is a major risk factor for dementia [61, 62]. Thromboembolic occlusion of cerebral blood vessels, a major complication of DIC, can cause a wide spectrum of neurological deficits, including cognitive impairment or dementia. Single or multiple infarcts, as a result of thromboembolism affecting major cerebral arteries, are estimated to account for approximately 20% of dementia cases associated with stroke [63]. It is probable that acute large cerebral vascular occlusion associated with hypercoagulability in severely affected COVID-19 patients will increase the risk of dementia to some extent.

Small vessel disease (SVD) accounts for about 20% of all strokes [64] but about 80% of stroke-related dementia cases [63], and is the most common cause of vascular cognitive impairment. SVD-associated neuroimaging abnormalities of the white matter and arteriolosclerosis of cerebral microvessels can be demonstrated in about 50% of all patients with dementia [65, 66]. Co-morbidities of SVD include hypertension and diabetes [67] (both also risk factors for severe COVID-19). Hypercoagulability and disseminated intravascular coagulation that affect many patients with severe COVID-19 are likely to reduce perfusion through small intracerebral vessels more than through larger ones. SARS-COV-2 induces endothelial dysfunction [23] and infects vascular beds in multiple tissues [24]. Cerebral white matter is particularly vulnerable to changes in cerebral blood flow, as would be expected in association with diffuse small vessel dysfunction and has been reported in COVID-19 [68–70]. The integrity of subcortical white matter is critically important for maintenance of cognitive function [71, 72], and one of the consequences of white matter damage in COVID-19 is likely to be cognitive impairment. This was highlighted by the neuroradiological demonstration that damage to white matter and disruption of functional integrity in brain regions such as the hippocampus, at 3-month follow-up in recovered COVID-19 patients, was associated with memory loss [9].

The pathophysiology of SVD remains incompletely understood but damage to endothelial cells and pericytes, as well as BBB leakiness, are contributors to SVD-related brain injury (reviewed [66, 73]), which are likely to be exacerbated in severe COVID-19. Endothelial dysfunction and pericyte loss are associated with the cerebral influx and accumulation of toxic constituents of the plasma, such as fibrinogen, leading to oligodendrocyte damage and myelin loss [74]. Fibrinogen-mediated activation of the bone morphogenetic protein signalling pathway prevents maturation of oligodendrocyte precursor cells and restricts oligodendrocyte maturation and remyelination [75]. The infiltration of immune cells across a damaged BBB may also contribute to white matter damage and cognitive decline in dementia and perhaps COVID-19 (reviewed [47]). In addition, endothelial dysfunction and loss of pericytes are likely to impair the clearance of cerebral metabolites, including Aβ peptides, that are toxic when present in excess. Impaired drainage of metabolites including Aβ is implicated in the development of cerebral amyloid angiopathy and Alzheimer's disease [76, 77] and ineffective drainage of solutes probably accounts for enlargement of perivascular spaces in patients with SVD (reviewed [78, 79] and cerebral amyloid angiopathy ([80–82]).

Several other factors may impact on cerebral perfusion during systemic infection. Increased blood viscosity tends to slow capillary transit and limit oxygen delivery. Damage to the glycocalyx, a carbohydrate-enriched matrix on the luminal side of capillaries may impair perfusion and exacerbate ischaemia [83, 84]. Many of the deleterious alterations to small vessels in systemic infection are exacerbated by the same risk factors that predispose to severe COVID-19, such as ageing, hypertension, diabetes, and obesity.

