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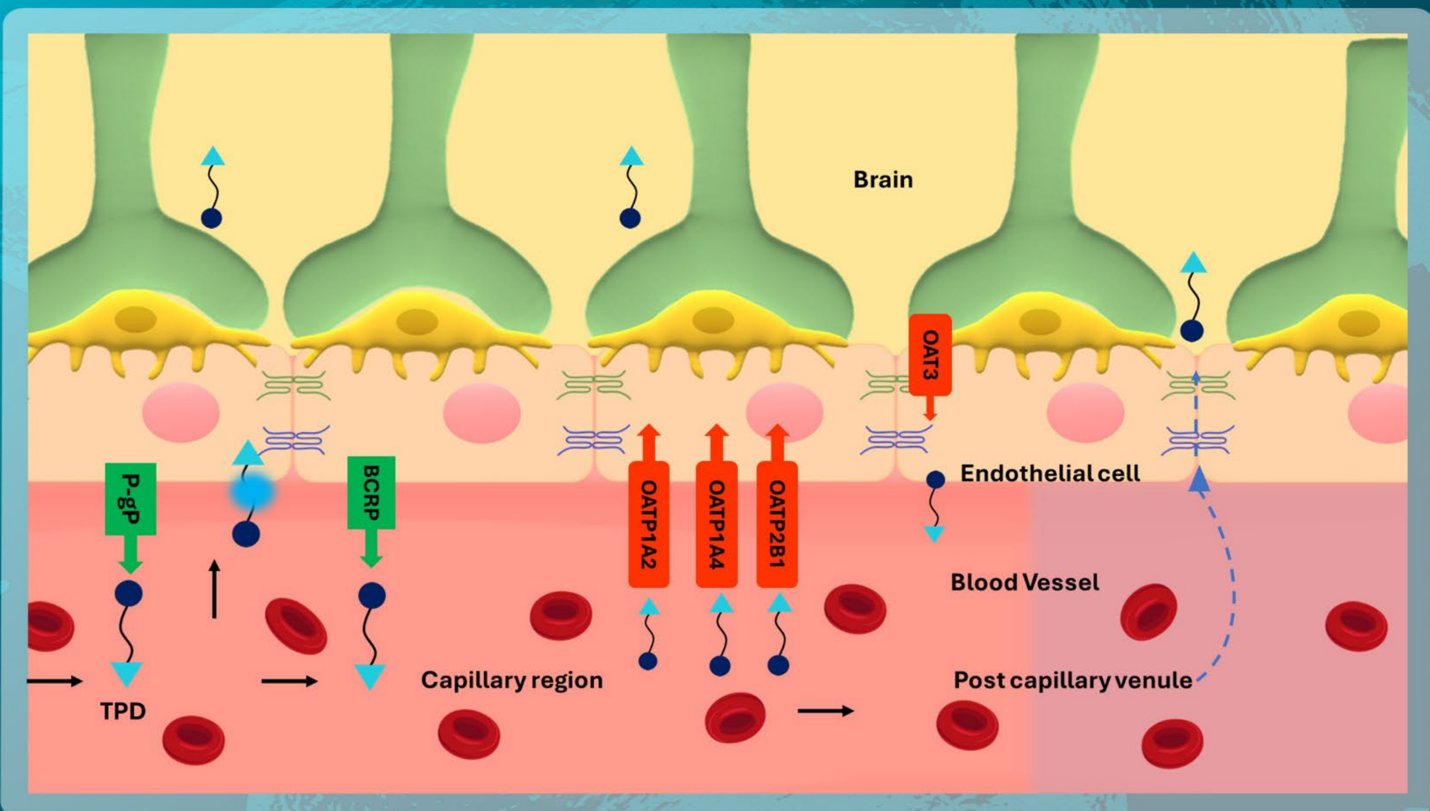

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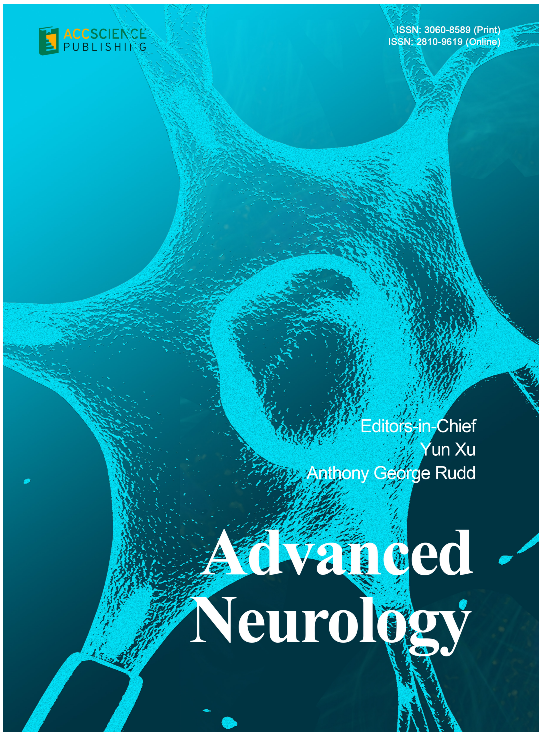

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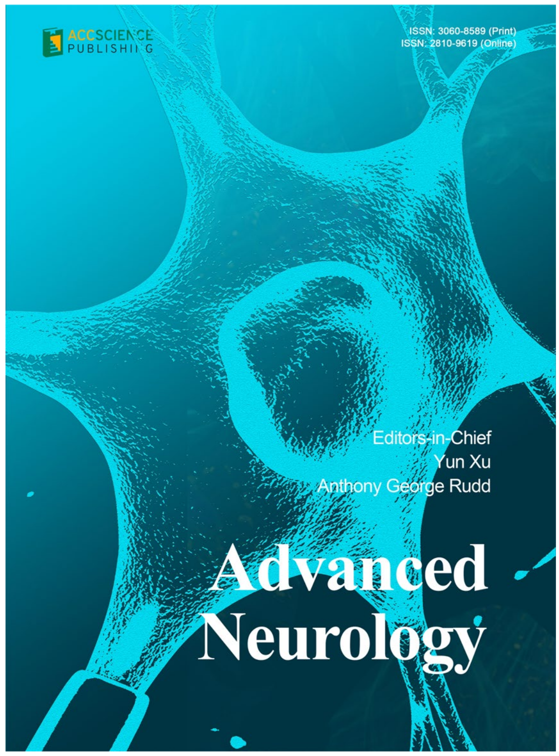

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REVIEW ARTICLE

Lipid droplets in neurodegenerative disorders: Molecular insights and therapeutic intervention

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Abstract

Lipid droplets (LDs) are dynamic lipid storage organelles, derived from phospholipid monolayer membrane of the endoplasmic reticulum. LDs are composed of neutral lipids, mainly including triglycerides, sterol esters, and other non-polar lipids like cholesterol. The functions of LDs differ from different cell types in the central nervous system. The major role of LDs is to maintain lipid homeostasis, provide energy supply, and play a protective role in response to intracellular oxidative stress in the brain. Oxidative stress can lead to lipid dyshomeostasis, particularly the accumulation of LDs, followed by abnormal myelination, neuroinflammation, and cognitive decline. Neuroinflammation, aging, and age-related neurodegenerative disorders (NDDs) are characterized by the excessive accumulation of LDs in the brain. Although the LDs accumulation in the brain of the NDDs mouse model has been reported as early as a century ago, the role of LDs in the brain remains largely unknown. It is still unclear regarding the involvement of LDs accumulation in the pathogenesis of NDDs. In this review, we provide an overview of LDs biogenesis, the characteristics and functions of LDs from a cell-type perspective, and the pathological roles of LDs in NDDs on recent advances. In addition, we discuss the emerging LDs-based therapeutic strategies for NDDs, which may provide promising clues to develop novel therapeutic approaches for NDDs.

Keywords: Lipid droplets; Neurodegenerative disorders; Aging; Neuroinflammation; Neuroglia

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1. Introduction

Lipid droplets (LDs) serve as dynamic organelles for the storage of neutral lipids, which play critical roles in the regulation of cellular lipid metabolism, homeostasis, and energy utilization. In central nervous system (CNS), highly energy-demanding neurons and glial cells are heavily reliant on lipid metabolism, which can provide nutrition, metabolism, and immunoregulation to neural networks. Although lipids represent around 50% of the dry weight in CNS, the brain has a limited capacity for the storage and oxidation for lipid metabolism.[1] Lipid dyshomeostasis is associated with neuroinflammation, brain aging, and neurodegenerative disorders (NDDs). LDs, as the neutral lipid storage organelles derived from endoplasmic reticulum (ER), are distributed in various cell types, including neurons, microglia, astrocytes, oligodendrocytes, and ependymal cells.[2-4] LDs show different functions in those cells. Recent advances revealed increased accumulation of LDs in brain aging and aging-related NDDs.[5] It is still unclear how dysregulated LDs are involved in overall homeostasis in brain aging and NDDs. Further understanding of the regulatory role of LDs may provide new insights to reveal the pathogenesis of NDDs. Here, we review the current research on the biogenesis processes and cellular specificity of LDs in CNS. Then, we provide an overview of molecular mechanisms governing the LDs in neuroinflammation, aging, and NDDs, focusing on the disturbance of LDs biology in neuroglia. Finally, we discuss the growing interest on the potential therapeutic treatments for NDDs in the management of restoring LDs balance and lipid metabolism pathways.

2. Biogenesis of LDs

Microscopically, LDs size ranges between 0.2 μm and 1 μm in diameter, even ranging up to 100 μm. The size and number of LDs vary with different cell types, metabolic conditions, and nutrient status.[6] LDs surface is bound with phospholipid monolayer membrane. The LDs core is composed of neutral lipids, including triglycerides, sterol esters, acylceramide, and other non-polar lipids such as cholesterol, diacylglycerol, and monoacylglycerol.[7] The dysregulation of neutral lipids synthesis may cause lipotoxicity, even inducing cellular inflammation, autophagy, and apoptosis. A large amount of proteins are embedded in or coat the LDs surface, which are mainly classified into Classes I and II. Class I proteins, "ERTELLED," including acyl-CoA synthetase long-chain family member 3, acyltransferases GPAT4, triglyceride lipase ATGL/PNPLA2 and UBX structural domain-containing protein 8, *etc.*, are generally distributed within ER membrane to feature a V-like hairpin configuration.[7,8] Class II proteins, "CYTELLED," are synthesized within the cytoplasm and target LDs surface through hydrophobic domains, including the perilipin (PLIN 1-5) family, CIDEA, and CCT.[8,9] All those proteins play pivotal roles in the regulation of LDs homeostasis and metabolism.

The biogenesis of LDs is a complex but conserved process, including nucleation, growth, budding, and maturation of a separate organelle (Figure 1). There are cellular proteins involved in regulating LDs biogenesis, including fat storage-inducing transmembrane proteins 1 and 2 (FIT1 and FIT2), seipin, and lipin. The relative LDs proteomic involved in biogenesis are termed as LD-related proteins, which can be divided into four types: perilipin family proteins (PLIN1, PLIN2, PLIN3, PLIN4, and PLIN5); lipid and energy metabolism-associated proteins (HSL, HSC79, ATGL and ACSL); and signaling proteins and membrane-trafficking proteins (Rab19 and vimentin).[7] These proteins are closely associated with LDs formation, budding, and maturation.

The neutral lipids, including triacylglycerols and sterol esters, are primarily synthesized within the ER. Then, triacylglycerols accumulates with the ER, and nucleate into an oil phase, leading to the lens formation. LDs nucleation of neutral lipid and PLIN proteins initially occurs in the ER.[10] After initial nucleation, neutral lipids gradually gather and expand into spherical droplets. Multiple proteins are involved in budding process. Seipin regulates the nucleation to form a LDs assembly complex. Seipin also controls the size of LDs and marks the formations sites of LDs in ER, inducing the separation of LDs from ER to cytoplasm.[10-12] FIT2 is responsible in partitioning neutral lipids during LDs budding. Loss of FIT2 prevents LDs from leaving from ER to cytosol, and suppresses triacylglycerols synthesis. Diacylglycerol acyltransferases are responsible for the conversion of diacylglycerol to triacylglycerol to modify LDs formation, which is the final step of triacylglycerols synthesis.[13] These enzymes also play an important role in axon regeneration by regulation of the phospholipid synthesis. After budding, LDs form membrane bridges with ER, allowing triacylglycerol transport into LDs. As the wrapped-triacylglycerol gradually accumulates, nascent LDs become separated from the ER, translocate into cytoplasm as a new organelle, and fuse with other LDs.

3. Pathophysiology of LDs in neurons and neuroglia

In CNS, LDs are actively involved in lipid metabolism and homeostasis, which is important for the maintenance of normal brain function. The characteristics and functions of LDs of various cell types in CNS are remarkably different (Figure 2).

In neurons, there are low triglyceride levels and limited LDs formation in normal conditions. It has been reported that LDs can be found in primary neuron cultures under appropriate conditions.[14,15] The accumulation of LDs is susceptible to the cellular stress. In neuron, lipids can be synthesized *de novo*.[16] When under oxidative stress conditions, neurons can synthesize lipids and store them in LDs to prevent reactive oxygen species (ROS) toxicity. Neurons can also absorb lipids from the external environment. Neuroglia secretes various lipids, including cholesterol, fatty acids, and phospholipids. All those lipids bind to extracellular proteins and can be ingested into neurons by low-density lipoprotein (LDL) receptors and fatty acid transporters. Cholesterol serves important roles in remodeling of membranes and synaptogenesis to maintain a high efficiency of neurotransmitter. It can be transported from astrocytes to neurons through apolipoprotein E (ApoE) particles and later endocytosed

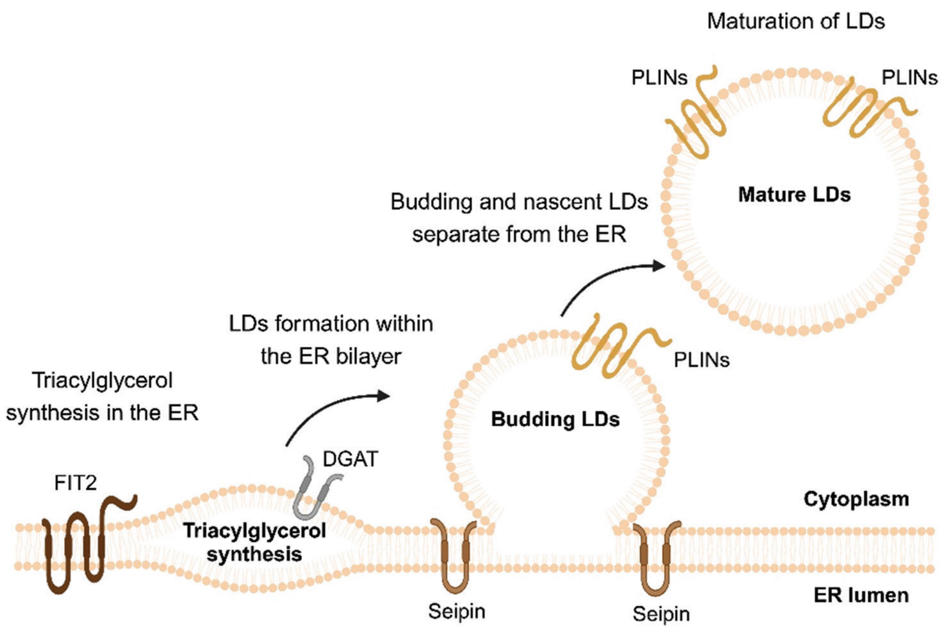

Figure 1. Biogenesis of LDs. Triacylglycerol is synthesized within the ER. LDs formation happens within the ER bilayer. After budding, the nascent LDs are separated from the ER and become matured organelles. Image created by the authors using Biorender.
Abbreviations: DGAT: Diacylglycerol acyltransferase; ER: Endoplasmic reticulum; FITs: Fat storage-inducing transmembrane protein; LDs: Lipid droplets; PLIN: Perilipin.

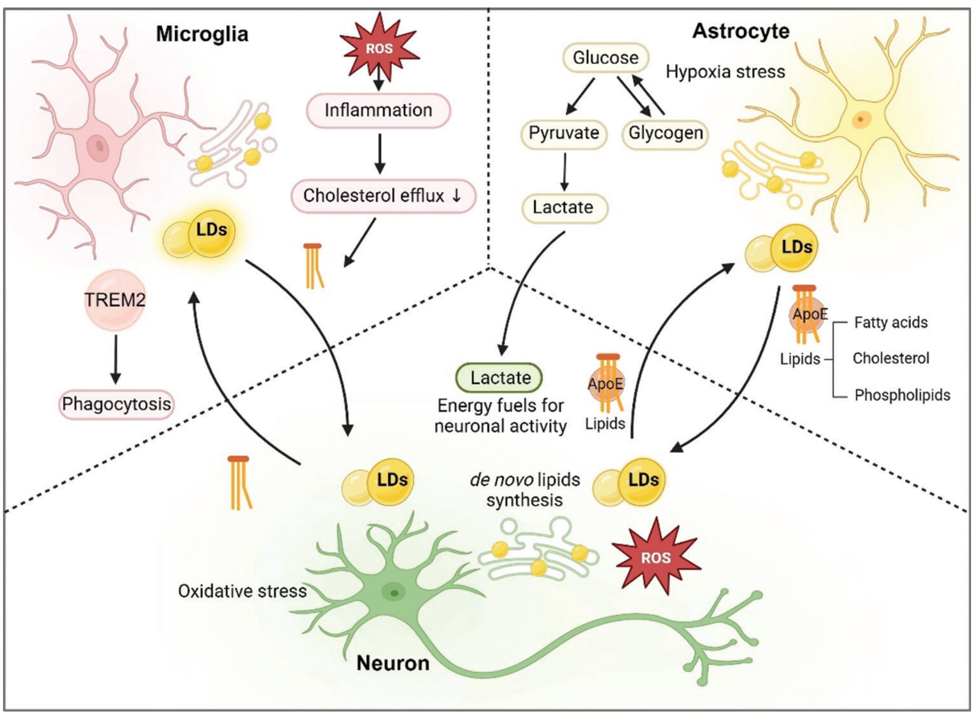

Figure 2. Pathophysiology of LDs in neurons, microglia, and astrocytes. There are various lipids crosstalk, including neuron-to-glia and glia-to-neuron, among these cells in the brain. Image created by the authors using Biorender.
Abbreviations: ApoE: Apolipoprotein E; LDs: Lipid droplets; TREM2: Triggering receptor expressed on myeloid cells 2.

by neuron through LDL receptor.[17,18] Abnormal delivery of cholesterol from neuroglia to neurons may result in defect of axon regeneration and synaptogenesis. Importantly, lipids can also be transported from neuron to other neuroglia. When under metabolically stressed conditions, increased ROS can drive *de novo* synthesis of fatty acids in neurons, leading to the accumulation of lipotoxicity.[19] Due to the low capacity for the formation of LDs and fatty acid metabolism, hyperactive neurons would deliver toxic fatty acid to neighboring neuroglia.

Microglia, the resident immune cells within CNS, are associated with the regulation of neuroinflammation and oxidative stress to maintain neuronal homeostasis.[20] There are several ways of LDs formation in microglia, including endocytosis of dead cells via triggering receptor expressed on myeloid cells 2 (TREM2)-mediated myelin phagocytosis, oxidized-phospholipid-containing particles, and neuronal-derived LDs.[1,21-24] Microglia are highly sensitive to environmental changes, including increased ROS production, lipotoxicity, inflammation, and intracellular stress. Under stress conditions or during the aging process, "resting" microglia transform into the activated form. A novel subtype of activated microglia with accumulated LDs, termed as "lipid-droplet-accumulating-microglia" (LDAM), exhibits a unique transcriptional profile, which is linked to dysregulation of phagocytosis, production of ROS, neuroinflammation, and decreased cholesterol efflux.[25] LDAM are abundant in the aging brain, which can either prevent from or contribute to the neuroinflammation and NNDs. Recently, it has been reported that LDAM with *ApoE4* genotype promotes the microglial status to a more maladaptive and damaging states, which is more likely contribute to Alzheimer's disease (AD) pathogenesis.[26-28] Future studies targeting the harmful roles of LDAM in AD might better uncover the intricate regulatory mechanism of NDDs.

Astrocytes are the predominant cell type accounting for around 40% in CNS, which are associated with a plethora of functions, including the formation of blood-brain barrier, synaptogenesis, regulation of neurotransmission, and metabolic regulation. Astrocytes also serve as an important role of LDs formation and metabolism. In fact, neurons have limited capacity for LDs and glycogen storage, so that energy is quickly consumed. Astrocytes can synthesize lipids, including fatty acids and cholesterol, through *de novo* way, which play important roles in neuronal metabolisms. Fatty acids released from membranes of astrocytes can shuttle into neurons via endocytosis of fatty acids transporters or ApoE particles. These fatty acids are mostly applied to the building sources of membrane for axonal growth and CNS myelination.[29] Cholesterol is primarily synthesized in astrocytes, and then secreted as ApoE-containing particles, which can be endocytosed by neurons through LDL receptor and lipoprotein receptor-related protein. Astrocytic glycogen metabolism is pivotal to maintain the neuronal activity after depletion of energy substrates. In astrocyte, glucose can be synthesized by glycolysis or glycogen shunt to pyruvate, and lately degraded to lactate, which is then transported into neurons and oxidatively metabolized as emergency fuels.[16,30] In addition, glycogen in astrocytes can also be converted to glutamine, which is a precursor of neurotransmitter glutamate.[31] In response to hypoxia and metabolic stress, astrocytes prefer anaerobic metabolism of glycolytic production of L-lactate. L-lactate is used for *de novo* fatty acid synthesis and ATP production in neurons under oxidative stress condition. Excess L-lactate could trigger LDs accumulation in astrocytes, which leads to pathogenesis of NDDs.[32]

The intercellular crosstalk of lipid metabolism in LDs across neurons and neuroglia plays essential roles for brain function, including metabolic homeostasis, synaptogenesis, and neurotransmission. Further investigations are required to elucidate how trafficking pathway of LDs integrates within various cell types, and how they might evolve in the pathogenesis of aging-associated NDDs.

4. LDs in neuroinflammation

The brain is highly susceptible to the oxidative stress. Oxidative stress is considered a key modulator of various NNDs during the aging process. The excess level of ROS is prone to induce oxidative stress and initiates the generation of multiple inflammatory cytokines. It is important to maintain a low level of ROS for intracellular homeostasis in the brain. When neuronal cells are exposed to high concentration of ROS for a long-term, various cellular macromolecules, including DNA, RNA, proteins, and lipids, may suffer from the ROS, in turn cause neuronal apoptosis and death. The excessive accumulation of ROS and lipotoxicity can result in neuroinflammation and even NNDs in brain. LDs formation can buffer cellular stress and restrict the levels of ROS. Lipopolysaccharide, which can initiate a cascade of proinflammatory mediators that are necessary to activate immune response, induces LDs formation through Toll-like receptor 4 (TLR4)-depended response pathway in macrophages.[33,34] Activation of TLR4 triggers the downstream signal pathway, including mitogen-activated protein kinases cascade, nuclear factor kappa-B (NF-κB) cascade, inhibitor of NF-κB cascade and c-Jun N-terminal kinase signaling pathway,[35] which then induces the activation of transcription complex activator protein-1 to promote the expression of proinflammatory cytokines.[34]

In the brain of inflammation, microglia suffer from the oxidative stress and ROS. Following the technologically-advanced genomic research, single-cell transcriptome sequencing has identified "disease-associated microglia" (DAM) as a subset of the resident phagocytic microglia population, which plays a protective role. According to human genome-wide association studies (GWASs), DAM shows unique transcriptional profiles which are highly associated to AD and other NDDs.[36] In response to damage-associated molecular patterns, DAM produces ROS from damaged cells, leading to lipid peroxidation, protein oxidation, and mitochondrial dysfunction.[37] Recent studies have identified another subgroup of microglia named "LDAM" in both aging mouse and human brain samples, which exhibit impaired phagocytosis, increased oxidative production of ROS, and superabundant proinflammatory cytokines.[38]

5. LDs in brain development and aging

As the second most lipid-rich organ, the brain is abundant with phospholipids and neutral lipids serving as energy supply. The lipid molecules comprise nearly a half of the dry weight of brain. Under normal physiological conditions, the presence of LDs is essential for the brain development. Neurons and neuroglia are derived from neural stem cells and progenitor cells, undergoing three types of division, including symmetric proliferative division, symmetric neurogenic division, and asymmetric neurogenic division.[39] During brain development, LDs protect the neural stem cells from oxidative stress. Hypoxia is a modulator of neuronal proliferation and enhances cellular proliferative capacity. The neural stem cells residing in unique hypoxic microenvironment named niches, can activate hypoxia-inducible factor and increase the production of ROS, and then in feedback trigger LDs formation and metabolic programing in neuron and neuroglia.[3,32] The LDs are mostly distributed around the niche glia, which acts as a protective event to limit ROS production and inhibit the oxidation of polyunsaturated fatty acids during the development of neural stem cells.[3]

Cholesterol stored in LDs is especially enriched in synaptic and myelin membranes. Cholesterol is closely involved in brain myelination, neuronal differentiation, synaptogenesis, and neurotransmission. The demands of cholesterol biosynthesis in CNS vary with development periods. During the brain development stage, cholesterol is highly demanded to build up the major portion of myelin sheaths for rapid saltatory neuron-to-neuron conduction. In adult brains of rodent animals, there is around 80% of cholesterol in myelin. Cholesterol and myelin membrane contact and restrain each other. The acyl-coenzyme A: cholesterol acyltransferase 1 and 2 (ACAT1 and ACAT2), also termed as sterol O-acyltransferases, can convert excess fatty acid to cholesteryl esters and lead to cholesterol ester storage in LDs, thus playing essential roles in neuronal cholesterol homeostasis.[40,41] The deficiency of ACAT1 in macrophages may reduce the responsiveness to cholesterol loading and alleviate proinflammatory responses.[42] Furthermore, ablation of *ACAT1* gene in triple transgenic AD mice causes more reduction in full-length human APPswe and attenuates cognitive impairments, which emerge as potential target for AD therapy.[43]

Another important role of LDs during brain development is to buffer the histone levels of transcription in neural stem cells. Various epigenetic elements, including histone deacetylases (HDACs), have been found to coordinate with stem cell pluripotency, self-renewal, neuronal proliferation, and differentiation to neural stem cells through the manner of chromatin remodeling and epigenetic modifications. Among HDACs, the HDAC1, HDAC3, HDAC5, and HDAC7 are highly expressed in neural stem cells.[44] Plethoric histones H2A, H2B, and H2Av are toxic for brain development. Although the molecular mechanism of how LDs regulate the histone storage remains unclear, in *Drosophila* embryos, concurrent accumulation of histones and LDs happens during oogenesis.[45,46] The histone-docking anchor Jabba can directly interact with histones and recruit them to LDs. Jabba can bind to LDs-localized H2A, H2B, and H2Av to stabilize these histones for chromatin assembly in the early *Drosophila* embryogenesis.[46]

Brain aging is a comprehensive process with an accumulation of unpredictable programmed and random damage to the neuronal cells. The hallmarks in the brain during aging are described as: intracellular accumulation of oxidative stress, damaged proteins, nucleic acids and lipids, and mitochondrial dysfunction. It has been found that LDs of microglia accumulate in 20-month-old mice brain during aging, showing abundant BODIPY+ positive signals. Similarly, additional analysis showed that there were more PLIN2+ and Iba1+ microglia in aging postmortem brain tissues than in young individual.[25] In 18-month-old mice, there was an increased density of LDs in cerebral Oil Red O-positive lipid-laden cells (LLCs), which appear to distribute in the cortex and striatum region. It has been found that LCCs are also spread out in the pia mater and blood vessels in the aging brain. This suggests that LLC may experience the migration from adjacent connective tissue and perivascular region to neural parenchyma during the aging process.[47]

The novel state of microglia, LDAM, has been defined as a hallmark of brain aging or neurodegeneration. Impaired phagocytic activity has been determined in LDAM compared to LDs- microglia in aging brain. It is unclear whether defective phagocytosis is a cause or consequence of LDs. *In vitro* studies showed that pharmacological inhibition of LDs production with Triacsin C, an inhibitor of long-chain acyl-CoA synthetase to inhibit endogenous synthesis of glycerolipids, can promote phagocytosis and inhibit ROS formation in BV2 cells.[25] Treating BV2 cells with proinflammatory endotoxin lipopolysaccharide increased phagocytic activity and promoted LDs formation. Moreover, culturing BV2 cells with 5% plasma from 18-month-old aged mice can induce a higher density of LDs.[48] Given these studies, there shows a close interplay between aging and LDs.

6. LDs in AD

In recent years, numerous studies have shown the connections between LDs accumulation and NDDs, such as AD, Parkinson's disease (PD), and amyotrophic lateral sclerosis. AD is the most common type of dementia, which is characterized by extracellular deposition of β-amyloid (Aβ) plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau, and neuron loss in the brain. In recent studies, more other pathologies have been revealed in the AD process, such as dysregulation of glucose metabolism, oxidative stress-induced neuroinflammation, mitochondrial dysfunction, and abnormal lipid metabolism.[49,50] In 1907, when Alois Alzheimer first described the AD case of Auguste Deter, he also noted the presence of "adipose saccules" or LDs inclusions in microglia.[51] Until recent years, the phenomenon of increased lipid accumulation in AD has been widely noticed.[52] In LDs, cholesterol can be converted to cholesteryl esters by ACAT during normal LDs biogenesis. Excess cholesterol and cholesteryl esters within LDs regulate Aβ and tau pathology independently. Intracellular cholesterol accumulation impairs mitophagy by disrupting optineurin recruitment and lysosomal clearance, leading to decreased Aβ clearance. This blockage of autophagic flux also leads to the increased HDAC6+ aggregates in AD brains samples, which have been reported to be increased by 90% in AD hippocampus.[53] As for tau-related pathology, inhibition of neuronal cholesterol esterification through deletion of *ACAT1* gene may induce tau degradation by regulation of ubiquitin-proteasome levels in APP- and Aβ-independent manner.[54]

ApoE is responsible for lipid metabolism and cholesterol homeostasis in CNS. The human ApoE gene has three polymorphic forms of alleles, including ApoE *ε*2, *ε*3, *and* *ε*4, which are translated into three isoforms of ApoE2, ApoE3, and ApoE4.[55] The different ApoE isoforms have different binding capacity to interact with lipids, receptors, and Aβ. The *ε4* allele of ApoE has been identified as the strongest genetic risk factor to develop AD, while *ε*2 and *ε*3 alleles are protective roles in brain.[56,57] The ApoE4 carriers bearing either in a heterozygous or homozygous genotype are linked to have about 9- to 15-fold increased risk of AD,[58,59] which have more severe AD pathologies, including increased Aβ accumulation and neurofibrillary tangles, and elevated levels of inflammatory cytokines. Given ApoE is important for lipid transport and metabolism in neuron and neuroglia, it is not surprising that ApoE can alter the LDs accumulation in the AD brain. ApoE serves distinct roles in the regulation of lipid transport within different neuronal cells. Astrocytes are the initial site of oxidation of fatty acid and are thought to be major sites expressing ApoE. Astrocytes are responsible for transportation of fatty acids away from hyperexcitable neuronal cells and storing them in LDs to provide energy to brain during starvation. ApoE4 in astrocytes can promote the expression of PLIN-2, whose levels are highly correlated with LDs abundance,[60] and inhibit oxidation of fatty acid, and increase the volume but decrease size of LDs. It has been reported that ApoE shows binding affinity for complement component 1q (C1q) on LDs in choroid plexus niche. C1q is the initial protein of the classic complement cascade of the immune system.[61] It has been suggested that ApoE may regulate the complement pathway within the choroid plexus during the progression of AD.

The neurons also express ApoE but only under the circumstance of oxidative stress to facilitate their lipid transfer and fatty acid clearance. The neurons also participate in the regulation of LDs metabolism through the same manner. A recent single-nucleus RNA sequencing has found that acyl-CoA synthetase long-chain family member 1, a key enzyme of LDs biogenesis, is abundant in the AD microglia with ApoE4 genotype.[26] This finding indicates that damaged LDAMs could affect the AD pathogenesis in an ApoE4-dependent way. In addition, there is a TREM2-APOE pathway that can induce microglia state switch from homeostatic to detrimental in AD.[62] TREM2, a transmembrane receptor expressed in microglia, can be activated by multiple ligands including lipids. TREM2 might promote the buildup of LDs by an ApoE4-dependent regulation on lipid metabolism, and thus affect the AD pathogenesis.[63,64]

7. LDs in PD

PD, the second most common NDD after AD, is characterized by the loss of dopaminergic neurons in the substantia nigra and intracellular accumulation of α-synuclein. The disturbance in lipid metabolism has also been reported in PD. According to GWASs, it has been validated that a lipid-associated pathway leads to PD development. The vesicle trafficking and lipid homeostasis-associated genes are regarded risk factors of PD. α-synuclein is a lipid-binding protein that directly binds with fatty acids and phospholipids under physiological

Table 1. Lipid droplets-based therapeutic strategy of neurodegenerative disorders

LDs-based therapeutics	Experimental model	Mechanism	Drug target	References
YTX-465	iPSC model	Inhibit α-synuclein-induced toxicity	α-synuclein	69
CMS121	APP/PS1 mice	Inhibit fatty acid synthase	Fatty acid synthase	70
Seipin interference	hESC model	Rescue a-synuclein toxicity with inhibition of oleic acid and diglyceride	Seipin	67,71
Mcc1274	SH-SH5Y cell line	Decrease the expression of PLIN4	PLIN4	72
Kaempferol	Primary neuron cultures	Promote cellular autophagy	ATG5	73
CP113818	APP mice	Inhibit generation of the Aβ peptide	ACAT	75
CI-1011	APP mice	Decrease the mature/immature ratio of human APP	ACAT	76

Abbreviations: ACAT: Acyl-coenzyme A: cholesterol acyltransferase; APP: Amyloid precursor protein; ATG5: Autophagy-related gene 5; hESC: Human embryonic stem cell; iPSC: Induced pluripotent stem cell; LDs: Lipid droplets; PLIN: Perilipin; PS1: Presenilin-1.

conditions.[65] Excess α-synuclein induces accumulation of LDs and impairs vesicle trafficking of dopaminergic neurons.[66] In neurons, deletion of seipin, which serves as a scaffolding protein located on ER/LD to regulate LDs biogenesis, can rescue α-synuclein toxicity by inhibiting oleic acid overproduction.[67] There are two mutant forms of α-synuclein: A53T and A30P. Both A53T and wild type-α-synuclein exhibit LDs binding ability in yeast, whereas the LDs binding ability of A30P is significantly lower.[68] Additional studies have focused on the dysregulated lipids homeostasis in PD. The conserved enzyme stearoyl-CoA desaturase (SCD) has been identified as a target for synucleinopathies. Inhibition of SCD expression could suppress pathological interactions between α-synuclein and lipids, and then prevent α-synuclein-induced toxicity in neurons and dopaminergic degeneration in mouse models.[67] Taken together, these studies indicate a potential role of LDs in the regulation of PD pathogenesis and provide a promising therapeutical intervention for PD.

8. Therapeutic intervention of LDs on NDDs

NDDs have become a major health problem worldwide. Yet, there are little effective disease-modifying therapies against NDDs. Elucidation of the complex regulatory mechanism of NDDs may provide helpful guidance for drug exploration and design. In view of the important role of LDs in NDDs, LDs-based targets provide a promising therapeutic strategy to NDDs (Table 1).

Vincent *et al.*[69] have used unbiased phenotypic screening and screened out a series of 1,2,4-oxadiazoles, which can protect yeast against α-synuclein toxicity. After multiple rounds of analog synthesis and biochemical assays in induced pluripotent stem cell-derived neurons, YTX-465 was found to strongly inhibit fatty desaturation with a 50% reduction. Further biochemical test revealed that YTX-465 directly inhibit Ole1, a homolog of SCD, to suppress α-synuclein toxicity in neurons.[69] This suggests that 1,2,4-oxadiazoles can be the promising compounds to treat PD. CMS121 is a small molecule derived from flavonoid fisetin. Ates *et al.*[70] demonstrated the therapeutic effects of CMS121 on memory decline in APP/PS1 mice. CMS121 can inhibit lipid peroxidation in both neurons and microglia. CMS121 also showed anti-inflammatory function *in vivo*, suggesting its inhibitory effect on lipid peroxidation. It has been identified that fatty acid synthase is a target of CMS121, which was proposed as a potential target for AD treatment. In neuronal culture, knockout of seipin can rescue α-synuclein toxicity with decreased levels of oleic acid and diglyceride.[67,71] Bernier *et al.*[72] found that the extracts from *Bifidobacterium breve* significantly inhibited the mRNA expression level of *PLIN4*, a key regulator of LDs biogenesis, and reduced the LDs accumulation in MCC1274 cells. Han *et al.*[73] found that kaempferol, a small molecule from natural flavonoid, suppressed accumulation of LDs in an autophagy-dependent way, consequently rescued neuronal death. Murphy *et al.*[74] found knockdown of *ACAT1* gene in the brain of AD mouse model decreased Aβ accumulation. Both the ACAT inhibitors, including CI-1011 and CP-113818, can regulate the APP processing to affect APP protein content.[43,74] Taken together, taking LDs as potential therapeutic targets provides a novel treatment strategy for NDDs.

9. Conclusion and future perspectives

LDs are recognized as functionally dynamic organelles with various functions in neurons and neuroglia cells. The function of LDs varies between cell types. In neuroglia, LDs are not only a site for lipid metabolism but also a mediator to initiate the onset and development of NDDs. Several essential knowledge gaps regarding the role of LDs in NDDs pathogenesis exist, warranting further investigations. First, a precise imaging map of the distribution of LDs in different brain regions is necessary for us to better understand the spatiotemporal alteration of LDs. Second, given the complexity and diversity of cell types in CNS, a deeper investigation into the functions of LDs in different cell types and transport crosstalk across neuroglia-to-neuron or neuron-to-neuroglia can enrich our understanding of the comprehensive regulatory roles of LDs in CNS. Finally, whether LDs can serve as a rational therapeutic target for improving the consequence of NDDs in the future requires additional research. For clinical application, the detection method of LDs should be more sensitive and specific. As for LDs-based therapeutics, it should be noted that LDs may have both protective and detrimental functions in the brain cells. How to maintain the balance of maximizing the protective role or minimizing the detrimental role of LDs would be a challenging but meaningful work to deal with.

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ACCSCIENCE PUBLISHING

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REVIEW ARTICLE

SARS-CoV-2 and its long-term neurological impact: Unraveling the mechanisms of neurodegeneration and cognitive decline

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for coronavirus disease 2019 (COVID-19), is associated with neurological effects that persist beyond the acute phase, collectively referred to as post-acute sequelae of SARS-CoV-2 infection (PASC) or "long COVID." This article discusses the neurological impacts of PASC, which can occur regardless of the initial illness's severity. Studies indicate that most patients continue to experience symptoms for at least 3 months post-infection. Long-term effects include neurocognitive deficits, sleep disturbances, and the exacerbation of pre-existing conditions. Proposed mechanisms underlying these effects include neuroinflammation, microvascular damage, and autoimmune responses, while direct viral neuroinvasion remains a topic of ongoing debate. SARS-CoV-2 may also worsen pre-existing neurological disorders and increase the risk of developing neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease. The article highlights the need for longitudinal studies to better understand the variability in outcomes and the mechanisms driving these persistent effects. In addition, it explores the inflammatory pathways linking long COVID to AD. Both conditions are characterized by chronic inflammation, activation of shared markers such as the NLR family pyrin domain containing 3 inflammasome, and alterations in amyloid-beta production. The apolipoprotein E4 gene, a known risk factor for AD, is also associated with more severe COVID-19 outcomes. Neuroimaging studies reveal brain changes in COVID-19 survivors, particularly in regions related to cognition and memory, further emphasizing the need for long-term research to assess the potential role of long COVID in exacerbating neurodegenerative diseases.

Keywords: SARS-CoV-2; Long COVID; Neurodegenerative diseases; Cognitive decline; Inflammatory pathways; Autoimmune responses; Alzheimer's disease; Neuro-post-acute sequelae of SARS-CoV-2 infection

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1. Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, has predominantly been linked to severe respiratory outcomes, such as acute respiratory distress syndrome (ARDS), respiratory failure, and, in extreme cases, death.[1] However, ARDS associated with COVID-19 has also been found to trigger various neurological manifestations, including encephalopathy, confusion, agitation, and corticospinal tract dysfunction. Early in the pandemic, symptoms such as anosmia (loss of smell) and other neurological disturbances were commonly reported.[2] Since then, the phenomenon of "long COVID," or post-COVID syndrome, has emerged, characterized by prolonged symptoms following the acute phase of infection.[3,4] This condition manifests through a wide range of neurological, cognitive, and mental health symptoms. Among the most frequently reported is "brain fog," a non-specific term encompassing symptoms such as headaches, cognitive impairment (CI), and generalized mental fatigue, all of which contribute to diminished concentration and executive functioning. Patients often experience overwhelming fatigue, further exacerbating cognitive difficulties, and commonly report mood disturbances, including anxiety and depression. Sleep disturbances, ranging from insomnia to non-restorative sleep, also significantly impact quality of life and cognitive functioning.[5-8] Despite its growing prevalence, the precise underlying mechanisms of long COVID remain poorly understood.

Recent studies examining the cognitive effects of long COVID have revealed deficits in global cognition as well as specific cognitive domains.[9,10] For instance, a multicenter study found significant cognitive slowing in patients with post-COVID conditions.[11] Similarly, a large observational study conducted in England documented more severe CIs in COVID-19 survivors, particularly among those infected with early variants or those who had been hospitalized.[12] Neuropsychological evaluations conducted several months post-infection have identified declines in memory recall, executive function, and processing speed.[13] However, the short follow-up periods in these studies underscore the need for extended research to better understand these CIs.[10-12] Moreover, approximately 35% of patients developed neurological or psychiatric complications within 6 months of infection.[14] Although similar neurological complications have been observed following other respiratory infections, the specific pathophysiology and long-term consequences of COVID-19-related neurological effects remain unclear.[15] Multiple large-scale studies have demonstrated an association between COVID-19 and cognitive deficits, including memory and concentration impairments during the post-acute phase. Survey data from United States working-age adults also indicated a growing incidence of memory and concentration difficulties linked to the virus.[16,17]

SARS-CoV-2 primarily enters host cells through the angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) receptors, with other potential entry pathways involving neuropilin and vimentin, which are highly expressed in neurovascular tissues.[18-20] Although the extent of viral neuroinvasiveness remains debated,[21-23] recent evidence suggests that viral RNA may persist in tissues, including the brain, long after the infection has resolved, much of which is derived from postmortem studies.[5] The mechanisms driving cognitive dysfunction in COVID-19 patients are still under investigation. Preliminary findings suggest that SARS-CoV-2 may induce neuronal fusion, alter normal neuronal activity, and lead to chronic neuroinflammation and premature brain aging, even in mild cases.[5,24,25] In addition, gut dysbiosis and disruptions in serotonin pathways are believed to contribute to post-infection cognitive deficits.[26]

This article discusses the neurological impact of COVID-19, with a particular focus on long COVID and its cognitive consequences. It examines the mechanisms underlying the neurological manifestations of COVID-19, considering both viral and host factors. In addition, the article explores current insights into how SARS-CoV-2 affects brain function and structure, drawing on recent research and emerging evidence. By synthesizing existing findings and identifying knowledge gaps, this article aims to provide a comprehensive overview of COVID-19's neurological implications and guide future research in this critical area.

2. The neurological impact of SARS-CoV-2: From acute infection to post-acute sequelae

The neurological impacts of SARS-CoV-2 extend beyond the acute infection phase, manifesting in a wide range of symptoms during the post-acute sequelae of SARS-CoV-2 infection (PASC) (Table 1). These effects are observed across the spectrum of illness severity, and while the exact mechanisms remain unclear, research is uncovering patterns of persistent neurological and psychiatric disturbances.[27] This section elaborates on the acute and long-term impact of COVID-19 on the nervous system, focusing on symptom prevalence, risk factors, and the broader implications for public health.

2.1. Acute neurological symptoms and post-acute sequelae

PASC refers to the persistence or emergence of new symptoms following the acute phase of the infection, affecting individuals across a spectrum of initial illness severity, ranging from mild to critical cases.[28] Early studies, including those involving both hospitalized and non-hospitalized patients, have shown that nearly all participants

Table 1. Key aspects, critical findings, implications, and knowledge gaps in the current understanding of post-acute sequelae of SARS-CoV-2 infection

Aspect	Key findings	References
Definition of PASC	PASC refers to the persistence or emergence of new symptoms following the acute phase of COVID-19. These symptoms can affect individuals across a broad spectrum of initial illness severity, ranging from mild to critical cases.	28
Prevalence of symptoms	• A vast majority of patients, both hospitalized and non-hospitalized, continue to experience symptoms 3 months after COVID-19 infection. • The most commonly reported symptoms include fatigue and dyspnea, with only approximately 1% of individuals reporting complete resolution of symptoms.	29
Neurological sequelae	• Both acute and chronic neurological symptoms have been documented, including anxiety, sleep disturbances, myalgia, and memory impairments. • Rare but severe neurological syndromes, such as seizures, vasculitis, encephalopathy, and acute inflammatory demyelinating polyneuropathy, have also been reported.	30-36
Focus of existing research	• Existing research predominantly focuses on the neurological outcomes of severe COVID-19 cases. • There is a notable paucity of studies examining long-term neurological outcomes in individuals who experienced mild or moderate infections.	32-34
Risk factors for PASC	• The risk factors for developing PASC are not yet fully understood, necessitating further research. • Severe acute illness is associated with a higher likelihood of developing PASC; however, emerging evidence suggests that individuals with mild or moderate COVID-19 may also face a substantial risk. • The role of pre-existing neurological conditions in the development of PASC remains underexplored, representing a critical research gap.	37-39
Impact on quality of life	• Neurological symptoms, particularly fatigue and headache, have significantly impacted the quality of life in approximately 80% of COVID-19 survivors. • An Italian self-reported survey indicated that around 75% of participants experienced persistent fatigue, muscle pain, and joint pain 3 months post-infection. • At 6 months, cognitive impairments, such as memory deficits and difficulty concentrating, became more prominent.	31,40,41
Mortality and long COVID severity	• The risk of mortality associated with long COVID appears to correlate strongly with the severity of the acute COVID-19 infection. • Notably, even patients who experienced mild illness during the acute phase have been found to have persistent symptoms, with one-third of mild cases reporting fatigue, dyspnea, cognitive disturbances, and anosmia up to a year post-infection.	42
Diagnostic criteria	• While formal diagnostic criteria for PASC remain undefined, it is generally accepted that symptoms persisting for 4 – 12 weeks after infection can be classified as PASC. • Follow-up studies indicated that between 25% and 75% of patients report symptoms extending up to 6 months, even among those who had mild cases.	43-46
Neurological and psychiatric symptoms	• Common neurological and psychiatric symptoms associated with PASC include persistent headaches, anosmia (loss of smell), sleep disturbances, fatigue, and cognitive dysfunction, such as difficulties with concentration, language, and executive function. • Depression and anxiety have been frequently reported among PASC patients. • Autonomic dysfunction, including orthostatic intolerance, has also been observed in some individuals.	47-53
Research needs	• There is an urgent need for the development of more robust diagnostic criteria for PASC, along with a deeper understanding of its risk factors and effective treatments. • Comprehensive longitudinal studies are crucial to unravel the long-term neurological and psychiatric impact of COVID-19, particularly in relation to neurodegeneration and cognitive decline.	54,55

Abbreviations: COVID-19: Coronavirus disease 2019; PASC: Post-acute sequelae of SARS-CoV-2 infection.

exhibited symptoms 3 months post-infection, with fatigue and dyspnea being the most prevalent, while only approximately 1% of individuals reported being symptom-free.[29] Similar findings have been reported in other studies documenting both acute and chronic neurological sequelae, such as anxiety, sleep disturbances, myalgia, and memory impairments.[30,31] Although most research has focused on severe cases, fewer studies have explored the long-term neurological outcomes in patients with mild infections.[32-34] Case reports have described rare neurological syndromes, including seizures, vasculitis, encephalopathy, and acute inflammatory demyelinating polyneuropathy (Table 1).[35,36] However, larger cohort studies are lacking, highlighting the need for comprehensive longitudinal research.

