



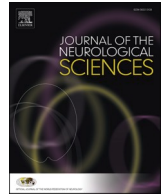
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Clinical short communication

Susceptibility-weighted imaging reveals cerebral microvascular injury in severe COVID-19

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ABSTRACT

We evaluated the incidence, distribution, and histopathologic correlates of microvascular brain lesions in patients with severe COVID-19. Sixteen consecutive patients admitted to the intensive care unit with severe COVID-19 undergoing brain MRI for evaluation of coma or neurologic deficits were retrospectively identified. Eleven patients had punctate susceptibility-weighted imaging (SWI) lesions in the subcortical and deep white matter, eight patients had >10 SWI lesions, and four patients had lesions involving the corpus callosum. The distribution of SWI lesions was similar to that seen in patients with hypoxic respiratory failure, sepsis, and disseminated intravascular coagulation. Brain autopsy in one patient revealed that SWI lesions corresponded to widespread microvascular injury, characterized by perivascular and parenchymal petechial hemorrhages and microscopic ischemic lesions. Collectively, these radiologic and histopathologic findings add to growing evidence that patients with severe COVID-19 are at risk for multifocal microvascular hemorrhagic and ischemic lesions in the subcortical and deep white matter.

1. Introduction

Hundreds of thousands of people worldwide have survived severe coronavirus disease 2019 (COVID-19) since the beginning of the pandemic in December 2019 [1]. For survivors, recovery of brain function may lag behind that of other organs [2,3]. Hypercoagulability, inflammation, hypoxia, and endothelial cell infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are potential mechanisms contributing to global brain dysfunction [4]. Although brain imaging studies in patients with neurological manifestations of severe

COVID-19 are rapidly emerging [5–14], performing and interpreting MRI in this critically ill population remains challenging, and fundamental questions about the pathogenesis of brain injury in these patients remain.

We investigated the imaging characteristics of COVID-19 brain injury by performing ultrafast high-resolution susceptibility-weighted imaging (SWI) MRI [15] on a clinical MRI scanner located in our Neurosciences Intensive Care Unit (ICU). We focused on SWI because emerging evidence suggests that COVID-19 patients are at risk for microvascular lesions [8–10,16], and the SWI sequence provides

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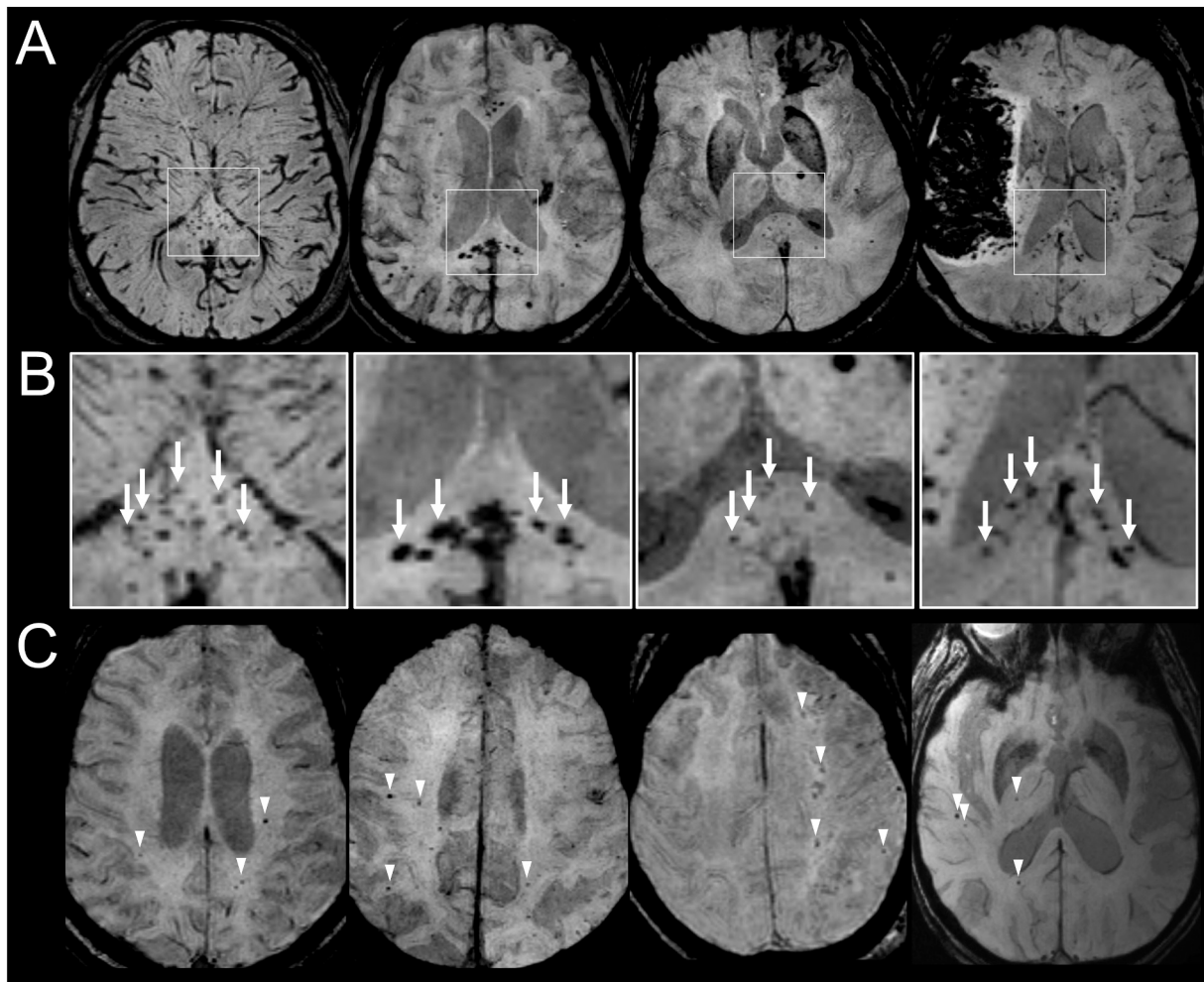