Post-mortem and neuroimaging studies indicate that ischaemic damage to the cerebral white matter is present in up to two thirds of patients with AD [85–87]. Although cerebral amyloid angiopathy may contribute to the damage, in most cases the ischaemia-related damage probably results from a combination of arteriolosclerotic SVD and non-structural vascular dysfunction (reviewed [88]). A series of recent neuroimaging studies has shown that ischaemic white matter damage occurs at a very early stage of AD, accelerates progression of the disease and contributes to the cognitive decline [89–93]. These clinical observations are supported by experimental studies showing that brain ischaemia accelerates Aβ accumulation, through a combination of dysregulated processing of amyloid-β precursor protein (APP) and impaired Aβ clearance (reviewed [87]) and that, in turn, Aβ peptides mediate vasoconstriction, by inducing contraction of pericytes [94] and vascular smooth muscle cells [95]. Studies of microvascular endothelial cell monolayers [96, 97], human APP transgenic mouse models [98], and

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on human post-mortem brain tissue [99] indicate that Aβ peptides also impair BBB function, in part by reducing expression of tight junction proteins. Pericyte degeneration in AD is associated with BBB breakdown [100–102]. Pericyte loss accelerates Aβ pathology and induces tau pathology and cognitive decline in human APP mice [103]. In human brain tissue from AD patients, a decline in the level of the pericyte marker, platelet-derived growth factor-β (PDGFRβ), was associated with increased Aβ level and reduced cerebral perfusion [104]. Aβ peptides are toxic to human brain pericytes in culture [105] and analysis of cerebrospinal fluid indicates that the level of soluble PDGFRβ, a marker of pericyte injury and BBB leakiness, is elevated in the elderly in association with the earliest detectable changes in cognitive performance [89, 90].