2.2. Risk factors and pre-existing conditions

The risk factors for developing PASC remain poorly understood and require further investigation to achieve a more thorough understanding. While severe acute illness has been associated with a higher risk of developing PASC,[37] emerging evidence suggests that individuals with mild or moderate COVID-19 may also be at considerable risk.[38,39] Moreover, there is a lack of comprehensive data on the risk of developing PASC in individuals with pre-existing neurological conditions, highlighting a critical gap that warrants further investigation (Table 1).

Many individuals who contracted COVID-19 have reported a worsening of pre-existing symptoms. Neurological symptoms, particularly fatigue and headache, have significantly affected the quality of life in approximately 80% of patients.[40] In line with these findings, an Italian self-reported survey found that 3 months post-infection, about three-quarters of participants experienced persistent fatigue, and around 60% reported muscle pain and joint pain.[31] By 6 months post-infection, the dominant symptoms had shifted from fatigue and headache to memory impairment and reduced concentration.[40] Similarly, a cross-sectional online survey identified post-exertional malaise, fatigue, and cognitive dysfunction as the most prevalent symptoms at the 6-month mark.[41]

2.3. Long-term neurological and psychiatric symptoms

The risk of mortality associated with long COVID appears to correlate with the severity of the acute infection. However, even individuals with mild COVID-19 are at risk of developing PASC. A year-long study reported that one-third of patients with mild illness experienced persistent symptoms, including fatigue, dyspnea, cognitive disturbances, and anosmia.[42] Although formal diagnostic criteria for PASC have yet to be established, it is generally defined as symptoms persisting for 4 – 12 weeks following infection.[43] Follow-up studies have demonstrated that between a quarter and three-quarters of patients report symptoms lasting up to 6 months, even in cases of mild illness.[44-46]

The neurological and psychiatric symptoms associated with PASC include headaches,[47] anosmia,[48] sleep disturbances,[49] and CIs, such as difficulties with concentration, language, and executive function.[50] Other commonly reported symptoms include depression and anxiety.[51] In addition, autonomic dysfunction, such as orthostatic intolerance, has been reported (Table 1).[52,53]

While much remains to be understood about PASC, its neurological and psychiatric impacts are clear, and warrant continued research to better define diagnostic criteria, risk factors, and effective treatments. The growing body of evidence highlights the need for comprehensive, longitudinal studies to address the long-term consequences of COVID-19 on the nervous system.[54,55]

3. Persistence, the emergence of new disorders, and long-term risks of neurological impact associated with SARS-CoV-2

3.1. Persistence of neurological disorders and the emergence of new conditions

Recent evidence has indicated that the severe acute phase of SARS-CoV-2 infection can both initiate the development of and persist within the nervous system throughout both the acute and post-acute phases of infection.[5,56] COVID-19 has been linked to the emergence of new neurological disorders and the exacerbation of pre-existing conditions. Individuals with pre-existing neurological conditions, such as dementia, Parkinson's disease (PD), and epilepsy, experience higher mortality rates when infected with COVID-19.[57-59] Furthermore, the development of new neurological conditions in hospitalized COVID-19 patients has been associated with increased morbidity and mortality that extends beyond the acute phase of infection.[60,61]

Studies on post-SARS-CoV-2 neurological sequelae, often centered on hospitalized patients without appropriate controls, frequently report headaches and cognitive dysfunction in both the acute and chronic phases. Less common conditions include movement disorders, cerebrovascular disease, neuropathies, and seizures.[62,63] Large-scale studies comparing COVID-19 with other viral illnesses or SARS-CoV-2-negative controls have shown an increased risk of neurological complications, though these studies often suffer from issues related to timing and geographical mismatch (Table 2).[6,14,17]

There has been increasing attention on PASC, characterized by symptoms that persist beyond 3 months after the initial infection. Neurological manifestations of PASC include neurocognitive deficits, autonomic dysfunction, pain, mood disorders, and anosmia.[64-67] Recent studies have observed a higher risk of encephalopathy, stroke, movement disorders, neuropathy, neurocognitive dysfunction, anxiety, fatigue, seizures, and headaches in the post-acute phase compared to SARS-CoV-2-negative or historical controls.[17,68-70] However, replication of these findings has been inconsistent.[71,72]

3.2. Long-term risks and challenges in studying post-COVID neurological impact

Studies have identified elevated risks for various neurological conditions following SARS-CoV-2 infection,

Table 2. Summary of key findings on the persistence of neurological disorders and the emergence of new conditions following COVID-19 infection

Phase/time post-infection	Neurological conditions	Observations	Key findings	References
Acute phase (0 – 30 days)	Headaches, cognitive dysfunction, movement disorders, cerebrovascular disease, neuropathies, seizures	• Increased risk compared to controls • Hospitalized patients at higher risk	COVID-19 has been associated with a wide range of neurological conditions, with headaches and cognitive dysfunction being the most commonly reported. Less frequent conditions include movement disorders and seizures	68,69
Post-acute phase (1 – 3 months)	Neurocognitive dysfunction, encephalopathy, stroke, movement disorders, neuropathy, anxiety, fatigue, seizures, headache	• Higher risk compared to SARS-CoV-2-negative controls • Associated with higher morbidity and mortality	Persistence of neurological symptoms like cognitive dysfunction and fatigue; elevated risks for encephalopathy, stroke, and seizures; the consistency of these manifestations remains mixed	56,76
Post-acute phase (3 – 9 months)	Encephalopathy, dementia, seizures, brain fog, myelitis, inflammatory myopathy, coma	• Prolonged neurological sequelae • Ongoing risk of cognitive dysfunction, brain fog, and seizures	Continued neurological symptoms, with increasing incidences of encephalopathy, seizures, and dementia; a higher risk for inflammatory myopathy and coma; the prolonged impact of brain fog and dementia remains a significant concern	73-75
Long-term (9 months onwards)	Cognitive deficits, encephalopathy, dementia, myopathies, stroke, parkinsonism (inconsistent findings)	• Varying results depending on the observational period • Inconsistent findings on stroke and parkinsonism	Longer-term complications, particularly cognitive dysfunction and encephalopathy, persist. However, studies on stroke and parkinsonism have shown inconsistent results, suggesting the need for standardized research methodologies	17,56,81
Stroke risk (post-infection, 1 month – 1 year)	Stroke risk, especially in individuals with peripheral inflammatory diseases	• Mixed findings across studies • Varying observational periods yielded different results	Conflicting data regarding stroke risk post-COVID-19: some studies reported an elevated stroke risk in the early months following infection, while others showed no increased risk. Standardized methodologies are required to clarify these discrepancies	56,86
PASC (>3 months)	Neurocognitive deficits, autonomic dysfunction, mood disorders, anosmia	• High incidence of persistent neurological symptoms • Need for further investigation	Neurological manifestations of PASC are diverse, including cognitive deficits, mood disturbances, and autonomic dysfunction. While studies indicate increased risk of these symptoms, replication of findings remains inconsistent	64,65

Abbreviations: COVID-19: Coronavirus disease 2019; PASC: Post-acute sequelae of SARS-CoV-2 infection; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

including increased incidences of encephalopathy, dementia, seizures, brain fog, and myelitis within 1 week – 3 months post-infection. In the 3 – 9-month post-acute phase, higher rates of inflammatory myopathy, coma, and continued increases in brain fog, seizures, and dementia have been observed. These findings underscore the prolonged risk of neurological diseases following COVID-19 infection, highlighting the need for ongoing medical follow-up (Table 2).[73-76]

Similarly, other large cohort studies have documented increased incidences of post-COVID-19 encephalopathy, cognitive deficits, dementia, and myopathies. However, findings related to stroke and epilepsy/seizures have been inconsistent, with no clear evidence linking COVID-19 to Parkinsonism.[6,14,17,56,70-72,77-81] Variations in study methodologies – including differences in the neurological sequelae assessed, observation timelines, control group selection, variable adjustments, and outcome measurements – complicate comparisons across studies. Future research should aim to standardize methodologies and outcome measures to facilitate more reliable comparisons and enhance understanding of the range and impact of neurological sequelae associated with PASC. Establishing consistent protocols and comprehensive assessment criteria will improve the ability to draw meaningful conclusions and inform effective interventions.

Several studies, including both clinical and basic research, have revealed that patients with peripheral inflammatory and immune-related diseases may have an increased risk of stroke, highlighting the importance of monitoring these individuals.[82,83] However, two large studies assessing stroke risk from 1 month – 1 year post-infection have yielded conflicting results compared to contemporary COVID-19-negative controls.[17,72] In addition, studies with different observational periods have reported varying findings: increased stroke risk was observed within 30 days,[79] 1-year,[84] and between 3 weeks and 4 months,[70] while other studies found no increased risk within 2 weeks – 6 months.[71] Comparisons with other respiratory illnesses showed mixed results, with some studies indicating an elevated stroke risk up to 6 months that resolved by 2 years,[6,14] while others did not observe similar findings within a 90-day period (Table 2).[56,85,86]

These discrepancies may arise from several factors: variations in cohort age, differences in case and control selection, and the presence of comorbidities. In addition, differences in inclusion criteria – such as whether only hospitalized patients or also milder cases were considered – and variations in observation timelines further complicate the interpretation. These factors make it difficult to fully understand the association between COVID-19 and stroke risk, as they can influence reported outcomes and affect the generalizability of the findings. Future research should address these issues by standardizing methodologies, including diverse patient populations, and establishing uniform observation periods. This approach will enhance the understanding of stroke risk in patients with COVID-19 and its long-term impact on health.

4. Pathophysiological mechanisms of neuroinflammation, immune dysregulation, and long-term cognitive decline associated with COVID-19

4.1. Mechanisms of neurological PASC

Several mechanisms have been proposed to explain neuro-PASC, including direct viral invasion of the central nervous system (CNS), microglial activation, and microvascular damage (Table 3).[87] However, the hypothesis of direct viral entry into the CNS has been challenged due to a lack of evidence supporting widespread CNS invasion.[88,89] Instead, autoimmune mechanisms have been posited to play a more significant role. One study identified anti-SARS-CoV-2 antibodies with antineuronal properties, alongside a compartmentalized immune response in the cerebrospinal fluid,[87] suggesting that autoimmunity may contribute to neuro-PASC. Although these findings vary across studies,[90] they suggest that a robust immune response during acute infection may reduce its severity, while increasing the risk of autoimmune neuro-PASC, particularly in younger individuals and women.[36]

Key mechanisms underlying neurological damage in acute COVID-19 include systemic and neuroinflammation, microvascular injury, blood-brain barrier (BBB) disruption (further discussed in Section 5), and microthrombosis.[50,91] Evidence for direct viral neuroinvasion remains scarce (Table 3).[92,93] Autopsy studies have linked innate immune responses to subacute neuro-COVID,[94,95] while brainstem dysfunction or vascular injury may contribute to autonomic symptoms in neuro-PASC.[96] Immune dysregulation appears to be central to PASC, with reduced expression of effector molecules in memory T-cells correlating with CI and a diminished quality of life.[97]

4.2. Genetic factors and inflammatory responses

Genetic polymorphisms in ACE2 and TMPRSS2 have been implicated in the development of long COVID. Variations in how ACE2 interacts with the SARS-CoV-2 spike protein, the cleavage sites of TMPRSS2, and ACE2 expression levels are associated with both susceptibility to and the severity of COVID-19. Certain ACE2 variants, for instance, may increase the risk of severe disease by up to 28-fold.[98,99] For patients hospitalized due to COVID-19, these polymorphisms are linked to both the severity of the disease and the persistence of long COVID symptoms (Table 3).[100]

Systemic inflammation has been strongly associated with CI in neurological long COVID. While SARS-CoV-2 does not persist in neurons, it can infect and activate astrocytes and microglia, leading to localized brain atrophy and cognitive deficits.[24,101,102] Inflammatory mediators such as tumor necrosis factor (TNF), interleukin (IL)-6, IL-1β, and interferon-gamma have been detected in the cerebrospinal fluid of PD patients,[103] with IL-6 emerging as a potential biomarker for severe COVID-19.[104] Despite these findings, there remains insufficient evidence of SARS-CoV-2 replication within the CNS, emphasizing the need for further research.

4.3. Pathological mechanisms of long-term cognitive implications and neurodegenerative risks

Emerging mechanistic evidence suggests that COVID-19 may induce neuronal damage and increase the risk of chronic neurodegenerative diseases.[105] Individuals who have recovered from COVID-19 are at an increased risk of developing conditions such as multiple sclerosis (MS), PD, and Alzheimer's disease (AD) within 6 months of infection, compared to those with influenza or other respiratory infections.[14]

In MS, elevated levels of proinflammatory cytokines and heightened B lymphocyte activity contribute to increased neuroinflammation, particularly in postmortem cases with significant gray matter damage.[106] A United Kingdom MS registry study reported that about one-third of MS patients

Table 3. Mechanisms and implications of neurological impact in COVID-19 and long COVID

Mechanism	Key findings	Implications	References
Direct viral invasion	• Evidence for direct CNS invasion is limited. • Autoimmune mechanisms and compartmentalized immune responses in CSF suggest an indirect pathway	The findings suggest that autoimmune responses may play a more critical role than direct viral invasion in neuro-PASC.	87-89
Microvascular injury	• Linked to microglial activation and microthrombosis • Autopsy studies reported innate immune responses and microvascular damage, particularly in the olfactory bulb • Spike protein compromises BBB integrity.	The findings indicate that systemic inflammation and vascular injury are primary drivers of neuro-COVID and cognitive impairments	50,91,119
BBB dysfunction	• Elevated inflammatory markers (e.g., IL-6, S100β, TGF-β) in both acute and long COVID • Evidence of fibrinogen leakage, endothelial damage, and gray matter volume reduction in COVID-19 patients • Persistently elevated inflammatory markers in long COVID associated with cognitive impairments	BBB integrity may serve as a biomarker for neuro-COVID severity and a therapeutic target for managing chronic symptoms, such as brain fog	121-123
Neuroinflammation	• Activation of astrocytes and microglia has been linked to localized brain atrophy and cognitive deficits • Elevated levels of pro-inflammatory cytokines (e.g., TNF, IL-6) in CSF • SARS-CoV-2 does not replicate in neurons but triggers neuroinflammatory cascades	The findings highlight the central role of neuroinflammation in cognitive impairments and ADRD-like symptoms	24,101,103
Genetic polymorphisms	• Variants of ACE2 and TMPRSS2 were associated with increased susceptibility to severe disease and long COVID • Certain ACE2 variants can increase the risk of severe disease by as much as 28-fold • Linked to persistent symptoms in hospitalized patients	Genetic predisposition may help inform personalized risk assessments and guide targeted interventions	98-100
Neurodegenerative risks	• Recovered patients showed increased risks for MS, PD, and AD • Neuroinflammatory and synaptic dysfunctions overlapped with ADRD • Elevated biomarkers, such as tau and neurofilament light chain, correlate with disease severity and risk	The findings suggest potential exacerbation of pre-existing neurodegenerative conditions or triggering of subclinical diseases	14,105,113
Astrocyte reactivity	• Reactive astrocytes observed in long COVID share features with ADRD pathophysiology • EEG abnormalities were associated with synaptic dysfunction and cognitive decline	The findings indicate potential therapeutic overlap between ADRD and COVID-related cognitive impairments	124,125
Spike protein persistence	• Detected in immune cells up to 15 months post-infection • Triggers inflammatory cytokine production and induces coagulation dysregulation • Induces neurodegeneration when introduced into brain tissue in animal models	• The findings suggest the spike protein as a potential therapeutic target for alleviating symptoms of long COVID • Administration of mRNA COVID-19 vaccines, which encode the SARS-CoV-2 spike (S) protein, may lead to leakage from the injection site. This leakage may result in the accumulation of spike proteins in various organs, potentially contributing to adverse side effects	131-133

Abbreviations: ACE2: Angiotensin-converting enzyme 2; AD: Alzheimer's disease; ADRD: Alzheimer's disease and related dementias; BBB: Blood-brain barrier; CNS: Central nervous system; COVID: Coronavirus disease; CSF: Cerebrospinal fluid; EEG: Electroencephalogram; IL-6: Interleukin-6; mRNA: Messenger RNA; MS: Multiple sclerosis; PASC: Post-acute sequelae of SARS-CoV-2 infection; PD: Parkinson's disease; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; S100β: S100 calcium-binding protein B; TGF-β: Transforming growth factor-beta; TMPRSS2: Transmembrane protease, serine 2; TNF: Tumor necrosis factor.

who contracted COVID-19 experienced symptoms lasting beyond 4 weeks, with fatigue being the most prolonged symptom, persisting in one-eighth of patients for over 12 weeks. This finding suggests that pre-existing neurological conditions may increase susceptibility to long-term COVID effects. However, no worsening of clinical disease activity was observed in long-term relapsing-remitting MS patients post-COVID-19.[107] Larger studies are required to determine whether SARS-CoV-2 influences the progression of MS.

A 15-month cohort study by Zenesini *et al.*[108] found that PD patients had a higher risk of SARS-CoV-2 infection and hospitalization for Parkinsonism compared to healthy controls. Although COVID-19 prevalence was higher among PD patients, mortality rates were comparable to those of non-PD patients.[109] Comorbidities such as hypertension and diabetes may complicate the severity of COVID-19 in PD patients.[110] While direct evidence linking SARS-CoV-2 to PD is lacking, ACE2 receptors, which are widely expressed in the CNS, including regions related to PD, may play a role in susceptibility.[111] The SARS-CoV-2 E protein may activate Toll-like receptor 2 in microglia, potentially influencing both AD and PD.[112]

COVID-19 may exacerbate pre-existing conditions or trigger subclinical neurodegenerative diseases. For instance, individuals with AD were particularly vulnerable to SARS-CoV-2, experiencing a higher mortality risk.[113] This increased susceptibility may be attributed to pre-existing neuroinflammation and the inflammatory response triggered by COVID-19. Biomarkers associated with AD, such as serum total tau and neurofilament light chain, were positively correlated with infection severity,[113] suggesting poorer outcomes among hospitalized COVID-19 patients. Neuroinflammation driven by SARS-CoV-2 may contribute to degenerative lesions and an increased risk of AD (Table 3).[114]

Electroencephalogram (EEG) abnormalities may provide valuable insights into neurological complications and cognitive decline in long COVID patients. Understanding the overlapping pathophysiological mechanisms, such as neuroinflammation and astrocyte reactivity, can deepen our understanding of AD and the effects of COVID-19 on cognitive function (Table 3). Insights from neurophysiology and AD and related dementias (ADRD) research could inform how reactive astrocytes contribute to neurovascular comorbidities observed in COVID-19, highlighting research gaps related to cognitive EEG findings and mild CI in long COVID.[115]

Some individuals recovering from COVID-19 exhibit CIs similar to those seen in neurodegenerative diseases, particularly ADRD. Evidence suggests that COVID-19 and ADRD share common impacts on synaptic and neurovascular dysfunctions, involving astrocyte reactivity and neuroinflammation. Cognitive symptoms associated with COVID-19 may be driven by neurophysiological abnormalities akin to those observed in ADRD, which may be detectable through routine EEG exams.[115]

Given the shared pathophysiological mechanisms between COVID-19 and neurodegenerative diseases, including synaptic and neurovascular dysfunctions, further research is essential to elucidate the long-term impact of SARS-CoV-2 on cognitive health. Understanding these overlaps may help identify biomarkers for the early detection and guide the development of therapeutic interventions to mitigate neuro-PASC and related neurodegenerative conditions. Collaborative studies exploring immune responses, genetic factors, and neuroinflammatory pathways will be crucial in advancing our understanding of how COVID-19 influences both short- and long-term neurological outcomes.

5. BBB disruption as a key driver of CI in COVID-19 and long COVID

Recent research highlights the critical importance of BBB integrity in the neurological effects of SARS-CoV-2 infection.[25,116] The BBB, primarily composed of endothelial cells lining the cerebral vasculature, acts as a selective barrier that regulates molecular exchange between the bloodstream and the brain (Table 3).[117] This protective function is maintained by the coordinated efforts of astrocytes, pericytes, microglia, neurons, and the basement membrane, which together ensure barrier integrity through mechanisms such as tight junctions, transporters, and efflux systems.[118]

Postmortem examinations of tissues from COVID-19 patients have revealed significant microvascular damage, including fibrinogen leakage and thinning of the endothelial basal lamina, particularly in the olfactory bulb.[91] Spatial transcriptomic studies further indicated alterations in the vascular and immune systems, characterized by serum protein leakage and platelet accumulation.[119] These findings, along with similar observations in animal models, suggest that SARS-CoV-2 or its spike protein may compromise the BBB. However, the precise cerebrovascular pathology, particularly in long COVID, remains incompletely understood.

Unlike other zoonotic coronaviruses, such as severe acute respiratory syndrome and Middle East respiratory syndrome, which rarely cause neurological complications, SARS-CoV-2 appears to induce more frequent BBB disruption, particularly in patients with CIs like brain fog.[120] This association suggests that BBB integrity may serve as a potential biomarker for neurological damage in COVID-19, with therapies aimed at preserving or restoring the BBB offering a promising approach for managing long COVID-related symptoms.[121]

Systemic inflammation during SARS-CoV-2 infection is a major driver of BBB dysfunction. Elevated levels of serum proteins, including S100 calcium-binding protein B (S100β), IL-6, basic fibroblast growth factor, and IL-13, have been observed in actively infected patients.[122,123] S100β, a marker commonly associated with neurological conditions such as epilepsy and traumatic brain injury, is particularly elevated in COVID-19 patients experiencing cognitive symptoms (Table 3).[122,123] In long COVID, BBB permeability appears to persist independently of age, emphasizing its potential role in chronic neurological symptoms. Structural changes, such as reduced gray matter volume and increased cerebrospinal fluid, have also been correlated with BBB dysfunction in long COVID patients.[121]

Increased levels of several inflammatory biomarkers, including IL-8, glial fibrillary acidic protein (GFAP), and transforming growth factor-beta (TGF-β), have been observed in patients with long COVID, particularly among those experiencing brain fog.[124,125] TGF-β, known for its role in BBB disruption and structural brain changes, has also been implicated in chronic fatigue syndrome, a condition with clinical similarities to long COVID.[126,127] In addition, GFAP, typically associated with cerebrovascular damage, is elevated in individuals with long COVID and related neurological impairments.[124,128]

Animal models and postmortem studies provide further evidence of BBB compromise in COVID-19, revealing fibrinogen leakage and coagulation dysregulation in brain tissue.[91,119] Mouse models, in particular, displayed abnormalities such as "string vessels," indicative of blood vessel pathology.[129] Research consistently demonstrates that inflammation and coagulation dysregulation are pivotal factors in the pathophysiology of disease in patients recovering from COVID-19.[130]

The long-term neurological effects may be partly attributable to the SARS-CoV-2 spike protein itself. Studies have shown that the spike protein can induce coagulation dysregulation and neurodegeneration when injected into the brain, and it has been detected in immune cells up to 15 months post-infection (Table 3).[131,132] Its persistent presence may underlie symptoms of long COVID, such as brain fog. *In vitro* studies also indicate that spike protein exposure activates brain endothelial cells, leading to increased inflammatory cytokine production and elevated expression of cell adhesion molecules, which may exacerbate BBB dysfunction.[131,133]

Growing evidence underscores the crucial role of BBB integrity in the neurological manifestations of SARS-CoV-2 infection, particularly with respect to CIs like brain fog observed in long COVID.[121] The persistent disruption of the BBB, driven by systemic inflammation and coagulation dysregulation, presents a significant challenge in understanding and treating the long-term effects of COVID-19. Future research should prioritize longitudinal studies to better elucidate the mechanisms contributing to BBB dysfunction and to develop therapeutic strategies aimed at preserving or restoring BBB integrity, which could alleviate chronic neurological symptoms in post-COVID patients.

6. The role of astrocytes and microglia in COVID-19-associated neuroinflammation and CI

6.1. Astrocytes: Mediators of neuroinflammation and CI in COVID-19

Astrocytes are key players in the neuroinflammatory response to COVID-19. Studies have shown that astrocytes in COVID-19 patients exhibit reduced process complexity, shortened process lengths, and enlarged cell bodies.[101,134,135] Furthermore, clusters of astrocytes associated with COVID-19 demonstrated upregulation of inflammatory and astrogliosis-related genes, such as interferon-induced transmembrane protein 3 and GFAP. These astrocytes also secrete neurotoxic factors like chitinase 3-like 1, contributing to neuronal death, which parallels astrocytic involvement in AD.[136,137]

Activated microglia can induce the formation of neurotoxic A1 astrocytes by secreting cytokines such as IL-1α, TNF-α, and complement component 1q (C1q). These A1 astrocytes promote neuronal and oligodendrocyte death, further contributing to neurodegeneration in conditions like AD. On the other hand, astrocytes also participate in amyloid-beta (Aβ) clearance by facilitating its transport across the BBB, which may help prevent its accumulation (Table 4).[138-142] However, the exact mechanisms by which astrocytes and microglia interact during COVID-19-induced CI remain unclear and warrant further investigation.

Astrocytes play essential roles in neurotransmitter recycling, maintaining synaptic transmission, and regulating neuronal excitability.[143,144] They modulate glutamate levels to prevent excitotoxicity. In SARS-CoV-2-infected mixed glial cultures, a significant reduction in L-glutamine levels has been observed, impairing neuronal metabolism and synaptic function. Inhibition of L-glutamine reduced viral replication and the inflammatory response, further disrupting neuronal homeostasis.[134] In addition, SARS-CoV-2 infection in astrocytes led to metabolic changes, such as reduced lactate levels, depriving neurons of crucial energy sources and contributing to CI and neuronal death.

6.2. Microglia: Dual roles in neuroinflammation and cognitive decline

Microglia, the brain's resident immune cells, play a complex role in neurodegenerative diseases such as AD, exhibiting both protective and harmful functions.[145,146] While moderate microglial activation is beneficial for clearing Aβ in the brain, overstimulation by Aβ or amyloid precursor protein (APP) can trigger excessive inflammatory responses, potentially accelerating neurodegeneration

Table 4. Inflammatory, genetic, and molecular pathways linking long COVID to Alzheimer's disease

Pathway	Mechanism	Key observations	Implications	Reference
Neurological symptoms	Persistent symptoms in long COVID overlap with those observed in AD, including fatigue, memory loss, depression, and brain fog	Both conditions share symptoms such as anosmia, insomnia, and cognitive impairment. AD involves amyloid-beta accumulation and hyperphosphorylated tau proteins, leading to neuroinflammation and neuronal damage	The shared features suggest common mechanisms, highlighting the potential role of viral infections in contributing to long-term brain dysfunction and neurodegeneration	171-173
APOE4 and NLRP3 inflammasome	*APOE4* genotype is a risk factor for both severe COVID-19 and AD. The NLRP3 inflammasome contributes to neuroinflammation in both conditions	APOE4 is associated with increased susceptibility to infections and serves as a receptor for SARS-CoV-2. NLRP3 inflammasome activation is implicated in tau aggregation, amyloid-beta production, and neurodegeneration	The findings highlight genetic and inflammatory pathways that converge to drive cognitive decline	163-165
ACE2 expression	ACE2 mediates SARS-CoV-2 entry and is overexpressed in AD-affected brain regions, such as the temporal lobe and hippocampus	Elevated ACE2 levels in AD may facilitate SARS-CoV-2 entry into neurons and glial cells. Viral binding to ACE2 reduces its availability, disrupting homeostasis and increasing inflammation and oxidative stress	The findings link viral entry mechanisms to exacerbation of neurodegenerative processes in AD	176-178
Blood-brain barrier compromise	SARS-CoV-2 may infiltrate the central nervous system via the olfactory nerve, particularly when the blood-brain barrier is compromised	AD patients often exhibit impaired blood-brain barrier integrity, which facilitates viral invasion and exacerbates neuroinflammation	The findings highlight the increased vulnerability of individuals with neurodegenerative diseases to viral-induced neurological damage	132,180-182
Inflammatory biomarkers	Shared biomarkers like interleukin-6, TNF, and interleukin-1, are elevated in both COVID-19 and AD, driving chronic inflammation.	Elevated inflammatory responses are associated with neurocognitive decline in both conditions. COVID-19 triggers widespread inflammation, even in patients without prior neurological symptoms	The findings suggest inflammation as a critical mediator of long-term neurodegenerative outcomes post-infection	139-142
Amyloid-beta and antimicrobial peptides	SARS-CoV-2 may induce amyloid-beta production as part of the immune response, potentially increasing the risk of AD	Amyloid-beta functions as an antimicrobial peptide but contributes to synaptic dysfunction and neuroinflammation in AD	The findings indicate a potential direct connection between viral infection and AD-related pathologies	149-152
Oxidative stress and cytokine imbalance	COVID-19 exacerbates ACE/ACE2 imbalance, promoting oxidative stress and inflammatory cytokine production	Dysregulated renin-angiotensin system activity and elevated angiotensin II levels in COVID-19 patients contribute to endothelial dysfunction and neuroinflammation	The findings enhances our understanding of the systemic contributions to neurodegenerative disease progression	171,187,188

Abbreviations: ACE: Angiotensin-converting enzyme; ACE2: Angiotensin-converting enzyme 2; AD: Alzheimer's disease; APOE4: Apolipoprotein E4; COVID: Coronavirus disease; NLRP3: NLR family pyrin domain containing 3; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; TNF: Tumor necrosis factor.

in AD.[147-152] Studies indicate that significant microglial activation occurs in the brains of rats with COVID-19-related pneumonia. This activation was characterized by reduced microglial branch length, diminished nearest neighbor distances, decreased stem areas, and enlarged cell bodies, signaling a shift toward a more pro-inflammatory state.[153,154]

Similarly, even mild respiratory infections caused by SARS-CoV-2 have been shown to activate microglia in the subcortical white matter of mouse models. This activation led to the loss of oligodendrocyte precursors and mature oligodendrocytes, followed by myelinated axon degeneration, impairing the structural and functional integrity of neuronal networks. Moreover, microglial activation in the hippocampus has been linked to suppressed neurogenesis, contributing to memory deficits and CI.[155]

Recent findings have also highlighted the role of C1q-mediated microglial phagocytosis as a mechanism underlying long-term CI induced by the SARS-CoV-2 spike protein. Genes enriched in microglial clusters associated with COVID-19-related pneumonia overlap with those found in AD-associated microglia.[156] Furthermore, genes related to neuroinflammation, such as receptor-interacting serine/threonine kinase 1, have been identified in COVID-19-affected microglia, revealing distinct yet overlapping inflammatory pathways between COVID-19 and neurodegenerative conditions.[157]

Neurodegeneration has been associated with inflammatory factors secreted by activated microglia, including TNF-α and IL-1β. These pro-inflammatory cytokines impair microglial endocytosis of pathological Aβ and tau proteins, exacerbating neuronal damage. In contrast, anti-inflammatory microglia secrete cytokines such as IL-2, IL-4, IL-10, and TGF-β, which promote repair and recovery of learning and memory functions through multiple signaling pathways (Table 4).[146,158] Understanding the regulatory mechanisms that modulate microglial activation and their shift toward an anti-inflammatory phenotype could provide new therapeutic targets for mitigating CI.

Both astrocytes and microglia play pivotal roles in the neuroinflammatory responses observed in COVID-19. Their activation not only mirrors processes seen in neurodegenerative diseases like AD but also introduces unique inflammatory pathways related to SARS-CoV-2 infection.[159] The interaction between microglia and astrocytes, along with their impact on neuronal survival and function, represents a key area for future research. Understanding the regulatory mechanisms that modulate glial activation in response to SARS-CoV-2 could inform potential therapeutic strategies for mitigating long-term CI in COVID-19 survivors. Further studies exploring the molecular crosstalk between microglia and astrocytes may pave the way for novel interventions aimed at reducing the neurotoxic effects of COVID-19.

7. Inflammatory pathways linking long COVID to neurodegeneration in AD

7.1. Neurological symptoms in long COVID and AD

Given the multisystemic nature of long COVID, characterized by the persistence or emergence of symptoms beyond the acute phase of infection, numerous hypotheses have been proposed to elucidate its underlying pathophysiological mechanisms.[3] The neurological symptoms associated with long COVID, such as insomnia, fatigue, brain fog, anosmia, memory loss, depression, and anxiety, bear a striking resemblance to those observed in AD.[160,161] This overlap suggests potential shared mechanisms between long COVID and AD, prompting further investigation into how viral infections may influence long-term brain function and neurodegeneration (Table 4).

AD is characterized by the accumulation of Aβ plaques and hyperphosphorylated tau proteins, which damage neurons. Aβ interferes with synaptic transmission, while hyperphosphorylated tau disrupts nutrient transport within neurons. Both proteins activate microglia, leading to chronic inflammation and reduced brain volume. AD is the leading cause of dementia, manifesting in cognitive decline, memory loss, impaired judgment, and changes in personality, mood, and behavior.[162]

7.2. Shared roles of apolipoprotein E4 and NLR family pyrin domain containing 3 inflammasome in AD and neurodegenerative consequences of COVID-19

During the COVID-19 pandemic, individuals with AD were five times more likely to die from COVID-19 compared to those without AD.[161] The apolipoprotein E4 (*APOE4*) gene, a major risk factor for AD, has been identified as a potential biomarker for severe COVID-19.[160,162] Specifically, the apolipoprotein E (APOE) $\varepsilon 4$ allele has been implicated as a susceptibility factor for both AD and COVID-19 (Table 4).[163-165] Research has shown that individuals with the *APOE* $\varepsilon 4/\varepsilon 4$ genotype face a higher risk of severe COVID-19 and are more likely to test positive compared to those with the *APOE* $\varepsilon 3/\varepsilon 3$ genotype.[162]

The *APOE* genotype plays a critical role in susceptibility to pathogens in various infectious diseases.[166] APOE proteins, which serve as receptors for viruses like herpesvirus and hepatitis C, may also act as receptors for SARS-CoV-2.[167] Pre-existing conditions such as dementia and delirium increase the risk of severe COVID-19 outcomes. Both SARS-CoV-2 and AD lead to neurocognitive disorders, anxiety, excessive fatigue, and olfactory dysfunction. Autopsies of COVID-19 patients have revealed widespread brain inflammation and degeneration, even in those without prior neurological symptoms. Studies have highlighted gene overlaps between AD markers and genes upregulated during COVID-19 infection.[23,168] Inflammatory biomarkers, such as IL-6, TNF, galectin-3, and IL-1 have, been proposed as shared prognostic indicators for both SARS-CoV-2 infection and AD.[169]

The activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome, a crucial component of the immune system, has been linked to both tau aggregation and neurodegeneration in the context of SARS-CoV-2 infection.[170] The NLRP3 inflammasome plays a pivotal role in regulating inflammatory responses, and its dysregulation can contribute to neuronal damage and cognitive decline. Furthermore, it is hypothesized that SARS-CoV-2 may trigger the production of Aβ, an antimicrobial peptide, thereby potentially heightening the risk of AD in COVID-19 patients (Table 4).[171-173] The potential link between SARS-CoV-2, NLRP3 inflammasome activation, and Aβ production underscores the need for further research to elucidate the mechanisms by which viral infections may contribute to neurodegenerative diseases.

7.3. The intersection of AD and COVID-19: ACE2 as a mediator of viral entry and neurodegeneration

In AD patients, there is an observed increase in the expression of ACE2, which serves as the cellular receptor for SARS-CoV-2, facilitating viral entry into host cells.[169] ACE2 is notably expressed in neurons and glial cells, with higher concentrations in the temporal lobe and hippocampus – regions critically involved in AD pathology. Although ACE2 expression does not exhibit significant age-dependent variation, evidence suggests a correlation between elevated ACE2 levels and the development or progression of AD.[169] In the context of COVID-19, ACE2 plays a crucial role by mediating the entry of SARS-CoV-2 into cells, contributing to the virus's pathogenic effects.[174] Beyond its role in neuronal and glial cells, ACE2 is expressed in epithelial and endothelial cells, which is essential for understanding the mechanisms by which SARS-CoV-2 infiltrates the body and exacerbates disease progression.[175] The increased ACE2 expression in AD-affected brain regions may not only facilitate viral entry but also contribute to the neurodegenerative effects observed in COVID-19, highlighting a potential intersection between viral infection and neurodegenerative disease pathways (Table 4).[176-178]

In the nervous system, ACE2 expression is generally low compared to other tissues. However, SARS-CoV-2 may still infiltrate the brain through the olfactory nerve, particularly in individuals with compromised BBB integrity – a common feature of neurodegenerative disorders such as AD.[132,179-182] This potential route of entry underscores the vulnerability of the CNS to viral invasion in the presence of pre-existing neurological damage.

Immunofluorescence staining and single-cell gene atlas studies revealed that ACE2 is predominantly expressed in type II alveolar epithelial cells and airway epithelial cells. These findings provide critical insights into the mechanisms of viral infection and highlight the role of ACE2 in facilitating SARS-CoV-2 entry into host cells.[183] Furthermore, the presence of ACE2 in saliva has been suggested as a potential diagnostic marker for COVID-19, reflecting its widespread expression and involvement in viral entry.[184]

In patients with comorbid conditions such as diabetes and hypertension, there is often an imbalance between ACE and ACE2, which exacerbates endothelial dysfunction.[185] Elevated plasma levels of angiotensin II, a key mediator in the renin-angiotensin system, have been observed in COVID-19 patients. SARS-CoV-2 binds to ACE2, reducing its availability and leading to an increased ACE/ACE2 ratio. This imbalance heightened inflammatory responses, promoted the production of ACE-dependent cytokines, induced oxidative stress, and diminished the protective effects typically mediated by ACE2.[171,186-188]

As a result, elevated ACE2 expression, particularly in the context of neurodegenerative diseases like AD, may represent a significant risk factor for increased susceptibility to SARS-CoV-2 infection.[189] The intersection of these factors emphasizes the need for further research into the role of ACE2 in both the pathophysiology of neurodegenerative diseases and the mechanisms of viral infection.

8. Potential therapeutic approaches for neurological complications of COVID-19

At present, no proven therapeutic regimen specifically targets the neurological complications associated with COVID-19, despite growing recognition of these issues. However, various potential therapies are being explored to address conditions such as CI, stroke, encephalopathy, and Guillain–Barré syndrome (GBS) that may arise following SARS-CoV-2 infection. Promising therapeutic strategies are under investigation, with the potential to mitigate the neurological impact of COVID-19.

8.1. CI and AD drugs

CI associated with COVID-19, particularly in long-haul cases, is a significant concern, as affected individuals often experience persistent brain fog or memory problems.[190] Some researchers are exploring the repurposing of AD drugs, such as aminoadamantane and memantine, as potential treatments for cognitive deficits caused by the virus (Table 5).[191,192] These drugs, which act on glutamatergic pathways, might improve cognitive function by reducing excitotoxicity,[193] a mechanism implicated in both AD and COVID-19-induced brain damage.[194] While these options are still speculative, they represent a key area of future research.

8.2. Revascularization and erythrocyte metabolism restoration

Another promising approach to addressing COVID-19-related neurological complications involves improving cerebral blood flow and oxygen delivery to damaged brain tissue.[195] Hyperbaric oxygen therapy (HBOT) is one technique under investigation, which delivers 100% oxygen under pressure to stimulate brain recovery. HBOT has demonstrated potential in improving revascularization and restoring erythrocyte metabolism, both of which

Table 5. Potential therapeutic approaches for managing neurological complications of COVID-19

Neurological condition	Proposed therapeutic approach	Mechanism of action	Current status	References
Cognitive impairment	Repurposing Alzheimer's disease drugs (e.g., aminoadamantane, memantine)	Targets glutamatergic pathways to reduce excitotoxicity, potentially improving cognitive function	Speculative; Currently under investigation for their potential efficacy in mitigating COVID-19-related cognitive deficits	190-194
	Hyperbaric oxygen therapy	Improves cerebral perfusion and oxygen delivery, promotes neurogenesis and angiogenesis, and restores erythrocyte metabolism	Promising case studies; requires larger trials to validate benefits for post-COVID-19 cognitive impairment	195-198
Neuroinflammation	Immune-modulating therapies (e.g., JAK inhibitors like baricitinib, anti-IL-6, and anti-IL-1β treatments)	Regulates the inflammatory response, particularly cytokine storms, and reduces neuroinflammation by targeting key inflammatory mediators	Early promise in reducing cognitive impairment; clinical trials ongoing to determine safety and efficacy	159,199-201
	Natural compounds (e.g., quercetin, ginkgolide, bilobalide)	Potentially reduces long-term neuroinflammation and cognitive complications through neuroprotective and anti-inflammatory properties	Experimental stage; larger studies are needed to establish their therapeutic potentials	202
Stroke (ischemic and hemorrhagic)	Established stroke interventions (e.g., thrombolysis, thrombectomy)	Maintains reperfusion and reduces clot burden in ischemic events related to COVID-19.	Standard care remains effective for COVID-19 patients; no significant differences in outcomes compared to stroke management for non-COVID-19 patients	86,203,204
Encephalopathy	Corticosteroids (e.g., methylprednisolone), IVIG, plasma exchange, rituximab	Reduces inflammation and modulates immune responses to address acute brain dysfunction and altered mental states in COVID-19	Reported benefits in clinical practice, but further evidence is required to confirm their effectiveness for COVID-19-related encephalopathy	205-208
Guillain–Barré syndrome	IVIG and plasma exchange	Standard treatments to mitigate progression, particularly in cases with respiratory insufficiency that is disproportionate to the pulmonary findings	Established care for GBS applies to COVID-19-related cases; ongoing monitoring of outcomes is essential	209
General neuroprotection	Development of tailored neuroprotective interventions	Focuses on preventing long-term neurological damage while minimizing the risk of overtreatment in low-risk patients	Represents a key area of future research, with an emphasis on personalized therapeutic strategies	210

Abbreviations: COVID-19: Coronavirus disease 2019; GBS: Guillain–Barré syndrome; IL-1β: Interleukin 1 beta; IL-6: Interleukin-6; IVIG: Intravenous immunoglobulin; JAK: Janus kinase.

are crucial for brain tissue repair (Table 5).[196,197] A recent case report highlighted the benefits of HBOT in a patient with post-COVID-19 CI, noting improvements in cerebral perfusion and the preservation of white matter microarchitecture in regions such as the frontal, parietal, and limbic areas.[198] This suggests that HBOT may promote neurogenesis and angiogenesis, aiding cognitive recovery in COVID-19 survivors.

8.3. Neuroinflammation and immune-modulating therapies

Neuroinflammation is believed to play a significant role in brain injury following COVID-19.[159] Consequently, immune-modulating therapies that can cross the BBB and target inflammatory processes are being explored for their potential to reduce CI.[199] Cytokine antagonists and pathway modulators, such as Janus kinase inhibitors like baricitinib, have shown early promise in this context. These drugs aim to regulate the intense inflammatory response, often referred to as a cytokine storm, which characterizes severe cases of COVID-19.[200] Therapies targeting specific inflammatory mediators, such as anti-IL-6 and anti-IL-1β treatments, are being investigated for their ability to modulate cytokine activity and may help prevent cognitive decline after COVID-19 (Table 5).[201]

In addition, natural compounds with neuroprotective properties, such as quercetin, ginkgolide, and bilobalide, are under consideration for their potential to reduce long-term cognitive complications associated with COVID-19.[202] However, the efficacy and safety of these interventions remain uncertain, and larger, well-designed clinical trials are needed to comprehensively evaluate these therapies.

8.4. Stroke and seizure management: Adhering to established standards of care

COVID-19 has been linked to an increased risk of stroke, with patients experiencing both ischemic and hemorrhagic events due to the virus-induced prothrombotic state.[86] Current evidence suggests that standard stroke interventions, such as thrombolysis or thrombectomy, remain applicable to COVID-19 patients, with no significant differences in the risk-benefit ratio compared to non-COVID-19 stroke patients (Table 5).[203,204] As such, the management of ischemic and hemorrhagic strokes in these individuals should follow established protocols to ensure timely evaluation and intervention.

8.5. Encephalopathy and Guillain–Barré syndrome

COVID-19 has also been associated with cases of encephalopathy, an acute condition characterized by altered mental status and brain dysfunction.[205] Treatments for COVID-19-associated encephalopathy have included corticosteroids (e.g., methylprednisolone), intravenous immunoglobulin (IVIG), plasma exchange, and rituximab, with the goal of reducing inflammation and modulating immune responses in affected patients (Table 5).[206-208]

Guillain–Barré syndrome, a rare but severe condition linked to COVID-19, is typically treated with IVIG or plasma exchange, which is the standard treatments for GBS caused by other factors.[209] These therapies can help mitigate disease progression, particularly when respiratory insufficiency appears disproportionate to pulmonary findings, a hallmark of GBS.

While several promising therapeutic options are under investigation to address COVID-19-associated neurological complications, their safety and efficacy remain uncertain. Larger clinical trials are essential to determine whether interventions such as anti-Alzheimer's agents, cytokine antagonists, or HBOT can effectively treat COVID-19-related CI. Similarly, the management of strokes, encephalopathy, and GBS should continue to follow established standards of care until more targeted COVID-19 therapies become available. As research progresses, neuroprotective interventions may play a key role in reducing the long-term neurological impact of COVID-19,[210] but a tailored approach will be crucial to avoid overtreatment in patients who are not at significant risk.

9. Long-term neuroimaging evidence of structural and functional brain changes in COVID-19 survivors

9.1. Acute phase neuroimaging findings

Neuroimaging research has consistently identified brain abnormalities in both the acute and recovery phases of COVID-19.[24,211-213] During the acute phase of the illness, MRI scans have detected signal abnormalities in approximately one-third of patients, highlighting early neuroimaging markers of the disease.[24] In addition, brain CT scans have revealed acute lesions, particularly in severe cases requiring intensive care unit admission, underscoring the severity of brain involvement in critical cases (Table 6).[214-218]

As patients progress to the recovery phase, neuroimaging studies have documented significant structural changes. These include reductions in cortical thickness, diminished cerebral blood flow, and alterations in white matter integrity, with notable effects observed in the frontal and limbic regions.[213,219-221] Such changes reflect the lasting impact of COVID-19 on brain structure and function, extending beyond the acute phase of infection (Table 6).

9.2. Long-term consequences of COVID-19 on brain health

Long-term follow-up studies have further elucidated the enduring impact of COVID-19 on brain health. Persistent reductions in gray matter, particularly in the left temporal lobe, have been observed up to 2 years post-infection.[222] Resting-state functional magnetic resonance imaging (fMRI) studies have also identified significant alterations in brain activity in regions such as the precentral gyrus, angular gyrus, and thalamus, indicating ongoing functional disruptions (Table 6).[223-228] These findings highlight the necessity for comprehensive, long-term research to fully elucidate the ramifications of COVID-19 on brain health.