Fig. 1. Representative susceptibility-weighted images from critically ill COVID-19 patients. (A) Four patients with susceptibility-weighted imaging (SWI) lesions including clustered lesions involving the corpus callosum. Patient 1 is shown in the first column. (B) Magnification views of the splenium of the corpus callosum for each patient shown in A, showing multiple SWI lesions (arrows). (C) Four patients with diffuse SWI lesions (arrowheads) involving the subcortical, periventricular and deep white matter, without involvement of the corpus callosum.

optimal sensitivity for detecting microvascular lesions based upon their paramagnetic susceptibility effects in an MRI scanner's magnetic field [15]. In a cohort of 16 consecutively imaged critically ill patients with COVID-19, we tested the hypothesis that COVID-19 is associated with cerebral microvascular injury detectable by SWI. In one patient who underwent autopsy, we performed correlative radiologic-pathologic analyses and cerebrospinal fluid (CSF) SARS-CoV-2 polymerase chain reaction (PCR) to explore the mechanisms of COVID-19 cerebral microvascular injury.

2. Methods

2.1. Patients

Chart review identified all patients at our hospital who met the following inclusion criteria: (1) MRI performed on our Neurosciences ICU scanner between March 12 and May 14, 2020; (2) confirmed COVID-19 diagnosis by reverse transcriptase (RT)-PCR nasopharyngeal swab; and (3) severe COVID-19, as recently defined [17]. Specifically, severe Covid-19 is defined as dyspnea, a respiratory rate of 30 or more breaths per minute, a blood oxygen saturation of 93% or less, a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO₂:FiO₂) of less than 300 mmHg, or infiltrates in more than 50% of the lung field within 24 to 48 h from the onset of symptoms. There were

no exclusion criteria. Clinical characteristics and laboratory data were collected for all patients. This retrospective study was HIPAA compliant and was approved by the institutional review board with a waiver of consent.