There is increasing evidence that cerebral hypoperfusion is also linked to tau pathology. In clinically normal adults with positron emission tomography (PET) evidence of cerebral Aβ accumulation, those who also had an increased cardiovascular disease risk score were significantly more likely to show evidence of tau accumulation as well [106]. In patients with mild cognitive impairment, increased cerebrovascular burden was associated with elevated PET-Tau signal and worse cognitive performance, independently of Aβ-PET [107]. Several experimental studies have shown that modelling of cerebral hypoperfusion increases tau phosphorylation: in adult Wistar rats [108], transgenic mice with Aβ and tau accumulation [109], and rat and human brain slices exposed to oxygen and glucose deprivation [110]. In a recent autopsy study, elevated levels of soluble tau and insoluble phospho-tau were associated with lower levels of endothelial tight junction proteins, claudin-5 and occludin, in AD [111]. Tau-overexpressing mice were shown to have abnormal blood vessel morphology and increased vessel density [112]. A recent study found impaired neurovascular coupling, prior to neurodegeneration, in young (2–3 months) mice expressing mutant tau [113]. There are therefore clinical and experimental data suggesting a bidirectional relationship between cerebral vascular dysfunction and pathological tau. There is evidence from recent studies that TDP-43 pathology, likewise, is associated with cerebral vascular dysfunction, including pericyte loss and small vessel disease [114, 115]. Lastly, cerebral ischaemia induces phosphorylation of α-synuclein at serine-129; this is the predominant disease-related modification of α-synuclein in Lewy bodies and neurites in Parkinson disease, and dementia with Lewy bodies, and also is significantly associated with concomitant AD pathology in these Lewy body diseases [116].
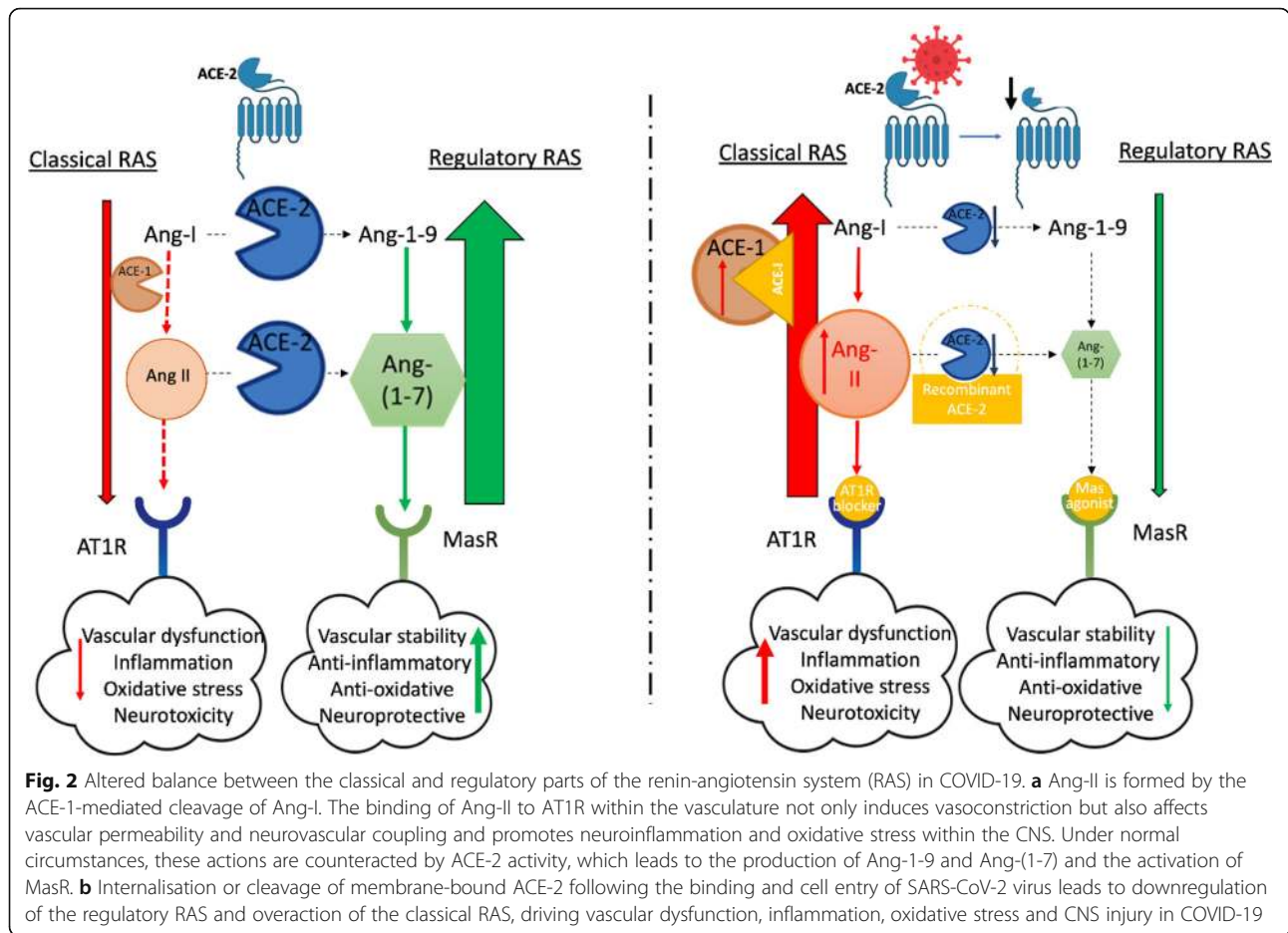
Both cerebral ischaemia and systemic inflammation can induce endothelial activation, with increased expression of integrins and selectins leading to adhesion and transendothelial migration of leukocytes into the brain parenchyma. Endothelial activation, with recruitment of leukocytes, has also been shown in AD [47, 117]. Leukocytes enter the brain through post-capillary venules within the parenchyma, and to a lesser extent, vessels in the leptomeninges and choroid plexus. Activated neutrophils in cerebral vessels and parenchyma were found to contribute to gliosis and cognitive impairment in human APP mice [118]. In APP/PS1 mice, respiratory infection increased infiltration of the brain by interferon γ- and interleukin-17-producing T cells and natural killer T cells, accompanied by gliosis and enhanced deposition of Aβ [119]. Monocytes are the most common type of peripheral immune cell that migrates via the BBB into the brain in AD. CCL2, the major ligand for CCR2 expressed on monocytes, is upregulated in microvessels in AD and plays a role in Aβ clearance (reviewed [47]). Whether endothelial activation predicts not only acute but also longer neurological complications (including dementia) in COVID-19 remains to be determined.