Moreover, fMRI studies have shown that individuals recovering from COVID-19 frequently experience cognitive deficits, neurological issues, and psychiatric symptoms.[229] Comparative analyses of brain imaging data, conducted over 2 years after infection, have demonstrated significant differences between COVID-19 survivors and healthy controls.[224,230-232] These insights are crucial for understanding CIs associated with long COVID, such as brain fog, and underscore the urgent need for targeted therapeutic interventions to address these persistent issues.

9.3. Structural and functional changes in specific brain regions

Neuroimaging studies of COVID-19 survivors have revealed increased amplitude of low-frequency fluctuations

Table 6. Neuroimaging evidence of structural and functional brain changes in COVID-19 survivors

Category	Neuroimaging findings	Affected brain regions	Functional/clinical implications	Current evidence and limitations	References
Acute phase	• MRI finding: Signal abnormalities detected in approximately one-third of patients. • CT finding: Acute lesions, particularly in severe cases requiring ICU admission.	• General brain regions with higher prevalence in critical cases.	• Early markers of COVID-19-related neurological involvement. • Indicators of disease severity and potential for acute brain injury.	• Predominantly observed in severe cases. • Limited longitudinal data tracking progression from acute to recovery phases. • Variability in imaging protocols across studies.	215-218
Recovery phase	• Structural change: Reduced cortical thickness. • Cerebral blood flow: Diminished perfusion. • White matter integrity: Alterations observed in diffusion tensor imaging.	• Frontal regions • Limbic regions	• Lasting impact on brain structure and function. • Potential for persistent cognitive and emotional impairments.	• Based on cross-sectional studies with varying follow-up durations. • Need for standardized longitudinal studies to assess long-term trajectory.	213,219-221
Long-term consequences	• Gray matter: Persistent reductions, especially in the left temporal lobe, observed up to 2 years post-infection. • Functional activity: Altered resting-state fMRI activity in the precentral gyrus, angular gyrus, and thalamus.	• Left temporal lobe • Precentral gyrus • Angular gyrus • Thalamus	• Ongoing functional disruptions contributing to cognitive deficits, neurological issues, and psychiatric symptoms. • Manifestation of long COVID symptoms such as brain fog.	• Long-term follow-up studies indicate enduring changes in brain structure and function. • Limited by small sample sizes and heterogeneity in study populations. • Potential confounding factors not fully controlled.	224-228
Structural and functional changes	• Amplitude of low-frequency fluctuations: Increased in the left putamen and right pallidum. • Connectivity patterns: Altered in the right insula and right putamen linked to cognitive failure. • Cortical thickness: Decreased in the left putamen. • Temporal lobe damage: Involving the STG and ITG. • Spontaneous activity: Decreased in the right SPG.	• Left putamen • Right pallidum • Right insula • STG • ITG • SPG	• Putamen and pallidum: Alters motor and cognitive functions. • Insula: Impacts emotional regulation and cognitive processing. • Temporal lobe: Affects memory, language comprehension, and sensory processing. • Parietal gyrus: Impairs visuospatial processing, attention, and working memory. • Potential compensatory mechanisms in response to brain damage.	• Findings suggest specific regional vulnerabilities. • Causality between COVID-19 and observed changes remains unclear. • Necessitates further investigation into underlying mechanisms. • Potential for overlapping symptoms with other neurological conditions.	223,224,237,238
Comparative analyses	• Differences in neuroimaging data: Significant disparities between COVID-19 survivors and healthy controls over 2 years post-infection. • Mendelian randomization studies: Association between severe COVID-19 and reduced cortical surface area in regions such as the superior parietal gyrus, pericalcarine cortex, and parahippocampal gyrus.	• Superior parietal gyrus • Pericalcarine cortex • Parahippocampal gyrus	• Highlights unique neurobiological impact of COVID-19 compared to other viral infections. • Enhances understanding of specific cognitive impairments like brain fog.	• Comparative studies are essential for isolating COVID-19-specific effects. • Need to account for confounding variables such as pre-existing conditions and other infections. • Further research is required to generalize findings across diverse populations.	224,230-232

Abbreviations: COVID: Coronavirus disease; CT: Computed tomography; fMRI: Functional magnetic resonance imaging; ICU: Intensive care unit; ITG: Inferior temporal gyrus; MRI: Magnetic resonance imaging; SPG: Superior parietal gyrus; STG: Superior temporal gyrus.

(ALFF) in the left putamen and right pallidum compared to healthy controls, indicating altered brain activity (Table 6). A 1-year follow-up fMRI study also found elevated ALFF values in the left putamen.[223] These observations align with a task-based fMRI study that showed altered connectivity patterns in the right insula and right putamen, which were linked to cognitive failure and may contribute to subjective cognitive decline.[233] In addition, a 3-month follow-up study reported decreased cortical thickness in the left putamen of COVID-19 survivors, suggesting potential structural damage.[219] These findings might reflect compensatory brain mechanisms in response to damage, although the exact cause of putamen impairment remains uncertain.

Various levels of damage to the temporal lobe, including the left superior temporal gyrus (STG) and right inferior temporal gyrus (ITG), have been observed in COVID-19 survivors.[224] The temporal lobe plays a key role in emotional regulation, sensory processing, memory, and language comprehension.[234] Damage to the STG has been linked to cognitive conditions such as subjective CI, mild CI, and dementia.[235,236] These findings align with structural MRI studies, which reported decreased cortical thickness in the STG of COVID-19 survivors 3 months after recovery.[219] In addition, increased ALFF values in the ITG were identified in a 1-year follow-up resting-state fMRI study, suggesting altered activity in this region post-COVID.[223,224,237,238]

In addition, decreased spontaneous brain activity in the right superior parietal gyrus has been observed in COVID-19 survivors.[224] The superior parietal cortex is essential for visuospatial processing, attentional control, and working memory.[239] A review on COVID-19-related CI indicates that survivors may experience deficits in memory, attention, and executive function, suggesting widespread brain damage.[81] Supporting this, a recent Mendelian randomization study found an association between severe COVID-19 and reduced cortical surface area in the superior parietal gyrus, pericalcarine cortex, and parahippocampal gyrus.[230] These findings suggest that the superior parietal gyrus may be particularly vulnerable to COVID-19-related damage, though the precise mechanisms remain unclear (Table 6).

Neuroimaging studies have identified significant brain abnormalities associated with COVID-19, particularly in severe cases, where acute signal disruptions and lesions are evident. Long-term structural changes, including reduced cortical thickness, alterations in white matter integrity, and diminished cerebral blood flow, have been prominently observed in the frontal and limbic regions. To further elucidate the impact of COVID-19 on brain health, future research should prioritize long-term neuroimaging follow-ups, investigate the underlying mechanisms of brain damage, develop targeted therapeutic interventions, and explore potential recovery pathways. Comparative studies with other viral infections may also provide critical insights into the unique neurobiological consequences of COVID-19.

10. Conclusion and future directions

The neurological impacts of SARS-CoV-2 span both the acute and post-acute phases, presenting a complex and evolving landscape of symptoms and sequelae.[240] The profound and multifaceted influence of SARS-CoV-2 on neurological health ranges from the acute infection phase to long-term outcomes. Persistent symptoms observed in PASC emphasize the need for ongoing surveillance and research (Table 1). Initial studies have highlighted common manifestations such as fatigue, CI, and mood disorders. However, substantial variability and uncertainty persist, particularly regarding mild infections and emerging neurological disorders.[27]

The complexity of neurological damage post-COVID-19 is further compounded by systemic inflammation, immune dysregulation, and potential autoimmune responses (Table 3).[241] Although direct viral invasion of the CNS is debated, evidence suggests that immune-mediated mechanisms and disruption of the BBB play central roles in the neurological sequelae associated with long COVID.[242] Genetic factors may also influence susceptibility and severity, underscoring the need for personalized approaches in both managing and studying these effects.[243]

There is an urgent need for well-designed, long-term cohort studies to monitor the progression of neurological symptoms over time, especially in cases of mild and moderate COVID-19. Such studies should encompass diverse populations and account for pre-existing neurological conditions to provide a comprehensive understanding of the spectrum of PASC. Comparative studies involving other viral infections and SARS-CoV-2-negative controls will help delineate specific neurological risks linked to COVID-19 and inform public health strategies.

Further research into the mechanisms underlying neurological symptoms is essential, focusing on immune dysregulation, systemic inflammation, and BBB integrity. Investigating how SARS-CoV-2 interacts with neural and immune systems will clarify the pathways involved in neuro-PASC. Identifying reliable biomarkers for the early detection and monitoring of neurological complications is also crucial. Biomarkers related to systemic inflammation and neurodegenerative diseases could offer valuable insights into the pathophysiology of PASC and aid in developing targeted interventions.

The shared neuroinflammatory pathways between COVID-19 and AD provide a promising framework for advancing our understanding of AD mechanisms, disease progression, and long-term cognitive decline

(Table 4). Both conditions involve glial cells – specifically astrocytes and microglia – which play a critical role in neurodegeneration. The inflammatory responses triggered by COVID-19, including microglial activation and astrocytic involvement, closely resemble those observed in AD, suggesting that viral infection may accelerate AD-like pathology in susceptible individuals. Future research focused on these shared mechanisms could lead to targeted therapies that not only address CIs resulting from COVID-19 but also offer novel strategies for treating AD. A deeper investigation into the molecular interactions between SARS-CoV-2 and neurodegenerative pathways, particularly the roles of the NLRP3 inflammasome and ACE2, could identify promising therapeutic targets to mitigate or prevent neurodegeneration in both conditions. Ultimately, the intersection of COVID-19 and AD underscores the need for integrated approaches to studying the impact of viral infections on neurodegenerative diseases, offering potential avenues for innovative treatments aimed at slowing or halting their progression.

Neuroimaging studies consistently demonstrate significant acute and long-term structural and functional brain changes in COVID-19 survivors (Table 6). Acute-phase imaging often reveals signal abnormalities and lesions, particularly in severe cases, indicating immediate neurological effects. In the recovery phase, persistent alterations such as reduced cortical thickness, diminished cerebral blood flow, and white matter disruption are prominent in the frontal and limbic regions. Long-term findings include gray matter loss, particularly in the left temporal lobe, and altered activity in regions such as the precentral gyrus and thalamus, with changes in the putamen, insula, and temporal gyri linked to cognitive and emotional impairments associated with long COVID. These distinct neurobiological impacts, compared to other viral infections, underscore the need for targeted therapeutic strategies. However, limitations such as small sample sizes and study heterogeneity call for comprehensive longitudinal research to clarify mechanisms and recovery pathways.

Advancements in neuroimaging and EEG may enhance our understanding of cognitive and neurophysiological abnormalities associated with COVID-19, facilitating early detection of cognitive decline and providing insights into neurovascular comorbidities in long COVID. Developing and evaluating both pharmacological and non-pharmacological treatment strategies tailored to these neurological impacts is essential for symptom management and improving quality of life. Engaging with patients to understand their experiences and outcomes will be crucial in shaping research priorities and ensuring interventions meet real-world needs.

While significant progress has been made in understanding the neurological impacts of SARS-CoV-2, substantial gaps remain. Addressing these gaps is crucial for mitigating the long-term effects of COVID-19 and improving patient outcomes.

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REVIEW ARTICLE

George Cotzias' achievements and levodopa therapy: Their contribution to medical science

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Abstract

George Cotzias was a tireless physician who conducted a variety of studies, focusing mainly on neurological diseases. After leaving his medical studies to serve the Greek army voluntarily, Cotzias was relocated to the United States of America where he continued his studies at Harvard University. His first research was on hypertension, metabolism, and energy balance issues. Later on, he became the chief director of a project on chronic manganese poisoning. It was there that he identified the common characteristics between chronic manganese poisoning and Parkinson's disease, which led him to be referred to the cyclotron. Furthermore, he noted the presence of dyskinetic, motor fluctuation abnormalities, and hypersensitivity caused by levodopa (L-DOPA). He was a pioneer in demonstrating the revolutionary practical benefits of L-DOPA therapy. This accomplishment was a consequence of his patience and insistence to monitor closely, even with cinematographic recording, the health condition of his patients while modifying the L-DOPA dose for optimal health benefit. Cotzias also developed drugs combining L-DOPA and dopamine agonists, defined the phenomenon of the brain's ability to store chemical memory, and established the correlation between L-DOPA and cancer. His work significantly improved the lives and longevity of many individuals. Before his death from cancer in 1977, Cotzias received numerous distinctions and awards for his outstanding contributions to medicine. After his death, his legacy was honored through the establishment of various scholarships, professorships, conferences, and a movie dedicated to his medical achievements.

Keywords: Cotzias; Levodopa; Parkinson's disease; Neurodegenerative diseases; Neuroscience; Neurology; Medicine

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1. Introduction

The investigation of Parkinson's disease (PD) began in 1817 when James Parkinson first described it as a separate neurological disease.[1-9] People with PD experience many endogenous and exogenous irregularities.[10-17] PD is estimated to be the second most prevalent neurodegenerative disease.[18-25] Its characteristic pathological features include injury to the substantia nigra (SN), neuronal loss, neuromelanin depletion, and dopamine deficiency within the SN.[21-33] The most widely recognized movement distortions of PD are bradykinesia and rigidity.[34-42] Yet, numerous other voluntary and involuntary movement impairments have also been documented.[43-51] The presence of gait disturbances represents another hallmark characteristic of PD.[39,42,44,47,51] These motor deficits affect the patient's quality of life (QoL).[20]

George Cotzias was born in Greece.[1,7,33,38] Cotzias studied at leading educational institutions in Greece and the United States of America (USA).[7,33,38,46] His first distinction was at Harvard University as one of the top graduates.[1,7,33,38,46] He received specialized training at New York's leading hospitals.[1,7,38]

Cotzias emphasized the importance of existing knowledge on levodopa (L-DOPA), identified novel L-DOPA-related properties, and developed combination therapies involving L-DOPA and dopamine inhibitors. He became the pioneer in uncovering the practical therapeutic effects and previously unrecognized side effects of L-DOPA in the treatment of PD.[52-56] Before Cotzias, many physicians have tried to translate the theoretical benefits of L-DOPA into clinical practice through extensive studies, but all had failed.[41,47,55-58] Cotzias used science and art to be sure and precise about L-DOPA features.[2,3,7,8,19,21,26,33,47,59] It was because of Cotzias that the resistance to neurodegenerative PD treatment was addressed.[1,7,30,33,46,55,60]

The conclusions of Cotzias on L-DOPA were verified by many other scientists.[61] L-DOPA was proven to be the standard, cheapest, and most successful PD therapy.[35,42,62-67] Providing L-DOPA to Parkinsonian patients has been proven to be a synonym for longer life expectancy and improved QoL.[35,37,66,68,69] It was a great gift for medicine, the health condition, and the lives of many PD patients.[46,66,68,70-72] They were "awakened" and found themselves from being breathing statues to having the ability to perform daily activities.[73,74] Oliver Sacks named his book "Awakenings" after being impacted by Cotzias using the term "awakenings" to describe L-DOPA's effect.[74] At present, the movie "Awakenings," based on the book by Oliver Sacks, reminds laymen of the importance of translating L-DOPA from theory to practice.[75] Certain allegations about the neurodegenerative and toxical properties of L-DOPA were not substantiated.[17,22,37,41,69] Another unique achievement of Cotzias was the formulation of carbidopa/L-DOPA medicine.[1,3,7,19,32] Within a year Cotzias published the results of carbidopa/L-DOPA, the first oral formulation of this therapy became commercially available.[3,5,32] Its efficacy in treating PD and gastroenterological problems led to the development of an intestinal gel formulation in Sweden.[5,10,19]

Later, a skin patch option of carbidopa/L-DOPA was produced.[2,5,8,32] Beyond its use in neurological disorders, L-DOPA was also effective in curing other diseases.[56,72] It was an essential tool for a further explanation of other illnesses, not always neurological.[7,56] At Brookhaven National Laboratory (BNL), where he focused on cyclotron research,[7,38] Cotzias began studying manganese poisoning[7,38,45,46] and catecholamines.[7,38,53] Cotzias discovered the ability of the brain to remember the chemical content of a drug and respond to it upon reintroduction.[7] Even while struggling with cancer, Cotzias never stopped working on challenging research studies.[7,46] He stated that L-DOPA deficiency could be an early indicator of future breast cancer.[7]

Cotzias received numerous international awards and distinctions.[1,2,7,38,46] After his passing, his body was returned to Greece,[3,46] where state-organized funeral was held.[46] Greece further honored him by issuing a commemorative stamp bearing his image and his name.[1,7] Nowadays, many academic courses, events, and scholarships are named after him.[7] The current article was written to provide a concise biography of George Cotzias as a mark of honor to his memory and all his great novel achievements. Furthermore, this article intends to provide a comprehension of how Cotzias created the successful formula of L-DOPA, the revolutionary therapy. The article also explores how Cotzias leveraged the new research models to combine L-DOPA with other substances, extending its application to the diagnosis and understanding of other diseases. In addition, this work seeks to highlight the significance of detailed scientific observation in achieving medical breakthroughs. Another aim of this article is to have a better comprehension of the nervous system and consider seriously the possibility of restoring health in PD patients. Finally, the article provides a comprehensive review of PD and its evolving treatment modalities, from past to present.

2. Brief history of PD

PD named Kampavata[1-3] was first mentioned in Ayurvedic texts between the 5th and 12th centuries.[1] However, James Parkinson, through his book "An Essay on the Shaking Palsy" published in 1817, was the first to define the standard symptoms of PD. His work also introduced the pathophysiological framework that was recognized by the medical community, classified PD as a distinct neurological disease, and inspired many researchers to pursue further investigations on the disease.[1-9]

PD is characterized by internal as well as external disorders.[10-17] Affecting no fewer than 10 million people globally, it was defined as the second most common and progressive neurodegenerative disorder.[1,18-25] The disease impacts the patient's QoL.[20] The distinguishable internal abnormality and the most studied and strongly damaged part of the brain in PD are the SN, a component of the basal ganglia.[5,11,15,21,26-28] The SN is composed of neuromelanin, over 1,200,000 neurons, and high levels of dopamine - a neurotransmitter crucial for regulating movement, which becomes dysregulated in PD.[10,17,18,23] Therefore, a wounded SN is accompanied by loss of neurons, neuromelanin, and dopamine within the affected region.[1-4,8,9,11,13-15,17-21,23,28]

The classic external features of PD are bradykinesia and rigidity.[34-42] It is important to address the existence of other voluntary and involuntary movement disorders, including tremors.[43-51] In many cases, the presence of gait complexities was also noted.[39,42,44,47,51] Many PD patients estimated rigidity to be the most disturbing symptom because it affects their daily activities, often more so than bradykinesia and dyskinesia.[12]

3. The young Cotzias in Greece

George Cotzias (Figure 1), the renowned pharmacologist and neuropathologist, was born on June 16, 1918, in Crete, Greece.[1,7,33,38] He was the eldest child of Caterina Stroumboli Cotzia and Constantinos Cotzias. His mother was a victim of cancer.[3] Unlike his mother, his father, Constantinos Cotzias, was continuously absent from home. The latter was a famous, successful, and courageous journalist, politician, lawyer, and businessman who supported the king of Greece during World War II.[7,38,46] In Athens, there is still a square named after Constantinos Cotzias, along with a statue of him.[7,38,46]

From his earliest years, George Cotzias, otherwise called "Zorba of Science"[1, p.10], studied at the leading educational institutions of Greece.

He began his medical studies in Greece.[7,33,46] At the age of 22, when Greece was defeated, he did not hesitate to risk his life.[1,7] It was at this age that his training under the supervision of the professor of surgery Xenophon Kondiades was interrupted because his application to serve as a sergeant volunteer of Greek army was successful.[1,7,33,38,46] In the Greek army, he again met Kondiades after Kondiades requested to collaborate with him at a hospital near the Albanian frontiers.[38] He had a brief military experience as his father could not reject the proposal from the King of Greece to represent the Greek government in the USA after Germans bombed a hospital when all the staff was inside.[7,38] Thus, all the family of Constantinos Cotzias had to travel to the USA.[7,38]

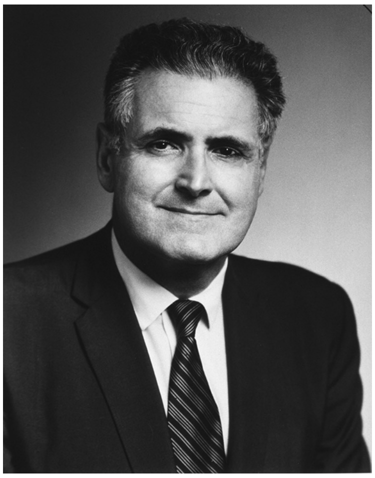

Figure 1. George Cotzias
Source: https://collections.nlm.nih.gov/catalog/nlm: nlmuid-101441400-img (Public Domain).

4. Cotzias in the USA

4.1. Early studies and collaborations

The life of Cotzias in the USA began in August 1941, when he intended to continue his medical studies. He submitted applications to several New York universities, including the University of Cornell, but all were rejected.[7,33,38] The universities rejected his applications citing Cotzias' low level of English language and insufficient knowledge of biochemistry, pharmacology, and physiology as reasons.[7,33,38] The rejections led Cotzias not to ignore his father's advice to apply to the top university in New York.[7,33,38] Thus, Cotzias applied to the Harvard University. Among the application procedures of Harvard University was an interview that Cotzias gave in fluent German with the Harvard medical professor and German refugee Soma Weiss.[38] Finally, Cotzias was accepted as a 3rd-year student at the medical school.[38] When Cotzias graduated as the second-best student of his class in 1943, he received the Summa Cum Laude distinction.[1,7,33,38,46]

In 1945, when his family went back to Athens, the physician stayed in the USA.[38] His first specialization as a pathologist was at Brigham Hospital.[7,38] He then pursued training in neurology at Massachusetts General Hospital, where he worked from 1944 to 1945 and collaborated with the hospital's chief specialist, Dr. Lewis Dahl.[1,7,38] In 1946, Vincent P. Dole decided to employ Cotzias as a researcher, after Dahl's positive recommendation, in the newly established research department at Rockefeller University.[38] During this collaboration, Cotzias added to his scientific portfolio by contributing to the studies on hypertension, exploring links between hypertension, salt metabolism instability, and energy balance.[3,7,38]

4.2. Steps toward the discovery of the distinguished therapy

In 1954, supported by the director of Rockefeller Hospital, Thomas Rivers, Cotzias and Dahl joined Van Slyke's research team in BNL.[7,38,46] It was there where Cotzias began his main studies, which led to the invention of L-DOPA.[38] He had his own research space and worked for 10 years as a senior scientist and head of the Department of Physiology.[7,45] Cotzias had knowledge of common precursors of dopamine and neuromelanin – one of which was L-DOPA.[7,38] This knowledge was informed by earlier findings: in 1951, Raab and Gigee identified catecholamine in humans, and in 1957, Kathleen Montagu's landmark paper in *Nature* first documented the existence of dopamine in the brain.[1,3,7,16,33,52] Knowing the presence of catecholamines and their function in humans, Cotzias began his studies on them. He suspected that catecholamine and vasomotor action were affected by diamine and histamine.[7,38] Cotzias detected the connection between catecholamine, manganese, and L-DOPA in the brain and opined that catecholamines, diamines, and histamine had vasomotor action.[38,53] In addition to linking angiomotor action with hypertension, Cotzias also focused on enzymes that limit their biological activity by oxidation.[7,38] These foundational studies prompted Cotzias to begin his first research on L-DOPA, laying the groundwork for his groundbreaking discoveries.[7,38]

When Cotzias accepted the proposal of the World Health Organization, he remained in BNL, but from 1963 and for the next 7 years, he and the professor of the Catholic University of Chile, Ismael Mena, were chosen to be the head chiefs of a scientific research team established in the Catholic University of Chile.[7,46] The team's primary objective was to conduct a longitudinal study investigating chronic manganese poisoning in Chilean miners.[38,45,46] The study included 13 adult miners, all under the age of 56.[45] The duration of manganese exposure among participants varied between 6 months and 21 years, while the onset of manganese intoxication varied between 3 and 25 years.[45] All participants experienced positive effects when treated with L-DOPA, also known as the amino acid L-dihydroxyphenylalanine.[7,8,10]

In the meantime, Cotzias considered seriously the relationship between the detailed activation of metal traces in tissue and blood samples using high-energy neutron beams.[7,38,45,46] As part of the longitudinal study, he identified common neurological factors – including injuries in SN due to reduced neuromelanin – between PD and manganese toxicity.[3,7,19,38,46] Later, Cotzias completed several studies explaining the distribution, absorption, kinetic elimination, and probable function of manganese.[38]

During this collaboration, Cotzias also identified the common neurological symptoms between PD and manganese toxicity, such as rigid facial expression, clenched hands, speech difficulties, balance disorders, hand tremors, facial rigid expression, slowed movements, speech tremors, lack of coordination, and difficulty in maintaining balance.[7,19,38,46,54]

5. The outstanding therapy

5.1. The discovery

Cotzias discovered a method to study manganese distribution in human tissues.[7,45] Despite the failure of previous researchers who focused on dopamine levels as a treatment for PD without demonstrating the practical therapeutic efficacy of L-DOPA,[41,47,55-57] Cotzias recognized the potential benefits of L-DOPA for PD patients. However, at the time, its utility was limited to theoretical dimensions due to its inability to cross the blood–brain barrier.[1,3,7,33,38,41,47,55,58] His incorrect – as he later self-acknowledged – hypothesis that the increase of neuromelanin levels in SN could ameliorate the health of Parkinsonian patients was the motivation to initiate the study of L-DOPA.[1,7,19,33,45] Thus, in 1966, Cotzias commenced a research project in BNL investigating the oral use of D-DOPA or otherwise named D, L-DOPA in 17 Parkinsonian patients treated in his clinic.[3,7,8,21,33,47] Cotzias was closely following the health progress of his patients.[2,3] He made a twice-per-day assessment and a cinematographic recording of the patient's health condition.[2,3,7,26,59] He began to increase the dose every 2 h over the course of several weeks until signs of health amelioration were observed.[2,7,38,41] The highest dose administered was 16 g/day.[4,5,14,17] The findings and characteristics of D-DOPA were presented in his article "Aromatic amino acids and modification of Parkinsonism," published in the *New England Journal of Medicine*.[7]

Cotzias also found that the inactive isomer, D-DOPA, which constituted 50% of the administered dose, caused toxicity and intense gastrointestinal and hematological injuries while lacking any therapeutic properties.[7,8,32,33,56] Driven by a priority to alleviate his patients' pain, Cotzias considered pain relief to outweigh concerns over potential drug dependency.[13] In 1967, knowing that L-DOPA did not cause toxicity, Cotzias initiated a study involving 28 adult participants diagnosed with PD.[2-5,7,19,32,33,35,38] The highest dose of L-DOPA administered to the patients was 8 g/day.[17,19,32,33] Cotzias attributed the success of this therapy to the gradual increase of the final high doses of L-DOPA and the continuous observation of his inpatients for months.[7,19] This marked the first successful attempt to achieve practical therapeutic results using L-DOPA. The participants of Cotzias' study became the first individuals to overcome the refractory nature of PD, which, until that time, had been unresponsive to all other treatments.[1,7,30,33,46,55,60] Although its curable features, Cotzias assessed L-DOPA not as a treatment *per se*, but as a step toward developing a definitive treatment.[38]

[1] He persuaded doctors to give morphine (a significantly addictive substance) to his mother till she would have a relief from severe cancer pain.[3]

His paper "Modification of Parkinsonism: chronic treatment with L-DOPA" published in the *New England Journal of Medicine*, in 1969, presented the findings of the study.[3,7,16,34] As Oliver Sacks mentioned in his book "Awakenings," it was in 1969 that Sacks, at the Mount Carmel Hospital, administered L-DOPA to his patients.[73] Sacks vividly described the unexpected and valuable impact of L-DOPA on the patients' health and lives.[73,74] He dedicated the book to the effects of L-DOPA, even choosing the title "Awakenings" in reference to Cotzias' use of the term to describe the transformative results of the drug.[74] The significance of L-DOPA eventually reached the cinema. In 1990, the movie *Awakenings*, based on Sacks' book, which was directed by Penny Marshall, with a screenplay written by Oliver Sacks and Steven Zaillian, was released to the public.[75]

The article and the study's video recordings by Cotzias fascinated Melvin Yahr to such a degree that in 1969, he and his research team started a study on L-DOPA therapy.[1,2,7,19,33] Yahr's study was the most important research of that time because it comprised the first prospective, double-blind, controlled clinical trial, which included a placebo to emphasize the effect of L-DOPA.[1,2,7,19,32,33] The study enrolled 60 patients,[1,2,7,19,33] of whom 56 were diagnosed with idiopathic PD, three with postencephalitic parkinsonism, and one with probable progressive supranuclear palsy.[2,7,19] The study clearly indicated that the beneficial dose of L-DOPA could vary between 3 and 8 g/day, dyskinesia was an inevitable side effect, while cardiac arrhythmia, vomiting, nausea, and hypotension were defined as self-limited adverse effects of L-DOPA.[2,7,19] Furthermore, according to the study, L-DOPA had unique positive results that a placebo could not provide.[7] At least 80% of the participants significantly benefited,[1,7] and more than 90% of the patients had at least 20% improvement not later than 4 months of treatment.[7,33] The conclusions of Yahr's study confirmed the positive therapeutic effects of Cotzias' earlier study.[1,7,19,21,29,32,33,61] After confirmation from Yahr and other scientific studies, L-DOPA was recognized as the most effective treatment for PD at all stages.[7,25,33,60] While it does not impede the progression of the disease,[35] in at least 50% of cases, it can significantly improve the patients' QoL.[20,35,37,66] L-DOPA remains the standard therapy for PD.[52,54,59,60,62-67]

In 1970, the Food and Drug Administration approved L-DOPA therapy.[3,7,19,28,32,34]

Cotzias *et al.* classified the invention of L-DOPA as "the most significant contribution to the medical treatment of neurological examinations in the past 50 years."[46] Nowadays, it is the cheapest and most prescribed antiparkinsonian drug (Figure 2).[42]

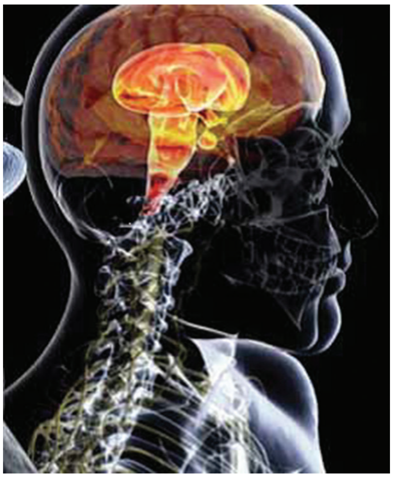

Figure 2. Internal L-DOPA and PD image. Copyright ©[2015] [Dr Dr Mylonas Anastasios][82]. Reprinted with permission of Dr Dr Mylonas Anastasios.

5.2. Further benefits of L-DOPA

Responsiveness to L-DOPA is considered a hallmark of PD diagnosis.[28,54] It was the only treatment that filled the dopamine gaps in the brains of PD patients.[46] It is worth noting that Cotzias himself in his scientific study had shown that L-DOPA has the capacity to stop the progression of PD.[35] L-DOPA helped millions of Parkinsonian patients worldwide.[7,46] Levodopa had impressive results, especially in the early stages of PD in improving tremor, bradykinesia, and rigidity.[4,34,66] Commencing L-DOPA since the early stages of PD means living a better and longer life because it offers a greater improvement in the 1st year of the disease, without side effects on later years, and keeps the subject active, physically and mentally, which means middle-age prepared for older.[68] L-DOPA was proven to be non-toxic, easy to administer, and a better-tolerated therapy, which could also ameliorate the health condition of manganese-poisoned people with no impulsive movements.[3,8,32,46,69] Another precious characteristic of the therapy was the ability to alleviate the consequences of PD, which, in certain cases, have been proved fatal.[7,15,18,41,50] In other words, L-DOPA saved the lives of many people with PD. Even though previous studies, with doubtful results, claimed that there were cases in which the use of L-DOPA therapy caused deterioration of neuronal degeneration and toxicity, there was no strong evidence to support their hypothesis.[17,22,37,41,69] Moreover, none of these studies failed to emphasize the positive influence of L-DOPA on Parkinsonian people's health and lives.[25,28,37,41,69] It has been proved that the revolutionary and the most effective therapy for PD, L-DOPA, possesses neuroprotective abilities and can halt the progression of the disease.[2,4,7,8,21,34,35,70] Yet, the unsatisfying L-DOPA response is not because of the drug *per se*, but due to other factors independent of it, like gut abnormalities, the presence of protein, and other pharmacokinetic or pharmacodynamic disorders.[28,36] In cases where the faster deterioration of PD after initiating L-DOPA therapy was attributed to the drug, several factors, like assessment of intestinal disorder, the neuroimaging before and after beginning L-DOPA therapy, protein accumulation, and many other factors, were overlooked.[20,66]

In many cases, there was the same death percentage in PD patients receiving L-DOPA and PD patients not receiving L-DOPA.[35] Indeed, the longevity of the patients who received L-DOPA was equal to the normal population.[34] In addition, in other cases, the patients with L-DOPA therapy lived longer than those who did not have the therapy.[17,34,37,69,71] Its impact on the conceptualization of neurodegenerative diseases and other neurological illnesses was more than significant.[7,56] Its therapeutic properties were also extended to non-neurological diseases.[56,72]

6. Cotzias' achievements

While being in BNL, Cotzias accomplished new studies on cyclotrons[7,38] and discovered a method to study manganese distribution in human tissues.[7,45]

In his 1964 and 1967 studies, it was obvious that rats receiving the high concentration of dopamine experienced a 50% longer lifespan compared to those without the treatment.[68] In September 1967, when speaking at the Second International Congress of Neurophthalmology in Montreal, Cotzias gave the first scientific findings on the practical therapeutic impact of L-DOPA for PD patients.[7] Cotzias was also the first to prove the emergence of dyskinetic and motor fluctuations as negative features of L-DOPA.[49,50,53,56,70,76]

Cotzias holds the title of the pioneer of the practical potency of L-DOPA, marking it as the first long-term treatment capable of crossing the blood–brain barrier and restoring dopamine deficiency in the brains of Parkinsonian patients.[7,19,34,46,76] Cotzias was also the first to prove that L-DOPA could defeat PD, whether it was caused by idiopathic, post-encephalitic, or manganese factors.[7] At this point, it is worth noting that Cotzias, in his cutting-edge scientific study, had shown that L-DOPA could stop the exacerbation of PD symptoms.[35] He was the first to establish the role of dopamine and L-DOPA in cerebrospinal fluid, and the interaction between L-DOPA, aging, and fertility, as well as identify the presence of dyskinesias linked to melatonin and growth hormone in patients undergoing L-DOPA therapy.[7] Furthermore, Cotzias proved that L-DOPA could improve the health of people suffering from chronic manganese poisoning without causing any involuntary movements.[3,46,69]

A lesser known but significant achievement of Cotzias was the discovery of severe L-DOPA-induced dyskinesia (LID), which, although not present in all cases, emerged as a consequence of nigrostriatal lesions and high doses of L-DOPA.[39,43,67,70,76] Other factors contributing to the development of dyskinesia were unconscious movements.[7] The fluctuation of the movements depended on the dose of L-DOPA.[7] The dyskinesias disappeared in the absence of L-DOPA and came back after restarting the therapy. It was Cotzias who proved that LID was a phenomenon related to the drug's chemical memory storage in the brain.[7] Cotzias, in collaboration with Lily Tang, named the mechanism of this phenomenon "L-DOPA-induced super-sensitivity."[7] This concept formed the foundation for understanding the "priming" phenomenon, initially named "neuroleptic-induced dyskinesias," which was later studied extensively to explain the molecular events that caused dyskinesias.[7] It is now obvious that dyskinesia is a common disability that comes as a consequence of excessive dopaminergic action in therapy.[23,65,66,77] Therefore, when Cotzias' patients used L-DOPA, it caused a high dopaminergic response. Cotzias was the first to note practical benefits from L-DOPA and no one had recorded the presence of dyskinesia before him. This suggested that dyskinesia was a reaction of the brain to the drastic change from dopamine depletion to dopamine adequacy, causing an intense dopaminergic response. In addition, the absence of dyskinesia corresponds to the ineffectiveness of L-DOPA.[36] The main factors that affect LID are the presence or absence of tremor as an initial manifestation of PD, disease duration, the age at onset, the duration of PD before treatment, the equivalent daily L-DOPA dose, the initial dose, dose duration, disease severity, the presence of motor fluctuations, and the severity of motor symptoms, especially those caused by prolonged L-DOPA therapy.[13,28,39,40,42,77]

Dyskinesia's presence was noted even in therapy change from dopamine agonist medicine to L-DOPA.[20] Yet, many physicians refuse to propose L-DOPA as a treatment for PD due to its side effects, ignoring the fact that even dopamine agents, which these physicians prefer, possess serious complications too.[28] Since dyskinesias is also present when treated with dopamine agonists, it is the dopamine stimulation that causes dyskinesias and not L-DOPA *per se*.[13] Another explanation for dyskinesias is that their occurrence can be influenced by the method of administration.[17] While LID can affect the QoL and, in many cases, increase the risk of falls,[42] it does not always affect QoL to a significant degree, though it can lead to increased healthcare costs.[13]

LID has led to disruptions of carbidopa/L-DOPA therapy and it has been the factor limiting the clinical use of L-DOPA.[28,39,78] It is suggested that the presence of resting tremor may be associated with secondary compensatory mechanisms that could mitigate LID, even with L-DOPA treatment.[76,79] In some cases, LID occurs as a progression following other motor fluctuations.[13]

Moreover, lower-than-expected rates of LID were observed when low daily doses of carbidopa/L-DOPA were administered.[39] The pathophysiology of L-DOPA-associated response fluctuations is complex and not yet fully understood.[28] Indeed, it is understood that choosing L-DOPA as a first-line therapy results in significant functional improvements for patients, particularly in terms of symptomatic relief, during the early years following the onset of PD.[28] During the final years of his life, Cotzias provided significant data on the relationship between cancer[7] and the features of L-DOPA.[7]

7. Development of new drugs by Cotzias

Although the adverse effects of L-DOPA therapy were significant, they could neither win over the exceptional potency of the therapy nor deter Cotzias from using L-DOPA to treat his patients.[3,7] On the contrary, the side effects of L-DOPA became a strong motivation for Cotzias to create new medications combining L-DOPA with various adjuncts like dopamine agonists – substitute compounds capable of crossing the blood–brain barrier without being converted by local enzymes.[3,36,38] The new drugs led to a reduction in the required L-DOPA dose and minimized irregularities associated with its use.[3]

Cotzias recommended the use of apomorphine, the dopamine agonist that could pass the blood–brain barrier without being converted by local enzymes. When selected as a treatment option, apomorphine stimulated all dopaminergic receptors, exhibited antidyskinetic evidence, and demonstrated an efficacy nearly equal to that of L-DOPA in improving PD symptoms.[7,32,38]

Apomorphine was a reliable substance to assess the dopaminergic receptor responsiveness and the presence of dyskinesia.[7,80] It cured various stages of PD before functional neurosurgery and was used as a treatment for patients who became disabled following deep cerebral stimulation or pallidotomy.[7,32,80] Later, the use of apomorphine was permitted in European countries and Canada.[80] Furthermore, it was estimated to be a successful drug for patients resistant to other therapies experiencing motor distortions during the last stages of PD.[3,7,80] At present, a sublingual form of apomorphine is available.[64] Moreover, apomorphine can serve as a temporary perioperative treatment during abdominal surgery for patients with PD.[80] An important parameter to assess the efficacy of dopamine agonists was the patient's reaction to L-DOPA.[80]

The efficacy of apomorphine as a treatment was closely linked to the patient's response to L-DOPA; only if the health condition of the patient was getting better by the use of L-DOPA, there would be amelioration by the use of apomorphine.[80] In addition, N-propylnoraporphine was shown to minimize certain L-DOPA-related adverse effects, though it was ineffective in reducing dyskinesias when their underlying cause was not L-DOPA.[7]

Moreover, the suspicion that carbidopa might be effective in the treatment of chorea led Cotzias to pioneer the revolutionary carbidopa/L-DOPA combination, also known as the peripheral amino acid decarboxylase inhibitor (DDI) medication.[1,3,7,19,32] This therapy is primarily used in the early stages of PD.[25] Cotzias tried for the 1st time the synthesis of this drug in 1968 by adding carbidopa to the already administered L-DOPA.[3,7]

The benefits of this medicine were reduced doses of L-DOPA,[1,5,8,23,28,32,36,48] faster efficacy of L-DOPA, and milder harms caused by the latter.[28,32,33,40,48,60] In 1972, Cotzias published the study of DDI in a scientific article.[7,48] The therapy's results were so extraordinary that the first oral form of the DDI, marketed under the trademark Sinemet, was released in 1973.[3,5,32]

Since then, various research studies have confirmed the valuable impact of the new formula on Parkinsonian patients.[5,17,23,36] Carbidopa/L-DOPA increases the level of L-DOPA in the brain from 1% in carbidopa's absence to 10% in carbidopa's presence.[23] L-DOPA/carbidopa is considered the most efficient symptomatic treatment for PD, the easiest drug to source, the most affording drug and can be easily monitored.[39] To address the issue of gastrointestinal problems, in the 1990s, the compound was released in Sweden as an intestinal gel.[5,10,19] This formulation has since become a cost-effective therapy not only for gastroenterological disorders but also for patients with acute abnormalities.[20] The gel formulation maintains nearly stable plasma concentrations of L-DOPA, contributing to its effectiveness.[28] It is commercialized in Europe under the trade name Duodopa™[28] and is considered safer and better tolerated compared to the immediate-release form of carbidopa/L-DOPA.[24] In 2006, an oral solution of carbidopa/L-DOPA was commercialized in the USA.[17] In addition, a skin patch of carbidopa was developed for patients with metabolism dysfunction, overcoming the skin barriers and improving the drug's duration and concentration.[2,5,8,32]

At present, carbidopa/L-DOPA is defined as the most impactful oral medicine in PD.[81] It is the most prescribed treatment as it improves PD symptoms, especially at the early stages of PD.[23] It offers mild and infrequent side effects, including dyskinesia, as well as a positive influence on the

QoL of PD patients.[24,28] In certain cases, it could have no adverse, including dyskinesia.[24] The efficacy of carbidopa/L-DOPA is not typically questioned. Any inefficacy observed is often related to an individual's tendency to overproduce aromatic L-amino acid decarboxylase (AAD) enzyme activity, rather than an issue with the drug itself.[36]

Another alternative formulation of carbidopa/L-DOPA involves nanoparticles (NPs) – small colloidal particles ranging from 10 and 200 nm.[23] These NPs can help overcome challenges related to drug delivery and potentially reduce toxicity.[23] The intranasal delivery of NP-encapsulated L-DOPA provides a non-invasive delivery strategy that is easy for PD patients to administer themselves.[23] Its use resulted in reduced adverse effects, minimized L-DOPA dose, fewer treatments, and better drug delivery to the central nervous system.[23]

Despite their efficacy, all the dopamine agonists and PD medication could not replace L-DOPA therapy.[7,11,19,25,34,47,52]

8. Awards received and the involvement in cancer research

In January 1977, when Cotzias traveled to Greece to help his colleagues, he was already a renowned figure.[7,46]

It was in 1954 that George Cotzias received the A. Cressy Morrison Award in the Natural Sciences.[38] In 1969, Cotzias was given an honorary degree from the Santiago Catholic University and the Albert Lasker Award in Clinical Medical Research.[1,2,7,38,46,82] The next year, came another honorary degree for Cotzias, this time from the Women's Medical College of Pennsylvania.[38] In the same year, he was elected to the American Academy of Arts and Sciences.[38] In 1971, he was awarded the Honorary Degree of St. John's University, New York.[38] The next year Cotzias was awarded the Borden Award of the American Medical College Association, while the Harvey Society honored Cotzias for his discovery that some or all of the main symptoms of PD could be reversed with dihydroxyphenylalanine (dopa).[7,82] Indeed, the Harvey Society defined the discovery as one of the greatest therapeutic contributions of that generation.[7,82] In 1973, he was elected to the National Academy of Sciences.[7,38] In 1974 came the annual prize from the American College of Physicians and the honorary degree from the National University of Athens.[38,82]

Cotzias was ranked among the great personalities in American medicine.[46] He has a distinctive place in the global history of 20th-century medicine.[82] The American medical journal *Family Health* classified Cotzias among the first 100 most famous leaders in the World of International Health.[82]

In 1973, Cotzias was diagnosed with lung cancer and battled with the illness till the last moment by continuing to visit the hospital.[1,7,46] Despite his personal struggle, cancer became a source of inspiration for Cotzias, prompting him to initiate scientific research on the disease.[7,46] His studies proved that depletion of dopamine-stimulated adenylate cyclase activity in the brain, which minimized spontaneous motor activities and motor responses to intravenous L-DOPA, could be important indicators of a high probability of future breast cancer.[7] Two years later, Cotzias was employed by Cornell University Medical College and Memorial Sloan-Kettering Cancer Center.[7,46] He even hypothesized that the proper dose of DOPA could cure cancer.[3]

9. Death of Cotzias

Cotzias died on June 13, 1977, at the age of 59 in New York.[1,7,38,46] The Sloan-Kettering Memorial Cancer Center arranged for his body to be sent to Greece.[3,46] His state funeral took place in Greece.[46] The next day, The New York Times published a commemorative article about Cotzias.[46]

10. After death

Six months after his death, the New York Scientific Community Establishment and the Memorial Sloan-Kettering Cancer Center organized an event in Cotzias' honor.[1,7] High-society members were present at this ceremony. Among them were Laurence S. Rockefeller, the permanent representative of Greece to the USA, and many Greek ambassadors.[1,7] The Archbishop of the North American Greek Orthodox Church was also present.[1,7] To honor Cotzias' memory, Greece issued a commemorative stamp in his name (Figure 3).[1,7]

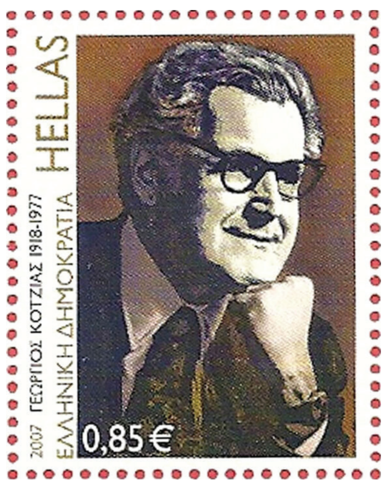

Figure 3. A commemorative stamp with a picture of George Cotzias. Copyright ©[2015] [Dr Dr Mylonas Anastasios][82]. Reprinted with permission of [Dr Dr Mylonas Anastasios].