2.2. Imaging

All patients underwent brain imaging on a 3 Tesla Skyra MRI scanner (Siemens Healthcare) using an ultrafast 3D SWI sequence [15] with the following parameters: TE/TR = 21.5/40 ms, voxel dimensions = $0.9 \times 0.9 \times 1.8 \text{ mm}^3$, Wave-CAIPI acceleration $R = 6$, total scan time 100 s. Conventional T1, T2, fluid-attenuated inversion recovery (FLAIR), and diffusion-weighted images (DWI) were also obtained. Two board-certified neuroradiologists (J.C., S.Y.H.) independently reviewed the SWI data. Foci of abnormal susceptibility signal were counted using an abbreviated version of the Microbleed Anatomical Rating Scale [18]. The raters counted up to 10 lesions per anatomical location, after which they recorded "greater than 10". We resolved discrepancies between the radiologist ratings by consensus.

2.3. Brain autopsy and microscopy

An autopsy was performed for Patient 1, who died on hospital day 16. Routine microscopic analysis of the cerebral and cerebellar gray

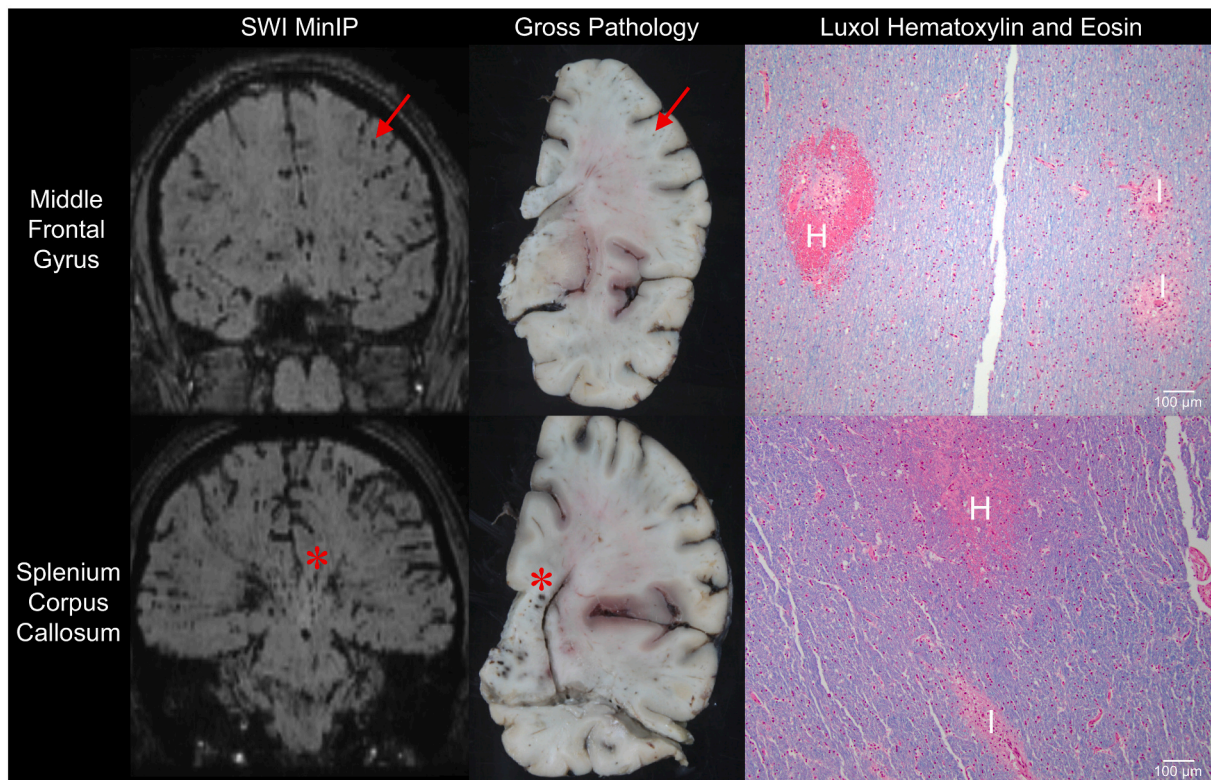


Fig. 2. Radiology-pathology correlations in a patient who died from severe COVID-19. Susceptibility-weighted minimum-intensity projection images (SWI MinIP) are shown for the Patient 1 in the coronal plane at the level of the middle frontal gyrus (top row) and splenium of the corpus callosum (bottom row). Representative punctate hypointense foci are indicated by a red arrow (middle frontal gyrus) and asterisk (splenium of the corpus callosum). Gross pathologic analysis reveals corresponding lesions in coronal tissue slabs, as indicated by the red arrow and asterisk, respectively. On microscopic analysis of tissue stained with Luxol hematoxylin and eosin (LH&E), the lesions seen on SWI MinIP and gross pathology are characterized by microhemorrhages (H) and microscopic ischemic lesions (I). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