Angiotensin-converting enzyme-2-mediated entry of SARS-CoV-2 into human cells and activation of the classical renin-angiotensin system

SARS-CoV-2 cell attachment and entry are initiated by binding of the virus to angiotensin-converting enzyme-2 (ACE-2) [120]. The expression of ACE-2 at the cell surface is therefore likely to be a critical determinant of viral tropism and pathobiology in COVID-19. ACE-2 is expressed in stem cell-derived neurons [121] and in neuronal and glial cells within the brain [33, 42], potentially enabling virus entry and spread through the cribriform plate by retrograde axonal transport along olfactory nerves [33], or from sensory fibres that pass from the lungs to the brain stem via the vagal nerve and nodose ganglion [4]. ACE-2 is also expressed in the temporal lobe and hippocampus—brain regions that are involved in cognition and memory and are affected in AD [122]. Neuronal uptake and spread within the brain were demonstrated in human ACE-2 transgenic mice infected with SARS-CoV-1 [123] and SARS-CoV-2 [124]. However, as noted above, ACE-2 is also highly expressed on endothelial cells and pericytes, and haematogenous spread followed by endothelial uptake or influx of infected peripheral immune cells are further possible routes of entry of virus into the brain.

During SARS-Cov-1 infection, ACE-2 is cleaved from the cell surface [125] by ADAM-17 [126] during viral entry [127]. Although likely, it remains to be determined whether SARS-CoV-2 similarly results in loss of cell membrane-associated ACE-2. Ordinarily, ACE-2 is a key effector of the regulatory RAS that counters the actions of the classical RAS and reduces the risk of cardiovascular disease (reviewed [128, 129]), stroke [130–132], and dementia [133] (Fig. 2). ACE-2 is reduced in Alzheimer's disease (AD) [42] and cognitive decline is pronounced in ACE-2 knock-out mice [134]. If SARS-CoV-2 entry leads

Fig. 2 Altered balance between the classical and regulatory parts of the renin-angiotensin system (RAS) in COVID-19. **a** Ang-II is formed by the ACE-1-mediated cleavage of Ang-I. The binding of Ang-II to AT1R within the vasculature not only induces vasoconstriction but also affects vascular permeability and neurovascular coupling and promotes neuroinflammation and oxidative stress within the CNS. Under normal circumstances, these actions are counteracted by ACE-2 activity, which leads to the production of Ang-1-9 and Ang-(1-7) and the activation of MasR. **b** Internalisation or cleavage of membrane-bound ACE-2 following the binding and cell entry of SARS-CoV-2 virus leads to downregulation of the regulatory RAS and overaction of the classical RAS, driving vascular dysfunction, inflammation, oxidative stress and CNS injury in COVID-19

to loss of ACE-2 (as in SARS), angiotensin II-mediated classical RAS activation would increase the risk of cerebrovascular and neurological disturbances in COVID-19 patients. This mechanism has also been proposed to explain other vascular and pulmonary manifestations of COVID-19 [135, 136]. Internalisation of ACE2, as a result of angiotensin II activation of angiotensin receptor type 1 (AT1R), may further exacerbate damage [137]. The available reservoir of ACE-2 may be an important determinant of clinical outcome in COVID-19. Studies in rodents indicate that ACE-2 expression declines with age and is lower in males [138–141]. In contrast, oestrogen upregulates ACE-2, which could help to protect pre-menopausal women against severe complications of COVID-19 [142, 143]. It may be relevant that most co-morbidities that increase the risk of complications in COVID-19, including hypertension, obesity, and diabetes, are associated overactivity of the classical RAS. Indeed ethnicity and genetic variations also influence baseline ACE-2 levels [144] and could provide a biological explanation as to why some ethnic groups are at higher risk of COVID-19 [145]. The hypothesis would explain why angiotensin receptor blockers (ARBs) and

angiotensin-converting enzyme-1 inhibitors (ACE-Is), which downregulate the classical RAS by either blocking angiotensin II (Ang-II) signalling or Ang-II synthesis, respectively, and upregulate ACE-2, may reduce mortality in COVID-19 patients [146, 147].

