Neurodegeneration and convergent factors contributing to the deterioration of the cytoskeleton in Alzheimer's disease, cerebral ischemia and multiple sclerosis (Review)

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Abstract. The cytoskeleton is the main intracellular structure that determines the morphology of neurons and maintains their integrity. Therefore, disruption of its structure and function may underlie several neurodegenerative diseases. This review summarizes the current literature on the tau protein, microtubule-associated protein 2 (MAP2) and neurofilaments as common denominators in pathological conditions such as Alzheimer's disease (AD), cerebral ischemia, and multiple sclerosis (MS). Insights obtained from experimental models using biochemical and immunocytochemical techniques highlight that changes in these proteins may be potentially used as protein targets in clinical settings, which provides novel opportunities for the detection, monitoring and treatment of patients with these neurodegenerative diseases.

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1. Introduction

The cytoskeleton of neurons is the primary structure that regulates cell shape, protein localization and transport from the soma to dendrites and axons or vice versa. This structure consists of microfilaments (MFs) (diameter of ~8 nm), intermediate filaments (IFs) (diameter ranging from 7-11 nm) and microtubules (MTs) (diameter of ~25 nm) (1).

MFs are primarily composed of actin, enriched in cortical regions near the cell membrane and are particularly concentrated in presynaptic terminals, dendritic spines and growth cones. MFs are also called actin filaments; they are formed during proliferation, are considered to be versatile due to their ability to create and destroy themselves, used as construction materials and bind to accessory proteins, regulating the creation and destruction of filaments (2,3).

IFs are components of the cytoskeleton that extend from the nucleus to the cell periphery, and their main function is to provide mechanical resistance. IFs show an unusual degree of cell specificity and are often used as markers of cell differentiation. These filaments are subdivided into multiple types, among which neurofilaments (neurons) and glial filaments (glial cells) are noteworthy structures in the current discussion (4). Neurofilaments (NFs) support axons and dendrites (5) and play an important role in determining the axonal caliber of myelinated fibers (1). These filaments have been a focus of research, as an increase in their presence is potentially related to axonal damage and the severity of neurological diseases (6).

MTs consist of dimers of α and β-tubulin, and these dimers subsequently bind from one end to the other to form a 'protofilament' of tubulin. Additionally, 13 protofilaments are aligned in parallel in a circular manner to form an empty tube that is nm-μm long. The tube is polarized: it has head (+) and tail (-) ends. Polymerization occurs by adding new subunits at the (+) end. In this process, the units are removed from the (-) end and added to the (+) end. This process requires various accessory factors; two of the most important accessory factors

are microtubule accessory proteins (MAPs) and tau protein. These accessory proteins appear to be involved both in the polymerization process and in the stabilization of tubulin once it is polymerized (7).

Due to the significant role of the cytoskeleton in cell transport, in addition to being essential for the normal functioning of neurons, cytoskeletal anomalies result in neuronal damage and cell death, which are the common denominators of neurodegenerative diseases (8,9). Although the causes of transport abnormalities may vary among the various pathological conditions, in many circumstances, deficiencies in transport are caused by a decrease in the stability of its components, such as alterations in neurofilaments, microtubule stability, loss of actin dynamics (9), and an increase in the phosphorylation rates of some proteins that comprise this network (10), as is the case for the tau protein (11). Based on these observations, cytoskeletal organization defects may be a common feature that contributes to neurodegeneration in pathologies such as Alzheimer's disease (AD), cerebral ischemia and multiple sclerosis (MS). Therefore, the potential benefits of cytoskeletal stabilizing agents in improving axonal transport and nerve function in patients with these diseases has been highlighted.

2. Cytoskeletal damage and neurodegeneration

The process of destabilization and aggregation of cytoskeletal proteins causes neurons to become unstable, and interferes with the antegrade and retrograde transport of biomolecules along their axons and dendrites, and, in some cases, generates protein aggregates that lead to neurodegeneration (12). For example, in AD and cerebral ischemia, the hyperphosphorylation of the tau protein leads to the formation of neurofibrillary tangles, axonal damage, dendritic damage and subsequent cell death (13,14). Axonal damage associated with the tau protein has also been reported in other pathologies, such as MS (15), although in the latter, neurofilaments are the central filaments contributing to deterioration due to demyelination (16,17). These proteins have become potential biomarkers, since they are related to the pathophysiology of the disease, and therefore have a significant association with diagnosis, treatment and prognosis, facilitating the diagnosis of individuals at earlier stages of the disease with less severe symptoms and long-term repercussions.

In this review, we discuss the current body of literature with regard to the abnormalities of the cytoskeleton and related proteins that are considered to play critical roles in the pathology of AD, cerebral ischemia and MS, such as microtubule-associated protein 2 (MAP2), tau and neurofilaments.