matter and white matter was performed after approximately two weeks of fixation in buffered formalin and standard paraffin embedding with Luxol and hematoxylin-and-eosin (LH&E) staining. Immunohistochemical staining was performed with a Leica Bond III automated stainer, with mouse monoclonal antibodies directed against neurofilament (Dako M0762, 1:3200), CD3 (Leica PA0553, used as supplied), CD163 (Leica Cat #PA 0090, used as supplied) and CD68 (Biocare Cat # IP-033-AA, also used as supplied).

2.4. SARS-CoV-2 cerebrospinal fluid PCR

For the Patient 1, extracted RNA was tested for the presence of SARS-CoV-2 using a probe-based quantitative RT-qPCR assay (see Supplementary Material for details). The RNA extraction from CSF was performed under a research protocol with IRB approval and waiver of patient consent. The CSF sample was obtained during a lumbar puncture that was performed as part of the patient's clinical care.

2.5. Statistical analysis

Clinical and laboratory variables were compared between (1) patients with >10 SWI hypointense lesions, and (2) patients with <10 SWI hypointense lesions, using an unpaired *t*-test for normally distributed variables and Mann-Whitney *U* test for variables that were not normally distributed. *P*-values less than 0.05 were considered significant.

3. Results

A total of 154 patients underwent brain MRI on our Neurosciences

ICU scanner during the study period. Twenty-one patients tested positive for COVID-19 prior to the time of brain MRI, and 16 met clinical criteria for severe COVID-19 and were included in the study (Supplementary Table 1). All 16 study patients had acute respiratory distress syndrome (ARDS) and were mechanically ventilated at the time of MRI. MRI was performed because of unresponsiveness in 11 cases, and focal neurologic deficits in five cases. Punctate foci of abnormal susceptibility signal were identified in 11/16 cases (69%), and 8/16 (50%) had >10 lesions (Fig. 1, Supplementary Table 2).

In 4/16 cases (25%), multiple clustered lesions involved the corpus callosum. In the remaining 4/16 cases with >10 lesions, the lesions showed a predilection for subcortical and deep white matter, with variable involvement of the brainstem and cerebellum. Additional MRI findings are summarized in Supplementary Table 3.

4. Clinical and radiologic-pathologic correlations for patient 1

Patient 1 was a 57-year-old man with a history of asthma, obesity, and hyperlipidemia who tested positive for SARS CoV-2 by nasopharyngeal RT-PCR swab. His only COVID-19 related symptom on admission was shortness of breath, and he walked into the urgent care clinic. His hospital course was notable for hypoxic respiratory failure requiring mechanical ventilation and pupillary dilatation during weaning of sedation on hospital day 13. Head CT showed diffuse white matter hypoattenuation with preservation of gray-white matter differentiation and no downward herniation. Lumbar puncture was performed on day 14, and CSF analysis showed normal white blood cell count (1 cell/ul) and elevated total protein (181 mg/dL). RT-qPCR for the SARS-CoV-2 nucleocapsid protein targets (N1 and N2) performed on CSF was