An imbalance in RAS contributes to acute ARDS (reviewed [148]), which develops in a high proportion of the COVID-19 patients who develop viral pneumonia [149, 150]. ARDS patients show overactivity of the classical RAS [151] and reduction of ACE-2 [152, 153]. Increased Ang-II-mediated AT1R signalling, which drives inflammation [154], is likely to contribute to the inflammatory storm in severe COVID-19. Lung tissue damage in ARDS can be reduced by ARBs and ACEIs [155] and by administration of recombinant ACE-2 [152, 156]. ACE-2 catalyses the formation of Ang-(1-7), which binds to Mas receptor; both ACE-2 activation [157] and Ang-(1-7) activation of Mas receptor attenuate lung injury in ARDS [158]. Ang-(1-7) activates ERK1/2 signalling and modulates interleukin (IL)-10 expression, protecting against lung damage (reviewed [159]).

Recombinant soluble ACE-2 (rsACE-2) shows therapeutic promise in severe COVID-19 infection; administration was reported to reduce viral titre and serum Ang-II,

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elevate serum Ang-(1-7) levels and markedly reduce pro-inflammatory cytokines [160]. In addition to preventing viral binding, rsACE-2-mediated reduction in Ang-II is likely to prevent AT1R-mediated ADAM-17 cleavage of membrane-bound ACE-2 and restore balance in the RAS [161].

Potential neurological consequences of increased classical renin-angiotensin system activation in COVID-19

The RAS is expressed and functions independently within the brain. Overactivation of the classical RAS, with elevated ACE-1 and Ang-II, has been demonstrated in post-mortem human brain tissue in AD [162–164]. Cerebroventricular infusion of Ang-II into adult Wistar rats promoted Aβ production and tau pathology [165, 166], and ARBs and ACEIs protect against cognitive decline and disease pathology in transgenic APP mouse models of AD (reviewed [167]). We previously reported that reduction of ACE-2 in brain tissue in AD correlated strongly with parenchymal Aβ and tau levels and with increased ACE-1 activity [42]. We and others have since shown that induction of ACE-2, or administration of Ang-(1-7) or peptide analogues, protects against Aβ-related cognitive decline in mice associated with reduced neuroinflammation and oxidative stress [133, 168, 169].

The RAS is a critical regulator of vascular function. Ang-II binds to AT1R on vascular smooth muscle cells [170, 171] to induce cerebral artery constriction [172], and on pericytes to cause constriction of microvessels [173, 174]. Ang-II also modulates BBB permeability: AT1R signalling induces leakiness in endothelial cell culture models of the BBB [175] and Ang-II infusion causes BBB leakiness in mice that can be reversed by adding a superoxide scavenger, indicating a role for oxidative stress [176]. Several mediators of BBB leakiness, including vascular endothelial growth factor and matrix metalloproteinase (MMP)-2 and MMP-9, are induced by Ang-II [177–179]. In mice, Ang-II was shown to impair neurovascular coupling (i.e. the blood flow response to increased neural activity) in the somatosensory cortex [180] and to interfere with cerebral autoregulation [181, 182]. The accumulation of ACE-1 in the extracellular matrix around cerebral arterioles (particularly in AD patients with cerebral amyloid angiopathy) suggests that locally produced (as well as circulatory) Ang-II participates in cerebrovascular dysfunction mediated by overactivation of the classical RAS [163].