To the memory of his brilliant achievements, the American Academy of Neurology added the "George Cotzias Lecture" to its annual meeting, and the American Parkinson Disease Association founded the "George C. Cotzias Memorial fellowships."[7] The Cornell Medical Center created the "George Cotzias Distinguished Professorship," and the Memorial Sloan-Kettering Cancer Center added the "G.C. Cotzias Neuro-oncology Division".[7] In Europe, the Spanish Neurological Society established the "Cotzias Lecture" at its annual meeting.[7]

11. Discussion

George Cotzias, a brilliant physician from Greece, stands as a great figure in the annals of international medical history, notably for his pioneering work in PD.[7,46,82] Being the son of highly educated parents, he had eminent academic qualifications and accomplishments.[7,38,46] His studies journey commenced in Athens and culminated at Harvard University.[7,33,38,46]

Cotzias' groundbreaking discovery on the therapeutic implications of L-DOPA for PD and manganese poisoning has been of enormous international benefit.[7,8,10] His methodology, encompassing innovative techniques like cinematographic recording and meticulous patient observation, was revolutionary in its own right.[2,3,7,8,19,21,26,33,47,59] Such techniques ensured accurate assessments of L-DOPA's efficacy and provided deeper insights into the disease progression and treatment response.[7,19] In addition, his revelations about the dyskinetic distortions associated with L-DOPA therapy and its potency on individuals poisoned with manganese comprise significant milestones in neuroscientific research.[7,8,10]

Each medication he formulated provided much-needed relief to patients grappling with PD, a disease once deemed refractory. In this light, L-DOPA stands as a pivotal stepping stone in neurology's evolution.[7,46,56] Cotzias demonstrated the brain's ability to retain the chemical "memory" of a medication.[7] It is noteworthy that Cotzias viewed L-DOPA not as an end but as a significant milestone in the journey toward a comprehensive solution for PD.[38] Given the invaluable impact of L-DOPA, bringing longevity[17,34,37,68,69,71] and alleviating the suffering of PD patients,[4,20,28,34,35,37,66] his vision of transforming PD into a mild, non-lethal ailment seems not only hopeful but also attainable. Beyond L-DOPA, Cotzias' endeavors led to the genesis of a new generation of drug synthesis to combat PD. To date, carbidopa/L-DOPA is the most effective and well-developed medication among the available options.[2,3,5,8,10,17,19,23,32,39,81]

His battle with cancer, while tragic, did not defeat his research spirit. Until his last breath, he remained an insistent seeker and established the connection between cancer and L-DOPA.[3,7,46]

His outstanding career, marked by numerous awards and recognition of his great achievements, is a priceless gift to science and many patients worldwide.[1,2,7,38,46,73-75,82] Today, his legacy endures, with numerous institutions worldwide hosting academic events bearing his name,[7] celebrating the indomitable spirit and unparalleled contributions of George Cotzias to medical history.

George Cotzias's legacy, along with the practical application of L-DOPA, stands as a testament to his immense contributions to the world of medical science.[73-75]

12. Conclusion

The Greek doctor George Cotzias started his medical studies in Athens and accomplished them at Harvard University. He had an extraordinary career in the USA and left an important scientific route behind. The immense discoveries of Cotzias were the practical and remarkable therapeutic use of L-DOPA in treating PD. Cotzias proved the efficacy of L-DOPA using tools that no one has used before, including cinematographic recording to closely monitor the patient's reaction to the therapy and adjust the L-DOPA dose. He also discovered new dyskinetic abnormalities associated with the therapy and demonstrated the therapy's positive effect on individuals poisoned by manganese. L-DOPA was a common substance in all the complex and beneficial drugs composed by Cotzias for PD patients. No one before Cotzias provided an impactful treatment for PD. L-DOPA was a hallmark in the advancement of neurology. Successfully advancing to this stage not only extended life expectancy but also made PD a more manageable condition. The hypothesis that future therapies could transform PD into a common, mild disease – one that no longer causes suffering or death – may not be an exaggeration. Cotzias died from cancer while conducting original scientific research on the disease. He received many awards in recognition of his contribution. Many academic events and awards have since been named in his honor at several institutions worldwide. In addition, a fictional movie illustrates the valuable influence of L-DOPA.

This paper gives a concise but clear image of George Cotzias' great achievements and the tools that could lead to breakthrough research in neuroscience, brain function, and brain response.

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Conflict of interest

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REVIEW ARTICLE

Targeted protein degraders: Blood–brain barrier permeability and central nervous system exposure

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Abstract

Innovative approaches are essential for treating central nervous system (CNS) diseases that present severe neurological manifestations and low survival rates. Delivering chemical or biological molecules across the blood–brain barrier (BBB) at therapeutically effective concentrations to treat CNS pathologies is a significant challenge. The urgent need for novel treatments targeting disease-causing proteins has propelled targeted protein degraders (TPDs) into the spotlight. TPDs have emerged as promising therapeutics for the treatment of CNS proteinopathies, characterized by the accumulation of misfolded protein aggregates. Given their structural features, the BBB permeability and CNS bioavailability of TPDs may seem improbable. However, several TPDs have demonstrated measurable concentrations in cerebrospinal fluid and the brain. Understanding the mechanisms behind their permeability across the BBB could open new avenues for the development of more effective TPD-based therapies for CNS proteinopathies. This review explores the absorption, distribution, metabolism, and excretion properties of TPDs in relation to brain pharmacokinetic parameters. It also delves into the likely interactions of advanced-stage TPDs with drug transporters and possibilities of disruption-propelled versus B-B barrier permeability-driven CNS bioavailability. Finally, it provides critical insights into the BBB permeability aspects of TPDs, uncovering new dimensions for future research.

Keywords: Targeted protein degraders; Central nervous system diseases; Blood–brain barrier; Proteinopathies; Pharmacokinetics; Permeability mechanisms

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1. Introduction

The central nervous system (CNS) druggability has been attributed to molecules with a molecular weight ≤400 Da, log partition coefficient (P) between 1.5 and 2.7, ≤2 hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs), ≤5 rotatable bonds, a total polar surface area (tPSA) of 60 – 70 Å, a net positive charge at pH 7 – 8 and a negative base 10 logarithm of the acid dissociation constant (pKa) between 4 and 10.[1] In addition, tertiary nitrogen groups improve blood–brain barrier (BBB) permeability efficiency.[2] On the contrary, highly protein-bound molecules (≥99.5%), polar hydrophilic compounds, or efflux transporter substrates exhibit poor BBB permeability. Despite these well-established metrics for CNS drug candidates, drug development for CNS indications remains challenging, as evidenced by the fact that only approximately 350 drugs out of over 7,000 in comprehensive medicinal chemistry databases have been approved to treat CNS diseases.[3] Furthermore, among this 5%, many CNS active agents, including antimigraine drugs, narcotic analgesics, mood elevators/antidepressants, sedatives, tranquilizers, and hypnotics, exhibit poor pharmacokinetic/pharmacodynamic (PK/PD) correlations.

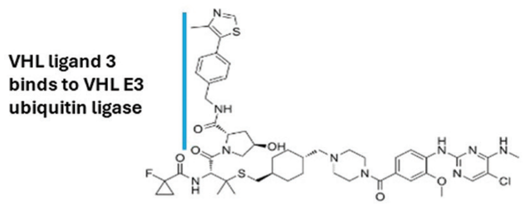
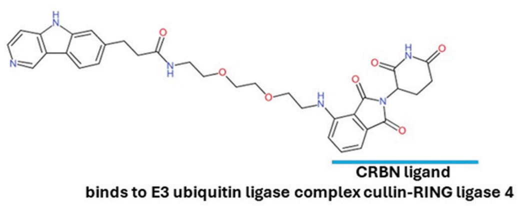
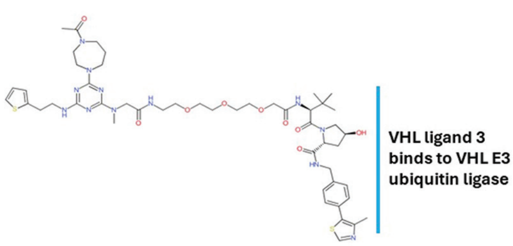
For targeted protein degraders (TPDs), achieving good BBB permeation efficiency is even more challenging due to their higher molecular weight (800 – 1,200 Da), increased number of HBAs, HBDs, and rotatable bonds. Despite these obstacles, several TPDs in preclinical settings have demonstrated quantifiable concentrations in cerebrospinal fluid (CSF) and the brain, and are being developed for CNS indications. A few notable examples are listed in Table 1.

This review outlines the different routes that macromolecules (both chemical and biological) use to cross the BBB and the likely mechanisms by which TPD crosses BBB. It also discusses the physiochemical properties of TPDs that may regulate their CNS bioavailability. Furthermore, the best approaches to assess the BBB permeability of TPDs are discussed, along with their importance. Finally, the review explores the mechanism of CNS bioavailability driven by BBB disruption rather than by BBB permeability.

2. The complex organ: Brain

The brain regulates thoughts, emotions, memory, sensory and motor functions, vision, lung functioning, temperature, satiety, and the body's homeostasis mechanisms. Together with the spinal cord, it constitutes the CNS. The adult brain is approximately 60% lipid, which includes phospholipids such as phosphatidylcholine, phosphatidylethanolamine, omega-3 fatty acid (docosahexaenoic acid, alpha-linolenic acid, and eicosapentaenoic acid), while the remaining 40% consists of water, carbohydrates, proteins, and minerals. The brain is composed of two distinct regions: white matter (the lighter, inner core) and gray matter (the darker, outer portion). In the spinal cord, however, the arrangement is reversed, with white matter forming the outer portion, consisting of axons (long fibers that connect neurons),

Table 1. Targeted protein degraders in development for central nervous system indications and their attributes

TPD ID	Structure	Indication and target	Properties	References
XL01126		Parkinson's disease; Leucine-rich repeat kinase 2 degrader	tPSA: 194.3; Mol wt: 1,019.7; XLogP: 5.05	4
QC-01-175		Alzheimer's disease; Degrader of pathogenic tau protein	tPSA: 171.82; Mol wt: 626.25; XLogP: 0.98	5
C004019		Alzheimer's disease selectively promotes tau protein degradation	tPSA: 302.39; Mol wt: 1,034.48; XLogP: 1.81	6

Abbreviations: CRBN: Cereblon; Mol. wt: Molecular weight; TPD: Targeted protein degraders; tPSA: Topological polar surface area; VHL: Von Hippel–Lindau; XlogP: Prediction of octanol/water partition coefficient.

wrapped in myelin (a protective coating), and gray matter forming the core, composed of neuron somas (the round, central cell bodies). The different composition of neurons in each part causes the brain to appear as separate shades of gray and white.

3. Cerebral vasculature

Capillary segments in the human brain range in size from 50 – 100 µm in length and 8 – 10 µm in diameter between bifurcations.[7-10] In comparison, the smallest capillaries in the mouse and rat brains have a diameter of about 3 µm and 4 µm, respectively.[11] Brain capillaries feature the largest and tightest junctional complexes, whereas venules have relatively looser junctional configurations. At the level of circumventricular organs (CVOs), capillaries are comparatively more permeable, with fenestrations and discontinuous tight junctions (TJs), particularly in their central regions. Notably, the CVOs and choroid plexus (CP) are two vascularized areas of the brain where the classical barrier phenotype is largely absent. The fenestrated microvessels of the CVOs and CP, therefore, allow molecules to diffuse into the brain parenchyma relatively easily.[12]

Blood is delivered to capillaries through arterioles, and metabolic products are primarily cleared by post-capillary venules (PCVs), which can have diameters of up to 200 µm.[13] The perivascular space separates the parenchymal and endothelial basement membranes in PCVs. Plasma proteins, immune cells, tumor cells, and parasites can extravasate through the intercellular connections of endothelial cells in PCVs.[14-22] PCVs primarily exhibit transcytosis-mediated transport.[23] While venules tend to express genes involved in inflammation-related processes more abundantly, capillaries preferentially express genes related to solute transport.[24] Pericytes are located on the abluminal surface of PCVs. Although they do not directly contribute to barrier function, pericytes, in conjunction with astrocytes, produce substances that aid in recovery and repair.[13] The brain vasculature exhibits varied spatial orientations and metabolic and energy requirements, depending on the brain region (white or gray matter).[25] An essential regulator of the transport of fluid and solutes along the walls of the cerebral microvasculature is the mechanical stress exerted by blood flow.[26] Moreover, there are significant variations in the density and organization of tight junctions along the vascular tree,[27] which influence the regions and mechanisms through which chemical macromolecules can enter the brain. The BBB varies in shape and function across the microvascular network.

4. The barriers

There are three major barriers in the brain: the endothelial cell-based vascular (BBB), the ependymal cell-based blood–cerebrospinal fluid (B-CSF) barrier, and the arachnoid barrier. Figure 1 illustrates these three major barriers in the brain.

5. The BBB

The BBB is the interface for the exchange of substances between blood and brain.[28,29] It is formed by microvascular endothelial cells, pericytes, astrocytic endfeet, and neurons.[30,31] Brain microvascular endothelial cells

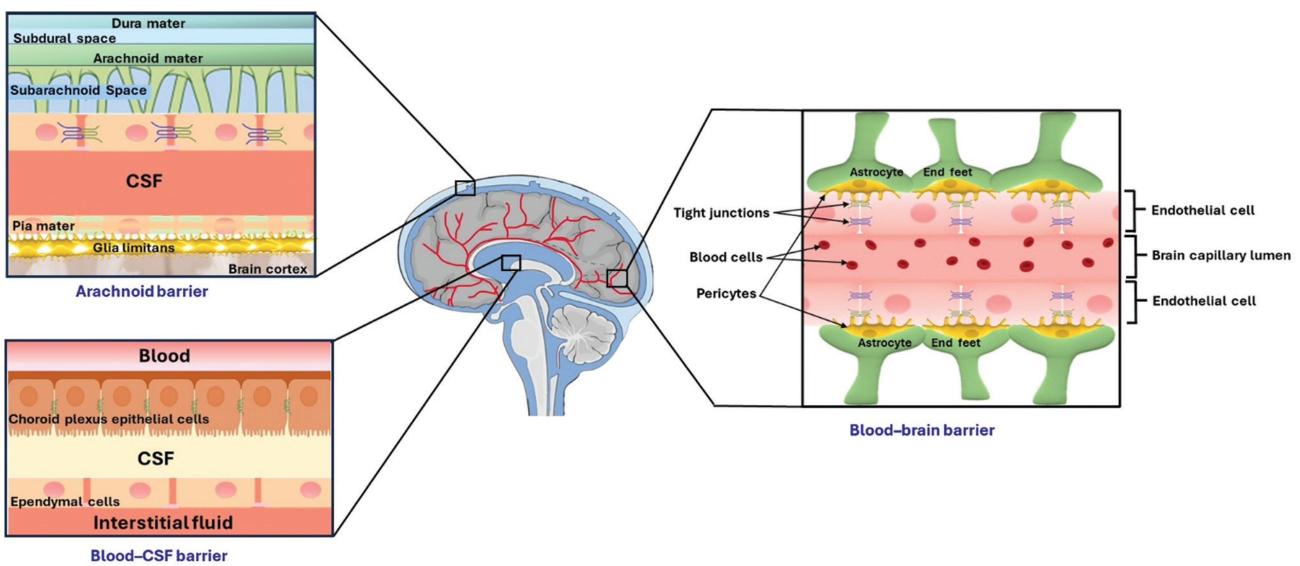


Figure 1. The three barriers protecting the brain. The arachnoid barrier, the blood-CSF barrier, and the blood–brain barrier. Image created by the authors.
Abbreviation: CSF: Cerebrospinal fluid.

(BMECs) differ from other endothelial cells in that they have a relatively higher concentration of mitochondria and are virtually devoid of fenestrations.[32,33] As a result, except for small lipophilic molecules, BMECs prevent most biological and chemical molecules from entering the brain.[34] Gated by tight junction proteins, the BBB prevents hydrophilic molecules, charged moieties, peptides, and proteins from entering the brain, thus playing a crucial role in protecting the brain parenchyma from xenobiotics and other exogenous compounds.[35] Hydrophobic, low molecular weight compounds can diffuse across brain endothelial cells, but larger molecular weight compounds are predominantly shuttled back into circulation by efflux transporters, resulting in pharmacologically insignificant brain absorption.[36,37]

Brain microvascular endothelial cells contain efflux transporters such as breast cancer resistance protein (BCRP) and multidrug resistance transporter 1 (MDR1)/P-glycoprotein (P-gp), which efflux substrate compounds from the brain.[38-40] Figure 2 depicts the key features of the BBB.

Despite these checkpoints, a significant number of drugs do cross the BBB. Many of these interact with organic anion transporting polypeptides (OATPs), such as OATP1A2/solute carrier organic anion transporter (SLCO) 1A2 and OATP1A4/SLCO1A4, as well as transmembrane organic anion transporter (OAT) proteins, such as OAT1/solute carrier (SLC) 22A6. OATP1A2 assists in the entry of various solutes and drugs into the brain. While predominantly

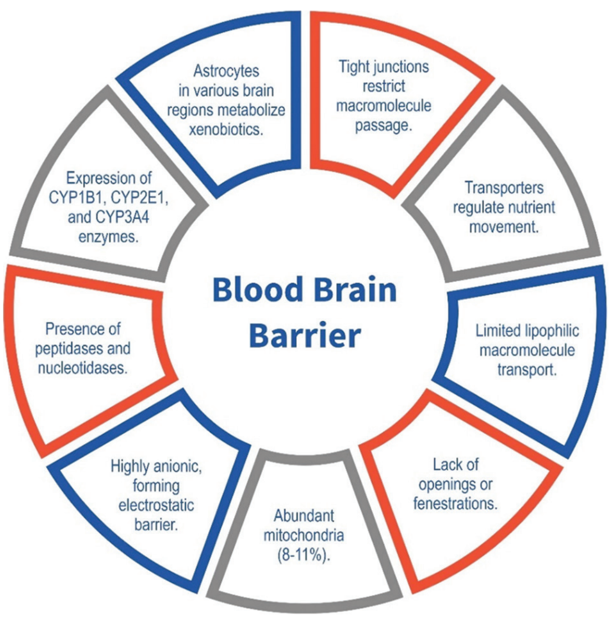


Figure 2. Key features of the blood–brain barrier. Image created by the authors.

expressed in the liver, OATP1A4 has also been detected in both the apical and basolateral sides of the endothelial cells of the BBB. It mediates the uptake of drugs from both the brain and the blood compartments.[41,42] Predominantly expressed in the kidneys, small amounts of OAT1 are also present in the brain cortex, hypothalamus, hippocampus, and cerebellum. OAT1 interacts with a broad spectrum of drugs, including β-lactam antibiotics (e.g., penicillins, benzylpenicillin, carbenicillin), immunomodulators (e.g., methotrexate), and anti-hypercholesteremic drugs (fluvastatin, pravastatin, bezafibrate).[43]

It is noteworthy that the BBB was once conceived purely as a neurovascular unit. However, following the discovery that the cytochrome P450 enzymes and transporters in endothelial cells regulate the rate and extent of substances reaching the brain parenchyma – primarily through the transcellular route – the BBB has been redefined as a pharmacological checkpoint.[44] The blood-spinal cord barrier is more permeable than the BBB due to a lower expression of TJ proteins and fewer pericytes covering the capillaries' outer surface.[45]

6. The blood-cereberospinal fluid barrier

The B-CSF barrier is formed by the epithelial cells of the CP and is structurally and functionally distinct from the BBB. While the BBB serves as the interface between the blood and interstitial fluid (ISF), the B-CSF barrier is confined to the epithelial cells of the CP.[36] The secretion of CSF into the ventricular brain is regulated by the CP epithelial cells.[46] The proteins in CSF are similar to those in serum but are present at much lower concentrations. Albumin, for instance, is the most abundant protein, though its concentration is approximately 500 times lower than that in serum.[47]

The ISF, which consists of the remaining brain extracellular fluid (ECF), is partially derived from secretion across the capillary endothelium of the BBB.[48-51] The ISF interfaces with CSF at several locations, and its contribution to CSF varies in the range of 10 – 60%.[12] Drug transport from the CSF to the brain is generally slow and predominantly diffusion-driven, with the rate of diffusion tapering as the distance increases.[36] Compounds with moderate-to-high cellular permeability and low efflux liability tend to accumulate more in CSF compared to those with high efflux liability. An increased distribution of immunoglobulin G (IgG) into the CSF, as opposed to the brain, has been attributed to the relatively greater permeability of the CP compared to the BBB.[52]

Drug distribution into the CSF is sometimes considered an indicator of BBB permeation and CNS bioavailability. This notion arises from the assumption that the BBB and

B-CSF barriers are interconnected and nearly identical. However, it is important to recognize that the B-CSF barrier is more permeable than the BBB, and the molecular weight of circulating molecules (inversely proportional to their ability to cross) remains a key determinant of their entry into the CSF.[53] Thus, the distribution of a compound in the CSF primarily reflects transport through the CP at the B-CSF barrier, rather than serving as a direct indicator of BBB penetration ability.

When a drug is injected into the CSF, it first permeates into the bloodstream before crossing the BBB to re-enter brain tissue, subjected to molecules' physiochemical properties. This process is illustrated in Figure 3.

On the contrary, the drug rapidly equilibrates throughout the entire volume of the brain after crossing the BBB from the blood. This phenomenon suggests that drugs injected directly into the CSF may exhibit transient pharmacological benefits, but only after the drug is transported from the CSF to the blood and then re-enters the brain through the BBB, enabling a full pharmacological response.[54] This notion is further supported by a study on barbiturate intracerebroventricular administration in dogs. Regardless of whether the barbiturate was administered by intravenously (IV) or through the intracerebroventricular route, the amount required to maintain the anesthesia for the necessary duration was the same: 0.2 mg/kg/min.[55] Hence, drug penetration into the CSF, when estimated for any molecule, does not necessarily correlate with the drug's penetration through the BBB at the endothelium level of the brain capillaries, nor with CNS bioavailability in general. CSF is sampled for compounds whose transporters are not yet known or identified. TPD concentrations quantified in the CSF are a good predictor of the unbound concentration in the brain, unless specific transport mechanisms exist between the brain and CSF. When selecting clinical candidates, the concentration of TPD in the CSF should not be considered a direct indicator of CNS bioavailability due to the differing permeability characteristics of the BBB and the B-CSF barrier. In addition, the unbound brain-to-plasma partition coefficient ($K_{p,uu,brain}$) should be estimated.[56] However, at the early discovery stage, CSF concentrations can be considered a surrogate for $K_{p,uu,brain}$.

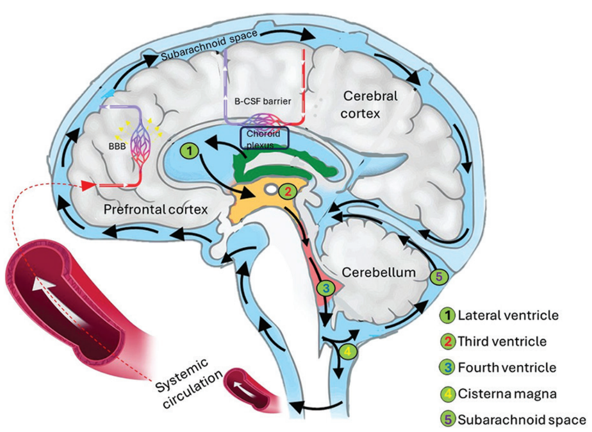


Figure 3. Drug kinetics from blood-cereberospinal fluid to blood–brain barrier. Image created by the authors.

7. The arachnoid barrier

The avascular arachnoid epithelium, located underneath the dura mater, forms the arachnoid barrier. The dura mater, part of the meninges, lies just beneath the bone and envelops the CNS, thus forming a seal between the CNS's ECF and the rest of the body.[57] The arachnoid barrier plays an insignificant role in the exchange of xenobiotics between the blood and brain, primarily due to its avascular nature and its relatively small surface area compared to the BBB or B-CSF barrier.[58] Furthermore, the efflux transporter P-gp is expressed on the apical membrane toward the dura mater, and BCRP is expressed on both the apical membrane toward the dura mater and the basal membrane toward the CSF. Therefore, the pharmacological relevance of the arachnoid barrier in drug uptake is relatively insignificant and has not been discussed in detail in this review.

To facilitate blood-parenchyma exchange, endothelial cells express receptors and transporters. The active efflux pumps out brain materials by ATP binding cassette transporters for example P-glycoprotein and Breast Cancer Resistance Protein. The influx and efflux transporters and their respective substrates and inhibitors have been illustrated in Figure 4.

8. Influx transporters

Various SLC transporters are expressed by brain capillary endothelial cells to facilitate brain absorption.[59] SLC7A5/L-type amino acid transporter 1 (LAT1) is a transmembrane heterodimeric protein predominantly expressed in neurons, astrocytes, and microglia, and specifically at both the luminal and abluminal sides of the BBB.[60] LAT1 and SLCO2B1/OATP2B1 are reported to have the highest expression levels in microvessels. Furthermore, brain microvessels express significant amounts of SLC2A1/glucose transporter 1 (GLUT1), as well as the glutamate transporters SLC1A2/excitatory amino acid transporter (EAAT) 2 and SLC1A3/EAAT1.

Antioxidant ascorbic acid (AA) protects lipid membranes and proteins from oxidative damage. Two transport proteins in the brain can transport L-AA: GLUT1 and the sodium-dependent vitamin C transporter 2. GLUT1 transports the oxidized form of AA, that is, dehydroascorbic acid, which is then reduced to

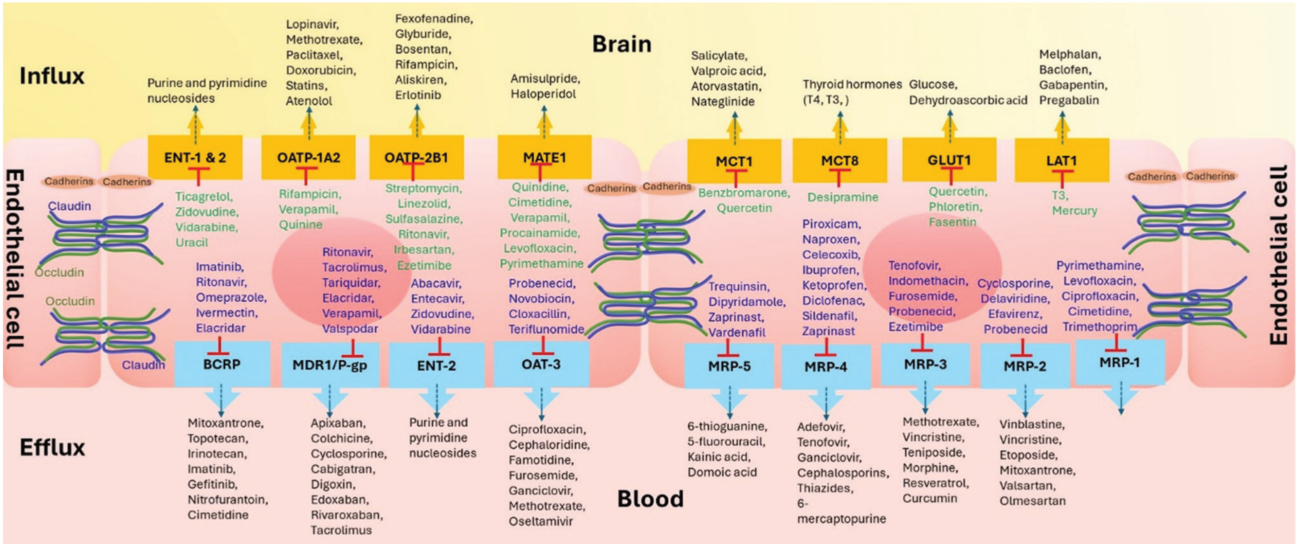


Figure 4. Influx and efflux transporters in brain and some of their substrate and inhibitors. Image created by the authors.

Abbreviations: BCRP: Breast cancer resistance protein; ENT: Equilibrative nucleoside transporter; GLUT1: Glucose transporter 1; MATE: Multi-antimicrobial extrusion protein; MCT: Monocarboxylate transporter; MDR: Multidrug resistance transporter; MRP: Multidrug resistance proteins; OATP: Organic anion transporting polypeptides; P-gp: P-glycoprotein.

AA and retained in the brain at higher levels.[61] GLUT1 is expressed in the endothelial cells of the BBB,[62] on the apical membrane of some regions of the ependymal epithelium, and on the basolateral membrane of the CP epithelium,[63] while sodium-dependent vitamin C transporter 2 is expressed in neurons, the epithelial cells of the CP, and hypothalamic glial cells.[64] The folate receptor, primarily present in venules, outperforms the lactoferrin receptor, which is almost exclusively expressed in capillaries, in aiding macromolecular delivery, despite its low expression levels.[65]

9. Efflux transporters

Efflux transporters, including adenosine triphosphate-binding cassette (ABC) transporters and OAT3/SLC22A8, extrude solutes from the brain endothelium into the circulation.[66-68] The most important ABC efflux transporters at the BBB are ABCB1/MDR1 and ABCG2/BCRP, while ABCC1 is prominent at the B-CSF barrier.[69] MDR1 expression in the human brain is highest in the capillaries, with little to no expression in the arterioles.[70] This transporter is located on the luminal membrane and restricts the uptake of several potential CNS drugs into the brain.[71]

Multidrug resistance transporter 1 functions as a "flippase," transporting lipophilic molecules from the inner leaflet of the plasma membrane to the ECF.[72] In contrast, ABCC1 at the B-CSF and BCRP at the BBB bind their substrates in the cell cytoplasm.[69] BCRP is widely expressed in the CP epithelial cells, the fenestrated vasculature of the plexuses, and in microvessels in the subarachnoid space.[37] A synergistic relationship has been reported between BCRP and MDR1, which together limit the transport of xenobiotics through the BBB.[73] Protein analyses revealed that the microvessels contain approximately twice as much BCRP as MDR1.[74] OAT3 is abundantly expressed in brain capillaries, specifically at the basolateral side of the plasma membrane in CP epithelial cells. It plays a crucial role in the efflux of drugs (e.g., statins, diuretics, antibiotics, and antivirals), as well as endogenous neurotransmitters and hormone metabolites from the brain.[43]

10. Macromolecule permeability across the BBB

An increase in the molecular weight of small molecules results in greater binding and decreased brain $K_{p,uu,brain}$.[75] For larger molecules, such as antibody fragments, biodistribution decreases, resulting in more drugs remaining in plasma than in tissues.[76] Due to the relatively low tissue binding of proteins and peptides, quantifiable concentration K_p typically equals $K_{p,uu,brain}$.

It has been suggested that the BBB permeability for macromolecules, such as 10 kDa dextran or solutes bound to albumin, is negligible (1×10^{-7} cm/s).[13] Although some estimates are quite low, immunoglobulins (with an average molecular weight of 150 kDa) have been shown to be transported into brain tissue.[77] According to published data, circulating biologics exhibit CNS exposure ranging from

0.1% to 0.4% of their respective serum concentrations.[78-80] Confocal microscopy of the brain reveals that the neonatal Fc receptor is the primary Fc receptor on brain capillaries.[81] However, the transfer of IgG molecules from blood to the brain is not mediated by the BBB. Instead, IgG molecules undergo asymmetrical transcytosis through Fc receptors in the brain-to-blood direction.[82]

The molecular weight of TPDs generally ranges between 700 and 1000 Da, which is a significant limiting factor for their use in CNS indications. Despite this, several TPDs are in the advanced pre-clinical development stage for CNS indications, with a few listed in Table 2.

It is important to note that for some of the TPDs mentioned in the table above, the reported plasma and brain concentrations were so disparate (double-digit μM versus sub-nM, respectively) that it potentially indicates vascular contamination rather than meaningful BBB permeation. Estimating the vascular-restricted atenolol could help rule out the possibility of blood contamination. In addition, washout or perfusion technique could be employed to flush out residual drug within the vascular volume.

Macromolecules, such as proteins, can enter the brain through vesicular transport or transcytosis. However, this process is highly selective and is actively repressed by recently discovered homeostatic mechanisms.[88] It has been reported that transcytosis of macromolecules primarily occurs through endocytic mechanisms, including receptor-mediated transcytosis (RMT), adsorptive-mediated transcytosis (AMT), or carrier-mediated transport (CMT).[89]

In RMT, ligands bind to specific receptors, such as the transferrin receptor (TfR), insulin receptor, or low-density lipoprotein receptor-related protein 1, on the luminal surface of endothelial cells. This binding triggers an endocytic cascade, followed by vesicle-mediated transport across the BBB. These "Trojan horses" transport large biomolecules such as insulin (5734 Da), transferrin (80,000 Da), insulin-like growth factor 1 (7,649 Da), or antidiuretic hormone (1,084.24 Da).[90] Lipoparticles or iron-bound transferrin bind to specific receptors on brain endothelial cells and are transported across the BBB.[91] RMT at the BBB endothelium primarily occurs at clathrin-coated pits, with a marginal contribution from caveolae. The BBB endothelium contains a large number of clathrin-coated pits, most of which are located on the luminal membrane, indicating that clathrin-dependent transport primarily occurs in the blood-to-brain direction.[92,93] A well-known example of clathrin-dependent endocytosis is the transfer of iron across the BBB, which is mediated by transferrin and its receptor.[94,95] Caveolin-mediated endocytosis regulates insulin transport across the BBB in the hypothalamus, while clathrin-mediated mechanisms drive insulin binding in isolated brain microvessels.[96] The major facilitator superfamily domain containing 2a protein in the endothelial cells of the BBB modulates the lipid composition of the plasma membrane, thus limiting the formation of caveolae vesicles and restricting transcytosis.[97,98]

Antibody conjugates are also being explored as a means of achieving the desired CNS concentration of piggyback molecules for intended therapeutic efficacy.[99] Antibody variable domain binding has been used in RMT-based delivery techniques to target brain endothelial cell receptors.[100-103] Some BBB receptors facilitate endocytosis into endothelial cells but do not mediate transcytosis across the endothelial barrier.

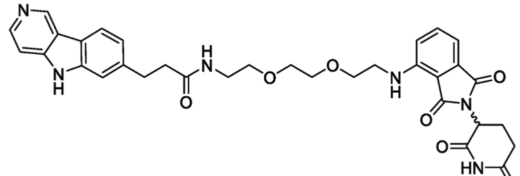
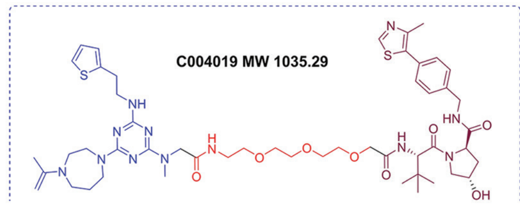
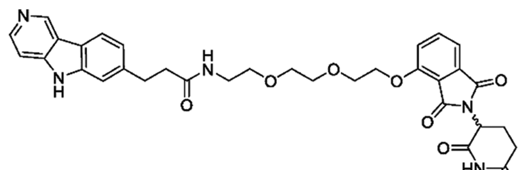
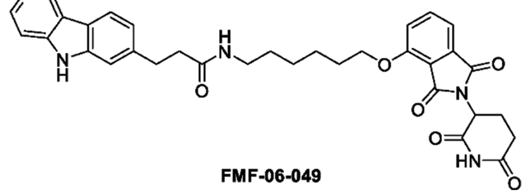
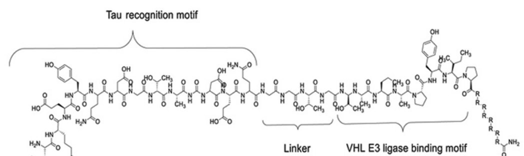
The surface of brain endothelial cells contains approximately 10% of the total receptor pool for the recycling receptor TfR.[104] Anti-TfR antibodies have been shown to traverse the BBB.[105-107] These studies suggest that if drugs intended for CNS indications are tagged with TfR ligands, such as antibody fragments, their brain delivery is enhanced.[108-110]

To harness RMT, protein therapies have been designed to bind these receptors, which are abundantly expressed on brain endothelial cells, thereby delivering the therapeutic payload to the CNS. A few examples of such therapies are listed in Table 3.

RMT of biotherapeutics conjugated to a receptor-binding ligand has shown promise, although the net quantity transported remains relatively low. The same applies to drugs conjugated to Angiopep-2 and activatable cell-penetrating peptides.[111] Antibodies or endogenous protein receptor ligands have been shown to be transported across endothelium at the capillary level.[12] Brain vasculature hemodynamics vary across arteries, arterioles, capillaries, venules, and veins,[110,112] with the TfRs being most abundant in capillaries. It is therefore conceivable that the slowest blood velocity in capillaries could prolong the time TfR substrates interact with the endothelial surface, thereby increasing the likelihood of internalization. However, it is important to note that red blood cells (RBCs) also express TfR1, and the high affinity of TfR1 may lead to drug accumulation in RBCs, potentially causing hemolysis in some instances.

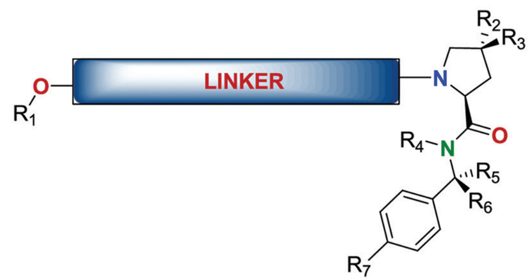
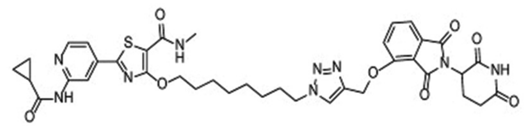
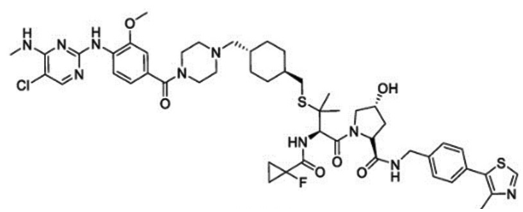
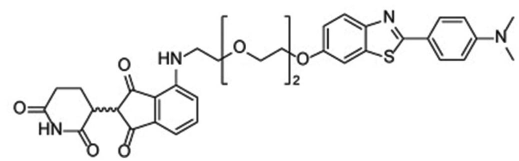
Adsorption-mediated transcytosis is a saturable mechanism of transport.[94,113] AMT requires the molecule to be positively charged at physiological pH 7.4 for electrostatic interactions with cell surface binding sites.[34] The positive charge is necessary due to the highly charged

Table 2. Targeted protein degraders under development for central nervous system indications

TPD	Indication	Development stage	Targeted protein	E3 ligase ligand	Structure	References
QC-01-175	Frontotemporal dementia; tauopathies	Preclinical	Tau	CRBN	 QC-01-175	5
C004019 (240)	Alzheimer's disease; tau-related diseases	Preclinical	Tau	VHL		6
FMF-06-038	Frontotemporal dementia; tauopathies	Preclinical	Tau	CRBN	 FMF-06-038	83
FMF-06-049	Frontotemporal dementia; tauopathies	Preclinical	Tau	CRBN	 FMF-06-049	83
TH006	Alzheimer's disease; tauopathies	Preclinical	Tau	VHL	 10	84

(*Contd...*)

Table 2. (*Continued*)

TPD	Indication	Development stage	Targeted protein	E3 ligase ligand	Structure	References
α-Synuclein Degrader	Parkinson's disease; α-synucleinopathies	Preclinical	α-synuclein	CRBN; VHL		85
PG-21	GSK-3β degradation	Preclinical	GSK-3β	CRBN		86
XL01126	Parkinson's disease	Preclinical	LRRK2	VHL		4
JMF4560	Amyotrophic lateral sclerosis; TDP-43	Preclinical	TDP-43	CRBN		87

Abbreviations: CRBN: Cereblon; GSK-3β: Glycogen synthase kinase-3 beta; LRRK2: Leucine-rich repeat kinase; TDP-43: Transactive response DNA binding protein 43 kDa; TPD: Targeted protein degraders; VHL: Von Hippel–Lindau.

Table 3. Leveraging transferrin receptors to enhance central nervous system penetration of biologics

Drug	Company	Indication	Composition	Status
Tividenofusp alfa	Denali Therapeutics	Hunter syndrome	huIDS fused through peptide linker (GGGGS) to anti-TfR Ab	Phase II; Accelerated approval sought from the FDA
TAK-594	Takeda	Frontotemporal dementia	Recombinant progranulin protein fused with anti-TfR Ab	Phase I/II
TAK-920	Takeda	Alzheimer's disease	Trem2 fused with TfR binding sequence	Discontinued Phase I due to narrow therapeutic window
Trontinemab	Genentech	Alzheimer's disease	Bispecific mAb: Anti-amyloid beta Ab (gantenerumab) fused to TfR1-binding module	Phase I
Pabinafusp alfa	JCR Pharmaceuticals	Hunter syndrome	huIDS fused with anti-TfR Ab	Phase III
RO 7121932	Genentech	PPMS, SPMS, RRMS	Anti-CD20 Ab fused with anti-TfR Ab	Phase I

Abbreviations: Ab: Antibody; CD20: B-lymphocyte antigen CD20; FDA: The United States food and drug administration; huIDS: Human iduronate-2-sulfatase; mAb: monoclonal antibody, PPMS: Primary progressive multiple sclerosis; RRMS: Relapsing-remitting multiple sclerosis; SPMS: Secondary progressive multiple sclerosis; TfR: Transferrin receptor; Trem2: Triggering receptor expressed on myeloid cells 2.

pericellular matrix, or "glycocalyx," on the blood side of the endothelium wall, which facilitates binding with positively charged molecules while repelling those with a net negative charge at physiological pH. This is a high-capacity, low-affinity process that leads to increased binding and extensive biodistribution, rather than being confined to the CNS. Polycationic proteins, such as protamine from salmon sperm (molecular weight ~4500 Da), can also penetrate the BBB through AMT.[96] Cationized albumin (isoelectric point = 8.5 – 9) can be conjugated to liposomes to facilitate interaction with endothelial cells.[114,115] In addition, the lectin wheat germ agglutinin can enter the brain through AMT.[95] However, the cationic modification also increases systemic clearance. In the B-B barrier endothelium, Non-specific transcytosis happens very seldom and would need distinct molecular signatures than those found in peripheral endothelial cells.[88] In both RMT and AMT, the payload is internalized from the plasma membrane of endothelial cells into endosomes, followed by the fusion of late endosomes with lysosomes and the digestion of the cargo through the endolysosomal pathway. This process ultimately reduces the transcytosis rate into the brain parenchyma.[12]

CMT is a saturable, active transport mechanism that facilitates the movement of polar molecules across the BBB. It is involved in the transport of relatively smaller molecules such as glucose (180 Da) through GLUT1, large neutral amino acids through LAT1, and gamma-aminobutyric acid throu12gh GABA transporter 2. Polar drugs, such as levodopa (197.2 Da), gabapentin (171.24 Da), hormones, and fatty acids are also transported through CMT.[12,100,116] These transporters are highly stereoselective. While harnessing CMT for drug transport is appealing, the development of small molecules that effectively engage CMT remains challenging. The drug must fit well -in the transporter pocket while maintaining its target binding affinity within the CNS.

Fluid-phase endocytosis (micropinocytosis) provides transporter-independent entry to the brain for larger, hydrophilic molecules.[88] This process involves the uptake of ECF and dissolved solutes, rather than requiring the molecule to interact with the endothelial plasma membrane. For instance, albumin can cross the BBB through fluid-phase endocytosis.[95] Under homeostatic conditions, albumin passes through the endothelial layer from the luminal side to the interstitium through an energy-dependent process mediated by vesicles. This transport can occur through vesiculo-vacuolar organelles or caveolae.[117,118] Albumin binds to the albondin receptor, which facilitates its endocytosis by endothelial cells.[119,120] The binding of albumin to albondin triggers receptor clustering and interaction with caveolin-1, a multifunctional protein critical for caveolae formation.[121] It is important to note that plasma proteins, particularly albumin, are toxic to brain cells,[122] and disruption of the BBB may lead to the influx of plasma into the brain.

11. Extent of plasma protein binding and CNS bioavailability

The percentage of plasma protein binding helps assess the clearance and volume of distribution of a drug and predict the likelihood of drug-drug interactions and the PK/PD relationship.[123,124] Like other plasma proteins, albumin does not readily traverse through the BBB, and its complexation with a drug molecule makes this process even more difficult. According to the "free drug theory," drugs that extensively bind to plasma proteins typically have lower CNS bioavailability.[125,126] In other words, high protein binding is undesirable for molecules targeted at brain indications, as a lower free fraction (f_u) would be available for BBB permeation. However, there are notable exceptions, such as long-acting, non-steroidal anti-inflammatory agents, such as isoxicam and meloxicam, tricyclic antidepressants such as imipramine and desipramine, calcium channel blockers, such as isradipine and darodipine, and benzodiazepine antagonists like flumazenil and iomazenil.[127-130]

TPDs, in general, exhibit high plasma protein binding and non-specific binding, resulting in very low f_u. Therefore, to meet the regulatory requirement of reportable f_u values ≥0.01 for drug-drug interaction prediction, the dilution method is commonly used to estimate the extent of plasma protein binding for TPDs. In this approach, a diluted f_u, or f_{ud} ≥0.01, can be obtained by adjusting the dilution factor, taking care not to saturate the potential binding sites. This dilution method provides f_u levels comparable to those estimated by the pre-saturation approach without plasma dilution. The equilibrium is achieved rapidly, and the impact of non-specific binding is reduced.[131,132]

In the case of TPDs, the percentage of unbound f_u in plasma does not appear to correlate reasonably with the concentrations quantified in the brain. Various mechanisms may contribute to this phenomenon. One possibility is the interaction of the TPD-bound protein with the brain capillary walls, leading to conformational changes and the release of the TPD from the protein.[133-135] Another probability is protein-mediated transport, similar to what is observed with neutral and basic drugs, such as diazepam, disopyramide, and chlorpromazine. Their binding to alpha-1-acid glycoprotein enhances BBB permeability.[126] Yet another plausible mechanism is the enhanced extraction of the drug-protein complex into CNS regions with more permeable capillary endothelium.[127,136] Another potential mechanism could involve transporter-induced shifts in protein binding. In this scenario, the TPD may detach from the protein through transporters present on the luminal membrane before it dissociates and achieves binding equilibrium. High protein binding affinity, thus, would not restrict the TPD's permeation into the brain, making it incorrect to use *in vitro* f_u values obtained at equilibrium to predict the drug available for brain penetration. The two predominant drug transporters in the brain are OATP1A2 and OATP2B1. OATP1A2 (SLCO1A2) is expressed in endothelial cells of the BBB and the apical (brush border) membrane of enterocytes in the duodenum, where it facilitates the oral absorption of xenobiotics. OATP1A2 has a broad range of substrates, including bile salts and acids, steroid conjugates, thyroid hormones, and xenobiotics such as methotrexate, fexofenadine, erythromycin, imatinib, lopinavir, and some β-blockers. The high molecular weight dopamine D2 receptor agonist bromocriptine (654 Da) is a substrate of OATP1A2. OATP2B1 is localized in the sinusoidal membranes of hepatocytes and enterocytes and the apical membrane of endothelial cells of the BBB. OATP2B1 substrates include statin, prostaglandin E2, thyroid hormones, and sulfonylureas. Although the crystal structures of OATP1A2 and OATP2B1 are not available, the crystal structure of OATP1B1 is available from the Protein Data Bank. OATPs share a conserved transmembrane helix fold that harbors a druggable binding pocket.[137] Given that a significant number of TPDs have been reported to be orally bioavailable, the likelihood of TPD binding with OATP1B1 can be assessed though docking studies using SwissDock. OATP1A2 shares ~58% identity with OATP1B1, and OATP2B1 shares ~68% identity with OATP1B1. The sequence identity is spread across the entire sequence and is not concentrated in any specific region. Therefore, docking studies with OATP1B1 may provide a reasonable estimate of TPD's likely affinity toward OATP1A2 and OATP2B1.