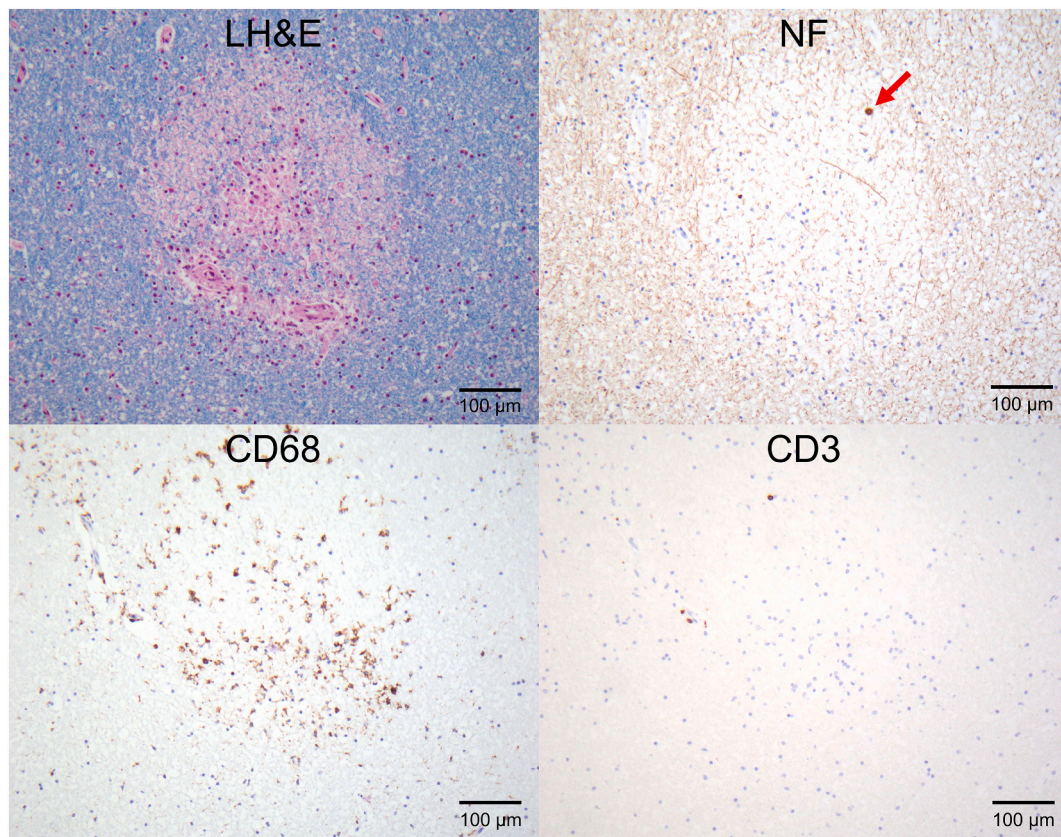


Fig. 3. Pathology of ischemic lesions in a patient who died from severe COVID-19. Microscopic analyses of cerebellum at 200 \times magnification are shown for Patient 1. In the top left panel, a representative focus of microscopic ischemic lesion with significant loss, but not absence, of myelin is seen with Luxol hematoxylin and eosin (LH&E) stain. In the top right panel, neurofilament (NF) immunohistochemistry stain of the same lesion shows a significant loss, but not absence, of axons with rare axonal spheroids (red arrow). In the bottom left panel, a CD68 immunohistochemistry stain demonstrates aggregates of macrophages associated with the ischemic lesion. In the bottom right panel, a CD3 immunohistochemistry stain shows only rare CD3-positive T cells, indicating an absence of significant inflammation associated with the lesion. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

negative.

Brain MRI on day 15 revealed numerous SWI hypointense foci (Fig. 1, left column), predominantly involving the subcortical and deep white matter, e.g., corpus callosum, internal capsules, and cerebellar white matter, as well as diffuse DWI and T2/FLAIR hyperintensity throughout the white matter. Life-sustaining therapy was withdrawn on hospital day 16, and he died shortly thereafter (see Supplementary Material for clinical details).

At autopsy, gross pathology revealed a diffusely edematous brain weighing 1410 g (within normal range), with uncal and tonsillar herniation and diffuse discoloration of the gray-white matter junction. Numerous punctate hemorrhages were observed at the cortical gray-white matter junction and in the deep white matter.

Radiologic-pathologic correlations of the microvascular lesions are shown in Fig. 2. Focused evaluation of the microvascular lesions showed microscopic foci of extravasated red blood cells associated with apparently necrotic blood vessels and foci of ischemic injury. The latter demonstrated significant loss, but not absence, of both axons and myelin, with occasional scattered axonal spheroids within the lesions and an associated macrophage/microglial response without other significant inflammatory cell components (Fig. 3). Associated with the vessels nearest to the ischemic lesions, there was a moderate accumulation of microglia (CD163) and macrophages (CD68) (Fig. 3). Detailed pathological findings are reported in the Supplementary Material.