Overactivation of the classical RAS may also reduce clearance of Aβ. Intra-mural peri-arterial drainage (IPAD) and para-vascular glymphatic channels have been implicated in the removal of Aβ from the brain [183–185]. The functioning of these drainage pathways depends on the polarised expression of aquaporin-4 in astrocytic endfeet [186], which is regulated by pericytes [187]. Focal absence of pericytes results in the redistribution of aquaporin-4 to the cell soma [51]. The RAS modulates aquaporin-4 expression in astrocytes [188], and Ang-II was shown to act via AT1R to reduce ACE-2 expression in astrocyte cultures [189]. These alterations in pericyte and astrocyte function are likely to impair the clearance of Aβ. It remains to be determined whether this occurs in COVID-19.

Neuroinflammation is strongly implicated in the development of AD. Genome-wide association studies have identified several inflammatory pathway genes as risk factors for AD [190, 191]. Complement [192] and inflammasome activation [193, 194] are likely to contribute to cerebrovascular dysfunction, neuronal toxicity, and the accumulation of Aβ and tau in AD. Ang-II activates the complement system [195, 196] and the NLRP3 inflammasome [197], and both complement [198] and inflammasome activation [11] have been proposed to contribute to neurological disease in COVID-19 patients. A recent in silico study implicated SARS-CoV-2 activation of Toll-like receptor 4 (TLR4) as a major contributor to the inflammatory response in COVID-19 [199]. Ang-II upregulates TLR4 [200], which is a critical determinant of Ang-II-mediated vascular remodelling [201]. Blocking of TLR4 signalling delayed the development of Ang-II-mediated hypertension in rats and was associated with a dramatic increase in ACE-2 [202]. Pericytes express high levels of TL4R, activated by free long-chain fatty-acids [203]. The spike protein of SARS-CoV-2 has been shown to bind to linoleic acid, affecting the conformation of the protein and possibly the binding of the virus to ACE-2 (pre-published study [204]). It may therefore be relevant that linoleic acid is reduced in both COVID-19 [205] and AD [206], potentially influencing the progression of both diseases. Ang-II also acts as a molecular switch regulating microglial phenotype—switching between an M1 (pro-inflammatory) and M2 (immunoregulatory) protective phagocytic phenotype [207] which is relevant to AD pathogenesis [208]. The role of microglia in neurological manifestations of COVID-19 has yet to be fully explored.

Ang-II-mediated endothelial activation promotes the binding and diapedesis of leukocytes across the BBB; these effects are mitigated by Ang-1-7 [209]. Pericytes too have immune-regulating properties (reviewed in [210]) and their localisation within the cerebral vasculature suggests that they may serve a 'gate-keeper' role in regulating immune cell infiltration. Although pericytes express ACE-2, it remains to be established whether they are targeted by SARS-CoV-2. Because of the pivotal role of pericytes in regulation of cerebrovascular function (and perhaps immune cell infiltration), it is likely that virus-induced pericyte damage would compromise cerebral perfusion, BBB integrity, and immune regulation. In

addition to vascular effects, angiotensin peptides derived from Ang-II, including Ang-IV and Ang-(1-7), have neuromodulatory [211] (reviewed [212]) and neuroprotective properties (reviewed [213]). Ang-(1-7) activation of Mas (regulatory RAS) receptor and Ang-IV activation of the c-Met and insulin-regulated aminopeptidase receptors (reviewed [214]) limit tissue damage in models of stroke (reviewed [215, 216]). Similarly, ACE-2 activation and/or Ang-(1-7) infusion prevents cognitive decline and disease pathology in animal models of Aβ accumulation [133, 169, 217, 218], independent of changes in blood pressure. There is therefore a wide range of mechanisms through which reduced regulatory RAS signalling may exacerbate brain damage in COVID-19.

Is APOE ε4, an established risk factor for AD and vascular dysfunction, also a risk factor for COVID-19?