3. Alzheimer's disease

AD is considered the most prevalent cause of dementia worldwide (18). AD is characterized by two proteinopathies that result in a loss of cell and tissue homeostasis, leading to progressive neuronal degeneration and loss of cognitive functions in patients (19). Amyloidopathy is a product of the abnormal cleavage of amyloid precursor protein (APP), which leads to the accumulation of the Aβ peptide in senile or amyloid plaques, and tauopathy, which is due to hyperphosphorylation and aggregation of the tau protein (14). This protein is normally associated with MTs, but when it is abnormally phosphorylated, it dissociates and aggregates, leading to the formation of paired helical filaments (PHFs) and resulting structures called neurofibrillary tangles (NFTs) (20) (Fig. 1).

Tau and MAPs in Alzheimer's disease. Tauopathy in AD is responsible for a set of abnormalities that lead to neuronal degeneration, such as defects in axonal transport and mitochondrial and lysosomal function, among other functions associated with MTs (21,22). These abnormalities are due to the overactivation or loss of inhibition of various kinases and phosphatases that regulate the binding of MAPs and NFs to the neuronal cytoskeleton and their functions (23). Under pathological conditions, the tau protein itself leads to a loss of function, preventing the binding or shedding of MTs and accumulating in the cytoplasm along with other MAPs, forming PHFs and subsequently resulting in extracellular accumulation in NFTs, which leads to brain deterioration (14,24,25).

In mice deficient in the tau protein, decreases in the numbers of MTs and small caliber axons, muscle spasms and behavioral deficits have been identified, although MAP2 partially compensates for the tau deficit (26). In neurons, the tau protein regulates the establishment of MT dynamics in axons and has been linked to the formation of cytoplasmic extensions, axonal transport and protection against compounds that are deleterious to the cell (13). *In vitro* models related to AD, such as glutamate excitotoxicity, generate a cellular environment that resembles the conditions in the neurons of a patient (27). This model shows the disassembly of MTs, the retraction of dendritic and axonal processes and the hyperphosphorylation and aggregation of PHFs in the cell soma in the short term. Increased intracellular calcium levels, abnormal activation of signaling cascades, and loss of cellular functions trigger cell death signals that represent the neurodegenerative process of the disease (28).