12. Cereblon ligands and linker chemistry to augment BBB permeability

While the protein-of-interest (POI) ligand depends on the target, there is a certain degree of flexibility in selecting E3 ligase ligands, such as CRBN, VHL, integrin-associated protein, and mouse double minute 2 homolog, as well as the linker chemistry. Furthermore, within each class of E3 ligase ligands, there are various options to choose from. For example, in the case of CRBN, one can choose between phenyl glutarimide, chiral lenalidomide, phenyl dihydrouracil, or benzoimidazolone, depending on the stability, potency, and druggability of the TPD. Phenyl glutarimide, for example, offers good aqueous solubility, cellular permeability, and *in vitro* plasma stability, but it lacks stability in hepatocytes. Regarding linker chemistry, rigidification of linkers (e.g., linear to piperidine, piperazine, and spiro) has been shown to improve the absorption, distribution, metabolism, and excretion (ADME) profile, such as aqueous solubility, cellular permeability, and plasma and hepatocyte stability. Polyethylene glycol (PEG) linker's exhibit improved aqueous solubility, while PEG amide linkers tend to impart poor cellular permeability. O-linked acetamide PEG displays good aqueous solubility but poor plasma and hepatocyte stability, as well as low cellular permeability. Conversely, the conversion of amide into alkyl linker results in poor aqueous solubility, low cellular permeability, and diminished plasma and hepatocyte stability.

13. TPD bidirectional permeability assessment in MDCK-MDR1 and Caco-2 cell lines

MDCK-MDR1 and Caco-2 cell lines are widely used to assess membrane permeability and efflux liability of TPDs. Despite the canine origin of MDCK cells, MDCK-MDR1 is preferred over Caco-2 due to its high expression of human P-gp and faster turnaround time (7 days versus 21 days).[138,139] Endogenous (canine) *Mdr1* and *Mrp2* (Abcc2) mRNA expression is significantly lower in MDCK-MDR1 cells compared to MDCK wild type (WT).[140] Both MDCK-MDR1 cells and Caco-2 cells express comparable levels of the 170 kDa isoform of P-gp.[141] However, the 150 kDa isoform is overexpressed in MDCK-MDR1 cells compared to Caco-2 and MDCK-WT cells. The readouts generated from the Caco-2 cell line may differ from the apparent kinetic constants and affinities of substrates determined in MDCK-MDR1 cells.[139]

In MDCK-MDR1 bidirectional permeability assays, TPDs often exhibit a high efflux ratio and low permeability, which may not be relevant to BBB permeability. The addition of 0.5% bovine serum albumin in the donor well and 2% in the receiver well improves solubility slightly and reduces non-specific binding. However, this does not significantly improve permeability in the absorptive direction. Regardless, TPDs still exhibit quantifiable concentrations in the brain. This raises questions regarding the suitability of MDCK-MDR1 cell line permeability indices for assessing BBB penetration. The efflux liability of TPDs may be overestimated in the MDCK-MDR1 assay. The human MDR1-transfected porcine-kidney-derived LLC-PK1 cell line, with a similar turnaround time (7 days), is a relatively better indicator of TPDs' BBB permeability. LLC-PK1 cells express multiple endogenous transporters, including P-gp and members of the MRP/ABCC family of efflux transporters.[142] MDR1-transfected LLC-PK1 cells exhibit comparable *Mdr1* levels but significantly higher *Mrp2* mRNA levels than WT cells.[140] Alternatively, BBB permeability can be assessed using human brain microvascular endothelial cells in astrocytes co-culture. Another option is to utilize primary porcine brain endothelial cell-astrocyte co-culture or porcine endothelial cells, pericytes, and astrocytes culture for BBB permeability assessment.[143,144] However, the validation, repeatability, and reproducibility of the primary co-culture approach can be challenging, and high inter-experiment variation is difficult to address.

14. Diffusion

The impact of efflux is potentially low for small molecules with a high propensity for passive diffusion. For P-gp substrates, the $K_{p,uu,brain}$ typically ranges from moderate to high (≥0.3). For TPDs, both uptake and efflux transporters play a significant role in BBB permeability, and $K_{p,uu,brain}$ values range from 0.3 – 0.05.

High-molecular-weight oligonucleotides, peptides, monoclonal antibodies, and proteins primarily rely on transcytosis, with passive diffusion being negligible. The tissue concentrations of these macromolecules are very low compared to plasma, with $K_{p,uu,brain}$ values being extremely low (<0.01).

The mechanisms of intra-tissue penetration remain largely unknown. For small molecules, diffusion in brain regions is effective only over short distances, spanning a few cell bodies (~10 – 100 uM), but is extremely low and limiting over larger distances (≥1 mm). This slow distribution is a limiting factor for the effective distribution of drugs into the brain's affected regions. For TPDs, due to their large size, intra-tissue diffusion is expected to be even more restricted.

15. Wider pharmacokinetic/ pharmacodynamic disconnect

For small molecule non-covalent inhibitors, the therapeutic effects often depend on maintaining adequate trough concentrations. In contrast, for TPDs, the concentration-time profile has less relevance to the observed therapeutic effects over time. This disconnect is primarily due to the time lag between target engagement and degradation, coupled with an event-driven, catalytic mechanism.

The therapeutic effects of TPD are driven by the degradation of disease-causing proteins by cellular proteosomal machinery. The TPD transiently binds to a target protein and directs it for enzymatic degradation through ubiquitination.[145] The PD effect persists even after the TPD has been cleared off from systemic circulation and its levels fall below the lower limit of quantitation. Thus, the systemic exposure of TPDs exhibits a pronounced disconnect with the therapeutic response. A high maximum concentration therefore is not necessary for TPDs and may instead contribute to unwanted toxicities. Consequently, infrequent dosing regimens at very low doses may still provide sustained efficacy, particularly in cases where the protein synthesis rate is low.[146,147]

This PK/PD disconnect is expected to be more pronounced for CNS therapeutics due to the presence of the BBB, low diffusion propensity in the CNS, difficulties with neuronal cell transfection, and altered proteasomal machinery. To modify the optimal amount of POI degradation, dosing regimens may need to be adjusted, as POIs with long half-lives will influence the duration of the therapeutic effect.

16. BBB disruption-driven CNS bioavailability

TPDs exhibit the chameleon-like ability to adopt a folded confirmation, reducing their tPSA and achieving higher permeability than would be expected based on their molecular weight and calculated tPSA.[148] However, the membrane permeation efficiency of TPDs and their attainment of equilibrium across the plasma membrane of endothelial cells is expected to be lower than that of typical small molecules. In some cases, however, the reported brain concentrations at different time points following IV injection have been comparable to those observed for small molecules. This raises the possibility of BBB disruption-driven CNS bioavailability rather than permeability-mediated bioavailability. Crizotinib and lorlatinib are both indicated for anaplastic lymphoma kinase-positive non-small cell lung cancer. Their structures, physicochemical properties, and ADME/PK profiles are summarized in Table 4.

The cyclized version, lorlatinib, has been found to be effective in reducing the progression of metastatic disease, including brain metastasis. It was assumed to have good BBB permeability. Consequently, lorlatinib, but not the linear version of crizotinib, has been approved for anaplastic lymphoma kinase-positive metastatic non-small cell lung cancer.

However, a single and repeat-dose study of lorlatinib in Sprague Dawley rats revealed that lorlatinib administration increased Evans blue (EB) dye concentration in the brain, indicating damage to the BBB, whereas crizotinib did not exhibit this effect. Furthermore, SPP1, VEGF, TGF-β, and Claudin genes were downregulated in the lorlatinib group, strongly correlating with the abrupt disruption of BBB.[149] Thus, lorlatinib brain exposure is largely attributed to BBB disruption-driven penetration rather than a permeability-driven mechanism. Another anti-cancer drug, docetaxel, has also been implicated in destabilizing the BBB, likely driven due to its mechanism of action.[150] In addition to active ingredients, excipients and inhalant anesthetics can transiently perturb the BBB and increase the CNS bioavailability of drugs.

17. Increasing BBB permeability of chemical macromolecules

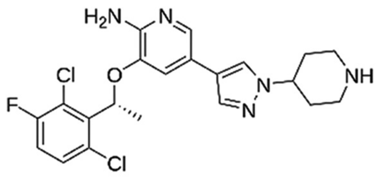
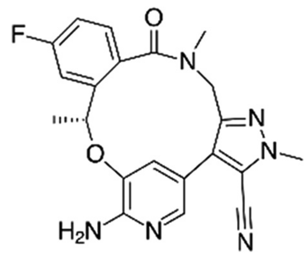
The brain penetration of compounds primarily depends on lipophilicity, affinity for efflux and influx transporters, molecular weight, electric charge, extent of plasma protein binding, tPSA, number of rotatable bonds, HBDs, and HBAs. As the drug's molecular weight increases, its ability to traverse the BBB does not increase proportionally with its lipophilicity. Moreover, as the drug's surface area increases from 52 Å (for a molecule with a molecular mass of 200 Da) to 105 Å (for a drug with a molecular weight of 450 Da), BBB permeability decreases by 100-fold.[151,152] In addition to size, the compound's composition, which determines its physiochemical properties, also regulates BBB permeability. Given that targeted proteins are primarily intracellular, aqueous solubility and cellular permeability are critical for achieving the desired therapeutic effect. TPDs, by design, can be considered chemical macromolecules. The high molecular weight of TPDs is often associated with low aqueous solubility and poor cellular permeability due to a high tendency for aggregation or precipitation. This property of TPDs presents significant ADME challenges in the development of orally bioavailable formulations. Table 5 highlights some of the obstacles encountered during the oral delivery of TPDs, challenges in estimating plasma protein binding of TPDs, and major mechanisms of their elimination.

18. Structural modifications of TPDs to increase BBB permeability

Typically, CNS active drugs have ≤3 HBDs, significant rigidity, fewer rotatable bonds, and a tPSA in the range of 60 – 70 $Å^2$ (with a maximum of 90 $Å^2$). Ideally, for effective CNS penetration, the number of HBAs and HBDs should be ≤2; however, TPDs often have far more HBAs and HBDs. Despite this, a substantial number of TPDs are advancing pre-clinical and clinical development for neurological indications, for example, XL01126, an Arvinas molecule (Figure 5).

XL01126 has a molecular weight of 1,019.7 Da and calculated water: octanol partition coefficient of 4.44. The Caco-2 permeability values are A–B <0.74 and B–A <1.43 $\times 10^{-6}$ cm/s. Despite possessing unfavorable structural and physiochemical attributes for BBB penetration, quantifiable concentrations of XL01126 in the CNS have been reported in the literature. XL01126 has demonstrated the ability to penetrate the BBB and induce degradation of leucine-rich repeat kinase 2.[4] This is particularly intriguing, given the tPSA of XL01126 (194.3 $Å^2$), which far exceeds the maximum suggested for steady CNS drug entry (90 $Å^2$).[153] The formulation excipients included 10% 2-hydroxypropyl-β-cyclodextrins in 50 mM citrate buffer (pH 3.0) and the dose strength was 1 mg/mL for IV injection. Male C57BL/6 mice were administered a single dose of XL01126 either orally (30 mg/kg), intraperitoneally

Table 4. Physicochemical, absorption, distribution, metabolism, excretion, and pharmacokinetics properties of crizotinib and lorlatinib

Particulars	Crizotinib	Lorlatinib
Structure		
Molecular formula	$C_{21}H_{22}Cl_2FN_5O$	$C_{21}H_{19}FN_6O_2$
Molecular weight (Da)	450.34	406.41
HBA	6	7
HBD	2	1
Rotatable Bond	5	0
tPSA	78 Å²	110 Å²
Appearance	White to pale-yellow powder	White to off-white powder
pKa	5.6	4.92
Aqueous solubility (mg/mL)	>10 – <0.1 (pH 1.6 – pH 8.2).	32.38 – 0.17 (pH 2.55 – pH 8.02)
Log D	1.65 (pH 7.4)	2.45 (pH 9)
Protein binding	91%	66%
Volume of distribution (Vss)	1,772 L	305 L
Blood: plasma ratio	1	0.99
P-gp substrate	Yes	No
P-gp inhibitor	Yes	Yes
Oral bioavailability	43%	81%
Half life	42 h	24 h
Clearance (CL/F)	100 L/h (single dose); 60 L/h (steady state); Autoinhibition	11 L/h (single dose); 18 L/h (steady state); Autoinduction
Excretion	63% feces (53% unchanged); 22% urine (2.3% unchanged)	41% feces (9% unchanged); 48% urine (<1% unchanged)
Indication	Anaplastic lymphoma kinase/ROS1-positive non-small cell lung cancer	Anaplastic lymphoma kinase-positive metastatic non-small cell lung cancer
Dose	250 mg BID	100 mg OD
Dosage form	Hard gelatin capsule	Tablet
Excipients	Microcrystalline cellulose, dibasic calcium phosphate anhydrous, sodium starch glycolate, and magnesium stearate	

Abbreviations: BID: Twice a day; CL/F: Clearance of the drug from plasma; Log D: Water: octanol partition coefficient; OD: Once daily; P-gp: P-glycoprotein; pKa: Negative base -10 logarithm of the acid dissociation constant; ROS1: Proto-oncogene tyrosine-protein kinase; Vss: Steady-state volume of distribution; HBA: Hydrogen Bond Acceptors; HBD: Hydrogen bond donors; tPSA: Total polar surface area.

(30 mg/kg), or IV (5 mg/kg), and the concentrations of XL01126 were measured in plasma, brain tissue, and CSF at seven-time points. The brain-to-plasma ratio was estimated to be <0.035, which is relatively low.

However, given the substoichiometric/catalytic mechanism of action, which is distinct from the occupancy-driven mechanism of inhibitors, TPDs are expected to achieve targeted protein degradation in the affected tissue

Table 5. Challenges associated with the ADME profiling of targeted protein degraders and major mechanisms of elimination

Most significant ADME/physicochemical property challenges facing the development of orally bioavailable (>30% F) degraders		Main challenges faced with measuring PPB of TPD	Major mechanisms of TPD elimination	
Primary	Secondary		Metabolism	Other pathways
Permeability	P-gp	Non-specific binding and poor recovery	Pathways	Biliary elimination
Solubility	Metabolism/stability	Analytical sensitivity limitations	CYP450	Renal elimination
MW	>3 HBDs and HBAs	Solubility limitations	Amidase	Intestinal secretion
IVIVC	Polarity/lipophilicity	Equilibrium issues	Esterase	P-gp efflux
	PPB	Slow binding kinetics	UGT	OATP uptake
	Absorption	Plasma instability	Hydrolysis	
			Aldehyde oxidase	
			GST	

Abbreviations: ADME: Absorption, distribution, metabolism, and excretion; CYP450: Cytochrome P450; F: Oral bioavailability; GST: Glutathione S-transferase; HBA: Hydrogen bond acceptors; HBD: Hydrogen bond donors; IVIVC: *In vitro in vivo* correlation; MW: Molecular weight; OATP: Organic anion transporting polypeptide; P-gp: P-glycoprotein; PPB: Plasma protein binding; TPD: Targeted protein degraders; UGT: UDP-glucuronosyltransferase.

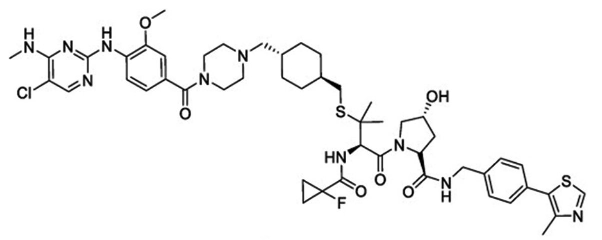


Figure 5. XL01126 chemical structure

even with low exposure, leading to amelioration of disease conditions and symptomatic relief. Despite very low CNS bioavailability, XL01126 selectively degrades more than 95% of pathological tau in the mouse brain 24-h after parenteral administration, while sparing the WT tau. Other tau-targeting TPDs have exhibited even lower BBB permeability.[4,154]

To treat CNS neuropathies, small-molecule drugs must traverse the BBB. Achieving reasonable permeability necessitates careful optimization of druggability properties while retaining the molecule's potency to achieve sufficient CNS concentration for the desired therapeutic efficacy. In the case of TPDs, the challenge is significantly amplified due to their high molecular weight and structural composition. To create a TPD with reasonable BBB permeability, medicinal chemistry efforts may focus on masking polar functional groups, which can be particularly difficult given the size and composition of TPDs.

There are three primary approaches to increase the exposure of drugs in the brain: augmenting diffusion, curtailing efflux, and engaging non-saturable career transport.

Many CNS-targeted drug discovery studies have attempted to enhance the CNS delivery of hydrophilic compounds through optimal lipidation of the polar parent molecule. While increasing lipophilicity enhances drug delivery to the brain, this may not always translate into increased efficacy. A plausible explanation is that increased lipophilicity can lead to enhanced binding to brain tissue, which in turn reduces the amount of free drug available to interact with its therapeutic target within the brain parenchyma.[155] This consequence may also affect properties, such as low aqueous solubility, rapid metabolism, and cellular quenching.[156]

Water: octanol partition coefficient does not appear to be a reliable metric for predicting BBB permeability. Instead, the experimental polar surface area has shown a relatively better correlation (data not shown) with BBB permeability.

For CNS-targeted therapies, molecular weight alone cannot be considered a major determinant of BBB permeability for TPDs. Instead, structural composition—especially the linker—and malleability appear to compensate for the high molecular weight, with polar functional groups (hydroxyl [-OH], amino [-NH^2, or carboxyl [-COOH]) groups enhancing aqueous solubility. In addition, alkyl/aryl groups or fluorine substitution adjacent to the -OH group can confer lipophilic traits. Among the three components of TPDs (POI, linker, and E3 ligase ligand), the POI is unique based on the targeted protein, and the E3 ligase ligand is selected according to the abundance of E3 ligases at the site of action. Thus, identifying BBB-permeable linker motifs represents an essential strategy for optimizing TPDs' CNS druggability properties.

Incorporating flexible PEG linkers is a common approach in TPD designing. However, PEG linker's exhibit low solubility, poor permeability, and poor plasma, chemical, and metabolic stability due to the presence of many non-polar ethylene groups and high HBDs. Alternatively, incorporating nitrogen-containing triazole rings in the linker can improve aqueous solubility due to the electron-donating nitrogen group, which enhances its ability to form hydrogen bonds. On the contrary, rigidified piperidine and piperazine linkers have demonstrated improved aqueous solubility and permeability due to their constrained conformations while maintaining reasonable plasma, chemical, and metabolic stability.

Efforts to increase a TPD's plasma half-life, such as linker rigidification and the enhancement of lipophilic properties, can amplify its interaction with BBB endothelial cell membranes. However, these modifications may also increase its interaction with other endothelial cells in the body and lead to binding with blood cellular components' membranes. Consequently, while CNS exposure may improve, undesirable tissue distribution of the TPD could also increase in multiple organs/compartments, potentially leading to off-target toxicity.

If structural tailoring enhances CNS exposure, it must be demonstrated beyond a reasonable doubt that decreased hepatic metabolism resulting in reduced plasma clearance and increased systemic presence is not the underlying cause. To establish this, the amount of administered dose (AD) that actually reaches the brain can be compared across different approaches (% AD/g of brain).

Generally, drug action in the brain is primarily driven by the amount of drug uptake, which is measured as % AD/g of brain tissue. This depends equally on the plasma area under the curve and the BBB permeability-surface area product:

% AD/g = (BBB-surface area product) × (Plasma area under the curve)

While the % AD/g of a TPD indicates its ability to cross the BBB, it is also influenced by TPD's plasma concentration.[3]

19. Formulation excipients and inhalant anesthetic selection for increasing target protein degraders' BBB permeability

Administering TPDs in a vehicle that transiently opens the BBB and facilitates their delivery into the brain parenchyma is a plausible strategy for crossing the BBB, particularly in pre-clinical settings. Solvent-mediated disruption of the BBB can result from doses of solvents, such as ethanol or dimethyl sulfoxide (DMSO) at levels ranging from 1 – 4 g/kg.[157,158]

For instance, administering just 50 μl of 50% DMSO to a 20 g mouse equates to a dose of 1.25 g/kg of DMSO.[3] In mice, even a low dose of 3 mg/kg of Tween 80/polysorbate-80 can transiently disrupt the BBB, allowing formulated drugs to cross.[159] An infusion of the non-ionic surfactant Tween 80 helped kyotorphin, an oligopeptide that typically does not penetrate the BBB, to cross and induce analgesia.[160] Anionic surfactants, such as sodium lauryl sulfate (also known as sodium dodecyl sulfate, SDS), are typically included in microquantities in formulations targeting CNS indications. Short-term BBB disruption can be triggered by SDS doses as low as 1.0 g/kg. TPDs can therefore be dissolved in solvents such as ethanol or DMSO and co-formulated with SDS PEG hydroxystearate, which transiently destabilizes membranes and temporarily disrupts the BBB to facilitate brain penetration.

One commonly used formulation excipient, β-cyclodextrin, has been reported to inhibit the caveolae-mediated pathway and prevent macromolecular substance endocytosis/extravasation through the BBB.[161] However, another study suggests that 2-hydroxypropyl-β-cyclodextrin may enter endothelial cells.[162]

Diuretic mannitol has been shown to induce osmotic shrinking of endothelial cells and disrupt the BBB architecture when infused at a 2M solution through the carotid arteries.[163] In addition, selective agonists that induce adenosine receptor signaling transiently enhance BBB permeability, facilitating the entry of otherwise impermeable macromolecules into the CNS.[164]

The inhalant anesthetic isoflurane has been reported to disrupt the membrane lipid nanodomains and trigger caveolar transport in brain endothelial cells, thus augmenting cisplatin delivery to the brain for treating glaucoma. This disruption is reversible, with the BBB regaining tightness after the cessation of anesthesia.[165] Therefore, it should be crucial to evaluate whether the observed CNS bioavailability is due to the solvent/excipient, high doses of the anesthetic agent, or inherent drug properties.

20. The way forward

A high dose of CNS-targeted investigational molecule can breach the neurotoxicity threshold, disrupt the BBB, and exhibit brain bioavailability. It is therefore imperative to distinguish between the BBB disruption-triggered penetration and permeability-driven pathways by employing the EB dye brain staining approach. Using the EB dye method, the parenteral dose of optimized CNS-targeted lead candidates can also be titrated (or kept as low as possible) to identify the optimal dose for assessing BBB permeability upon parenteral administration. Further, it would be interesting to determine whether XL01126, when administered at doses as high as 30 mg/kg intraperitoneally or 5 mg/kg IV, penetrates the BBB through disruption-driven mechanisms, breaching the neurotoxicity threshold, or enters the brain through permeability-driven pathways.

However, simply crossing the BBB is not sufficient for TPDs to disease conditions. Efficient penetration into brain cells, which are typically difficult to transfect, is necessary. Furthermore, enhancing the selective accumulation of TPD in the affected brain regions remains a challenge. We have yet to conclusively demonstrate that TPD activity is region-specific, that is, concentrated in the affected areas of the brain. Finally, a major obstacle in achieving the desired therapeutic benefit is overcoming the downregulation of proteasome machinery in various CNS diseases. Thus, *in vitro* efficacy screening should encompass a cellular environment that mimics the senile state (e.g., neurons and astrocyte primary cultures derived from 16-week-old rats or 24-week-old mice), and establish a PK-PD correlation in experimental animals.

21. Discussion

The limitations posed by the BBB present an enduring challenge in delivering macromolecular therapeutics to the CNS. TPDs are large molecules that belong to the "beyond rule of 5" chemical space. Due to their size and composition, achieving reasonable BBB permeability is a major roadblock. Nonetheless, TPDs are being considered as effective CNS therapeutics. In our preliminary experiments, we found quantifiable concentrations (K_p) of TPDs despite demonstrating poor MDCK-MDR1 cell permeability, high efflux potential, and >98% plasma protein binding in *in vitro* studies. Therefore, it would be valuable to investigate the mechanism(s) and route(s) by which TPDs cross the BBB. Several approaches could be explored:

(i) Although the likelihood of passive diffusion of macromolecule TPDs is low, the parallel artificial membrane permeability assay can be employed as a Tier 1 assay to assess the passive diffusion potential of TPDs. Molecules with an apparent permeability value of $>4.0 \times 10^{-6}$ cm/s can be considered to possess passive diffusion potential.

(ii) The possibility of passive diffusion of TPDs through PCVs should be evaluated, as PCVs offer less resistance at the BBB. Compared to capillaries, they are the primary site for transcytosis-mediated brain delivery of therapeutic nanoparticles. Moreover, there is substantial evidence supporting the notion that PCVs are the preferred site of extravasation for leukocytes, tumor cells, and parasites. Capillaries' slower blood velocity may increase the time that TPDs interacting with venular endothelial cells, which contain intracellular machinery that could aid in TPD internalization.

(iii) The passive diffusion potential of TPDs may also be assessed *in vivo* by comparing brain concentrations of TPDs in young (2 months) versus aged (16 months) rats. In aged rats, the number of tight-junction proteins and the total length per capillary decrease. Alternatively, 1 week old neonatal mice may be used to determine the passive diffusion ability of TPDs. The route of administration however will be limited to intraperitoneal or subcutaneous only, as it is relatively difficult to administer through oral or IV route in 1 week old mice.

(iv) The novel mechanism(s) of BBB permeation by TPDs could be investigated using a unilateral internal carotid artery ligation rat model. Specific brain regions could be isolated, and free fractions of TPDs measured to assess region-specific distribution. In addition, the BBB membrane may be subjected to next-generation sequencing for differential gene expression profiling, with gene regulation confirmed through reverse transcription polymerase chain reaction.

(v) The ability of OATP1A2, OATP2B1, and OATP1B1 to transport TPDs across the plasma membrane could be evaluated using transporter-overexpressing cell lines.

(vi) To assess the brain-specific turnover of TPDs, the liability of CYP1B1 can also be evaluated. CYP1B1 metabolizes several endogenous substances, including retinol, prostanoids, estradiol, and melatonin. Assessing the CYP1B1 liability of investigational TPDs will provide insights into their metabolic stability in the CNS and offer a comprehensive view of brain pharmacokinetics.

22. Conclusion

The clinical therapeutic efficacy of drug candidates relies in part on the earliest possible detection of neurological diseases and the initiation of therapy. Most CNS diseases have a poor prognosis once they reach advanced stages. Early treatment of neuroinflammation or neurodegeneration may help prevent or slow the progression of BBB deterioration to an irreversible stage. In addition, early intervention could restore the BBB's protective function, shielding the brain from inflammatory cytokines, toxins, metastatic cancer cells, and xenobiotics. With the advent of advanced imaging techniques, artificial intelligence-powered technologies for detecting pathological changes in the CNS, improved diagnostic tools, and an expanded battery of biomarkers, it has become relatively easier to identify CNS diseases at early stages. TPDs are making significant inroads into the CNS disease space, with high hopes pinned on advanced pre-clinical leads. Compared to late-stage diseases, TPD delivery across the BBB in younger subjects exhibiting early-stage disease manifestations could be more challenging due to the anatomically tight and intact BBB, characterized by the high density of tight junctions and proteins. The BBB in younger individuals offers high resistance to macromolecule permeation, posing a significant challenge in achieving sufficient TPD delivery across the barrier – an issue that has historically hindered many small molecule pre-clinical drugs from reaching their CNS targets, thus limiting their therapeutic efficacy.

However, there is still a long way to go before we can conclusively identify the underlying mechanisms active at the BBB within the therapeutic window, as well as assess any potential spillover into the neurotoxicity zone if any, while evaluating BBB permeability. Unraveling the mechanisms and routes that TPDs use to permeate the BBB could help address this issue. Given the number of TPDs undergoing pre-clinical and clinical evaluation for CNS indications, understanding the underlying mechanisms will pave the way for developing effective TPD-based therapies that can more effectively penetrate the BBB and preferentially accumulate in brain regions affected by neurological disorders.

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Conflict of interest

Satinder Singh, Satish Kumar, and Pratima Srivastava are employees of Aragen Life Sciences Limited. Vyas M. Shingatgeri is the Dean of the School of Biosciences at Apeejay Stya University. The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article.

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ACCSCIENCE PUBLISHING

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PERSPECTIVE ARTICLE

On disclosing Alzheimer's disease: A need of empathy

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Abstract

The disclosure of an Alzheimer's disease (AD) diagnosis is not always carried out following recognized bioethical principles. Inappropriate disclosing attitudes may induce unfortunate psychological impacts on the patients' well-being, depriving them of an emotionally balanced adjustment to the condition. We present and discuss some examples of such inappropriate professional behavior, contradicting traditional medical teaching that "there are no diseases but patients." We emphasize patient singularity and the need for a caring and empathy-driven approach to diagnostic disclosure. We underline the need to avoid emotionally charged terms at the time of disclosure and to provide cognitive, physical, and social interaction guidelines as prevention and containment strategies. We call for a revised approach on the appropriate disclosure of an AD diagnosis.

Keywords: Alzheimer's disease; Diagnostic disclosure; Bioethics

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1. Introduction

New-onset psychiatric disorders are more common among patients with dementia both before and after diagnostic disclosure, including depression, anxiety, and stress-related disorders.[1] In a previous report,[2] we raised the subject of bioethical considerations on the diagnostic disclosure of Alzheimer's disease (AD) from the clinical standpoint of the doctor/patient relationship, through several examples of professional attitudes resulting in a negative emotional impact, ultimately damaging to patients, relatives, and to a proper approach to the clinical situation. Given the ominous prospect of short-term mental breakdown, a diagnosis of AD entails, the aim of the present paper is to reassert the appropriate ethical principles that should be followed by medical professionals at the time of the diagnostic disclosure of the disease.

A face-to-face diagnosis of AD, "thrown across" with indisputable certainty in its early stages, may negatively affect patients, engendering a cascade of harmful behaviors, such as feelings of insecurity, anxiety, loss of self-esteem, obsession with cognitive performance, hopelessness, depression, and despair, thus depriving them of an emotionally balanced attitude to the condition and disrupting family ties as a result.[1-4] These consequences call for careful consideration of the appropriate approach on the disclosure of such a diagnosis, its social and family impact, and its progression prognosis. Defined patterns of disclosure behavior, such as pre-diagnostic counseling, establishing patient preferences for disclosure, integrating family members, reducing the gap between the information to be disclosed and patient beliefs and expectations, "speaking the patient's language," avoiding the use of medical jargon, including information on prognosis as well as diagnosis beyond simply naming the disease, recognizing that the patient may find it difficult to understand the meaning and consequence of dementia, fostering a sense of hope by emphasizing preserved abilities and skills, and avoiding excess disability by unnecessarily curtailing social activities, all may help patients adjust to the diagnosis.[5,6]

The inclusion of the subjective dimension in disease should not be overlooked. Traditional medical teaching has taught us that "there are no diseases but patients," thereby emphasizing the singularity of patients undergoing different disease processes and evolutions.

Before the introduction of diagnostic biological markers, many patients with AD did not show enough biological changes to account for their cognitive symptoms.[7,8] Although the current paradigm requires diagnostic confirmation with biological markers in the presence of cognitive decline,[9] there is evidence that 30 – 50% of elderly adults who meet the neuropathological diagnostic criteria for AD do not have dementia.[8,10] Consequently, the clinical features of such a decline should also be contemplated, as they may be due to alternative causes other than AD.[3,4,11,12]

However, if the detection of an amyloid or Tau biomarker in cerebrospinal fluid, blood, or positron emission tomography (PET) is followed by prompt communication of AD to the patient and family without due consideration of its psychological consequences, the use of such an emotionally charged term may introduce a turning point that the patient will be unconsciously conditioned to address.[11,13] In addition, the uncertain predictive value of a biomarker-based diagnosis without full certainty whether or when they will develop further symptomatic decline is another potential cause of disclosure anxiety.[12,14,15]

As a result, the patient may experience worsening memory and cognitive symptoms, even in the absence of biological evidence, and this phenomenon increases with time, as only symptoms that support the deficit are taken into account. Psychological approaches suggest that people tend to build up specific biases of themselves and others on the basis of limited or misunderstood information.[7] In other words, people tend to construct their own theories of disease, thereby biasing information and emphasizing features that support the diagnosis. Addressing these powerful unconscious processes is essential to an adequate understanding of the cognitive and behavioral features of patients and relatives.[11,16-18]

Our previous report addressed the following issues:[2] Should a diagnosis of AD be disclosed to the patient? When and how? What are the consequences for the patient and family of such a revelation? Should it be presented as a certified diagnosis? Should the important role of cognitive reserve in disease prognosis be explained?

Cognitive reserve (CR) may protect patients against cognitive decline even in the presence of neuropathology. It is based on the concept that socio-behavioral factors, such as education, intellectually engaging occupations, and other activities, facilitate the development of neuronal networks that preserve cognitive function, even if AD biomarkers point to progressing neuropathology. CR may regulate the cause-effect relationship between neuropathology and cognitive decline. A higher CR may result in a strengthened cognitive performance against AD dementia progression. Although the measurement of CR has been controversial, there is evidence that higher CR associates with a lower relative risk of mild cognitive impairment (MCI) or dementia progression above and beyond biomarkers, showing that CR delays the onset of MCI and dementia in the presence of AD neuropathology and, subsequently, provides potential targets for preventive interventions.[19-21] These data show that the concept of CR has by now been widely developed and recognized, and though it may not yet be commonly applied in everyday clinical practice, whether because of lack of appropriate measuring techniques, or because patients' histories do not include relevant premorbid information to draw upon as a resource, it should be carefully addressed and explained to patients and families to identify possible therapeutic enhancement strategies.

Regarding diagnostic communication,[17] an interesting point of view has been proposed by Robles *et al.*[22,p.166]: "Diagnostic disclosure should avoid upsetting the patient's and family's life. In other words caution is called for, especially when it is solely based on an isolated memory loss. The term 'Alzheimer' evokes strong emotional associations, beyond the rational, that can generate sudden unfortunate changes in mood and behavior."[1,17,18,22]

Herein we share three cases dealing with the regrettable consequences of improper diagnostic disclosure for patients and families, further underlining the need of a revised approach on the appropriate communication of an AD diagnosis. Our cases were selected according to the diagnostic approach adopted by the professionals involved and included if we deemed it contrary to the Principles of Biomedical Ethics as defined by Beauchamp and Childress.[23]

2. Case 1

G.H., a 72-year-old female patient, consulted her treating physician on memory symptoms. He recommended her to consult a neurologist. She did so, accompanied by her husband, and the specialist proposed to include the patient in a pharmacological trial that could benefit her in case her symptoms should be consistent with the diagnosis of AD. She was told that the inclusion criteria of the trial protocol required proof of the presence of amyloid in her brain, for which a brain PET scan was needed. The proposal was accepted and G.H. was included in the trial.

At the time of consultation, the patient led a normal everyday life. As a housewife, she carried out household chores properly, was spatially orientated, went shopping, and could travel around the city on her own. Together with her husband, she traveled often out of town for leisure trips, as they owned property 500 km away from Buenos Aires.

The patient's Mini-Mental State Test (MMST) score was 27/30; she showed a mild deficit in executive functions and had undergone some periods of low mood or drive. The PET scan revealed brain amyloid, consistent with the diagnosis of AD. The doctor phoned and transmitted this information to the patient's husband, who was then driving through a provincial route accompanied by his wife. On receiving it, he stopped the car at the roadside, told the patient about the diagnosis, and that she was required to return to Buenos Aires as soon as possible to start her participation in the trial.

Back in town, the couple informed their sons about the AD diagnosis. Immediately, a radical change in the couple's everyday life ensued. The husband was profoundly dismayed and controlled all his wife's movements, stopped her from performing regular shopping, and would not let her stay at home or go out on her own. She consequently developed a very low mood, became depressed, got out of bed late, did not make up the bed anymore, and only got dressed late in the morning. She had almost no more social interactions with others.

Together, they consulted one of us. The therapeutic intervention aimed to clarify the situation to the patient and her husband, suggesting MCI as an alternative diagnosis, given her previous cognitively independent performance in everyday life, not necessarily implying rapid progression to AD. They were told that the time elapsed between MCI and its eventual progression is not easily predictable, but should be closely monitored and properly addressed as it may induce undesirable emotional consequences that may worsen the prognosis and should be avoided.[2,17,21] We provided cognitive, physical, and social interaction guidelines as prevention and containment strategies.

After 10 months, G.H. consulted us on her own, twice a week. She had started to exercise with a personal trainer, stayed at home under the care of her daughter, and could manage meals and shopping when her husband was away. Regular cognitive follow-ups have not shown any progression, with cognitive scores remaining stable so far.

3. Case 2

E.D., a 73-year-old male patient, was referred to one of us by his neurologist for cognitive rehabilitation. He attended in the company of his wife. He has a university degree and is a business manager. He had consulted a physician four years before because he confused his children's names and showed forgetfulness and unstable gait. On that occasion, his MMST score was 28/30, and the remaining neurocognitive assessment showed impairment of episodic memory, and cortical-type denomination deficit. A diagnosis of MCI was made, and follow-up and cognitive rehabilitation were prescribed.

A second cognitive assessment was carried out 12 months later, showing mild temporal disorientation, MMST 26/30, very low memory and denomination scores, and milder impairment in executive functions. The results were communicated by a neurologist to the patient and his wife, telling them that such a cognitive profile was consistent with AD. His wife, a psychologist, openly rejected such a diagnosis, considering it "impossible," and decided to seek a second opinion. A new specialist prescribed brain magnetic resonance imaging (MRI). Hydrocephalus was then diagnosed, and the patient underwent valve shunting, which was probably ventriculoperitoneal.

The patient consulted his physician again three years later. His wife said that he had quit using his cellphone except for answering incoming calls, and no longer managed his finances. He also experienced exacerbation in memory deficits, and could not follow television films or programs. He had quit driving, after having experienced two car crashes. He consequently did not travel alone nor went out for shopping anymore. A third cognitive assessment showed progression, with aggravated executive and visuoconstructive impairment. Cognitive rehabilitation was prescribed.

His wife ascribed his impairment to the hydrocephalus and reported unsolved shunt valve malfunctioning. She stated that he was otherwise doing "alright," that they were planning a trip abroad, and that "luckily he doesn't have AD." She did not look distressed and planned to consult further about the shunting, apart from tackling rehabilitation.

E.D.'s conversation was coherent and lively, albeit with anosognosia of his predicament. He felt well, went out with friends, and traveled, but admitted eventual forgetfulness.

4. Case 3

M.G., a 63-year-old, high-school-educated female patient, was treated by her psychiatrist upon a diagnosis of minor depression, secondary to a relevant clinical history: she underwent subtotal gastrectomy for high gastrointestinal bleeding and a benign polyp, with deep vein thrombosis and good recovery. She had been prescribed antidepressants and tranquilizers. She denied high blood pressure, diabetes, or cardiac disease, but admitted smoking 20 cigarettes daily. She denied any neurological symptoms.

In a brain MRI, prescribed before as part of her psychiatric work-up, a cortical frontotemporal retraction was described. Consequently, a fluorodeoxyglucose (FDG) PET scan was prescribed. It showed low dorsofrontolateral, orbitalfrontal, bitemporal and temporalmesial FDG uptake. On the basis of these findings, a diagnosis of frontotemporal dementia (FTD) was suggested in the PET report.

Neither the patient nor her psychiatrist reported any behavioral changes suggesting disinhibition, apathy, low empathy, stereotyped, perseverative, compulsive, inappropriate oral or unexpected eating behaviors, or weight gain, which are the features and criteria described for the diagnosis of FTD.[24-26]

The neurological examination showed normal gait, cranial nerve functions, strength, muscle tone and trophysm, taxia, tendon reflexes, and sensibility. No abnormal grasp, snout, or sucking reflexes were found. Her blood pressure was 140/80 mm Hg.

Her Montreal Cognitive Assessment (MoCA) score was 26/30, with delayed recall defects. The Trail Making Test (TMT), cube drawing, and clock tests showed normal scores (7/7 in the clock test). Attention, calculation, repetition, and abstract thinking were preserved. Phonological and semantic fluency were 15/15. Her full Boston Naming Test score was 58/60.

The patient was told that her cognitive examination showed overall normal scores and that she had some memory defects in her delayed recall liable to be followed. She did not meet clinically established criteria for FTD, as such a diagnosis should not be made solely on the basis of imaging findings. She was told that the results do not have diagnostic value unless there is a reasonable clinical and anatomical correlation, which was not found in her case, and that a PET-^{18}F-FDG by itself did not certify a diagnosis of FTD. At the time of writing this paper, the patient was still on follow-up with her psychiatrist without showing any further significant symptoms.

5. Ethical considerations

All three cases presented show relevant features concerning ethical issues related to diagnostic disclosure.

Recognized bioethics principles that must be respected by all health professionals are autonomy, beneficence, non-maleficence, and justice.[23] These principles do not appear to have been respected, let alone considered, in our presently reported cases.

In case 1, the diagnosis was disclosed only on the basis of a biological marker. The finding of brain amyloid in a PET scan may be consistent with the diagnosis of AD if it coexists with an *evolving* cognitive cortical impairment pattern. On the contrary, this patient had a reasonable daily cognitive performance, carrying out activities of daily living without aid, and showing only mild impairment in cognitive tests. The diagnosis of AD, based only on the presence of amyloid in the PET scan and delivered by phone, elicited a rather desperate and extreme attitude in her husband, who locked her up at home and controlled every aspect of her daily routine, inducing distress, anxiety, and depression in the patient, and ultimately leading to despondency, self-abandonment attitudes, and worsening of her symptoms. The inappropriateness of this attitude, triggered by the above-mentioned diagnosis and ill-advised disclosure, was shown later when the patient was reevaluated, offered the alternative diagnosis of MCI, and adequately compensated by advice, counseling, and way of life guidelines.

A diagnosis of AD should not be assumed solely on the basis of a PET finding if the clinical features do not warrant it. Extreme caution is needed when disclosing a diagnosis of AD to patients and families.

Furthermore, the diagnosis was disclosed on the basis of the professional's proposal to the patient to enter a randomized controlled trial to test the *possible* benefit of a yet unproven pharmacological agent. The patient's main reason for consulting was to seek an adequate diagnosis and treatment of her symptoms, not to be recruited for research purposes. The proposal to enter a trial of a drug that could eventually be of yet to be proven benefit, even if assigned to the active arm of the trial, probably did not meet the patient's expectations and conditioned her autonomy and independent decision-making to possible third-party interests. As beneficence was not assured, in this way the ethical principle of beneficence was also compromised. As the diagnosis was not disclosed personally in the proper circumstances of respect for the patient's expectations, with complete information on prognosis, CR, or support and prevention strategies, but untimely over the phone and during a leisure trip, it resulted in emotional distressing consequences, disrespecting the principle of non-maleficence.

In case 2, the diagnosis was made solely on the basis of a progressive cortical-type cognitive decline, without taking into consideration any further studies. The underlying diagnosis (hydrocephalus) was only confirmed after an MRI. The wife's adamant denial of the initial diagnosis revealed its emotional impact, as well as her later disregard of the patient's worsening, due to possible shunt malfunctioning, even though he seemed to have improved situationally and socially. Once again, improper communication attitudes during diagnosis disclosure would generate undesirable emotional fallouts.

In case 3, a diagnosis of FTD was made solely on the basis of ^{18}F-FDG findings, not upon any clinically ascertained clinical correlation. Regrettably, an emotionally and anxiety-charged term – dementia – was used, but fortunately, the patient remained compensated. As a result, an unwarranted diagnosis was provided, once again disrespecting the principles of beneficence and non-maleficence. One cannot but wonder what might have happened in another case, with a patient finding such an ominously sounding diagnosis upon reading her report.

The key element for a diagnosis of FTD is the pattern of behavioral changes the patient undergoes[24-26] and its reasonable correlation with the imaging findings. Those changes occur in general initially. The cognitive decline generally does not appear until in later stages. Therefore, follow-up is essential. Imaging studies may be used to confirm low marker uptake areas in the brain, or also the presence of amyloid or Tau, which in the case of FTD has less relevance than in AD.

The central message in disclosing a diagnosis of AD or FTD (or of other less frequent types of cortical dementia, such as primary progressive aphasia or corticobasal degeneration) is that the patient should be emotionally respected and not faced with a suddenly presented scenario of mental breakdown. Respect for patients' expectations is essential in disclosing diseases such as AD.

6. Conclusion

The disclosure of an AD diagnosis should be done following the recognized ethical principles of autonomy, beneficence, non-maleficence, and justice, in an empathy-driven approach respecting patients' beliefs and expectations, feelings and singularity, emphasizing CR and helping them adjust to the diagnosis. In the cases reported in this paper, these guidelines do not seem to have been followed.

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ACCSCIENCE PUBLISHING

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ORIGINAL RESEARCH ARTICLE

Specificity of multiple performance validity tests in a mixed neurological patient sample from Indonesia

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Abstract

Performance validity testing (PVT) is crucial in contemporary neuropsychological assessment. This study aimed to validate several PVTs— – the Reliable Digit Span (RDS), the Longest Digit Forward-1 Trial, the Longest Digit Forward-2 Trials (LDF-2), the *Tes Memori Jangka Pendek Indonesia* (TMJPI), and the Non-Verbal Medical Symptom Validity Test – in an Indonesian mixed neurological sample. We recruited 141 patients through convenience sampling, divided into three groups: Neurocognitive disorder due to possible neurodegenerative disease (ND; n = 49), post-stroke (n = 42), and mixed etiology (n = 47). Data were collected prospectively. The PVT cut-off scores were adjusted when specificity rates fell below 0.90. Intercorrelations between Mini-Mental State Examination (MMSE) scores, demographic variables, and PVT scores were computed. The cut-off scores were modified for each group due to unacceptable specificity rates, with the most substantial adjustment made for the TMJPI cut-off score in the ND group. Three variables (MMSE, education, and age) were significantly correlated with PVT scores, with the exception of RDS and LDF-2. The PVTs were also significantly intercorrelated. We conclude that the previously developed Indonesian PVTs can be validly applied in neurological patients. However, clinicians should exercise caution when selecting PVTs and consider the demographic backgrounds of patients to minimize false-positive results.