APOE polymorphism greatly influences the risk of developing AD: the risk is increased with *APOE* ε4 and decreased with *APOE* ε2 [219, 220]. The physiological roles of the encoded apolipoprotein (apolipoprotein E, ApoE) have still not been fully defined. Recent studies indicate that possession of *APOE* ε4 is associated with cerebrovascular dysfunction, including BBB leakiness and pericyte degeneration [221] and cerebral amyloid angiopathy with capillary involvement [222]. A recent UK study reported that there was a higher prevalence of COVID-19 in people who were carriers of *APOE* ε4 [223]. We previously showed that *APOE* ε4 individuals also have lowest ACE-2 activity [42]. Pericyte expression of *APOE* ε4 was reported to promote BBB leakiness because of deficient basement membrane formation [224]. Moreover, possession of *APOE* ε4 is associated with reduced cerebral blood flow and increased subcortical ischaemic white matter damage [225, 226], as well as neuroinflammation in AD (reviewed [227]). Future studies should aim to clarify the relationships between *APOE* ε4, COVID-19, and cerebral vascular dysfunction and AD.

Potential upregulation of ADAM-17 in COVID-19

ACE-2 is cleaved by ADAM-17 upon SARS-Cov-1 entry into cells [125–127]. It seems likely that this also occurs upon SARS-Cov-2 cell entry, although the data are not yet available. Ang-II-mediated activation of ADAM-17 and shedding of ACE-2 points to a positive feedback loop in which increased Ang-II level is associated with loss of ACE-2 [228]. Yet, it is worthy of note that ADAM-17 cleaves many cell-associated proteins that are required for proper function of the vasculature including the ApoE receptor, low-density lipoprotein receptor-related protein 1 (LRP-1), involved in transendothelial clearance of Aβ (facilitated by ApoE), and PDGFRβ, needed for maintenance of pericytes. Upregulation of ADAM-17 may therefore potentially exacerbate vascular

dysfunction in COVID-19. ADAM-17 also acts as one of the α-secretases and cleaves APP, preventing the generation of Aβ (reviewed [229]). The complex divergent roles of ADAM-17 in AD and potentially COVID-19 require further investigation.

Clinical management, clinical trials and possible future targets for therapeutic intervention in COVID-19 patients

In severe COVID-19, patients present with pneumonia and those most severely affected develop ARDS with features of septic shock and multiple organ failure, requiring oxygen treatment and/or mechanical ventilation. Infection-induced inflammatory and vascular changes associated with coagulopathy and thrombosis, including venous thromboembolism (VTE), DIC and thrombotic microangiopathy with thromboembolic microvascular complications, are common complications of severe COVID-19, as illustrated by reports of VTE in 25–27% hospitalised patients [230, 231]. The International Society on Thrombosis and Hemostasis (ISTH) recommends measuring levels of d-dimer, prothrombin time, partial thromboplastin time and platelet count, in hospitalised COVID-19 patients [232]. A retrospective study in Wuhan, China, at the beginning of the pandemic, found that mortality rates were lower in patients given low-molecular weight heparin [233]. Clinical management of severe COVID-19 patients now routinely includes low-dose sub-cutaneous heparin [233] and/or thrombotic prophylaxis [234], unless patients are at increased risk of bleeding.

Lymphopenia with marked loss of regulatory T and B cells and natural killer cells, reduction in monocytes, eosinophils and basophils, and an increase in neutrophils are typical in severe COVID-19 (reviewed [235]). There are also elevated levels of pro-inflammatory cytokines, sometimes marked (a so-called cytokine storm). Convalescent plasma [236] and plasma exchange [237, 238] improve survival rates in severe disease, and immunomodulatory therapies such as tocilizumab, a monoclonal antibody against the IL-6 receptor [239], and sarilumab, an IL-6 receptor antagonist [240], may offer protection and are currently undergoing clinical trials. Neutralising antibodies targeting other pro-inflammatory cytokines (IL-1, IL-17) may also offer protection, as too may potential inhibitors of complement system activation. Intravenous transplantation of mesenchymal stems cells was shown to improve the outcome of COV-19 patients with pneumonia in 7 COVID-19 patients in Beijing, China [241]. It seems possible that mesenchymal stem cells might also ameliorate brain injury in severe COVID-19, given their immunomodulatory and anti-inflammatory properties, and ability to attenuate BBB damage and neuroinflammation after cerebral ischaemia [242–244].