5. Discussion

In this study of 16 critically ill patients with COVID-19 who

underwent brain MRI because of persistent unresponsiveness ($n = 11$) or focal neurologic deficits ($n = 5$), we detected diffuse microvascular injury involving the subcortical and deep white matter in 69% of patients. Microvascular lesions manifested as punctate and linear hypointense foci on SWI, with a neuroanatomic predilection for the corpus callosum and the subcortical and deep white matter. There were no significant differences in clinical or laboratory variables between patients with and without SWI lesions. In one patient who died and underwent brain autopsy, the lesions showed mixed pathology: microhemorrhages and microscopic ischemic lesions. While the hemorrhagic lesions could be identified by SWI, no definitive imaging correlate to the microscopic ischemic lesions was observed, likely due to their small size relative to the spatial resolution of clinical DWI. Collectively, these findings suggest that cerebral microvascular lesions are common in severe COVID-19 patients who have neurologic deficits and that the pathogenesis of the microvascular lesions involves both hemorrhagic and ischemic etiologies.

These correlative radiologic-pathologic findings add to the growing literature on the neurological effects of COVID-19 and offer insight into the mechanisms of brain injury in severe COVID-19. The neuroanatomic distribution of the microvascular lesions, particularly the callosal and capsular predominance, has been reported as a rare complication of ARDS, high altitude exposure, and extracorporeal membrane oxygenation (ECMO) – all of which are associated with cerebral hypoxia [19,20]. Endothelial cell infection and endotheliitis have been described in extracerebral organs of patients with COVID-19 [4], presumably mediated via the ACE2 receptor, which is also found in brain endothelial cells [21]. These findings thus suggest a potential role for hypoxic

microvascular injury or endothelial dysfunction in the pathogenesis of cerebral microvascular injury in severe COVID-19.

It is important to acknowledge that the presence of microvascular lesions does not necessarily imply that such lesions are directly caused by COVID-19, or that all lesions reflect an acute etiology of brain injury. A subset of the lesions could be due to pre-existing microvascular lesions for example due to amyloid angiopathy or chronic hypertension, although the observed distribution including prominent callosal involvement would be unusual for these entities. Further, the relationship between microvascular injury and other neuroimaging findings observed in these patients (e.g., large artery infarcts, other types of intracranial hemorrhage, and parenchymal restricted diffusion suggesting hypoxic-ischemic injury) remains uncertain. Future work is needed to elucidate the inter-relationships between these pathophysiological processes.

If replicated, these observations have clinical relevance at the individual patient and population levels. For individuals, detection of diffuse microvascular injury may be clinically actionable. Differentiating microhemorrhage from microthrombosis remains a diagnostic challenge because both appear hypointense on SWI. Nevertheless, identification of SWI lesions could alter the risk-benefit analysis of anticoagulation, a particularly important consideration given the hypercoagulability observed in COVID-19 patients [22].

At the population level, these imaging findings raise fundamental questions about neuroprotective strategies in COVID-19 patients undergoing mechanical ventilation for hypoxic respiratory failure. Could tracking serum-based biomarkers of bleeding, hypercoagulability, and COVID-related hyperinflammatory states or cytokine release syndrome [23] identify patients at risk for cerebral microhemorrhage and ischemia? Could COVID-19-related hypoxia contribute to the pathogenesis of microvascular injury, as suggested by reports of patients with ARDS and “critical illness-associated cerebral microbleeds”? [19,20] Finally, does cerebral microvascular injury contribute to the prolonged unresponsiveness being observed in survivors of severe COVID-19? [2,3,11] All of these questions warrant urgent, systematic investigation to optimize neurologic outcomes in patients with severe COVID-19.

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Conflict of Interest Disclosures

Dr. Huang has received research support from Siemens Healthineers. Dr. Branda has received research support from Zeus Scientific, bioMerieux, and Immunetics, and consulting fees from T2 Biosystems, Roche Diagnostics and DiaSorin for work unrelated to this study. Dr. Lev has received research support from GE Healthcare, and consulting fees from GE Healthcare and Takeda Pharmaceutical for work unrelated to this study. All other authors report no relevant disclosures.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jns.2021.117308>.

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