Keywords: Neuropsychological tests; Malingering; Cognitive dysfunction; Diagnosis; Motivation; Sensitivity and specificity

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1. Introduction

Performance validity testing (PVT) is essential in neuropsychological assessment, enabling clinicians to make accurate inferences about a patient's current cognitive abilities and functioning.[1-4] Initially referred to as "malingering," performance invalidity was primarily associated with medicolegal settings.[5] PVTs have also become important in general clinical settings,[6] where external incentives may play a role in neuropsychological assessments.[7] When external incentives are present, performance invalidity has been found to increase up to 40% compared to settings without such incentives.[8] Performance invalidity, however, is not solely attributable to external incentives. For instance, older individuals with dementia may lack insight into their cognitive deficits, making them unmotivated to undergo assessments, thereby increasing the risk of invalid test outcomes.[7] Therefore, administering PVTs is necessary for clinical assessments, regardless of the presence (or absence) of external incentives.[7,9,10]

Failure to establish performance validity can have serious consequences. First, patients exhibiting invalid performance may be classified as more cognitively impaired than their actual cognitive functioning reflects.[11] Second, and more importantly, falsely diagnosing patients with a neurodegenerative disease when they have no cognitive impairment could lead to adverse psychological outcomes.[7] Third, misclassifying genuine patients as individuals "feigning" their symptoms (i.e., false positives) can have both emotional and financial consequences.[1,12] In each of these cases, patients may fail to receive accurate diagnoses, necessary treatments, appropriate care, or essential recommendations (e.g., driving restrictions or the specific care or support they need).[5]

Consequently, incorporating PVTs into neuropsychological assessments is imperative for obtaining accurate results. In clinical practice, it is essential to avoid misclassifying patients with genuine cognitive impairments as having invalid performance. For this reason, the specificity of a PVT (i.e., its ability to classify valid performers as passing the PVT) should be high (≥0.90).[13,14] In addition, clinicians should use multiple PVTs to assess performance validity, as this practice enhances diagnostic accuracy by improving true-positive rates while minimizing the likelihood of false positives.[9,15]

Although PVTs are not designed to measure cognitive abilities, some patients with "actual" cognitive impairments may fail PVTs, particularly when standard, often conservative cut-off scores are applied.[16,17] The failure rates of PVTs in patients with mild to moderate cognitive impairments (e.g., traumatic brain injury [TBI], early-stage Parkinson's disease, or epilepsy) range from 0% to 20%, whereas failure rates in patients with severe cognitive impairments (e.g., dementia) may approach 100%.[16] These rates vary depending on the PVT used and the applied cut-off score.[16] Moreover, PVT cut-off scores derived from one clinical group may not only apply to other clinical groups.[7,13] Therefore, in clinical practice, it is essential to use PVT diagnostic accuracy data obtained from patients with clinical characteristics similar to the patient group under evaluation.[13,18] Thus, research on PVT failure rates in clinical populations and the refinement of cut-off scores' accuracy is essential for their validation.

Clinical neuropsychologists have suggested that PVT validation studies should include individuals with severe memory deficits (i.e., individuals with chance-level recognition memory performance), such as those with dementia due to Alzheimer's disease.[19] In these cases, prominent impairments in the memory domain are typically present.[20] In fact, even individuals with Alzheimer's dementia rarely score at or below 50% correct on PVTs based on a yes/no recognition paradigm.[21] Therefore, the PVT cut-off score obtained in individuals with dementia may represent the lowest possible false-positive rate. If patients with less severe cognitive impairment (e.g., mild TBI) score below this cut-off, this could be considered evidence of non-credible performance.[9,21]

In a previous study,[22] several PVTs, including the non-verbal medical symptom validity test (NV-MSVT), the *Tes Memori Jangka Pendek Indonesia* (TMJPI), the Reliable Digit Span (RDS), the Longest Digit Forward-1 Trial (LDF-1), and the Longest Digit Forward-2 Trials (LDF-2), were validated for use in Indonesia through a simulation study. However, this study involved only healthy participants, and therefore, the derived cut-off scores may not be applicable to individuals with clinical conditions.[23] The current study aims to evaluate the specificity of these PVTs in a mixed neurological sample in Indonesia, including individuals with neurocognitive disorder (ND) due to possible neurodegenerative disease, post-stroke (PS) patients, and patients with other brain disorders (e.g., epilepsy, TBI, or brain tumor). Specificity data on PVTs are essential for clinicians to interpret test results accurately. We hypothesize that the ND group will perform worse than the other two groups on all PVTs, as previous studies have shown higher failure rates in individuals with conditions similar to ND (e.g., dementia).[16] In addition, we hypothesize that the recommended cut-off scores from the previous study[22] will result in unacceptably high false-positive rates (i.e., poor specificity). Furthermore, we expect that the specificity and cut-off scores of the PVTs in the present study will differ from those in validation studies conducted in Western, high-income countries, as data derived from one culture may not automatically apply to others.[13,24,25] Finally, we anticipate a significant intercorrelation between PVT scores, as well as correlations between age, educational level, and overall cognitive function.

2. Materials and methods

2.1. Materials

2.1.1. NV-MSVT (Green, 2008)

The NV-MSVT is a computerized PVT that uses colored images as stimuli. Several scores are obtained from this test: immediate recognition (IR), delayed recognition (DR), consistency (CNS), DR variations (DRV), DR archetypes (DRA), paired associates (PA), and free recall (FR). More detailed information on these scores is provided in the test manual.[26]

The NV-MSVT manual[26] classifies "pass" or "fail" scores based on the examinee's performance on the "easy" subtests first, which are reflected in Criterion A (composed of Criteria A1 and A2). Criterion A1 is met if the mean of IR, DR, CNS, DRA, DRV, and PA is ≤90. Criterion A2 is met if the mean of DR, CNS, DRA, and DRV is ≤88.[26] In addition to these original cut-off scores, our previous simulation study[22] suggested lowering Criteria A1 and A2 to ≤87 and ≤81, respectively, to maintain a specificity of 0.90. If an examinee fails either Criterion A1 or Criterion A2, the patient's performance on Criterion B (composed of Criteria B1, B2, and B3) should be examined to determine whether the failure is due to invalid performance or severe cognitive impairment. This approach is also known as the "genuine memory impairment profile" approach.[26] Criterion B1 is met if PA - (DR + CNS + DRA + DRV)/4 is >−11. Criterion B2 is met if (IR + DR + CNS)/3 - (PA + FR)/2 is <20. Finally, Criterion B3 is met if the IR, DR, CNS, DRA, and DRV standard deviations are ≥12. Failure on at least two of these criteria indicates invalid performance.[26] We calculated Criteria A and B for all participants according to the formulas outlined above.

2.1.2. TMJPI

The TMJPI is a standalone, paper-and-pencil PVT constructed based on the Amsterdam Short-Term Memory (ASTM) test.[27,28] The TMJPI test booklet consists of two training items and 30 test items. Each item consists of three pages. On the first page, the examinee is presented with five stimulus words from a common semantic category, such as seafood (e.g., *ikan* [fish], *cumi* [squid], *kerang* [oyster], *kepiting* [crab], *udang* [shrimp]). The examinee is asked to read these words aloud and memorize them. On the next page, a distractor is shown in the form of a simple addition or subtraction task (e.g., 5 + 2 = ?), which must be solved. On the third page, the five words from the same semantic category as the ones on the first page (e.g., *udang* [prawn], *terasi* [prawn paste], *ikan* [fish], *kaviar* [caviar], *cumi* [squid]) are displayed. The examinee is then asked to identify and name three words that appeared on the first page (i.e., *udang* [shrimp], *ikan* [fish], and *cumi* [squid]). The test administrator provides feedback by stating the number of correct responses. All stimuli are presented in *Bahasa Indonesia*. Based on the original ASTM manual,[28] the cut-off for determining invalid performance is a score below 85. However, in a previous simulation study,[22] we suggested increasing the cut-off score to below 89.

2.1.3. RDS, LDF-1, and LDF-2

The RDS, LDF-1, and LDF-2 are embedded validity measures derived from the Digit Span (DS). This study used the DS from the Wechsler Adult Intelligence Scale-Fourth Edition-Indonesian version.[29] Although the RDS was initially developed for the WAIS-R, its diagnostic ability has been established and found comparable to later versions of the WAIS.[30] The RDS is the most commonly used embedded PVT in neuropsychological assessment.[31] The RDS score is calculated by summing the raw scores for the longest forward and backward DSs with no errors in both trials.[32] The LDF-1 is the longest span in the forward condition in which at least one trial of a given pair is repeated correctly, and the LDF-2 is the longest span in the forward condition in which both trials are repeated correctly.[33] The original cut-off scores for the RDS, LDF-1, and LDF-2 were ≤6, ≤4, and ≤3, respectively.[33,34] In a previous simulation study,[22] an increased cut-off score for the RDS of ≤7 was recommended, while the cut-off scores for LDF-1 and LDF-2 remained unchanged.

2.2. Participants

Our study was prospective in design. Eligible participants were adults (≥18 years) with neurological disorders. A total of 141 participants were recruited for this study, and they were divided into three groups: those with a neurocognitive disorder due to possible neurodegenerative disease (ND) (*n* = 50), patients with a history of stroke (PS; *n* = 42), and a mixed-etiology group (*n* = 47). The mixed-etiology group consisted of individuals diagnosed with epilepsy (*n* = 20), TBI (*n* = 7), brain tumor (*n* = 6), Parkinson's disease (*n* = 5), multiple sclerosis (MS; *n* = 4), cerebral small vessel disease (CSVD; *n* = 2), neuropathy (*n* = 2), and chronic migraine (*n* = 1). Participants with Parkinson's disease were included in the mixed-etiology group, as they constituted a small subgroup, and their cognitive status was likely better than that of the ND patients (e.g., Parkinson's disease participants were able to provide consent, while ND participants were not). Group classifications were based on the primary diagnosis made by the neurologist, considering comorbidity where applicable. Detailed diagnostic and more demographic characteristics of the participants are provided in Table 1. Only participants with no apparent external incentives (e.g., patients not involved in medicolegal proceedings) and without a history of psychiatric disorders were included in the study.

Data collection took place from 2022 to early 2023, with participants recruited through convenience sampling. Participants in the ND group were recruited from Alzheimer's Indonesia (a non-profit organization focused on dementia care and support),[35] several nursing homes in the Greater Jakarta area, and a halfway house for elderly transgender people in Jakarta. In addition, a small number of participants were recruited from Dr Cipto Mangunkusumo Hospital. We had not anticipated recruiting only two ND participants from the hospitals. Participants in the PS and mixed-etiology groups were outpatients from the Neurology Department of Dr. Cipto Mangunkusumo Hospital and Atma Jaya Hospital in Jakarta.

2.3. Procedure

This study was conducted in accordance with the Helsinki Declaration. Ethical approval was granted by the Research Ethics Committee of Atma Jaya Catholic University of Indonesia (No: 0032L/III/LPPM-PM.10.05/12/2021), the Ethics Committee of the Faculty of Medicine, University of Indonesia—Cipto Mangunkusumo Hospital (No: KET387a/UN2.F1/ETIK/PPM.00.02/2022), and Atma Jaya Hospital (No: 133/DIR-e/II/2022). Informed consent was obtained from the caregivers of participants with ND (i.e., family members or nursing home staff), while those in the PS and mixed-etiology groups provided their own consent.

Data were collected by trained psychologists and psychology graduates, who administered the PVTs individually and face-to-face to all participants. Before administering the PVTs, participants with ND, recruited from Alzheimer's Indonesia, nursing homes, and the halfway house, were screened using the Indonesian version of the Mini-Mental State Examination (MMSE).[36] Only candidates with MMSE scores of 26 or below were recruited. The MMSE score for the two ND participants recruited from the Dr. Cipto Mangunkusumo Hospital was obtained from the hospital. In addition to the ND group, the MMSE was administered to participants in the PS group before the PVTs. The MMSE was administered to the ND and PS groups only. The MMSE was administered first (to participants in the ND and PS groups), followed by the DS, TMJPI, and NV-MSVT. All tests were administered in a single session for each participant, with a break provided upon request. Participants were encouraged to complete the tests to the best of their ability. Participants received research compensation for their participation (e.g., food or money, depending on the institutions' requests). After data collection, the test administrators scored the PVTs for each participant.

2.4. Statistical analysis

The sample size was calculated using an online Sample Size Calculator (http://wnarifin.github.io), with an expected specificity of 0.90, a confidence level (1-α) of 0.95, and a precision of 0.10. The prevalence of poor symptom validity was estimated at 0.16, based on a previous meta-analysis[14] on the prevalence of PVT failure rates in clinical groups. Participants who dropped out were expected to be replaced (with no anticipated dropout). This calculation resulted in a minimum required sample size of 42 participants

Table 1. Demographic characteristics and MMSE scores of participants

Parameter	ND (*n*=49)	PS (*n*=42)	Mixed-etiology (*n*=47)	*P*-value
Mean age (SD)	69.4 (9.1)	57.0 (12.0)	48.2 (15.5)	<0.001[a]
Education (%)				0.26[b]
No/minimal education	8 (16.3)	3 (7.1)	2 (4.3)	
Elementary (6 years)	10 (20.4)	7 (16.7)	6 (12.8)	
Secondary (9 – 12 years)	24 (49.0)	27 (64.3)	25 (53.2)	
Tertiary (more than 12 years)	7 (14.3)	5 (11.9)	14 (29.8)	
Gender (%)				0.26[b]
Male	22 (44.9)	16 (38.1)	26 (55.3)	
Female	27 (55.1)	26 (61.9)	21 (44.7)	
MMSE mean score (SD)	21.7 (3.2)	27.0 (2.6)	-	<0.001[c]

Notes: [a]The statistic was analyzed using ANOVA; [b]The statistic was analyzed using the Chi-square test, [c]The statistic was analyzed using the Mann–Whitney *U*-test.
Abbreviations: MMSE: Mini-mental state examination; ND: Neurocognitive disorder due to possible neurodegenerative disease group; PS: Post-stroke group.

per subgroup for determining specificity.[37] There were no missing data in this study. Demographic variables (age, education, and gender) were compared between groups. MMSE scores in the ND and PS groups were calculated and compared. The mean PVT scores for each group were compared using the Kruskal–Wallis test due to the non-normal distribution of all PVTs across the groups (Shapiro–Wilk $P < 0.05$),[38] followed by *post hoc* Mann–Whitney U-tests with Bonferroni correction and their corresponding effect sizes (*r*).[39,40] In addition, participants with ND were divided into mild (MMSE score ≥22) and major (MMSE score <22) ND groups,[41] as distinct PVT cut-off scores might apply to these groups. Diagnostic accuracy was evaluated in each group by examining the specificity of the recommended cut-off scores from a previous simulation study.[22] Participants who performed below the specific cut-off score were considered false positives. If the obtained specificity was below the acceptable threshold (<0.90), the cut-off scores were adjusted (i.e., lowered) to ensure a specificity rate of at least 0.90, as higher specificity is essential for PVTs.[14,42,43] For the NV-MSVT, the specificity rate was calculated by dividing the number of participants failing at least one of the A criteria and at least two of the B criteria (indicating probable invalid performance) by the total number of participants in each group, as suggested by the NV-MSVT manual.[26]

The adjusted cut-off scores for each PVT were used to calculate the number of participants failing at least two PVTs. Failure on the embedded validities of DS (e.g., failure on RDS and LDF-2) was considered a single PVT failure, as they are derived from the same test,[13] as reflected by their high intercorrelation in this study. The demographic characteristics of the participants failing at least two PVTs were then presented.

Intercorrelation analyses were performed between PVT scores, demographic variables (i.e., gender, age, and education), and MMSE scores (for participants with ND and PS groups only). The point-biserial correlation was used for the gender variable, while the other variables were analyzed using the Spearman correlation due to their non-normal distribution.[44] The effect size was determined using *r*, with $r = 0.10$ indicating a small effect, $r = 0.30$ indicating a medium effect, and $r = 0.50$ indicating a large effect.[39] The data are available through the Donders repository. Analyses were performed using JASP 0.16.3.[45]

3. Results

As shown in Table 1, significant differences were observed between the clinical groups in terms of age. As expected, the ND group was the oldest and had a significantly lower MMSE score than the PS group. No significant differences were found between the clinical groups regarding educational level and gender distribution, although the ND group had a slightly lower educational level compared to the other two groups. The clinical groups also differed significantly on all PVT scores, as shown in Table 2. The ND group performed significantly worse than the other clinical groups on all PVTs, except for the RDS, with medium-to-large effects (*r* range = 0.29 – 0.55; Table 3). No significant differences were found between the PVT scores of the PS and mixed-etiology groups. In addition, the mild ND group scored significantly higher than the major ND group on all PVTs, with medium to medium-to-large effects (*r* range = 0.38 – 0.50; Table 4).

The results presented in Table 5 indicate that the cut-off scores from the previous simulation study resulted in unacceptably low specificity rates (<0.90) for all PVTs. Therefore, we adjusted the cut-off scores for use in clinical samples. For the RDS, LDF-1, and LDF-2, the PS and mixed-etiology groups achieved specificity rates of at least 0.90 using the adjusted cut-off scores of ≤4, ≤3, and ≤2, respectively. A more significant adjustment of the cut-off scores in the RDS, LDF-1, and LDF-2 was required for the ND group, with cut-off scores decreased to ≤3, ≤2, and ≤1, respectively. However, similar to the PS and mixed-etiology groups, the adjusted cut-off score for the LDF-2 in the mild ND group was ≤2. For the TMJPI, the mixed-etiology group required the least adjustment to

Table 2. Comparison of performance validity test scores between clinical groups

Statistical parameter	RDS			LDF-1			LDF-2			TMJPI			NV-MSVT					
													Criterion A1			Criterion A2		
	ND	PS	Mix	ND	PS	Mix	ND	PS	Mix	ND	PS	Mix	ND	PS	Mix	ND	PS	Mix
Mean	5.98	6.76	6.81	4.18	4.71	4.92	3.37	4.00	4.04	81.47	86.74	87.23	79.83	91.24	91.34	81.51	90.12	88.98
SD	1.82	1.48	1.78	1.22	0.74	1.12	1.22	0.94	1.00	9.63	5.41	6.03	16.08	9.95	10.89	13.88	11.27	14.94
H	7.97*			10.89*			9.30*			25.65***			21.35***			15.59***		

Notes: *P<0.05; ***P<0.001.

Abbreviations: H: Kruskal-Wallis index; LDF-1: Longest digit forward-1 trial; LDF-2: Longest digit forward-2 trials; Mix: Mixed-etiology group; ND: Neurocognitive disorder due to possible neurodegenerative disease group; NV-MSVT: Non-verbal medical symptom validity test; PS: Post-stroke group; RDS: Reliable digit span; SD: Standard deviation; TMJPI: *Tes Memori Jangka Pendek Indonesia*.

Table 3. *Post hoc* analyses of performance validity test scores between the neurocognitive disorder, post-stroke, and mixed-etiology groups

Test and group	*U* (*r*)
RDS	
ND versus PS	711.00* (0.31)
ND versus mixed-etiology group	853.50 (0.26)
PS versus mixed-etiology group	1029.00 (0.04)
LDF-1	
ND versus PS	725.00* (0.30)
ND versus mixed-etiology group	764.00* (0.34)
PS versus mixed-etiology group	901.00 (0.09)
LDF-2	
ND versus PS	714.00* (0.31)
ND versus mixed-etiology group	815.50* (0.29)
PS versus mixed-etiology group	991.00 (0.00)
TMJPI	
ND versus PS	552.50* (0.46)
ND versus mixed-etiology group	519.50* (0.55)
PS versus mixed-etiology group	910.00 (0.08)
Criterion A1 NV-MSVT	
ND versus PS	559.00* (0.46)
ND versus mixed-etiology group	590.50* (0.49)
PS versus mixed-etiology group	917.00 (0.07)
Criterion A2 NV-MSVT	
ND versus PS	604.50* (0.41)
ND versus mixed-etiology group	692.00* (0.40)
PS versus mixed-etiology group	930.50 (0.06)

Note: **P*<0.02 (after Bonferroni correction).
Abbreviations: LDF-1: Longest digit forward-1 trial; LDF-2: Longest digit forward-2 trials; ND: Neurocognitive disorder; NV-MSVT: Non-verbal medical symptom validity test; PS: Post-stroke; RDS: Reliable digit span; SD: Standard deviation; TMJPI: *Tes Memori Jangka Pendek Indonesia*.

the cut-off score (<84). Unexpectedly, the mild ND group required less adjustment to the cut-off score than the PS group (<79 vs. <78). To achieve a minimum specificity rate of 0.90, it was necessary to adjust the TMJPI cut-off score to <62 for both the major ND and ND groups. For the NV-MSVT, slightly lowering Criterion A1 (from ≤87 to ≤82) increased specificity for all clinical groups. However, when the B criteria from the NV-MSVT manual were applied, none of the clinical groups achieved a specificity rate of 0.90. In this case, the major ND group required only minor adjustments (i.e., changing Criterion B3 from ≥12 to ≥13), whereas the mixed-etiology group required the largest adjustment: decreasing B2 (from <20 to <12) and increasing B3 (from ≥12 to ≥32).

As shown in Table 6, only two participants from the ND group, one from the PS group, and one from the mixed-etiology group failed at least two PVTs using the new cut-off scores. This finding resulted in omnibus specificity rates of 0.96 in the ND group, 0.98 in the PS group, and 0.98 in the mixed-etiology group. Correlation analyses (Table 7) revealed that all PVT scores were significantly correlated. Except for the intercorrelation in the embedded validity measures of the DS and Criterion A in the NV-MSVT, the correlations ranged from 0.31 to 0.53, indicating medium-to-large effects. Furthermore, gender was the only demographic variable not significantly correlated with the PVT scores. Educational level (*r* range = 0.19 – 0.33) and age (−0.16 – −0.43) were significantly correlated with most PVT scores, with small-to-medium and medium-to-large effects. In contrast to the demographic variables, correlations with MMSE scores were medium to large (*r* range = 0.43 – 0.63).

4. Discussion

The aim of this study was to validate several PVTs in an Indonesian neurological sample. We compared a mixture of standalone and embedded PVT scores across groups

Table 4. Comparison of performance validity test scores between the mild and major neurocognitive disorder groups

Statistical parameter	RDS		LDF-1		LDF-2		TMJPI			NV-MSVT		
							Criterion A1			Criterion A2		
	Mild (*n*=28)	Major (*n*=21)	Mild (*n*=28)	Major (*n*=21)	Mild (*n*=28)	Major (*n*=21)	Mild (*n*=28)	Major (*n*=21)	Mild (*n*=28)	Major (*n*=21)	Mild (*n*=28)	Major (*n*=21)
Mean	6.59	5.14	4.59	3.62	3.69	2.91	85.14	76.38	85.59	72.10	85.03	76.62
SD	1.72	1.59	1.15	1.07	1.11	1.22	6.12	11.11	12.58	17.00	12.36	14.33
P-value	0.004		0.005		0.016		0.003		0.004		0.025	
r	0.47		0.45		0.38		0.50		0.49		0.38	

Notes: "Major" refers to major neurocognitive disorder (MMSE mean score=18.57 [SD=1.96], range=14–21); "Mild" refers to mild neurocognitive disorder (MMSE mean score=24.07 [SD=1.27], range=22–26).
Abbreviations: LDF-1: Longest digit forward-1 trial; LDF-2: Longest digit forward-2 Trials; MMSE: Mini-mental state examination; NV-MSVT: Non-verbal medical symptom validity test; RDS: Reliable digit span; SD: Standard deviation; TMJPI: *Tes Memori Jangka Pendek Indonesia*.

Table 5. Performance validity test specificity rates and cut-off scores

PVT	Specificity rate				
	ND (*n*=49)	ND		PS (*n*=42)	Mixed etiology (*n*=47)
		Mild (*n*=28)	Major (*n*=21)		
RDS					
≤7[a]	0.14	0.25	0.00	0.33	0.28
≤6[b]	0.35	0.50	0.14	0.57	0.56
≤4	0.84	0.89	0.76	0.93[f]	0.96[f]
≤3	0.94[f]	0.96[f]	0.90[f]	0.98	0.98
LDF-1					
≤4[a, c]	0.33	0.50	0.10	0.60	0.60
≤3	0.80	0.86	0.71	0.98[f]	0.96[f]
≤2	0.94[f]	0.96[f]	0.90[f]	1.00	0.98
LDF-2					
≤3[a, c]	0.53	0.64	0.38	0.76	0.70
≤2	0.84	0.93[f]	0.71	0.93[f]	0.96[f]
≤1	0.94[f]	0.96	0.90[f]	1.00	1.00
TMJPI					
<89[a]	0.24	0.36	0.10	0.57	0.64
<84	0.59	0.79	0.33	0.83	0.91[f]
<79	0.76	0.93[f]	0.52	0.88	0.94
<78	0.80	0.96	0.57	0.95	0.96
<62	0.94[f]	0.96	0.90[f]	1.00	0.98
NV-MSVT A Criteria					
A1: ≤87; A2: ≤80[a]	0.39	0.54	0.19	0.79	0.81
A1: ≤82; A2: ≤80	0.49[f]	0.61[f]	0.33[f]	0.83[f]	0.83[f]
NV-MSVT[d]					
B1: >−11; B2: <20; B3≥12[e]	0.86	0.86	0.86	0.88	0.85
B1: >−11; B2: <20; B3≥13	0.88	0.86	0.90[f]	0.88	0.87
B1: >−11; B2: <20; B3≥20	0.92[f]	0.89	0.95	0.90[f]	0.87
B1: >−11; B2: <20; B3≥22	0.94	0.93[f]	0.95	0.90	0.87
B1: >−11; B2: <12; B3≥32	0.94	0.93	0.95	0.93	0.91[f]

Notes: [a]Based on the simulation study by Adhiatma *et al.*[22] [b]Cut-off score from the original RDS.[32] [c]Cut-off score from the original development of LDF-1 and LDF-2.[33] [d]Specificity rate for the NV-MSVT as a single test, calculated by combining A and B criteria. Criterion A1=(IR+DR+CNS+DRA+DRV+PA)/6; Criterion A2=(DR+CNS+DRA+DRV)/4; Criterion B1=PA −(DR+CNS+DRA+DRV)/4; Criterion B2=(IR+DR+CNS)/3−(PA+FR)/2; B3=*SD* (IR, DR, CNS, DRA, DRV). [e]Based on the NV-MSVT manual.[26] [f]The selected specificity rate for each PVT in each clinical group.

Abbreviations: CNS: Consistency; DR: Delayed recognition; DRA: Delayed recognition archetypes; DRV: Delayed recognition variations; FR: Free recall; IR: Immediate recognition; LDF-1: Longest digit forward-1 trial; LDF-2: Longest digit forward-2 trials; ND: Neurocognitive disorder due to possible neurodegenerative disease group; NV-MSVT: Non-verbal medical symptom validity test; PA: Paired associates; PS: Post-stroke group; PVT: Performance validity test; RDS: Reliable digit span; TMJPI: *Tes Memori Jangka Pendek Indonesia*.

of patients with ND (n = 49), PS (n = 42), and a mixed-etiology group (n = 49) and then examined the specificity rates based on a previous simulation study.[22] Cut-off scores were adjusted for each clinical group until the specificity reached at least 0.90. Using the new cut-off scores, we then explored how many participants failed at least two PVTs. In addition, we analyzed the intercorrelation between PVT scores, as well as the correlations between the PVT scores, demographic variables, and MMSE scores.

Descriptive results indicated that all groups had lower PVT scores than the healthy participants in the previous study.[22] This finding is in line with other studies showing that

Table 6. Participants who failed at least two performance validity tests

Participant ID	Demographic information	MMSE score	Comorbid	PVT failure
ND				
32	Male, 79 years, elementary education	25	-	LDF-1, NV-MSVT
87	Male, 68 years, secondary education	26	Diabetes and high cholesterol	TMJPI, NV-MSVT
PS				
106	Male, 33 years, elementary education	26	-	TMJPI, NV-MSVT
Mixed-etiology				
130[a]	Female, 55 years, tertiary education	-	Post-stroke	RDS, TMJPI

Note: [a]The participant's primary diagnosis was brain tumor-related epilepsy (focal onset with impaired awareness).
Abbreviations: LDF-1: Longest digit forward-1 trial; MMSE: Mini-mental state examination; ND: Neurocognitive disorder group due to possible neurodegenerative disease; NV-MSVT: Non-verbal medical symptom validity test; PS: Post-stroke group; PVT: Performance validity test; RDS: Reliable digit span; TMJPI: *Tes Memori Jangka Pendek Indonesia*.

Table 7. Intercorrelation coefficients between performance validity tests and correlation coefficients between demographic variables, MMSE scores, and performance validity test scores

Parameter	RDS	LDF-1	LDF-2	TMJPI	NV-MSVT	
					Criterion A1	Criterion A2
Performance validity test						
RDS	-	-	-	-	-	-
LDF-1	0.61*	-	-	-	-	-
LDF-2	0.81*	0.67*	-	-	-	-
TMJPI	0.35*	0.47*	0.30*	-	-	-
Criterion A1 NV-MSVT	0.34*	0.38*	0.33*	0.52*	-	-
Criterion A2 NV-MSVT	0.31*	0.32*	0.29*	0.46*	0.97*	-
Demographic variables						
Gender	−0.03	0.09	0.07	0.03	−0.10	−0.08
Age	−0.14	−0.31*	−0.14	−0.34*	−0.42*	−0.36*
Education	0.27*	0.23*	0.21*	0.29*	0.21*	0.20*
MMSE[a]	0.43*	0.44*	0.42*	0.63*	0.56*	0.49*

Note: *$P<0.05$. [a]The correlational analyses for the MMSE were performed only for the neurocognitive disorder and post-stroke groups.
Abbreviations: LDF-1: Longest digit forward-1 trial; LDF-2: Longest digit forward-2 trials; MMSE: Mini-mental state examination; NV-MSVT: Non-verbal medical symptom validity test; RDS: Reliable digit span; TMJPI: *Tes memori jangka pendek Indonesia*.

patient groups generally perform worse on PVTs compared to healthy volunteers.[46] Specifically, compared to healthy participants,[22] the ND group scored disproportionately lower on the embedded PVTs than on the standalone PVTs. This may be due to the a posteriori approach used in embedded PVTs, which makes them more susceptible to failure in cases of severe cognitive dysfunction.[47] Some studies have shown that the diagnostic accuracy of standalone PVTs tends to be better than that of embedded PVTs.[17,48,49] Consistent with our hypotheses, the ND group performed significantly worse than the PS and mixed-etiology groups. In addition, the mild ND group scored higher than the major ND group, indicating that more severe cognitive dysfunction negatively affects PVT scores, particularly for embedded PVTs.[1,12,50-53]

Examination of the specificity rate for the RDS showed that the cut-off scores (≤7) from the previous simulation study[22] could not be applied to our clinical sample. This recommended RDS cut-off score was even higher than the traditional RDS cut-off score (≤6).[32] Our RDS cut-off score for the PS and mixed-etiology groups (≤4) was lower than those used in other studies with mixed neurological samples, which have suggested ≤5.[18,54,55] Further adjustment was needed for the RDS in our mild and major ND groups (i.e., ≤3). In some studies, the traditional cut-off score for the RDS has resulted in a specificity rate below 0.90 in people with dementia.[9,50-52] Consistent with our results, other studies have also suggested lowering the RDS cut-off score to ≤3 to achieve a specificity rate above 0.90.[21]

Our results for the LDF-1 and LDF-2 also indicated the need to lower the cut-off scores derived from the previous simulation study.[22] The ND group required lower adjusted cut-off scores than the other two groups. Again, our cut-off scores were lower than those used in previous studies (≤4 for LDF-1 and ≤3 for LDF-2), which found adequate specificity (≥0.90) in mixed psychiatric and neurological samples[33] and a slight specificity decrease in a probable Alzheimer's disease sample (0.83 for LDF-1 and 0.87 for LDF-2).[51] We argue that the need to lower the cut-off scores for the embedded validity measures of the DS may be due to the lower educational attainment of our samples, which we discuss in more detail later.

We hypothesized that the LDF-2 would be more appropriate as an embedded PVT than the RDS for participants with ND. First, the original cut-off score on the LDF-2 resulted in a higher specificity than that of the RDS (0.53 vs. 0.35). Second, the LDF-2 required only minor adjustments when applied to the mild ND group (from ≤3 to ≤2). A similar finding has been documented previously, where the LDF-2 also performed better than the RDS and maintained adequate specificity in participants with moderate Alzheimer's disease.[51] One possible explanation is that the RDS includes a backward trial that measures working memory, which may be more sensitive to severe memory impairment.[33,56] However, further research is needed to support this hypothesis.

The TMJPI cut-off score derived from the previous simulation study (<89) resulted in extremely low specificity rates across all clinical groups. This was not surprising, as the original ASTM on which the TMJPI is based suggests a cut-off score of <85 or <86.[27] The cut-off score obtained from the mixed-etiology group (i.e., <84) was comparable to those reported in studies involving MS patients, where cut-off scores around <85 or <83 were observed.[11,57] Furthermore, we did not expect that the PS group (<78) would require a slightly lower cut-off score than the mild ND group (<79), as the mean MMSE score suggested that the mild ND group was more cognitively impaired than the PS group. However, seven PS participants (16.7%) scored between 18 and 27 on the MMSE and below 83 in TMJPI, indicating that some patients with PS may have had mild-to-moderate cognitive impairment. Given the high language demands of the TMJPI,[23] we hypothesize that these participants with PS may have had residual language deficits. Unfortunately, we lack more detailed diagnostic information on other cognitive domains. Therefore, this hypothesis deserves further investigation.

The TMJPI resulted in very low specificity for the major ND group, which ultimately affected the specificity of the ND group as a whole. This finding raises concerns about the applicability of the TMJPI for individuals with severe cognitive impairment. A previous study reported a specificity rate of 0.10 on the ASTM for participants with Alzheimer's disease using a cut-off score of <84 or <85.[50] In a more recent study, 68% of patients with severe memory impairment due to Korsakoff's syndrome performed below the already lowered cut-off score of 82 on the ASTM.[58] Other studies have reported a significant correlation between the ASTM and working memory (as measured by the digit backward trial), which is severely impaired in people with ND or dementia.[50] Research has also shown that working memory significantly predicts reading comprehension in older adults.[59] Impairment in working memory among the major ND participants might hinder their ability to comprehend the TMJPI stimuli. However, the suggested cut-off score of <62 for the major ND and ND groups in our study is likely not practical for detecting invalid performance, as this cut-off score is very low.

For the NV-MSVT, previous studies have found that the original cut-off score for the NV-MSVT suggests perfect specificity in people with dementia while achieving a specificity of 0.98 in neurological participants without dementia.[19,46] These results were not replicated in our study sample. To improve specificity, we first adjusted Criterion A1, followed by adjustments to the B criteria, with different modifications applied to each clinical group. Criterion A1 required adjustment, but Criterion A2 did not, as Criterion A1 includes the PA subtest, which is considered more difficult for clinical patients.[26] Surprisingly, only a minor adjustment was needed for the B criteria in the major ND group (adjusting Criterion B3 from ≥12 to ≥13), while most adjustments were required in the mixed-etiology group. This finding supports Green's concept of the "genuine memory impairment profile,"[26] which is specific to individuals with severe cognitive impairment. We suspect that the need for more adjustments to the B criteria in the mixed-etiology group was due to the heterogeneity of the clinical diagnoses in this group.

In general, except for the TMJPI in the major ND and total ND groups, the PVTs examined in our study can be validly applied within our clinical groups, provided that the cut-off scores are adjusted. However, our cut-off scores were lower than those reported in previous studies from the United States, the United Kingdom, the Netherlands, and Germany. In addition to the severity of cognitive impairment, we suspect that these differences may be due to educational and/or cross-cultural factors. First, most previous studies included clinical participants with an average of 12 years of education,[9,11,12,18,21,46,51,52,57,60] which is equivalent to secondary education or higher. In contrast, most of our participants had secondary education or less.

In fact, nearly half of the ND group had completed only the lower level of secondary school (9 years of education) or less, which is common among older adults in Indonesia. Second, the results of our correlational analyses suggest that lower educational levels are associated with lower PVT scores. Despite this demographic difference, our study demonstrates that the specificity of the PVTs for use in Indonesia (a developing lower-middle-income country) and in clinical samples with low to moderate levels of education is adequate.

Only two participants with ND (Participants 32 and 87) failed at least two PVTs, even though their MMSE scores were not very low (25 and 26), and no participant with major ND failed at least two PVTs. One participant from the PS group failed the TMJPI and the NV-MSVT. This participant complained that the tests were "long and monotonous." We also observed that this patient appeared tired during these two tests, which were administered at the end of the session. Based on these observations, we argue that this participant should be classified as a true positive. Finally, one participant from the mixed-etiology group failed both the RDS and the TMJPI. This participant's primary diagnosis was brain tumor-related epilepsy (focal onset with impaired awareness), with PS as a comorbidity. This woman was cooperative and enthusiastic and seemed to exert her best effort in completing the tests. We suspect that her failure on the PVTs was due to her severe clinical condition, which likely reflects a false-positive outcome.

The intercorrelation between the RDS, LDF-1, and LDF-2 was high, which is not surprising, given that these tests were derived from a single test, the DS.[51] Similarly, the A1 and A2 criteria of the NV-MSVT were highly correlated, as the calculation of both criteria incorporates DR, CNS, DRA, and DRV. Furthermore, the other intercorrelations between PVTs were modest, indicating no redundancy between tests.[61] The modest intercorrelation between PVTs found in our study thus supports their convergent validity.

The MMSE scores in our study were most strongly associated with PVT scores compared to the demographic variables. This finding supports the evidence that more severe cognitive impairment is associated with lower PVT scores.[21,52] It is also important to note that in our sample, age and level of education were modestly associated with PVT scores. These findings suggest that clinicians should exercise caution when administering PVTs to older patients with severe cognitive impairment (e.g., ND or dementia) and low levels of education, as they are more likely to be classified as false positives.[14] Therefore, low PVT scores from individuals with this demographic and clinical background should not be immediately interpreted as invalid performance.

Our study has several limitations. Most of the participants in our ND group had not received a formal diagnosis based on a comprehensive diagnostic workup by a neurologist. Our sample included participants from nursing homes and a halfway house for elderly transgender people. Furthermore, in Indonesia, many older people with severe cognitive impairment do not consult medical professionals, resulting in a lack of formal diagnoses for those with probable dementia or ND.[62] Despite this, we consider the participants in our ND group to be cognitively impaired, based on their low MMSE scores and the everyday cognitive problems observed by nursing home staff and the head of the halfway house. In addition, the distinction between mild and major ND was based on MMSE scores (i.e., ≤26 and ≤21, respectively). Although the MMSE has faced criticism from a psychometric perspective,[63] it is extensively used for cognitive screening in Indonesia. Future research in neurological samples using PVTs should incorporate more comprehensive neuropsychological assessments. Another limitation of our study is the lack of information on dementia subtypes, which is likely to explain variation in PVT scores.[64]

The mixed-etiology group in this study consisted of participants with heterogeneous diagnoses, some of whom had comorbid disorders. Given that each neurological disorder or disease results in different cognitive symptoms,[18,55,65] this could ultimately affect the PVT cut-off scores as well.[13,55] Future should replicate our findings in prospective research using Indonesian samples with formal diagnoses (e.g., based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision criteria). Finally, this study examined the specificity of multiple PVTs only in a mixed neurological sample of participants who were assumed to be making their best effort. While lowering the PVT cut-off scores compared to those from our previous study improved the specificity, it inevitably impacted the sensitivity of the tests for detecting invalid performance.[51] Therefore, it is crucial to investigate the sensitivity of our adjusted cut-off scores in future research.

5. Conclusion

This study validated several PVTs, specifically their specificity rates, within an Indonesian mixed neurological sample. The cut-off scores from the previous simulation study[22] resulted in unacceptably low specificity rates in our clinical sample. As a result, the cut-off scores were adjusted. In general, more adjustments were required for the groups with the most severe cognitive impairment (i.e., the ND group). For individuals with severe cognitive impairment, using the LDF-1 or LDF-2 as embedded validity measures of the DS was preferred over the RDS, as the latter required a lower adjusted cut-off score. Moreover, the RDS includes the backward condition performance, which is sensitive to severe cognitive impairment. When clinicians encounter individuals with ND without prior specification of severity, the cut-off score for ND should be used instead of those for mild or major ND. In addition, the TMJPI is not recommended for individuals with severe cognitive impairment, as this test may result in an excessive probability of false positives, in line with previous findings.[50,58] Clinicians should also be cautious of invalid performance in older individuals with severe cognitive impairment who also have low levels of education. We recommend that clinicians use at least two PVTs in neuropsychological assessments to improve the accuracy of detecting performance validity.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

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Ethics approval and consent to participate

This study was conducted in accordance with the Helsinki Declaration, and ethical approval was granted by the Research Ethics Committee of Atma Jaya Catholic University of Indonesia (No: 0032L/III/LPPM-PM.10.05/12/2021), the Ethics Committee of the Faculty of Medicine, University of Indonesia—Cipto Mangunkusumo Hospital (No: KET387a/UN2.F1/ETIK/PPM.00.02/2022), and Atma Jaya Hospital (No: 133/DIR-e/II/2022). The last two institutional review boards were required for data collection at Cipto Mangunkusumo Hospital and Atma Jaya Hospital. Written consent was obtained from each participant, except for those with ND, for whom consent was provided by their caregivers (i.e., family members or nursing home staff).

Consent for publication

Patients consented on the publication of their data.

Availability of data

The data presented in this study are openly available in the Donders Repository at https://doi.org/10.34973/pyhj-0y27.

Further disclosure

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ACCSCIENCE PUBLISHING

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ORIGINAL RESEARCH ARTICLE

Postural orthostatic tachycardia syndrome in patients with tic disorders and functional tic-like behaviors

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Abstract

Postural orthostatic tachycardia syndrome (POTS) is an autonomic condition characterized by sustained excessive postural tachycardia, often accompanied by a range of other symptoms of orthostatic intolerance, including heart palpitations, light-headedness, weakness, tremulousness, blurred vision, nausea, headache, dizziness and syncope. POTS predominantly affects young females. Little is known about the exact pathophysiology of this condition; however, preliminary reports about comorbidity profile of POTS suggest a possible link with functional disorders. The striking increase in functional tic-like behaviors (a specific phenotype of functional movement disorder) during the COVID-19 pandemic offered a privileged opportunity to further explore the possible association between POTS and functional neurological disorder. In the present study, we retrospectively assessed the prevalence of POTS in two large patient populations from a specialist clinic for tic disorders: (1) patients with neurodevelopmental tics who received a diagnosis of Gilles de la Tourette syndrome (GTS), and (2) patients who developed tic-like behaviors and received a diagnosis of functional tics since the onset of the COVID-19 pandemic (April 2020 – December 2024). The prevalence of POTS was 0/638 in the GTS group and 4/177 (2.3%) in the functional tics group. The four patients with POTS were females, with age at assessment ranging from 17 to 24 years. In addition to describing their clinical characteristics, we discuss the significance of the selective association between POTS and functional tic-like behaviors, in the absence of comorbidity between POTS and neurodevelopmental tics.