There is continued debate on the role of systemic or inhaled corticosteroids in COVID-19 patients. Although earlier studies indicated a lack of benefit from corticosteroids [245], a randomised clinical trial reported by the RECOVERY collaborative group, Oxford, UK, found that systemic dexamethasone reduced mortality in severely affected COVID-19 patients [246]. Inhaled steroids had previously been shown to reduce inflammation and tissue injury in ARDS [247] (reviewed [248]). In addition to their inherent anti-inflammatory properties, steroids may have anti-viral properties [249]. Ciclesonide, an inhaled corticosteroid, was shown to suppress replication of MERS-CoV, SARS-CoV and SARS-CoV-2 in vitro [250].

The expression and activity of interferon-β (IFN-β), an endogenous protein with anti-viral and anti-inflammatory properties, is impaired in COVID-19 [251]. Interferon inhibits SARS-CoV-2 replication in vitro [252]. In a Phase II clinical trial, IFN-β in combination with anti-viral drugs shortened the duration of viral shedding and length of hospital admission [253]. A pharmaceutical company based in the UK, Synairgen, reported a lower risk of requiring ventilation, and reduction in mortality by about 79%, in a Phase II clinical trial of SNG001, an inhaled form of IFN-β (these data are currently unpublished).

Since ACE-2 is a receptor for SARS-CoV-2, and ACEIs and ARBs are predicted to increase ACE-2 expression, it was initially feared that the use of these drugs might exacerbate COVID-19 [254]. Recent meta-analyses have instead suggested that RAS-targeting medications are protective in COVID-19 [146, 147, 255]. This is likely to be a due to the protective role of ACE-2 in lowering or preventing overactivation of the classical RAS and minimising consequent Ang-II-mediated ischaemic and inflammatory damage, as outlined above. Several clinical trials have been registered with the National Institutes of Health (NIH) to test ARBs such as losartan in COVID-19 patients: NCT04335123, NCT04312009 and NCT04311177. Two studies are also investigating the impact of discontinuation of ACE-Is on COVID-19 (EudraCT numbers 2020-001544-26 and 2020-001206-35). Boosting the regulatory arm of the RAS may ameliorate COVID-19 because of the protective effects of ACE-2 and Ang-(1-7); interventional trials with recombinant human ACE2 (rhACE2) and Ang-(1−7) have also been registered (NCT04287686 and NCT04332666, respectively), although the rhACE2 study seeking to recruit people between the ages 18–80 years in China has since been withdrawn. A further rhACE2 study (2020-001172-15) is registered on the EU Clinical trials register. Other strategies being explored include several studies seeking to inhibit TMPRSS2. Studies that could include TL4R blockers and ADAM-17 inhibitors might also be worthy of future study. For a comprehensive review of the pharmacological targets that are currently being investigated as potential interventions and treatments in COVID-19, the reader should refer to recent reviews [256–258].

Conclusion

Cerebral vascular disease is emerging as a major complication of severe COVID-19. This is likely to cause lasting brain damage and to increase the risk of stroke and vascular cognitive impairment. Several of the metabolic abnormalities that affect COVID-19 patients may also increase the risk of developing AD. Dementia and COVID-19 share many co-morbidities and risk factors, including age, gender, hypertension, diabetes, obesity and possession of *APOE* ε4—most of which are associated with an overactive RAS, cerebrovascular dysfunction and neuroinflammation. These shared co-morbidities and similar mechanisms may also explain the high incidence and increased rates of mortality amongst people with dementia [59, 259, 260]. There is urgent need for research to better understand the pathogenesis of neurological disturbances in COVID-19, some of which have probably been covert and the prevalence of which may be considerably underestimated. This understanding is essential to establish the long-term consequences from the disease (including the potential for increased risk of dementia in some cases) and to identify means of preventing or ameliorating the brain damage.

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