Keywords: Postural orthostatic tachycardia syndrome; Tic disorders; Gilles de la Tourette syndrome; Functional tic-like behaviors

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1. Introduction

1.1. Postural orthostatic tachycardia syndrome (POTS)

POTS is a disorder of the autonomic nervous system characterized by sustained excessive postural tachycardia (an increase of ≥30 beats/min in adults or ≥40 beats/min in adolescents within 10 min of standing or >60° head-up tilt).[1] The increased heart rate is accompanied by multiple symptoms of orthostatic intolerance while standing, including heart palpitations, light-headedness, tremulousness, blurred vision, nausea, headache, dizziness, and syncope.[2-4] Orthostatic symptoms improve shortly after return to a supine position and occur in the absence of orthostatic hypotension (a decrease in blood pressure >20/10 mmHg with postural change).[1] In addition, patients with POTS may experience other persistent symptoms, such as non-specific generalized weakness, neurologic conditions (migraine, cognitive impairment often referred to as "brain fog," difficulty with concentration), dyspnea, fatigue, fibromyalgia, chest pain, gastrointestinal disturbances (unspecified vomiting, diarrhea, irritable bowel disease), exercise intolerance, sleep disorders (insomnia, sleep apnea, hypersomnia), genitourinary complaints (metrorrhagia, menorrhagia, dysmenorrhea, incontinence, dysuria) and psychiatric disorders (anxiety, depression, tic disorders and psychogenic non-epileptic seizures).[1,3] POTS has been reported in the context of autoimmune and connective tissue diseases, such as Ehlers Danlos syndrome, Raynaud's phenomenon, Hashimoto's thyroiditis, rheumatoid arthritis, and celiac disease.[5]

Although a heterogenous clinical sample can be referred to specialist assessments for POTS, this is not the only group of people who experience these symptoms. It has been estimated that 0.2 – 1.0% of the United States population is affected by POTS, which is equivalent to roughly 1 – 3 million people,[6] with substantial loss of productivity and healthcare costs.[7] This heterogeneous clinical syndrome[7] affects younger individuals (on average 15 – 45 years old), particularly in Caucasian populations, with a significant female predominance (up to 80%).[2] The connection with female sex remains poorly understood, although one potential link is the increased susceptibility to autoimmunity. A further possible explanation is the recognized association between female hormones, especially estrogens, and alterations in vascular function and blood volume.[8] About 10% of subjects have a family history of orthostatic intolerance or tachycardia,[5] underlining the genetic underpinnings of POTS,[8] although a monogenic cause is unlikely.[7] The age at onset of POTS is between 15 and 25 years in most cases.[4] This condition may be precipitated by immunological stressors such as a viral infection (typically upper respiratory or gastrointestinal), vaccination, physical trauma (*e.g.*, concussion), pregnancy, menarche, surgery or psychosocial stress.[1] Exacerbating factors include heat, exercise, and the postprandial state, and the perimenstrual period.[5] Two different developmental patterns have been identified: (1) acute onset after one of the abovementioned triggers or (2) slowly progressive symptoms over prolonged periods of time.[1]

With regard to the etiology of primary POTS, two main theories have been proposed: "partial dysautonomia" of neuropathic origin, possibly due to inadequate peripheral and splanchnic vasoconstriction with orthostatic stress, and "hyperadrenergic state," characterized by hyperactivity of norepinephrine pathways, possibly due to increased norepinephrine production and synaptic release, and/or reduction in norepinephrine re-uptake.[9] A third hypothesis was formulated as sympathetic denervation resulting in central hypovolemia and reflex tachycardia.[2] The influence of the autonomic nervous system on the development of POTS has gained prominence and extensive research focuses on alterations in the fight-or-flight reaction in subjects presenting with this condition. Autonomic dysfunction, of which POTS is an important subset, has been noted in more than half of the patients diagnosed with COVID-19 as part of their post-acute sequelae. Specifically, POTS symptoms have been included among the top three most impactful post-acute sequelae of COVID-19 according to the physician notes from the patient's initial visit.[10] The pathophysiology of SARS-COV-2 is thought to involve virus- or immune-mediated damage to the autonomic nervous system, due to autoantibody production against autonomic nerve fibers and/or sympathetic nervous system stimulation secondary to infection.[11] POTS has emerged as a relatively common manifestation of the long-COVID umbrella, affecting between 2% and 14% of survivors.[5] Post-acute sequelae at any age comprise a plethora of unspecific symptoms reported later than 4 weeks after a confirmed or probable infection with SARS-CoV-2: the most common symptoms are fatigue and post-exertional malaise, which can present in association with POTS.[12] Relatively little is known about the long-term prognosis of POTS, but it appears that about half of the patients spontaneously recover within 1 – 3 years. After diagnostic confirmation, patients should be thoroughly educated about non-pharmacological measures alleviating symptoms, such as graded exercise and increased fluid and salt intake.[3,4] The biopsychosocial model provides useful insights into the development of

POTS and plays a key role in the treatment approach to POTS, with the identification of a wide range of predisposing, precipitating and perpetuating factors. Pharmacotherapy also plays a role in the treatment of POTS: corticosteroids (fludrocortisone), alpha-agonists (midodrine), beta-adrenergic blockade, ivabradine, and – less commonly – immunotherapy, have been used for neuropathic POTS.[3] However, the overall effects of pharmacological therapy are modest.[2]

1.2. Neurodevelopmental tics and Gilles de la Tourette syndrome (GTS)

Neurodevelopmental tics are sudden, rapid, recurrent, and non-rhythmic movements (motor tics) or sounds (vocal tics), resulting from altered pathways of brain development.[13] Tic disorders are the most common hyperkinetic disorder in children.[14] GTS is characterized by multiple tics as well as comorbid psychiatric disorders – especially obsessive-compulsive disorder and attention-deficit hyperactivity disorder (ADHD) – in about 90% of cases.[13,15-17] The multiple phenotypes of GTS can affect different aspects of patients' health-related quality of life across the lifespan.[18,19] The prevalence of GTS in youth ranges from 0.3% to 1%, while isolated tics affect up to 5% of the general population at any point in life.[14] In most cases, the onset of neurodevelopmental tics occurs in childhood, between the age of 5 and 7, with a male-to-female ratio of about 3:1 – 4:1.[20]

Neurodevelopmental tics tend to spread to different body parts following a rostrocaudal direction over the years following their onset.[20] The most common simple motor tics include eye blinking, facial grimacing, and shoulder shrugging, while throat clearing, sniffing, and grunting are examples of simple vocal tics. More complex motor tics involve multiple body parts and even resemble intentional actions, such as palilalia (repeated gestures) and echopraxia (mimicking others' movements). Complex vocal tics involve full words or phrases, such as palilalia (repetition of own words) and echolalia (repetition of others' words). Socially inappropriate language (coprolalia) or gestures (copropraxia) are reported by a minority of patients (10 – 30%), typically in association with a range of more simple motor and vocal tics.[21] A hallmark of neurodevelopmental tics is the presence of premonitory urges, which are distressing sensory experiences that precede the expression of tics.[22] The sensory component is relevant to both diagnosis and treatment since awareness of these urges is often a prerequisite of behavioral interventions for neurodevelopmental tics.[23]

The exact pathophysiology of neurodevelopmental tics remains elusive, despite recent advances in understanding the sensorimotor processes behind the urge to tic and tic expression.[24] Dopaminergic neurotransmission is primarily implicated, although other neurotransmitter systems are believed to be involved. For example, research using animal models, such as knockout mice, has suggested a potential link to histidine decarboxylase deficiency.[25] Both genetic and environmental factors are thought to contribute to the onset of GTS, with studies indicating a heritability rate of 0.77.[26]

1.3. Functional tic-like behaviors

Functional tic-like behaviors are a subgroup of functional neurological disorder with motor manifestations that resemble neurodevelopmental tics and typically involve a complex clinical phenomenology, ranging from repetitive twitching and jerking movements to sounds and meaningful vocalizations.[27,28] By definition, these clinical manifestations are not consistent with a neurodevelopmental pathway and often correlate with psychological stressors.[29] Functional tics have traditionally been considered a condition with a relatively low prevalence, compared to other functional motor symptoms.[30] Comparisons with data collected before the COVID-19 pandemic show that the prevalence of functional tics in adolescents and young adults has increased dramatically since the pandemic.[29,31]

Despite their resemblance to neurodevelopmental tics, the clinical presentation of functional tic-like behaviors reported during the COVID-19 pandemic has some peculiar characteristics, which can assist the differential diagnosis. Functional tics are mainly diagnosed in adolescents and young adults, with a significant preponderance of the female gender. In most cases, there is no family history of tics.[28,30] Both case series[32-34] and controlled studies[35-37] have yielded consistent findings about phenotypical differences between the two conditions. Functional tic-like behaviors usually present with an acute or subacute onset, while neurodevelopmental tics have a gradual onset following a rostrocaudal distribution.[28] Moreover, the clinical phenomenology of functional tic-like behaviors is more varied, with repetitive movements involving the limbs and a higher prevalence of self-injurious tics, such as head banging or self-hitting.[34] Complex vocal tics in the form of random words or swear words (coprolalia) are frequently part of the repertoire of functional tic-like behaviors, while they are reported by a subset of patients with GTS or other neurodevelopmental tic disorders.[21,28,32,38] Data from a recent cross-sectional study showed that both simple head movements such as neck jerking and complex vocalizations (words/full sentences) were more likely to be reported by subjects with functional tic-like behaviors compared to those with neurodevelopmental tics.[34]

It has been observed that functional movement disorders are characterized by inconsistency and distractibility – clinical features that play an important role in differentiating them from organic movement disorders.[30] However, neurodevelopmental tics are often intermittent and distractible:[27,39] diagnostic agreement between experts can be suboptimal when based on clinical phenomenology alone, as showed in a recent study.[40] In general, functional tic-like behaviors become significantly more pronounced in social settings compared to when the person is alone; in addition to situation-specific manifestations, the presence of coprophenomena at onset, interference with the person's ability to carry out intended actions or communication, episodes of intense tic-like behaviors ("tic attacks"), and higher severity of the clinical presentation at initial diagnosis suggest a diagnosis of functional tic-like behaviors rather than neurodevelopmental tics.[41]

Members of the European Society for the Study of Tourette Syndrome (ESSTS) have proposed a set of diagnostic criteria for functional tic-like behaviors. The following three main criteria were suggested: (1) age at onset of at least 12 years, (2) rapid evolution of symptoms, and (3) presence of at least four out of nine specific clinical features (multiple types of tic-like behaviors, with a higher frequency of complex tics than simple ones; inconsistent tics that are not repetitive or stereotyped; complex motor tic-like behaviors including context-dependent or violent/offensive tics; evolution of tics not following the rostrocaudal progression; coprolalia; tics likely to be influenced by popular culture or social interactions; frequent fluctuations in intensity and frequency throughout the day; new tics emerging regularly). The same group of experts also proposed two minor criteria: (1) comorbidity with anxiety/affective disorders, and (2) other functional neurological symptoms. It has to be noted that the phenomenon of functional overlay might not be rare: in a large case series, around 40% of patients presenting with functional tics had a previous diagnosis of GTS.[36]

Little is known about the etiological and pathophysiological mechanisms of this relatively under-researched clinical phenotype. It has been hypothesized that the psychological repercussions of the COVID-19 pandemic, along with the lifestyle changes it brought about, may have contributed to the striking increase in functional tic-like behaviors.[30] Several studies showed that the most common comorbidities of functional tic-like behaviors are anxiety and depression: for example, multicenter data on functional tic-like behaviors from an international registry reported high comorbidity rates with anxiety (66%), depression (28%), autism spectrum disorder (ASD) (24%), and ADHD (23%);[33] similarly, a single-center study that involved 105 patients reported even higher rates of comorbid anxiety (70%) and affective disorders (40%).[36] The identification of predisposing, precipitating and perpetuating factors (biopsychosocial model) plays a key role in the clinical management of the different manifestations of functional neurological disorder.

The striking increase in functional tic-like behaviors since the COVID-19 outbreak[30] offered a privileged opportunity to further explore the possible association between POTS and functional neurological disorder. Specifically, little is known about the possible link between POTS and different types of tic disorders, including the newly described phenotype of functional tic-like behaviors. We therefore set out to conduct an original study aimed at assessing the prevalence and clinical correlates of POTS in two large clinical samples: patients with neurodevelopmental tics who received a diagnosis of GTS, and patients who developed tic-like behaviors and received a diagnosis of functional tics since the onset of the COVID-19 pandemic.

2. Methods

We conducted a retrospective chart review of two large clinical datasets from the specialist GTS Clinic, Department of Neuropsychiatry, National Centre for Mental Health, Birmingham, United Kingdom: (1) a clinical sample of 694 consecutive patients with GTS assessed between July 2008 and December 2024, and (2) a clinical sample of 233 consecutive patients who presented with tic-like behaviors and received a diagnosis of functional tics since the onset of the COVID-19 pandemic (April 2020 – December 2024).

Each patient was assessed by a behavioral neurologist with over 20 years of clinical experience with both primary tic disorders and functional neurological disorders (AEC). Comprehensive demographic and clinical data were systematically collected to confirm the diagnosis of either GTS (neurodevelopmental tics) or functional neurological disorder (functional tics) according to DSM-5 criteria.[42] The assessment was based on the National Hospital Interview Schedule for Tourette Syndrome,[43] a detailed semi-structured interview schedule originally validated in patients with neurodevelopmental tics and adapted for use in patients with functional tics by including key items relevant to functional movement disorders.[44] Specifically, we collected the following demographic and clinical data: gender, age at assessment, age and modality of onset, environmental/psychological triggers and phenomenology of tics, family history of tic disorder, psychiatric comorbidities, and treatment interventions. For the purpose of the present study, we systematically screened the medical records of the two groups of patients to determine the prevalence of POTS as a comorbidity in each group.

We excluded from the study 56 patients with GTS (neurodevelopmental tics) who subsequently developed a functional overlay (comorbid functional tics), as the clinical characteristics of this subgroup of patients appear to differ from those of patients with GTS only.[45] All patients provided informed consent and the study was approved by the local section of the National Research Ethics Service. Anonymized data were stored on Microsoft Excel 2019.

3. Results

3.1. Prevalence of POTS

None of the 638 eligible patients with GTS had a diagnosis of POTS in addition to neurodevelopmental tics. In the group of patients who developed functional tics since the COVID-19 outbreak, 4/177 (2.3%) had received a diagnosis of POTS. A summary of the demographic and clinical characteristics of the four patients with functional tics and comorbid POTS is shown in Table 1.

The four patients with functional tics and comorbid POTS fulfilled the ESSTS criteria supporting the diagnosis of functional tic-like behaviors. Specifically, each patient fulfilled all three major criteria plus at least one minor criterion (Table 2).

Neuropsychiatric comorbidities were common, including other functional neurological symptoms in two out of the four patients. Of note, none of them developed functional tic-like behaviors that were likely to be influenced by popular culture or social interactions.

3.2. Case 1

A 21-year-old female was referred to the specialist GTS Clinic for the assessment of her complex motor and verbal manifestations, in the form of severe and disabling repetitive movements and vocalizations. In addition to fluctuating neck jerks and other sudden movements involving her shoulders and arms, she reported self-hitting, hand clapping and flailing movements. Her complex vocalizations included humming, singing, swearing, and entire phrases (including "I'm faking it"), occasionally disrupting intended communication. She attended her consultation in a wheelchair. She reported the acute onset of her tic-like behaviors following a COVID-19 infection two years before her specialist assessment. In addition to her diagnosis of POTS, she reported a longstanding history of neurodevelopmental conditions. She was diagnosed with both high functioning ASD and ADHD, for which she was prescribed a central nervous system stimulant. Her other pharmacotherapy targeted her longstanding anxiety, depression, and insomnia, as well as behavioral manifestations linked to her previous diagnosis of borderline personality disorder. There was a family history of neurodevelopmental conditions, but no confirmed cases of tic disorders. On neurological examination, there was evidence of highly intermittent and distractible jerks, mainly affecting her neck and arms, as well as complex vocalizations, including random words and short sentences. There were no consistent premonitory urges.

3.3. Case 2

A 17-year-old female acutely developed her first tic-like behaviors after experiencing an increase in her anxiety

Table 1. Summary of the demographic and clinical characteristics of patients (*n*=4) with functional tics and comorbid POTS

Cases	Gender	Age at assessment (years)	Age at tic onset	Type of onset	Trigger (s)	Family history of tics	Comorbidities	Treatment
Case 1	Female	21	19	Acute	Stress, infection	No	ADHD, ASD, anxiety, depression, insomnia, EUPD	AA, SSRI, BZ, lisdexamfetamine
Case 2	Female	17	15	Acute	Stress	No	NEAD, ASD, anxiety, insomnia, eating disorder	AA, SSRI, mirtazapine, antihistamine, psychotherapy
Case 3	Female	21	18	Acute	Nil	Yes	ASD, anxiety, depression, eating disorder, EUPD, Ehlers-Danlos syndrome	AA, TCA, SNRI, BZ, gabapentin, antihistamine, psychotherapy
Case 4	Female	24	23	Acute	Stress	No	NEAD, functional weakness, functional dystonia, ASD, DCD	Psychotherapy

Abbreviations: AA: Atypical antipsychotic; ASD: Autism spectrum disorder (high functioning); BZ: Benzodiazepine; DCD: Developmental coordination disorder; EUPD: Emotionally unstable personality disorder; NEAD: Non-epileptic attack disorder; POTS: Postural orthostatic tachycardia syndrome; SNRI: Serotonin-noradrenaline reuptake inhibitor; SSRI: Selective serotonin reuptake inhibitor; TCA: Tricyclic antidepressant.

Table 2. ESSTS criteria supporting the diagnosis of functional tic-like behaviors in patients (*n*=4) with POTS

ESSTS criteria	Case 1	Case 2	Case 3	Case 4	Total cases
Major criterion 1 (age of onset≥12)	Yes	Yes	Yes	Yes	4/4
Major criterion 2 (rapid evolution of symptoms)	Yes	Yes	Yes	Yes	4/4
Major criterion 3a (multiple types of tic-like behaviors, with a higher frequency of complex tics than simple ones)	Yes	Yes	Yes	Yes	4/4
Major criterion 3b (inconsistent tics that are not repetitive or stereotyped)	Yes	Yes	Yes	Yes	4/4
Major criterion 3c (complex motor tic-like behaviors including context-dependent or violent/offensive tics)	No	No	Yes	Yes	2/4
Major criterion 3d (evolution of tics not following the rostrocaudal progression)	Yes	Yes	Yes	Yes	4/4
Major criterion 3e (coprolalia)	Yes	No	Yes	No	2/4
Major criterion 3f (tics likely to be influenced by popular culture or social interactions)	No	No	No	No	0/4
Major criterion 3g (frequent fluctuations in intensity and frequency throughout the day)	No	Yes	Yes	Yes	3/4
Major criterion 3h (new tics emerging regularly)	Yes	Yes	Yes	Yes	4/4
Minor criterion 1 (comorbidity with anxiety/depression)	Yes	Yes	Yes	No	3/4
Minor criterion 2 (presence of other functional neurological symptoms)	No	Yes	No	Yes	2/4
Total criteria	Major 1 Major 2 Major 3 Minor 1	Major 1 Major 2 Major 3 Minor 1 Minor 2	Major 1 Major 2 Major 3 Minor 1	Major 1 Major 2 Major 3 Minor 2	

Abbreviations: ESSTS: European Society for the Study of Tourette Syndrome; POTS: Postural orthostatic tachycardia syndrome.

and a relapse in her longstanding eating disorder, which resulted in her dropping out of school. In addition to multiple motor tics mainly affecting her face and neck (eye rolling, head jerks), she developed limb shaking, shouting, and complex vocalizations. She also reported occasional "tic attacks" and over time her functional tics became more episodic in nature, merging into non-epileptic attacks, described as prolonged (up to 20 min) episodes of stiffening and uncontrolled movements, often accompanied by unresponsiveness with preserved awareness. She reported a 2-year history of repetitive movements and vocalizations, in the context of her complex mental health issues. Her other diagnoses included POTS, high functioning ASD, anxiety and insomnia. There was no history of neurodevelopmental motor or vocal tics. Her local psychiatry team prescribed multiple pharmacological agents alongside regular therapy sessions. In her family, there were no confirmed cases of GTS or other tic disorders. During her consultation, there was evidence of intermittent and distractible jerking movements, mainly affecting her face and her limbs.

3.4. Case 3

A 22-year-old female reported a 3-year history of repetitive movements and vocalizations, with an acute onset of complex and severe manifestations mainly involving her upper limbs. Specifically, she described throwing, knocking, dropping objects, hitting, punching (objects, others, self), hair pulling, flailing movements, forced touching (hot surfaces), and situation-specific rude gestures. She also reported a few simple motor manifestations, including mouth pulling and neck tensing. In terms of her complex vocalizations, she reported coprolalia and echolalia, as well as random words in the form of non-obscene socially inappropriate utterances. In addition to her diagnoses of POTS and Ehlers-Danlos syndrome, she reported longstanding mental health issues, with previous diagnoses of high-functioning ASD, anxiety, depression, eating disorder, and borderline personality disorder. There was no history of neurodevelopmental motor or vocal tics. She received intensive input from the psychotherapy services, as well as complex pharmacotherapy for her disabling symptoms. In terms of family history, she reported that one of her cousins had mild tics. She attended her specialist consultation using a wheelchair. Her multiple repetitive movements mainly affected her face and her limbs. Her complex motor and vocal manifestations were noted to be highly intermittent and distractible.

3.5. Case 4

A 24-year-old female came to our attention with an eight-month history of highly variable and intermittent tic-like behaviors, characterized by uncontrolled movements and vocalizations, including occasional random words. Her symptoms had an acute onset following stressful changes in her life circumstances. She initially developed frequent inhaling spasms, which were followed by jerking movements affecting her face, neck, and limbs, including self-hitting, dropping objects, and occasional situation-specific rude gestures. Over the following months, she developed six prolonged episodes of bilateral rigidity with unresponsiveness and preserved awareness, prolonged episodes of fixed dystonia, intermittent leg weakness, fatigue, and joint pain. In addition to her diagnosis of POTS, she had a longstanding history of neurodevelopmental disorders (high-functioning ASD and developmental coordination disorder). She did not report neurodevelopmental motor or vocal tics. She received input from the psychotherapy services for her disabling functional neurological symptoms, which had caused her to quit her job in retail. She also required support with self-care and food preparation. There was no collateral history of childhood tics or family history of tics. At the time of her specialist assessment, she was using a wheelchair. Throughout the consultation, there was evidence of intermittent and distractible jerking movements mainly affecting her neck and limbs, as well as complex vocalizations.

4. Discussion

To the best of our knowledge, this is the first study assessing the prevalence and clinical correlates of POTS in patients diagnosed with neurodevelopmental tic disorders (GTS) or functional tic-like behaviors. In our clinical sample, the prevalence of POTS was 0/638 in the GTS group and 4/177 (2.3%) in the functional tics group. The four patients with POTS and functional tics were young females, with age at assessment ranging from 17 to 24 years. They all fulfilled the diagnostic criteria for functional tic-like behaviors proposed by the ESSTS and half of them presented with other functional neurological manifestations (non-epileptic attacks, functional weakness, and functional dystonia).

The possibility of shared mechanisms and symptoms between POTS or other autonomic disorders and functional neurological disorders has been the subject of research and recent scientific debates. Specifically, it has been reported that there is often a clinical overlap in functional neurological symptoms such as fatigue, dizziness, and non-specific pain, which are often reported by patients with POTS.[46,47] These clinical overlaps suggest the possibility of shared underlying mechanisms, as the autonomic nervous system imbalance associated with POTS presents with excessive tachycardia and symptoms of cerebral hypoperfusion in the upright position.[48] Although the exact relationship between autonomic dysfunction and functional disorders remains unclear, abnormalities in the autonomic nervous system have been proposed as a potential pathophysiological explanation for functional neurological symptoms.[49]

Despite extensive research, the exact pathophysiology of POTS remains elusive and treatment interventions need to be tailored to the individual patient.[50,51] Working hypotheses have mainly focused on baroreflex abnormalities and largely overlooked the possible role of cortical centers controlling the autonomic nervous system, despite their frequent involvement in autonomically mediated paroxysmal disorders.[52] In a recent study on the role of fear conditioning in POTS, Norcliffe-Kaufmann *et al.*[53] showed that patients with POTS respond to verticalization with increased heart rate, catecholaminergic secretion, hyperventilation and lower speed in middle cerebral arterial flow compared to controls. Crucially, they also showed that tachycardia can be induced by the mere announcement of imminent verticalization rather than the positional change itself, indicative of fear conditioning. Based on their findings, the authors concluded that patients with POTS suffer from a "fear-conditioning behavioral response" to the thought of standing, and that POTS could be conceptualized as a "functional psychogenic disorder."[53]

The notion that fear conditioning alone is sufficient to explain POTS has been questioned by other authors.[54-57] It has been pointed out that patients with POTS have shown signs of altered physiological parameters, including lower blood volume and lower stroke volume, especially when upright, compared to healthy participants.[56] The increased rate of entry of norepinephrine into the venous drainage of the heart while patients with POTS are supine, might be caused by multiple mechanisms beyond conditioned fear of orthostasis. Treatment interventions that counter orthostasis-induced decreased venous return to the heart have been shown to be at least partly effective in decreasing orthostatic tachycardia.[54] In general, POTS is a syndromic condition presenting with a range of multi-system abnormalities – small fiber neuropathy, slow gastric emptying, autoimmune markers, among others – that are at best distantly related to excessive orthostatic tachycardia. Moreover, it has been shown that POTS can develop among the sequelae of systemic infections, including COVID-19.[54]

When addressing the pathophysiology of POTS, it is important to distinguish between correlation and causation.[57] Patients with POTS commonly report anxiety and affective symptoms, but the mechanisms linking autonomic dysfunction and psychiatric symptoms is not well understood.[46,48] As with other chronic illnesses, patients with POTS are more likely than healthy controls to report anxiety and to score higher on questionnaires assessing awareness of physical sensations.[57] While fear conditioning might exacerbate symptoms, it does not necessarily account as the only responsible factor for the initial development of POTS.[55] Once again, the implementation of a biopsychosocial model provides a comprehensive framework for the clinical characterization and management of the heterogeneous group of people with this condition.

The association between POTS (and other autonomic disorders) and functional neurological disorders was recently questioned by Blitshteyn *et al.*,[58] based on differences in diagnostic criteria, pathophysiological mechanisms, and treatment interventions. Specifically, these authors highlighted the prominent role of pharmacotherapy for common autonomic disorders as opposed to the focus on psychotherapy and physical therapy for patients with functional neurological symptoms. In cases where a patient meets the diagnostic criteria for both disorders, their recommendation is to treat POTS first with appropriate pharmacological and non-pharmacological treatments, before implementing therapy for the functional neurological symptoms.[58] The exact extent to which POTS and functional neurological disorder interact as comorbid conditions with overlapping but distinct mechanisms is still poorly understood, highlighting the complexity of brain-behavior interfaces.

In our sample, the notable absence of functional tic-like behaviors that were likely to be influenced by popular culture or social interactions possibly reveals the existence of a distinct sub-phenotype of this condition. Another interesting finding of our study is that all patients presenting with both functional tic-like behaviors and symptoms of POTS had been formally assessed and diagnosed with high-functioning ASD (formerly referred to as Asperger syndrome). A recent prospective cohort study demonstrated a high frequency of ASD in children and young people with a wide range of functional neurological disorders.[59] As part of the methodology of this study, clinician-confirmed diagnoses for both conditions were obtained, and its findings indicated that patients with functional neurological symptoms were 3 – 11 times more likely to have ASD than the general pediatric population. Little is known about the possible association between POTS and ASD, but preliminary evidence suggests that intermittent neuro-cardiovascular autonomic dysfunction affecting heart rate and blood pressure could be over-represented in patients with ASD.[60]

A few notable limitations need to be taken into account when interpreting the findings of the present study. The study was characterized by a relatively large sample size; however, referral bias might have limited the generalizability of our findings to the wider community of patients with POTS and functional neurological disorder, since our clinical populations were recruited at a single specialist center. Moreover, our research predominantly relied on a retrospective analysis of medical records, which inherently limits the scope of variables that could be investigated as part of the clinical correlates of POTS in patients with functional tic-like behaviors. Finally, the diagnosis of functional tic-like behaviors was corroborated by the ESSTS criteria, but it has recently been pointed out that such criteria inevitably involve a degree of circular reasoning – which is likely to be shared by any operational criteria for the diagnosis of functional neurological disorders.[61] Despite these limitations, our study makes a contribution to the growing body of evidence regarding the comorbidity profiles of patient with POTS, with focus on the interface between POTS and the multiple phenotypes of functional neurological disorder. Specifically, a better understanding of the clinical characteristics and comorbidity profiles of patients with POTS and patients with functional tic-like behaviors has the potential to improve the diagnostic accuracy of healthcare providers dealing with these conditions. Our preliminary findings may therefore influence clinical practice by providing additional information for the assessment protocols of patients presenting with selected phenotypes of functional neurological disorders.

Moreover, researchers investigating the pathophysiology and clinical presentations of both POTS and functional neurological disorder might be informed by these results. Further research is needed to shed more light on the intricate relationship between POTS and functional neurological disorder. In particular, our preliminary findings should be validated in future studies involving culturally diverse populations, monitoring a broader range of demographic and clinical variables, and employing prospective data collection methods. Longitudinal data would also provide useful insights into the long-term prognosis of these conditions, including the possible presence of modifiable outcome predictors. There is a clear need for further research exploring the neurobiological underpinnings of both POTS and functional neurological disorder, to investigate the possibility of shared elements in their pathophysiological underpinnings. Finally, clinical and experimental data would provide key information to facilitate the development of more personalized care pathways to address the healthcare needs of challenging clinical populations.

5. Conclusion

Since the COVID-19 pandemic, the list of clinical phenotypes of functional movement disorders has expanded to include functional tic-like behaviors.[27]

Preliminary evidence pointed toward a possible association between POTS and functional neurological disorder, and the "pandemic within the pandemic" of functional tic-like behaviors opened up the opportunity of exploring the possible links between POTS and different types of tic disorders. In the present study, we retrospectively assessed the prevalence of POTS in two large patient populations from a specialist clinic for tic disorders: 638 patients with neurodevelopmental tics who received a diagnosis of GTS, and 177 patients who developed tic-like behaviors and received a diagnosis of functional tics since the onset of the pandemic. In our sample, the prevalence of POTS was 0% in the GTS group and 2.3% in the functional tics group. A significantly larger population is required to establish the actual prevalence of POTS in patients with different types of tic disorders and functional neurological symptoms. The four patients with POTS from the functional tic group were females, with age at assessment ranging from 17 to 24 years. Our preliminary finding about the selective association between POTS and functional tic-like behaviors, in the absence of comorbidity between POTS and neurodevelopmental tics, suggested a possible link between POTS and functional neurological disorder. This was in line with the clinical comorbidity pattern of the four patients with POTS and functional tic-like behaviors, which included high-functioning ASD (4/4), anxiety (3/4), and other functional neurological symptoms (2/4). Our findings require validation in further studies conducted across large samples of patients with different functional neurological phenotypes.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Andrea E. Cavanna
Data curation: Andrea E. Cavanna
Formal analysis: Andrea E. Cavanna
Investigation: Andrea E. Cavanna
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Writing–review & editing: All authors

Ethics approval and consent to participate

All patients provided informed consent to participate in the study, which was approved by the local section of the National Research Ethics Service (ethics approval code: 10/H1207/1).

Consent for publication

Consent for publication was obtained from all patients.

Availability of data

The anonymized study data are available from the authors upon reasonable request.

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ACCSCIENCE PUBLISHING

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ORIGINAL RESEARCH ARTICLE

Machine learning-based recognition of epileptic and non-epileptic EEG signals

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Abstract

Epilepsy is a chronic neurological disorder affecting approximately 50 million people worldwide. Accurate and efficient detection of epileptic seizures is crucial for effective treatment and management. Electroencephalogram (EEG) signals, being non-invasive and rich in temporal information, are widely used for epilepsy diagnosis. However, manual inspection of EEG data is time-consuming and relies heavily on the expertise of clinicians. Machine learning techniques offer promising solutions for automating the classification of epileptic and non-epileptic EEG signals. In this study, we investigate the performance of various machine learning models – including Light Gradient Boosting Machine, deep learning architectures, and convolutional neural networks (CNN)—in classifying EEG signals for epilepsy detection. Our experiments demonstrate that CNN outperform other models due to their ability to capture complex spatial and temporal patterns inherent in EEG data. The CNN model achieved higher accuracy and better convergence, as evidenced by the confusion matrix and learning curves. In contrast, Deep Neural Networks without convolutional layers showed lower performance, likely due to their limitations in capturing the intricate features of EEG signals. Similarly, the Light Gradient Boosting Machine model exhibited good initial results but failed to generalize well to unseen data, possibly due to overfitting and lack of convergence. These findings highlight the potential of CNN-based approaches in the automated recognition of epileptic seizures using EEG signals, paving the way for more efficient and accurate diagnostic tools.

Keywords: Epilepsy; Machine learning; Electroencephalogram; Dense Neural Network; Convolution neural network

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1. Introduction

Epilepsy is a chronic neurological condition characterized by having at least two unprovoked seizure episodes at least 2 h apart.[1] A seizure is a sudden burst of activity in the brain.[2] Seizures can have various causes, including strokes, head injuries, neoplasm, and infections. However, in many cases, the exact cause remains unknown. There are numerous types of seizures, each affecting different parts of the body and presenting unique symptoms. Most seizures can be separated into either generalized or focal seizures.[3] Focal seizures are caused by abnormal brain activity in a specific area of the brain and can either present with impaired awareness or without it.[4] Those with reduced awareness involve a change or loss of consciousness, where individuals may seem to be awake but unresponsive, often engaging in repetitive movements. In contrast, focal seizures without impaired awareness can alter emotions and sensory perceptions without causing a loss of consciousness, leading to symptoms such as emotional changes, sensory disturbances, or physical jerking. Generalized seizures affect all areas of the brain from the moment they begin, causing widespread disruption in brain activity.[5] There are various types of generalized seizures including absence seizures, tonic seizures, clonic seizures, and myoclonic seizures.

Epilepsy can be diagnosed through various methods, including a range of imaging techniques. These methods help assess brain activity and pinpoint abnormalities, contributing to a comprehensive diagnosis of the condition. The most common imaging technique used to diagnose epilepsy is the electroencephalogram (EEG).[6,7] An EEG measures the electrical activity in various parts of the brain by placing small electrodes on the scalp to detect abnormalities in brain wave patterns.[8] Abnormal brain wave patterns detected on an EEG can assist doctors in determining the type of seizure and identifying the specific area of the brain affected. These irregularities are key in diagnosing epilepsy and understanding its origins within the brain. High-density EEG is a variation of the standard EEG where electrodes are placed closer together, allowing for more precise identification of the brain areas affected by seizures.[9]

Diagnosing epilepsy involves several steps: first, determining if the episode was an epileptic seizure or another event, such as syncope or cardiac arrhythmias, which tend to present with similar symptoms.[10] Once a seizure is confirmed, the next step is to classify the seizure type, followed by identifying the underlying cause through additional tests such as EEGs, computed tomography (CT) scans, or blood tests. The final step involves classifying the epilepsy syndrome, which helps guide treatment choices, including the selection of antiepileptic drugs. This process can take weeks to months, depending on the complexity of the case and the need for further investigations. The diagnosis is often complicated by interobserver variability, as different doctors may interpret clinical descriptions and tests differently, leading to delays or errors.[11,12]

Overall, more efficient screening for epilepsy can lead to faster and more accurate diagnoses, allowing for earlier intervention and better-targeted treatment.[13] By identifying the specific type of epilepsy or seizure syndrome quickly, healthcare providers can tailor treatment plans more precisely, reducing the trial-and-error approach that often comes with antiepileptic drugs. This can improve patient outcomes by potentially reducing side effects and enhancing seizure control. In addition, timely screening can help prevent the progression of the condition, reducing the risk of complications and improving overall quality of life for patients.[14]

Recent developments in deep learning architectures, particularly those integrating temporal and spectral analysis techniques, have shown remarkable accuracy (up to 100% in binary classifications) for seizure detection.[15-18] This underscores the significant potential of combining various analytical approaches in enhancing detection capabilities. However, while studies reporting exceptional classification metrics highlight methodological promise, many lack comprehensive validation protocols to ensure clinical robustness. For instance, claims of near-perfect accuracy often omit critical evidence of model training dynamics (e.g., learning/loss curves) or class separation reliability, such as the balance between true positive rates (TPR, i.e., correctly identified seizures) and false positive rates (FPR, i.e., erroneous seizure alerts) across classification thresholds (i.e., receiver operating characteristic [ROC] curves), making it difficult to assess whether reported performance reflects generalizable learning or dataset-specific overfitting.[19] Moreover, in the context of assessing these learning architectures for tasks like seizure detection, it is important to compare training versus validation learning/loss curves, which many of these studies omit. This comparison provides insights into the model's training dynamics, allowing the reader to observe whether the curves converge, merge, and eventually plateau. Such behaviors are indicative of a model that is learning effectively and generalizing well to unseen data. Without demonstrating these dynamics, one cannot ascertain whether reported high accuracy (such as claims of up to 100% in binary classifications) holds any meaningful clinical significance. If the learning curves show divergence or erratic behavior, it may suggest overfitting to the training data rather than true generalization, thereby undermining the reliability of the model in real-world clinical applications.[19] Thus, transparent evaluation frameworks that incorporate both performance metrics and convergence behavior analyses are essential for translating algorithmic advancements into clinically robust solutions. This underscores the need for transparent evaluation frameworks that pair performance metrics with convergence behavior analyses when translating algorithms to clinical settings.[20]

2. Materials and methods

2.1. Data description

The dataset utilized for this study was derived from Kaggle, an open-source online database.[21] It consists of 2,216 rows, each representing an EEG examination where each row represents a different patient. It includes data from various channels across the scalp, capturing different frequency bands. The dataset comprises 668 columns, of which 253 columns represent specific frequencies recorded at different scalp locations. Additional columns provide statistical metrics (e.g., mean and standard deviation) for specific frequency bands at various times. Local sleep-wake transition band values are also recorded. The target column indicates whether the individual examined was epileptic (1) or non-epileptic (0). This comprehensive dataset was used to identify EEG features indicative of epilepsy and to develop predictive models capable of screening patients for epilepsy.

2.2. Preprocessing

The dataset was preprocessed to ensure suitability for machine learning modeling. Initially, the data were loaded into the Python environment and the target column was separated from the predictor variables. The dataset was then divided into three subsets for training, validation, and testing, with proportions of 70%, 20%, and 10%, respectively. Stratified sampling was employed to preserve the original class distributions in each subset, ensuring that both epileptic and non-epileptic cases were adequately represented. Predictor variables were standardized using a StandardScaler (class from the Python library scikit-learn) to normalize the feature values. This step was critical for neural network models to achieve consistent convergence during training. For the convolutional neural network (CNN), the standardized predictor data was reshaped to include a channel dimension, enabling the model to interpret each feature as part of a temporal sequence.

2.3. Machine learning models

Three distinct machine-learning approaches were implemented and evaluated in this study. Each model architecture was carefully designed to leverage the unique characteristics of the EEG dataset and optimize performance for epilepsy prediction.

2.3.1. Dense neural network (DNN)

The dense neural network (DNN) was constructed using the Keras framework. This model comprised an input layer configured to match the dimensions of the predictor variables, followed by three hidden layers. Each hidden layer employed rectified linear unit (ReLU) activation functions to introduce non-linearity and enhance the model's ability to capture complex patterns in the data. Dropout layers with a rate of 0.3 were incorporated after each hidden layer to mitigate overfitting by randomly disabling a fraction of neurons during training. The final output layer utilized a sigmoid activation function to produce a binary classification output – epileptic or non-epileptic. The DNN was trained using the Adam optimizer, which dynamically adjusts learning rates to accelerate convergence. The binary cross-entropy loss function was chosen to optimize classification accuracy. Training was conducted over 50 epochs with a batch size of 32, and early stopping was applied based on validation performance to prevent overfitting.

2.3.2. Convolution neural network

The CNN was designed to exploit the spatial and temporal relationships within the EEG data. The model architecture began with two convolutional layers, each equipped with ReLU activation functions and configured with 32 and 64 filters, respectively. These layers were followed by max-pooling operations to downsample feature maps and reduce computational complexity. Dropout layers, with a rate of 0.3, were included to enhance generalization by preventing overfitting. The CNN further included a flattening layer to convert the multi-dimensional feature maps into a one-dimensional feature vector. This vector was processed by a series of dense layers, culminating in an output layer identical to that of the DNN. Training for the CNN followed a similar protocol, employing the Adam optimizer and binary cross-entropy loss function, with model evaluation occurring at each epoch to monitor progress.

2.3.3. PyCaret machine learning

The third approach involved the use of PyCaret, an automated machine learning framework. PyCaret's classification module was configured to preprocess the data, evaluate multiple machine learning algorithms, and identify the best-performing model based on a variety of metrics. Following the automated model comparison, the selected model underwent hyperparameter tuning to optimize performance further. The final tuned model was then evaluated on the test set, with key metrics such as accuracy, precision, recall, and F1-score calculated to assess its predictive capability.

2.3.4. Model implementation and training details

All models were implemented in Python 3.8.10 using TensorFlow 2.7.0 (Keras API) for neural networks and PyCaret 2.3.10 for LightGBM. Hyperparameter optimization used: (1) manual grid searches to constrain ranges, then (2) Keras Tuner (20 trials) for CNNs/DNNs, targeting kernel sizes (3 – 15 samples), dropout (0.2 – 0.5), and L2 regularization (1e-4 – 1e-2). ReLU activation (hidden layers) and sigmoid (output) ensured non-linear separability and probabilistic outputs. The Adam optimizer (lr = 0.001) balanced convergence speed/stability.

2.4. Model evaluation and visualization

Model performance was rigorously evaluated using the validation and test datasets. For the neural network models, training history was visualized through plots of accuracy and loss against epochs. These plots provided insights into the models' learning processes, highlighting convergence behavior and potential overfitting. Confusion matrices were constructed to depict the classification performance, illustrating true positive, false positive, true negative, and false negative counts for both epileptic and non-epileptic cases. Additional metrics such as area under the curve (AUC), Cohen's kappa, and Matthew's correlation coefficient (MCC) were calculated to provide a comprehensive assessment of classification effectiveness.

Visualization played a pivotal role in interpreting model outputs. Training curves were developed for each model comparing training (70% of the data) versus validation (20% of the data). Visualizing these curves indicates if the model is learning effectively or if adjustments are needed.

Heatmaps were created from confusion matrices to identify patterns of misclassification. For each model, two confusion matrices were derived – a validation set using the 20% of validation data and a test set using 10% of the data – representing previously unseen data. The validation matrix helps in understanding how well the model is performing during training and determines if overfitting is occurring. The test matrix provides insight into the model's performance on unseen data. These visual tools facilitated the comparison of model performance and guided refinements to improve predictive accuracy.

3. Results

The performance of all models was evaluated using validation (20% of the dataset) and test datasets (10% of the dataset) to assess its predictive performance. The validation set comprised data previously seen by the model during training, while the test set contained entirely unseen data. Table 1 presents the performance metrics for the DNN, including accuracy, recall, precision, F1 score, Cohen's kappa, MCC, and AUC. In addition, the DNN and CNN were also evaluated on their performance on the test datasets using these metrics.

On the validation dataset (Table 1), the DNN achieved an accuracy of 76.98% with an F1 score of 75.83%, indicating robust performance on data it had previously encountered. On the unseen test dataset, the DNN maintained comparable performance, achieving an accuracy of 77.48% and an F1 score of 70.59%. The AUC values for validation and test datasets were 0.82 and 0.84, respectively, suggesting consistent classification capability across datasets.

Table 1. A comparison of model performance metrics on the EEG data

Model	Accuracy	AUC	Recall	Precision	F1	Kappa	MCC
CNN	0.8194	0.8716	0.7315	0.8778	0.7980	0.6371	0.6458
DNN	0.7698	0.8190	0.7407	0.7767	0.7583	0.5387	0.5393
LightGBM	0.8590	0.9143	0.8156	0.8679	0.8397	0.7141	0.7168
RF	0.8572	0.9117	0.8055	0.8727	0.8364	0.7101	0.7134
ET	0.8544	0.9111	0.7995	0.8733	0.8331	0.7044	0.7086
GBC	0.8442	0.9071	0.8055	0.8480	0.8245	0.6846	0.6876
ADA	0.8074	0.8570	0.7670	0.8047	0.7826	0.6100	0.6140
LR	0.8046	0.8260	0.7691	0.7979	0.7816	0.6050	0.6073
KNN	0.7853	0.8435	0.7307	0.7797	0.7571	0.5677	0.5711
DT	0.7271	0.7254	0.7227	0.7452	0.7338	0.4520	0.4556
Ridge	0.7115	0.7218	0.6558	0.6942	0.6730	0.4156	0.4173
LDA	0.6913	0.6957	0.6314	0.6691	0.6499	0.3743	0.3754
NB	0.5816	0.5786	0.7992	0.8573	0.1732	0.0391	0.1859
Dummy	0.5447	0.5700	0.0000	0.0000	0.0000	0.0000	0.0000
SVM	0.5107	0.5700	0.5285	0.0000	0.0000	0.0000	0.0000
QDA	0.4646	0.6525	0.9980	0.4599	0.6295	0.0154	0.0263

Abbreviations: ADA: Ada boost classifier; CNN: Convolutional neural network; DNN: Dense Neural Network; DT: Decision tree classifier; EEG: Electroencephalogram; ET: Extra trees classifier; GBC: Gradient boosting classifier; KNN: K-Nearest neighbors classifier; LDA: Linear discriminant analysis; LightGBM: Light gradient boosting machine; LR: Logistic regression; MCC: Matthews correlation coefficient; NB: Naive Bayes; QDA: Quadratic discriminant analysis; RF: Random forest classifier; Ridge: Ridge classifier: Dummy: Dummy classifier; SVM: Support vector machine (Linear Kernel).

The CNN achieved an accuracy of 81.94% and an F1 score of 79.80% on the validation set (Table 1), demonstrating superior performance compared to the DNN. On the unseen test dataset, the CNN maintained comparable performance, achieving an accuracy of 81.98% and an F1 score of 75.90%. AUC values for the validation and test datasets were 0.87 and 0.86, respectively, indicating strong classification performance across both datasets.

Multiple models from the PyCaret Library were trained and analyzed with cross-validation. Of these models, LightGBM showed the greatest overall performance. Table 1 highlights the performance metrics for LightGBM, which achieved an accuracy of 85.90% and an F1 score of 83.97% on the test dataset. The model also attained a high AUC value of 0.91, reflecting its excellent ability to distinguish between classes. Precision and recall values were 86.79% and 81.56%, respectively, confirming the model's balanced performance across both positive and negative classifications. These metrics underscore LightGBM's suitability for accurate and reliable classification tasks. Because of its superior performance, LightGMB was selected for further analysis to be compared to the CNN and DNN.

3.1. Learning curves

Learning curves are a fundamental tool used in machine learning (ML) to assess the performance of a model during training and validation. They provide insights into how well a model generalizes to unseen data and whether it is overfitting or underfitting. A typical learning curve plots training and validation accuracy (or loss) against epochs or the number of training instances. Ideally, the training and validation accuracy should increase steadily and plateau, with the validation accuracy consistently lower than the training accuracy, reflecting the model's ability to generalize while avoiding overfitting.

In the context of this study, learning curves are particularly relevant for evaluating the performance of ML models in distinguishing epileptic from non-epileptic EEG signals – a challenging clinical task requiring high diagnostic accuracy and generalization. Monitoring these curves ensures that the models are learning robust features from the EEG data rather than memorizing patterns specific to the training set, which is critical for reliable deployment in clinical settings. Figure 1 illustrates the training and validation performance of the DNN, CNN, and LGBM classifier models used for the classification of epileptic and non-epileptic EEG signals, highlighting their learning behaviors and generalization capabilities across epochs and training instances.

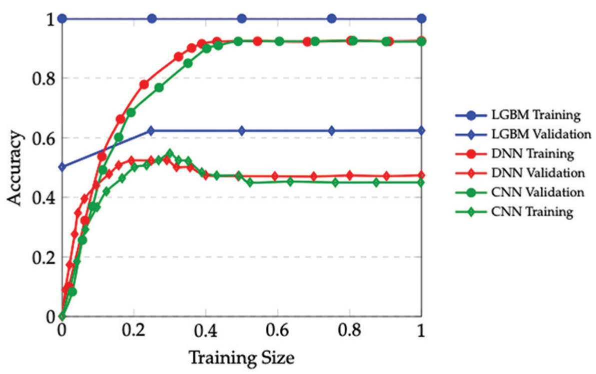

Figure 1. The graphs compare the performance of three models: DNN, CNN, and LGBM classifier. Both the DNN and CNN models exhibit a steady increase in training accuracy, eventually plateauing around 98%, while their validation accuracies stabilize at approximately 80% and 82%, respectively. This is a typical and expected behavior, where the training accuracy is slightly higher than the validation accuracy, reflecting proper learning without severe overfitting. In contrast, the LGBM classifier shows a perfect training score of 1.0 across all training instances, which is not ideal, as it suggests overfitting on the training data. The cross-validation score for the LGBM classifier stabilizes at around 85% but lacks the expected steady increase and plateau shape, which would indicate better generalization.

Abbreviations: CNN: Convolutional neural network; DNN: Dense Neural Network; LightGBM: Light gradient boosting machine.

The performance of the three models – DNN, CNN, and LGBM classifier – offers important insights into their applicability for distinguishing epileptic from non-epileptic EEG signals. Both the DNN and CNN models exhibit the expected trend where training accuracy steadily increases and plateaus around 98%. Similarly, their validation accuracies stabilize at approximately 82% and 80%, respectively, maintaining a reasonable gap from the training curves. This behavior indicates that both models are learning meaningful patterns from the EEG data without severe overfitting, making them suitable for clinical applications where generalization to unseen data is critical. The slight fluctuation in validation accuracy, particularly in the CNN model, may suggest sensitivity to hyperparameter selection or inherent variability in the EEG dataset but remains within acceptable limits for clinical decision-making.

The LGBM classifier, however, demonstrates a perfect training score of 1.0 across all training instances, which is a potential sign of overfitting to the training data. The cross-validation score stabilizes around 85%, with minimal improvement as the number of training instances increases. While the LGBM classifier achieves a slightly higher validation score, its inability to show a steady rise and plateau in both training and validation curves raises concerns about its ability to generalize effectively to new EEG data. This behavior may stem from insufficient regularization or the model's tendency to exploit spurious correlations in the data.

In the context of EEG-based classification for epilepsy diagnosis, models such as DNN and CNN, which show a more balanced trade-off between training and validation accuracy, may offer greater clinical reliability. Their performance aligns with the expectation that validation accuracy should trail slightly behind training accuracy, reflecting a model's ability to generalize while avoiding overfitting. Conversely, the LGBM classifier's results highlight the importance of rigorous evaluation and regularization in high-stakes clinical applications, as overly optimistic training performance does not necessarily translate to robust diagnostic capabilities. These findings underscore the need for careful model selection and tuning in machine learning-based approaches to EEG signal classification.

3.2. Confusion matrices

A confusion matrix is a widely used ML-based tool for evaluating the performance of classification models. It provides a detailed breakdown of the model's predictions by categorizing them into true positives, true negatives, false positives, and false negatives. This breakdown allows for a nuanced understanding of the model's strengths and weaknesses, particularly in high-stakes applications such as the classification of epileptic and non-epileptic EEG signals. In this study, confusion matrices were calculated for three models – DNN, CNN, and LightGBM – on both validation and test datasets. The validation set represents data the model has encountered during training (20% of the dataset), while the test set consists of unseen data (10% of the dataset). Comparing the performance on these two datasets provides insights into the model's generalization ability.

The purpose of calculating confusion matrices on both validation and test sets is to assess how well the model performs on data it has seen during training versus completely unseen data. A small decrease in accuracy (calculated as the sum of true positives and true negatives divided by the total number of predictions, which represents the proportion of correctly classified instances out of all instances) from the validation set to the test set is expected and indicates that the model has generalized well. However, a significant drop in performance suggests overfitting, where the model has memorized patterns specific to the training data rather than learning generalizable features. For instance, in the DNN model (Figure 2), the validation accuracy was 81.6%, while the test accuracy dropped slightly to 80.3%, indicating good generalization. Similarly, the CNN model (Figure 3) showed validation and test accuracies of 83.2% and 82.7%, respectively, demonstrating robust performance. In contrast, the LightGBM model (Figure 4) exhibited a more pronounced drop, with validation accuracy at 87.2% and test accuracy at 81.5%, suggesting potential overfitting.

From a clinical perspective, the confusion matrices provide critical insights into the reliability of these models for diagnosing epilepsy. False positives (non-epileptic signals misclassified as epileptic) can lead to unnecessary anxiety and potentially harmful interventions, while false negatives (epileptic signals misclassified as non-epileptic) may result in missed diagnoses and delayed treatment. For example, the DNN model had 46 false positives and 56 false negatives on the validation set, compared to 15 false positives and 35 false negatives on the test set. The CNN model showed slightly better performance, with fewer false positives and false negatives on both datasets. The LightGBM model, while achieving higher validation accuracy, had a higher number of false positives and false negatives on the test set, raising concerns about its reliability in clinical settings.

When comparing the models, the CNN appears to strike the best balance between validation and test performance, with minimal overfitting and fewer clinically significant errors. The DNN model also performs well but shows slightly higher false negatives, which could be problematic in a clinical context. The LightGBM model, despite its high validation accuracy, demonstrates a larger performance gap between validation and test sets, indicating overfitting and reduced generalizability. These findings underscore the importance of evaluating ML models not only on their overall accuracy but also on their ability to minimize false positives and false negatives, particularly in applications where diagnostic accuracy has direct implications for patient care.

3.3. ROC curves

The ROC curve is a graphical representation of a model's diagnostic ability, plotting the TPR (sensitivity) against the

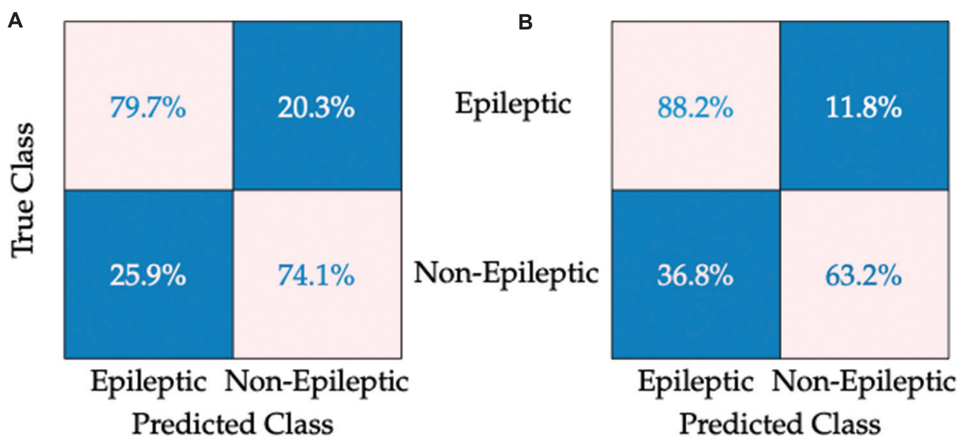

Figure 2. Confusion matrix of DNN performance (%). (A) The performance of the model on the validation data (20%) which the model has previously encountered. (B) The performance of the model on the test data (10%) which the model has not previously encountered.
Abbreviation: DNN: Dense Neural Network.

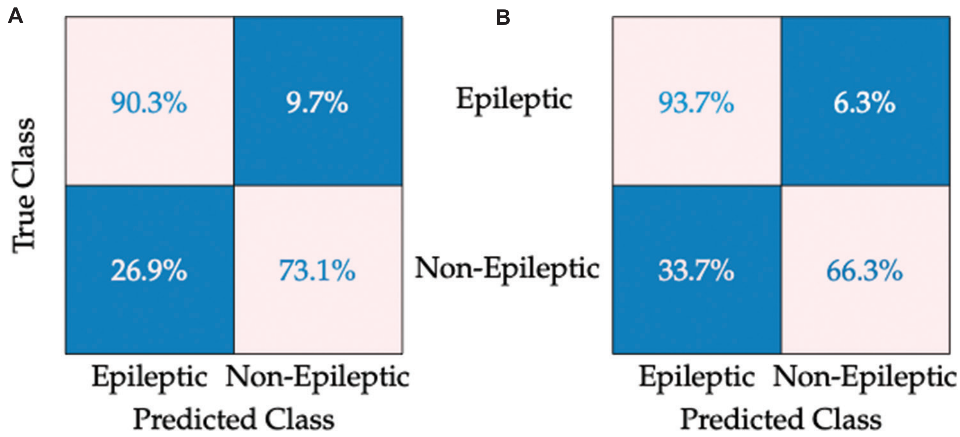

Figure 3. Confusion matrix of CNN performance (%). (A) The performance of the model on the validation data (20%) which the model has previously encountered. (B) The performance of the model on the test data (10%) which the model has not previously encountered.
Abbreviation: CNN: Convolutional neural network.

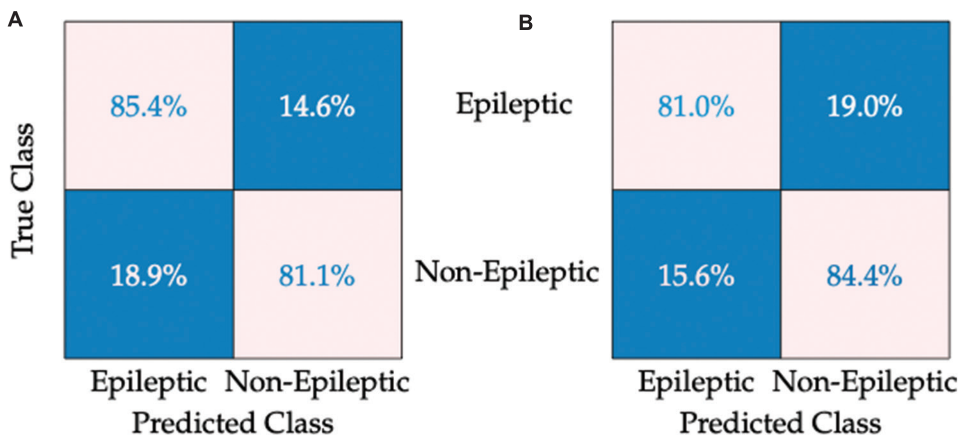

Figure 4. Confusion matrix of LightGBM performance (%). (A) The performance of the model on the validation data (20%) which the model has previously encountered. (B) The performance of the model on the test data (10%) which the model has not previously encountered.
Abbreviation: LightGBM: Light gradient boosting machine.

FPR (1-specificity) at various classification thresholds. The ROC curve provides a comprehensive view of a model's performance across all possible thresholds, making it a valuable tool for evaluating binary classification tasks, such as distinguishing between epileptic and non-epileptic EEG signals. The AUC quantifies the overall performance, with values closer to 1 indicating better discrimination.

In the context of epileptic seizure detection, ROC curves are particularly important because they allow clinicians and researchers to assess the trade-off between sensitivity (correctly identifying seizures) and specificity (avoiding false alarms). A high sensitivity is crucial in clinical applications to ensure that seizures are not missed while maintaining a low FPR is equally important to avoid unnecessary interventions or misdiagnoses. The ROC curves for the CNN and DNN models on both the testing and validation datasets are shown in Figure 5. The CNN model achieved an AUC of 0.86 on the testing set and 0.87 on the validation set, while the DNN model achieved an AUC of 0.84 on the testing set and 0.82 on the validation set. The interpretations of these results are given in the following.

The small differences in AUC values between the validation and testing datasets for both models indicate that the models are robust and generalize well to unseen data. This is critical in clinical applications, as it suggests that the models can reliably classify EEG signals from new patients. Robust models ensure consistent performance across different datasets, which is essential for clinical deployment. A model that generalizes well can be trusted to assist neurologists in diagnosing epilepsy, reducing the burden of manual EEG analysis and improving diagnostic

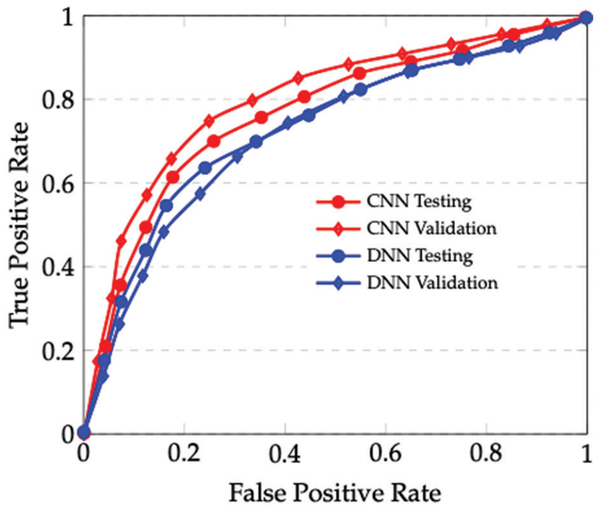

Figure 5. Comparison of ROC curves for CNN and DNN models on testing and validation datasets. The CNN model achieved an AUC of 0.86 on the test set and 0.87 on the validation set, while the DNN model achieved an AUC of 0.84 on the test set and 0.82 on the validation set. The ROC curves illustrate the trade-off between the true positive rate (sensitivity) and the false positive rate, with the diagonal line representing random performance.

Abbreviations: AUC: Area under the curve; CNN: Convolutional neural network; DNN: Dense Neural Network; ROC: Receiver operating characteristic.

accuracy. If the differences between validation and testing AUCs were large, it would indicate overfitting. In such cases, the model might capture noise or dataset-specific patterns rather than generalizable features, leading to unreliable predictions in real-world clinical settings. This could result in missed seizures or false alarms, undermining the model's utility in practice.

4. Discussion

This study evaluated the performance of three machine learning models – Light Gradient Boosting Machine (LightGBM), DNNs, and CNNs – in classifying epileptic and non-epileptic EEG signals. Each model demonstrated distinct strengths and limitations, with CNNs emerging as the most robust for this task.

Among the PyCaret models, LightGBM achieved the highest overall accuracy (85.9%) and AUC (0.91). Its confusion matrices for both the validation and test datasets demonstrated a low rate of false positives and negatives, indicating strong precision and recall.[22] However, the learning curve revealed a persistent gap between training and validation performance, indicating a lack of convergence.[23] This suggests that while LightGBM fits the training data well, it may not generalize effectively to unseen data.[24] Gradient boosting methods like LightGBM are known for their efficiency in structured data but can overfit when applied to high-dimensional datasets, such as EEG signals, without sufficient regularization.[25]

DNNs showed moderate performance, with lower classification accuracy and AUC compared to CNNs and LightGBM. The learning curves exhibited overfitting, as the model achieved near-perfect training accuracy but struggled to generalize to validation and test datasets.[26] DNNs lack the inherent ability to capture spatial or temporal dependencies in the data, which are critical for EEG signal analysis. Without convolutional layers, DNNs are less equipped to handle the intricate patterns required for this task, limiting their effectiveness in epilepsy screening.

While LightGBM exhibited strong initial validation performance, its lack of convergence on unseen data, evidenced by the disparity between training and test accuracies, highlights inherent challenges with overfitting in gradient-boosting frameworks applied to high-dimensional EEG data. Potential remedies such as enhanced regularization (e.g., L1/L2 constraints, reduced tree depth) or feature dimensionality reduction could mitigate these issues. Similarly, the underperformance of DNNs relative to CNNs suggests that architectural refinements (e.g., integrating convolutional layers or attention mechanisms) might improve their ability to model spatiotemporal EEG dynamics. However, a central tenet of ML is identifying and optimizing the model class best suited to the problem's intrinsic structure. The CNN's superior performance, stemming from its innate capacity to hierarchically extract localized spectral and temporal features without manual engineering, validates its prioritization for epilepsy-related EEG analysis. Accordingly, while theoretical avenues exist to improve LightGBM and DNNs, CNN's clinically aligned accuracy, generalizability, and convergence behavior justify its focus as the foundation for translational tool development.

CNNs demonstrated the best overall performance, achieving higher accuracy, AUC, and F1 scores compared to DNNs and LightGBM. The confusion matrices for both validation and testing datasets confirmed a balanced classification of epileptic and non-epileptic cases, with lower rates of misclassification. CNNs' ability to process spatial and temporal features makes them particularly well-suited for non-stationary signals like EEG data.[27] Their convolutional layers extract hierarchical features, capturing both local patterns and broader dependencies, which are crucial for detecting epilepsy. In addition, the CNN learning curves showed a better convergence trend than LightGBM, reflecting its ability to generalize more effectively.

A critical consideration for the clinical deployment of CNN-based EEG analysis is model interpretability.

While our results demonstrate that CNNs inherently amplify neurophysiologically discriminative features in intermediate layers (e.g., spectral asymmetries), their decision-making process requires explicit linkage to clinician-annotated biomarkers. Recent studies employing permutation entropy analysis have shown that CNNs trained on EEG data progressively enhance class separability through sequential convolutional layers, suggesting learned filters align with neuroanatomical seizure patterns.[28] Future implementations will integrate *post hoc* interpretability tools such as gradient-weighted class activation mapping to visualize spatiotemporal regions driving predictions (e.g., lateralized interictal spikes) and Shapley additive explanations to quantify feature importance across spectral bands. These methods will explicitly map model outputs to established epileptiform criteria (e.g., polyspike morphology, ictal rhythms), enabling clinicians to validate predictions against domain knowledge while preserving classification accuracy. Such interpretability not only validates CNN reliability but also bridges the gap between "black-box" deep learning and clinical trustworthiness, ensuring model decisions reflect neurophysiologically meaningful patterns rather than spurious correlations.

While LightGBM performed well on validation data, its lack of convergence highlights potential overfitting. CNNs exhibited only a slight gap between training and validation performance, indicating a much greater generalizability. DNNs struggled to match the performance of CNNs due to their inability to leverage spatial and temporal dependencies. This limitation underscores the importance of selecting architectures that align with the data's underlying structure. The high dimensionality and temporal characteristics of EEG signals pose unique challenges for ML models. CNNs' superior performance reflects their strength in addressing these challenges, but even they are not immune to misclassifications, particularly in borderline cases.

CNNs' ability to consistently outperform other models suggests their potential as a reliable tool for epilepsy detection using EEG signals. Their robustness in handling complex patterns ensures higher diagnostic accuracy, which is critical for timely intervention. The ROC curves for both the CNN and DNN models demonstrate strong performance in distinguishing epileptic from non-epileptic EEG signals. The small differences between validation and testing datasets highlight the robustness of the models, making them promising candidates for clinical applications. However, further validation on larger and more diverse datasets is recommended to ensure their reliability in real-world scenarios.

The implications of these findings in clinical applications are significant. CNNs, with their superior ability to capture spatial and temporal features, can serve as a reliable foundation for automated epilepsy screening tools. Such tools could enable faster and more accurate diagnoses, reducing the burden on neurologists and improving patient outcomes through earlier intervention. The balanced performance across both epileptic and non-epileptic cases also minimizes the risk of misdiagnoses, which is crucial for maintaining trust in clinical settings.[29] To further enhance clinical utility, additional efforts should focus on improving model interpretability and validating these approaches across diverse patient populations and clinical environments.[30] For CNNs, incorporating advanced architectures, such as attention mechanisms or recurrent layers, may enhance their ability to capture long-range temporal dependencies in EEG data.[31] Expanding the dataset and incorporating more diverse samples could help reduce bias and improve generalization across patient populations.[32] While CNNs demonstrated the most promise for EEG-based epilepsy detection, optimizing each model for its specific strengths could further enhance diagnostic accuracy and reliability.

This study employed a single publicly available EEG dataset to ensure methodological consistency during comparative model evaluation. While this approach controlled for confounding variables, we acknowledge that generalization across diverse clinical scenarios (such as pediatric vs. adult populations, focal vs. generalized seizure types, or EEG recordings from varying hardware) requires validation on multicenter, demographically heterogeneous datasets.[32] EEG signal characteristics inherently vary with age, pharmacological history, and comorbid neurological conditions, which are factors not fully captured in our dataset. Future work will expand to multicenter collaborations incorporating wearable EEG devices, which capture longitudinal data across real-world environments (e.g., sleep, stress).

5. Conclusion

This study highlights the potential of ML models, particularly CNNs, for classifying epileptic and non-epileptic EEG signals. CNNs outperformed other models in this study due to their superior ability to capture complex spatial and temporal patterns, making them well-suited for analyzing non-stationary EEG data. Critically, their training dynamics, evidenced by convergent training/validation learning curves and stable ROC metrics, demonstrate robust generalization, addressing concerns that high accuracy in prior studies might stem from dataset-specific overfitting rather than clinically transferable learning.

While LightGBM exhibited high initial accuracy and precision, its lack of convergence (i.e., divergent training-validation curves and unstable loss trajectories) indicates a need for further optimization to improve generalization. DNNs, though effective in some contexts, struggled to match CNN performance due to their inability to capture the intricate spatiotemporal dependencies inherent in EEG signals. These results align with broader methodological critiques: Studies relying solely on classification metrics (e.g., accuracy, F1-score) without transparently evaluating model dynamics (e.g., learning curve convergence, ROC threshold stability) risk overestimating clinical utility.

This work underscores that algorithmic performance claims must be paired with rigorous validation of training behaviors. CNNs not only achieved better accuracy but also demonstrated stable convergence patterns and balanced TPR-FPR tradeoffs across thresholds, fulfilling key criteria for clinical robustness. These findings solidify the promise of CNNs as a reliable foundation for automated epilepsy screening tools. Prioritizing architectures with verifiable generalization behaviors is necessary to bridge the gap between computational advancements and clinically deployable solutions.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: All authors
Formal analysis: All authors
Investigation: All authors
Methodology: Daniel Nasef, Milan Toma
Project administration: Milan Toma
Supervision: Milan Toma
Writing–original draft: All authors
Writing–review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The data utilized in this study is open-source and properly referenced.

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CASE SERIES

Autonomic storms and autonomic movement disorder associated with postural orthostatic tachycardia syndrome misdiagnosed as functional neurological disorder

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Abstract

Patients with postural orthostatic tachycardia syndrome (POTS) typically present with a constellation of symptoms, some of which may mimic other disorders and may lead to misdiagnosis, including autonomic storms and autonomic movement disorders. We discuss two cases in which patients with POTS were misdiagnosed as functional neurological disorder. In both cases, the patients were initially misdiagnosed on hospital admission and then referred to neurologists, who correctly diagnosed them with POTS. We also review video evidence of these patients' storms to showcase the visible presentation of POTS-related myoclonic episodes. Our study highlights the challenges of distinguishing POTS from other conditions due to overlapping, non-specific symptoms. In addition, this case series emphasizes the importance of a thorough clinical assessment, including an orthostatic assessment and tilt table testing, to ensure an accurate diagnosis. Misdiagnosis can delay appropriate management of POTS, highlighting the need for heightened awareness among clinicians when evaluating autonomic symptoms.

Keywords: Postural orthostatic tachycardia syndrome; Functional neurological disorder; Autonomic storms; Myoclonus; Misdiagnosis; Clinical video

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1. Introduction

Patients diagnosed with postural orthostatic tachycardia syndrome (POTS) typically have complex medical histories. POTS, a syndrome that presents with rapid heart rate on standing, can be spurred by several factors, including, but not limited to, stress, viral infection, trauma, pregnancy, and immunization.[1,2] Autonomic storms associated with POTS typically present with a constellation of symptoms including, but not limited to, alterations in levels of consciousness, increased posturing, dystonia, hypertension, hyperthermia, tachycardia, tachypnea, diaphoresis, agitation, tremor, and myoclonus. More commonly seen after traumatic brain injuries, these storms may be difficult to diagnose, especially in ambulatory care settings.[3,4] In the US, POTS affects up to 3,000,000 people, which represent approximately 1% of the nation's population.[5]

Historically, POTS patients are often misdiagnosed, with a previous study revealing that 83% of nearly 700 patients reported being misdiagnosed with a psychiatric diagnosis before being accurately diagnosed with POTS.[6]

Functional neurological disorder (FND) carries diagnostic challenges, with evidence of it being incorrectly misdiagnosed in patients with various neurological conditions, such as multiple sclerosis and POTS.[7,8] A previous study has shown that 12% of the included patients, having a neurological or an organic somatic disorder, were incorrectly diagnosed with FND.[9] Another systematic review suggests that FND demonstrates a misdiagnosis rate of 4%.[10]

FND and POTS are both prevalent conditions in neurological practice and have similar epidemiological patterns, as both conditions predominantly affect women more than men.[1,7] Myoclonus is a particular symptom that typically characterizes POTS-related autonomic storms and accounts for up to 20% of FND cases.[11] With a shared presentation of this symptom, it is important to differentiate between these two conditions and perform necessary tests of orthostatic vitals to avoid a missed diagnosis of POTS.

In this article, we discuss two cases of patients with POTS who, when presenting to the emergency department (ED) with autonomic storms, were misdiagnosed with FND. We also review video evidence of both patients' autonomic storms. Our aim in this review is to highlight the occurrence of autonomic storms associated with POTS being misdiagnosed as FND. We also highlight the detrimental impact of incorrectly attributing POTS to a psychogenic condition, including the delay in appropriate treatment for autonomic storms. This misdiagnosis not only affects patient care but also carries significant implications for future research.

2. Case presentation

2.1. Case 1

A 24-year-old female (she/her) initially presented to the hospital with a diagnosis of FND, reporting dystonia and a burning sensation throughout her muscles (Video S1). These episodes typically consisted of shaking for a few minutes, then 15 – 20 min of no activity, followed by subsequent episodes of shaking. The duration of these events ranged from an hour to an entire evening and would last over hours for 2 – 3 weeks. Thereafter, the patient reported that the movements would typically decrease in severity and duration until they disappeared and recurred a few months later. The patient could not find a consistent trigger for these episodes. Workup with magnetic resonance imaging (MRI) of the brain and spine was negative for structural changes, and electroencephalogram (EEG) was negative for epileptiform changes.

She was referred to our clinic for abnormal movements, and we noted her medical history of chronic back pain and fatigue. Her neurological examination was significant for POTS on orthostatic testing, poorly reactive pupils, hyperreflexia in the patella, and severe small fiber neuropathy. She was also noted to have postural tremors and occasional myoclonic movements involving her arms, trunk, and legs. POTS was initially suspected following an at-home orthostatic assessment indicating a normal heart rate (72 bpm) when supine and tachycardia (126 bpm) when standing. The patient received a confirmed diagnosis of POTS following a positive tilt table test, showing a >30-point increase in heart rate with standing, along with a negative 24-h urine test for metanephrine excretion. The patient was initiated on pyridostigmine with a reduction in tremor and myoclonus events. Further motor improvement occurred when she was diagnosed and treated for Ehlers–Danlos syndrome, neurogenic bladder, and tethered cord followed by tethered cord release surgery.[12] The pyridostigmine dose was adjusted and she was prescribed clonazepam to take at the onset of her symptoms. The storms decreased in frequency on the new regimen.

Two and a 1/2 years later, the patient started to experience episodes of limb weakness or paralysis. She presented to an external hospital ED and was diagnosed with a new FND. Concerned about another error in diagnosis, the patient presented again to her managing neurologist, who evaluated her and ran lab testing, which revealed a low potassium of 3.3 mmoL/L. Additional testing revealed hypokalemic periodic paralysis. She was initiated on potassium supplementation with a resolution of hypokalemic weakness events.

2.2. Case 2

An 18-year-old female (she/her) presented to our neurology clinic with Grave's disease (post-thyroidectomy), celiac disease, migraine, and supraventricular tachycardia. She had episodes of tachycardia with resting heart rates up to 140 bpm to 170 bpm. She also experienced increasing dizziness and frequent syncope.

Subsequently, the patient had her first episode of myoclonus that lasted about 24 h, characterized by substantial full-body twitching. These episodes would last about 20 – 30 min and would occur typically in the morning, one in the afternoon, and then one before bedtime, which was the longest. During these episodes, movement worsened the condition, and she noticed that a substantial amount of walking or exercise during the day would result in more severe myoclonic episodes. Right before the onset of these events, the patient reported feeling extreme nausea that did not lead to vomiting, dizziness, and extreme cold. Her right arm or right leg would start to twitch and within 2 – 3 min, she experienced full-body jerks (Video S2). After the events subsided, she claimed being extremely tired and sore. The twitching episodes at night were more violent. ED evaluation and hospital admission revealed a normal MRI of the brain and spine and a negative continuous EEG. Subsequently, she was diagnosed with FND.

The patient was referred to our neurology group for her abnormal movements. Her neurological examination revealed POTS, hyperreflexia in the patella, small fiber neuropathy, and frequent myoclonus involving arms and legs. Her POTS diagnosis was confirmed by a positive tilt table test, showing a >30-point increase in heart rate with standing, and autonomic evaluation. She was treated with pyridostigmine and valium and her twitching episodes resolved.

2.3. POTS symptoms in patients

Table 1 presents the list of common POTS symptoms affecting the patients in our case series. A categorized breakdown highlights the typical cardiovascular, neurological, and general symptoms of POTS-related autonomic storms and their occurrence in our patients.[3]

3. Discussion

In this retrospective case series of two patients affected by POTS, both were incorrectly misdiagnosed with FND on ED admission for autonomic storms. The patients in our study were both female and young, between 18 and 24 years old. One patient was previously diagnosed with POTS approximately 3 years before ED admission while the remaining patient was confirmed to have POTS after leaving the hospital, at our outpatient neurology clinic. Both patients were confirmed to have POTS on receiving positive tilt table testing results. In addition, both patients were advised to start on non-pharmacological treatment and lifestyle modifications, including optimization of hydration and electrolytes as well as wearing compression stockings. Neither patient in our case study had relevant personal or family history.

Table 1. Presence of clinical symptoms affecting patients with POTS at initial diagnosis

Clinical symptoms	Patient 1	Patient 2
Alterations in level of consciousness	No	No
Increased posturing	No	No
Dystonia	Yes	No
Hypertension	No	No
Hyperthermia	No	No
Tachycardia	Yes	Yes
Tachypnea	No	No
Diaphoresis	No	No
Agitation	No	No
Tremor	Yes	No
Myoclonus	Yes	Yes

Abbreviation: POTS: Postural orthostatic tachycardia syndrome.

POTS symptoms can be divided into three distinct grades. Grade 1 includes orthostatic symptoms that are infrequent, and the patients of this grade are able to stand for more than 15 min at a time and perform unrestricted daily living. Carrying a higher symptomatic burden, Grade 2 includes orthostatic symptoms developing at least once a week, commonly with orthostatic stress; an ability to stand for at least 5 min at a time; and some limitations in daily life. Finally, Grade 3 includes orthostatic symptoms that are common, an ability to stand for more than 1 but <5 min at a time, being severely incapacitated (being bed or wheelchair-bound), and syncope when the patient attempts to stand.[13,14] These three grades track orthostatic symptoms as a means of determining whether a patient has POTS. In our case series, both of our patients presented with tachycardia, and one of the patients presented with hyperthermia and dystonia, which are typical symptoms associated with POTS-related autonomic storms. Furthermore, both patients had episodes of myoclonus indicative of POTS (Table 1). At our clinic, we have seen tremor, myoclonus, and jerking movements that may be synchronous or asynchronous as manifestations of autonomic movement disorders.

The leading factor in the misdiagnosis of POTS is the lack of recognition of POTS symptoms by physicians. Making correct diagnosis of POTS on a patient's first visit is very rare, a factor causing delays in reaching a correct diagnosis, which may take 6 – 72 months from the time of presentation.[15] Among undiagnosed cases of POTS, many occur in the workplace, where patients present with syncopal and pre-syncopal episodes. Workers with POTS have symptoms that are associated with high occupational stress, poor quality of sleep, and disturbances in mental wellness, indicating the need for timely diagnosis of the condition.[16]

Furthermore, the accurate diagnosis of POTS is challenged due to the lack of universal consensus on diagnostic criteria. Many non-specific symptoms, such as myoclonus, fatigue, lightheadedness, and headache, overlap with a broad array of neurological and other clinical disorders. However, for a female patient presented to the ED or the clinic experiencing orthostatic symptoms as well as myoclonic episodes, performing a tilt table test may help to define and properly identify POTS to optimize management. The diagnosis can be reinforced by multiple heart rate and blood rate measurements, which can be conducted at home by patients, for a duration of approximately 3 – 6 months.[17]

There are several limitations to our study. First, the case series review was conducted with a small sample size, resulting in limited statistical power and precision. Second, our data included patient questionnaires, including subjective responses which may have been influenced by personal beliefs and assumptions. Despite these limitations, our study indicates that myoclonus is a symptom of POTS, and it should be seen as such to effectively decrease the time it takes for a patient to be correctly diagnosed and treated.

4. Conclusion

Due to its complex variety of symptoms and frequently associated comorbidities, POTS-affected patients are often misdiagnosed with psychological disorders, including FND. Considering the debilitating effects of POTS, our case series highlights the importance of performing a timely and accurate diagnosis of this condition. This can be facilitated by carefully recording the medical histories of patients who present with orthostatic symptoms and myoclonus, followed by tilt table testing. More clinical research is necessary to prevent neurological symptoms from being misattributed to psychological disorders, as more accurate diagnostics will improve clinical outcomes.

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The authors declare that they have no competing interests.

Author contributions

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Investigation: All authors
Writing – original draft: Anna D. Hohler
Writing – review & editing: All authors

Ethics approval and consent to participate

This case series received exemption from the Institutional Review Board. Informed written consent for publication was obtained from all patients.

Consent for publication

Consent for publication was obtained from all patients.

Availability of data

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ACCSCIENCE PUBLISHING

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CASE REPORT

Intracranial arachnoid cyst complicated with subdural hygroma in a 10-year-old child: A case report and literature review

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Abstract

Arachnoid cysts are abnormal collections of fluids within the arachnoid membrane after a doubling of the structure. These benign lesions represent only 1% of all intracranial space-occupying lesions. The affected patient can remain asymptomatic for several years, which is an incidental finding in most cases. Cysts have been reported to rupture after a minor head trauma, causing subdural hygroma or hematoma; however, spontaneous rupture complicated with subdural hygroma has been rarely reported. Herein, we report a rare case of a 10-year-old boy whose pregnancy follow-up, delivery, and parent's medical history were unremarkable. The patient was admitted for a temporal arachnoid cyst complicated with subdural hygroma, which was determined to be caused by intracranial hypertensive syndrome. He was successfully treated through endoscopic arachnoid cyst fenestration, wherein a pathway was created for the cyst to communicate with the subarachnoid space through the basal cisterns. The choice of treatment for arachnoid cysts remains controversial. Endoscopic treatment is considered when an ipsilateral subdural hygroma is present. Intracranial hypertensive syndrome subsided immediately after surgery. No post-operative complications occurred. The child recovered uneventfully in the post-operative period and was discharged 5 days after surgery. He underwent a follow-up computed tomography 1 month later, confirming a progressive regression of the hygroma as well as arachnoid cyst.

Keywords: Subdural hygroma; Pediatrics; Sylvian arachnoid cysts; Endoscopic treatment; Case report

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1. Introduction

Arachnoid cysts (ACs) are congenital, benign, and intra-arachnoid fluid collections. An AC contains cerebrospinal fluid (CSF) without connection to the ventricular system and is usually not accompanied with abnormal brain development.

ACs are leptomeningeal-lined CSF collections characterized by hyperplastic arachnoid cells. These congenital lesions represent approximately 1% of all non-traumatic intracranial mass lesions.[1] Although the exact mechanism of AC formation remains unknown, the dominant theory postulates that they arise from the splitting of the bilayer arachnoid membrane during development, followed by the expansion of the intra-arachnoid space through a ball-valve mechanism.[2] Although Mendelian inheritance of ACs has been reported in certain cases, such as mucopolysaccharidoses and acrocallosal syndromes, most cases are considered idiopathic or congenital. Furthermore, sex predominance, sidedness, and familial clustering support an underlying genetic mechanism, with genetic variants accounting for 20% of all ACs.[2,3] Sylvian ACs (SACs) are the most prevalent type of ACs in adult and pediatric patients. They are also more likely to occur on the left side and in men.[4,5] Additional sites include cerebral convexities, cerebellopontine angle, suprasellar cistern, quadrigeminal cistern, and cisterna magna.[6]

In rare cases, intracystic hemorrhage, subdural hematoma, or subdural hygroma can arise from the post-traumatic or spontaneous rupture of ACs. Spontaneous subdural hygroma has been an infrequent complication. Many authors have reported that most ACs are clinically asymptomatic. However, 60 – 80% of those measuring >5 cm develop symptoms.[6,7]

A literature review revealed that only 17 cases of ACs in children resulted in subdural hygroma.[8-24] The treatment for symptomatic ACs is still controversial. Herein, we present an additional case with comparable radiological and clinical findings that was successfully treated by endoscopic AC fenestration in which a pathway was created for the cyst to communicate with the subarachnoid space through the basal cisterns.

2. Case presentation

2.1. Patient information

A 10-year-old boy was admitted with a 15-day history of progressively worsening headaches unresponsive to standard analgesics and complicated by vomiting and visual disturbances. The child's pregnancy follow-up and delivery and his parent's medical history were unremarkable. He denied any history of head trauma.

2.2. Clinical findings

The patient was alert. He had a right convergent strabismus with Stage II papillary edema. No motor deficits were found in any extremities. Light touch and proprioceptive sensitivity were appropriate. Deep tendon reflexes were exaggerated bilaterally in the upper and lower extremities. Babinski and Lhermitte's signs were negative.

2.3. Diagnostic assessment

Head computed tomography revealed a left temporal CSF-like collection with hypodensity extending to the left hemispheric subdural space (Figure 1A and B). Head magnetic resonance imaging revealed a left temporal lesion measuring 45 × 57 mm. The lesion was hypointense on T1-weighted imaging and hyperintense on T2-weighted imaging, associated with the same magnetic resonance imaging signal anomalies observed in the ipsilateral subdural space. These characteristics and locations suggest a diagnosis of Sylvian fissure/middle cranial fossa AC associated with subdural hygroma (Figure 1C and D). The patient underwent surgery, and histological examination of the CSF-like material confirmed the diagnosis of intra-arachnoid CSF collection. The collected fluid had the same composition as the CSF.

2.4. Therapeutic intervention

The patient underwent an urgent surgical procedure for fenestration of a left temporal AC using an endoscope. The basal cisterns were opened to create a pathway for the cyst to communicate with the subarachnoid space.

The patient was placed in the supine position with the head secured in a Mayfield head holder under general anesthesia. Pressure points were padded to protect the nerves and reduce the pressure on the chest and abdomen.

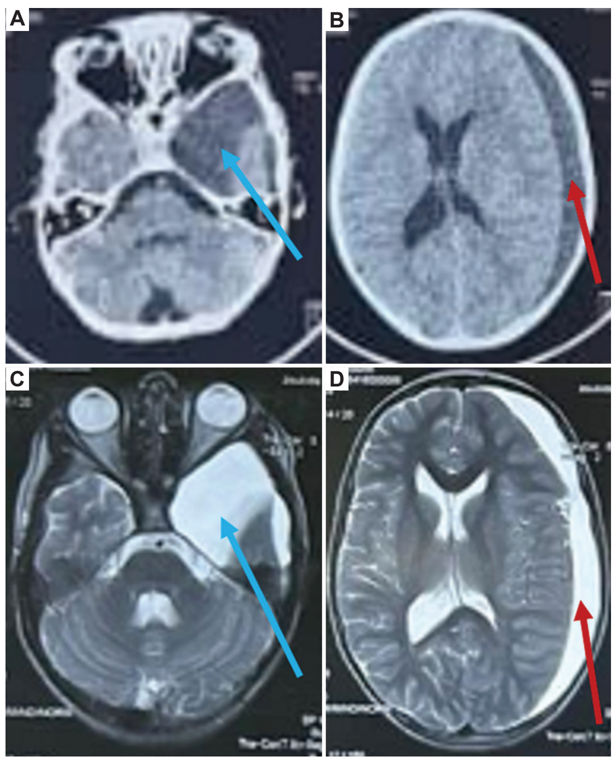

Figure 1. Pre-operative head computed tomography scan and magnetic resource imaging. (A and B) Head computed tomography showing a left temporal arachnoid cyst with ipsilateral hygroma. (C and D) Head magnetic resonance imaging confirming the diagnosis. Blue arrows, left temporal arachnoid cyst; red arrows, subdural hygroma.

The head was turned away from the surgeon, allowing for a 90-degree turn. An incision was made above the zygoma, behind the hairline. A 1.5 cm incision was made through the temporalis muscle to the underlying temporal bone. A small burr hole was created near the inferior aspect of the incision. Through the dura, which was cruciately incised and sutured outward, a dark blue tinge was observed. The CSF freely exited from the wound using pressure, and the endoscope was introduced. At this point, landmark identification was crucial, and the temporal floor and tentorium served as the main landmarks. The internal carotid artery and second and third cranial nerves should be identified. The arachnoid tissue, which was draped across and between the structures, was notably thicker and grossly abnormal, resembling billowing sheer curtains. These arachnoid layers were sliced to reveal the posterior communicating artery and internal carotid artery and permit CSF circulation. Before locating and opening the thickened membrane of Lillequist, the posterior communicating artery, and third nerve were completely fenestrated by the thickened arachnoid. The basilar artery, which was located directly below the thicker arachnoid segment, and all other perforating vessels needed careful protection. After the fenestration was made, a broad passageway leading into the basilar cisterns was constructed to facilitate the observation of the contralateral third nerve, posterior cerebral artery, superior cerebellar artery, and basilar artery with its perforators. At this time, good CSF flow was apparent. After the usual closure, the dura was closed, and the bone powder was used to seal the burr hole.

2.5. Follow-up

Postoperatively, the patient was doing well and still alert. He had no motor–sensory deficits. The intracranial hypertensive syndrome subsided. One month after surgery, the boy was in good health with satisfactory post-operative computed tomography findings (Figure 2).

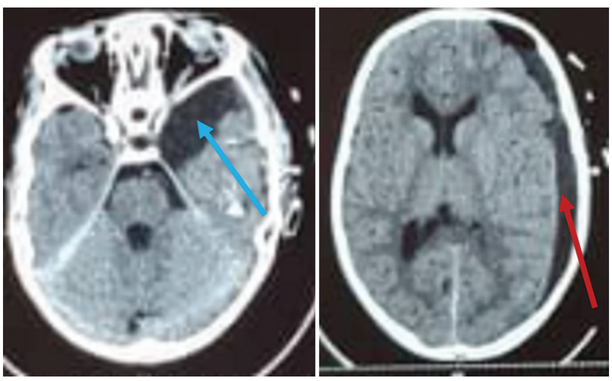

Figure 2. Post-operative head computed tomography. The blue arrow shows a progressive regression of the cyst, and the red arrow shows a progressive regression of the subdural hygroma.

2.5.1. Summary of the key points (Table 1)

Summary of the treatment and outcome of a temporal AC complicated with subdural hygroma in the pediatric population is shown in Table 1. Middle fossa AC cysts can experience the following:

- Spontaneous enlargement, followed by its disappearance without clinical symptoms.
- Spontaneous enlargement and post-traumatic rupture resulting in subdural hematoma or hygroma. They are typically asymptomatic.
- Spontaneous enlargement and spontaneous rupture resulting in a subdural hematoma or hygroma. They are typically symptomatic.
- The treatment for symptomatic ACs is still controversial.
- Endoscopic AC fenestration to create a pathway for the cyst to communicate with the subarachnoid space through the basal cisterns is now possible with good outcomes in experienced hands.

3. Discussion

Although middle fossa ACs rarely undergo spontaneous enlargement, disappearance, or rupture that results in a subdural hematoma or hygroma, they are typically asymptomatic.[25] In adults, the AC size usually increases by 2 – 3%, which is less than that of the pediatric population. Headache is the most typical sign of middle fossa ACs.[26] Interestingly, most complications have been associated with middle cranial fossa cysts, and all cases of cyst rupture resulting in subdural hygroma have been reported in the context of mild head injuries.[27,28] These findings could be explained by various factors, such as the disappearance of cysts following severe trauma or death of the patient from trauma, malignant complications such as severe subdural or intracystic hemorrhages concealing a prior hygroma, or failure to notice cysts in the presence of multiple injuries.[29]

Conversely, the case presented refuted any prior head injuries. Although the exact cause of the rupture is unknown, theories include direct damage to the ipsilateral sphenoid wing from the thinned temporal bone or compression of the Sylvian ACs against it. Cyst wall disruption and subsequent CSF leakage into the subdural space are believed to be the causes of hygroma.[30,31] According to some authors, the development of a hygroma following a cyst rupture disrupts the bridging veins, which in turn causes minor bleeding and a chronic subdural hematoma.[32] Because children experience symptoms earlier due to brain trophic changes, this could account for the higher prevalence of subdural hematomas in adult patients compared with that in pediatric patients.[31]

The most effective treatment approach for ACs in both pediatric and adult patients is still being debated. For

Table 1. Summary of the treatment and outcome of a temporal AC complicated with subdural hygroma in the pediatric population

Reference (year of publication)	Number of cases	Spontaneous/ Posttraumatic	Location	Treatment	Outcome
Motaghedifard *et al.*[8] (2016)	1	Post-traumatic	Sylvian AC and bilateral subdural hygroma	Craniotomy, fenestration, and marsupialization of the cyst into the subarachnoid space, and evacuation of the hygroma	Favorable
Singh. *et al.*[9] (2021)	1	Spontaneous	Sylvian AC and subdural hygroma	Craniotomy (performed to evacuate the subdural hygroma) and fenestration of the AC	Favorable
Maher *et al.*[10] (2013)	8	6 post-traumatic and two spontaneous	Sylvian AC and subdural hygroma	7 conservative treatment and one surgical treatment	Favorable
Ather *et al.*[11] (2016)	1	Post-traumatic	Sylvian AC and subdural hygroma	Conservative treatment	Favorable
Khilji *et al.*[12] (2016)	1	Spontaneous	Sylvian AC and subdural hygroma	The subdural hygromas were drained through the bilateral frontal burr holes. The left temporal AC was partially drained through the left temporal craniotomy. A left SDP shunt was placed for the drainage of any future collection	Favorable
Goswami *et al.*[13] (2008)	1	Post-traumatic	Sylvian AC and subdural hygroma	Cyst fenestration and evacuation of the right-sided subdural hygroma	Favorable
Hamidi and Hamidi[14] (2021)	2	Post-traumatic	Sylvian AC and subdural hygroma	Not treated	Lost to follow-up
Choong *et al.*[15] (1998)	1	Spontaneous	Sylvian AC and bilateral subdural hygroma	Oral acetazolamide (250 mg, three times a day) for 4 months	Favorable
Canty *et al.*[16] (2021)	1	Post-traumatic	Sylvian AC and bilateral subdural hygroma	Not treated	Lost to follow-up
Donaldson *et al.*[17] (2000)	2	Post-traumatic	Sylvian AC and subdural hygroma	Left temporal craniotomy, cyst fenestration, and evacuation of the left subdural hygroma	Favorable
Tamburrini *et al.*[18] (2002)	104	Post-traumatic	Sylvian AC and subdural hygroma	SDP and external parietal subdural drainage	Favorable
Arslan *et al.*[19] (2015)	1	Spontaneous	Sylvian AC and subdural hygroma	Conservative treatment	Favorable
Cullis *et al.*[20] (1983)	1	Spontaneous	Sylvian AC and subdural hygroma	Craniotomy was performed to evacuate the subdural hygroma	Favorable
Cakir *et al.*[21] (2004)	1	Spontaneous	Sylvian AC and subdural hygroma	Right frontotemporal SDP shunt	Favorable
Gelabert-González *et al.*[22] (2002)	3	Post-traumatic	Sylvian AC and subdural hygroma	After left temporal craniotomy, the subdural hygroma was evacuated, and the membranes were removed. The basal cisterns were opened to establish free communication between the cyst and subarachnoid space	Favorable
Yamanouchi *et al.*[23] (1986)	1	Post-traumatic	Sylvian AC and subdural hygroma	Conservative treatment	Favorable
Rakier *et al.*[24] (1995)	1	Spontaneous	Sylvian AC and subdural hygroma	Conservative treatment	Favorable

Abbreviations: SDP: Subduroperitoneal shunt; AC: Arachnoid cyst.

asymptomatic patients, treatment options are controversial, whereas symptomatic cysts can be managed through expectant management, cyst shunting, or microsurgical/endoscopic fenestration. The choice of the surgical method depends on the location of the cyst and its effect on nearby structures.[33] Better outcomes are seen with the endoscopic treatment of suprasellar cysts; however, the efficacy of using endoscopy for middle fossa cysts is still debated. Hall *et al.*[34] found that endoscopic and microsurgical methods yielded similar results. Shunting reduced the cyst volume more than other techniques, whereas endoscopic fenestration led to shorter hospital stays. The location of the cyst plays a major role in the response to treatment, with midline and posterior fossa cysts showing the best outcomes. Conservative management may be sufficient for asymptomatic middle fossa cysts with complications such as subdural hygroma and hematoma. Some studies have indicated that symptomatic hygromas associated with previously asymptomatic cysts can be managed without surgery, and cyst diversion or fenestration may not be necessary for all patients in these cases.[35,36]

The presented case has some important clinical findings. First, the spontaneous rupture of the cyst into the subdural space, without a history of head trauma, can be explained by temporal bone thinning on the same side, which allows for energy transfer to the structures below. This may be due to a shared neurodevelopmental abnormality contributing to the process. The thinned temporal bone is more likely to accommodate the increase in intracranial pressure caused by the cyst, leading to gradual CSF accumulation without immediate severe symptoms. Second, the absence of hemorrhagic transformation in the present case indicates that the chronicity of the hygroma alone does not necessarily lead to bleeding in children. Other factors such as the expansion rate, head trauma, and venous compliance may play a more significant role in this process. The wide opening of the cyst into the subdural space likely helps prevent bleeding by reducing the pressure gradient between different spaces in the brain.

Despite the lack of immediate reduction in cyst size after surgery, the patient experienced complete symptom resolution without recurrence. This may be due to the removal of any constraining effect on brain tissue, similar to cardiac tamponade, allowing for better outcomes despite the persistent presence of the cyst. The good outcomes reported in other cases with expectant management of similar conditions support this theory.[37-39]

4. Conclusion

In children, temporal ACs complicated with subdural hygroma are a rare comorbidity, and endoscopic fenestration should be considered for treatment to achieve rapid relief of the patient's symptoms.

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Ethics approval and consent to participate

Informed consent of the patient was obtained before his participation.

Consent for publication

The patient gave verbal consent to publish his data in this study.

Availability of data

Not applicable.

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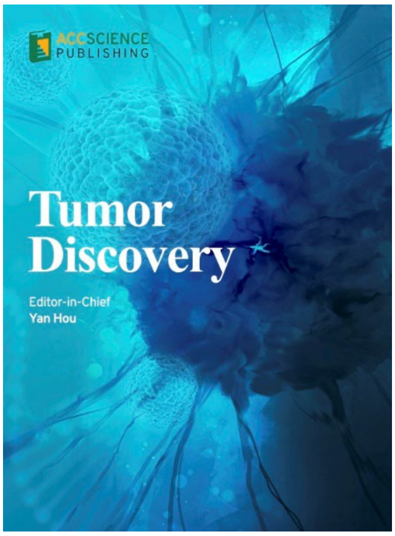

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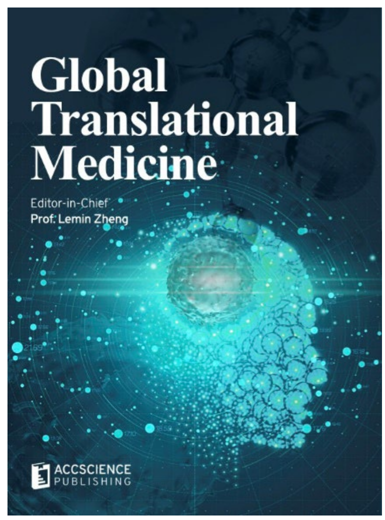

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