Studies have described how the inhibition of various cytoskeletal proteins is directly and indirectly involved in the disassembly of MTs, the accumulation of the tau protein and neuronal death (27,29). The tau protein kinases described here are grouped into three classes: proline-directed protein kinases (PDPKs), protein kinases (non-PDPKs) and tyrosine protein kinases (TPKs). Each kinase was assessed based on its structure, roles, regulation, involvement in tau phosphorylation and neurodegeneration linked to AD (30,31). Cyclin Dependent Kinase 5 (CDK5), a kinase related to the tau protein and its activators p35/p25, cytoskeletal modifying proteins such as p120 catenin, and enzymes related to the processing of β amyloid such as BACE1, have been linked to hyperphosphorylation of tau and neuronal death (Fig. 1). Silencing of the CDK5 protein, and the spatial and functional regulation of the activator p35, as well as its cleaved form p25 occupy central nodes in the mechanism regulating cellular signaling associated with the hyperphosphorylation of tau (23,28,32). CDK5 regulates NFs and MAPs either by forming protein complexes or by phosphorylating them directly, as in the case of NF or MAP2, MAP1b and the tau protein, which, when phosphorylated, induce the formation and stabilization of MTs (33,34). In turn, proteins associated with the processing of β amyloid, such
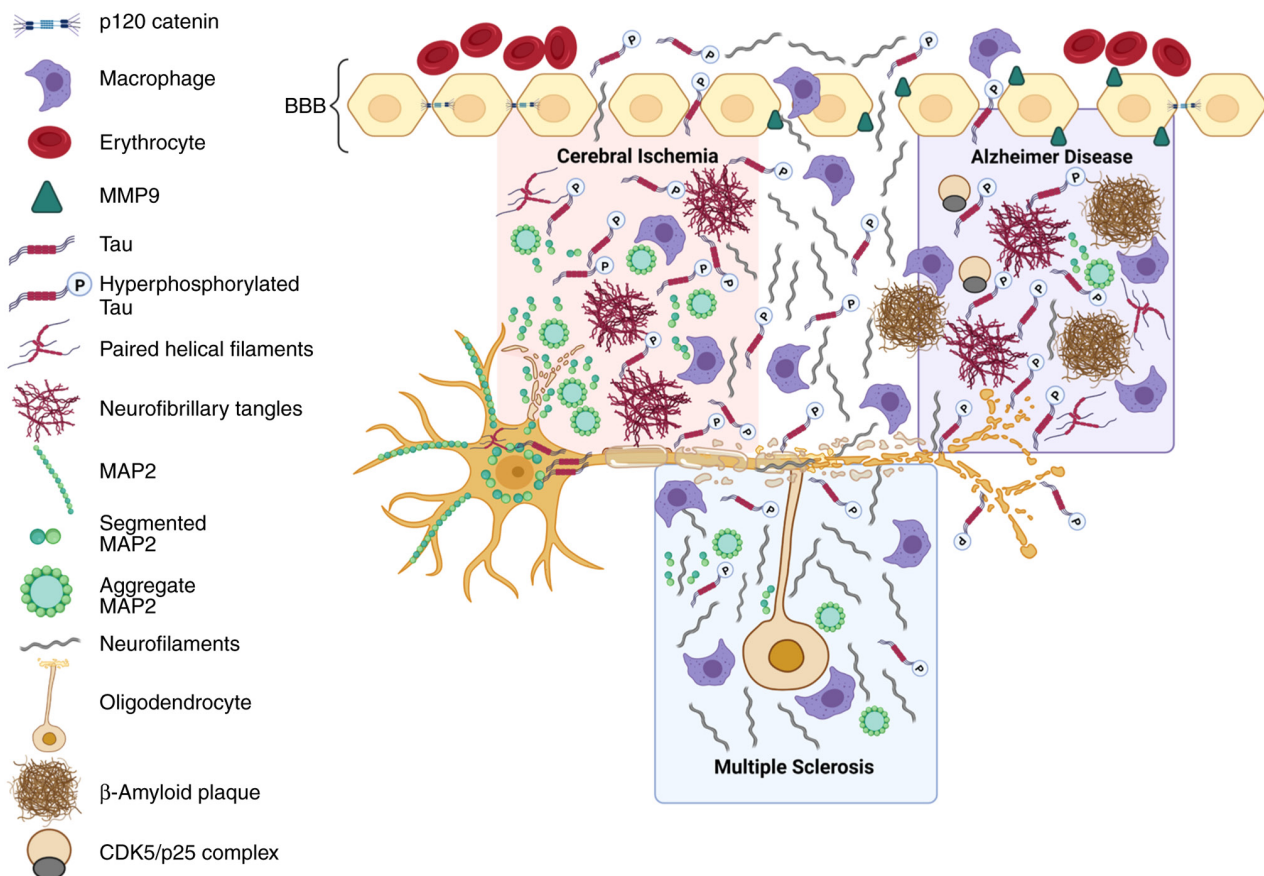
Figure 1. Cytoskeletal alterations in AD, cerebral ischemia and MS. In patients with AD, cerebral ischemia and multiple sclerosis, the tau protein is hyperphosphorylated by different kinases, such as CDK5, which leads to the formation of NFTs in patients with AD and cerebral ischemia. In individuals with AD, these tangles are accompanied by the formation of β amyloid plaques. The MAP2 protein is altered in dendrites, forming aggregates, and it translocates to the neuronal soma in individuals with cerebral ischemia. NFs are also a fundamental part of the neuronal cytoskeleton that are altered in patients with AD, cerebral ischemia and MS; this deterioration of the cytoskeleton and other events typical of neurodegeneration generate an immune response. The disruption of the blood-brain barrier mediated by MMP9 and the alteration of cell adhesion proteins such as p120 catenin allow these cytoskeletal proteins to be detected in peripheral blood. AD, Alzheimer's disease; MS, multiple sclerosis; CDK5, cyclin-dependent kinase; NFT, neurofibrillary tangle; NF, neurofilament; MMP9, matrix metalloproteinase 9; MAP2, microtubule-associated protein 2; p-, phospho.

as BACE1, participate in tau hyperphosphorylation as part of the cellular and molecular machinery that exerts a synergistic effect on the development of histopathological markers of the disease (35,36).

One of the main pathways for degradation of hyperphosphorylated tau is autophagy, which is a selective cellular catabolic process by which cytoplasmic material is transported from the cytoskeleton to lysosomes for degradation (37). This mechanism functions at basal levels in all cells and is required to maintain homeostasis (38). This process is particularly important in neurons, since they undergo cell division only at a low rate, and therefore must survive throughout the lifespan of the organism through the exchange of proteins and organelles (39). In neurodegenerative diseases such as AD, the cytoskeleton plays a key role in the degradation of PHFs, and when cytoskeletal integrity is compromised, PHFs are more likely to aggregate and accumulate (40). The current body of literature show an increase in autophagy in the early stages of AD in the 3xTg-AD model, which loses its effect as the pathological process progresses (41). The regulation of CDK5 and BACE1 and the effects on the cytoskeleton restore the degradation of the transgenically expressed tau protein and promote the functional recovery of the brain (23,35).

The contribution of other MT-associated proteins, such as MAP2, MAP1B and MAP1A, in the pathogenesis and progression of AD may vary as they generate different MT dynamics dependent on their location in the models described, despite sharing similar sequences and functions in stabilizing MTs with the tau protein (42). Unlike the tau protein, which is abundantly distributed in the axonal compartment, MAP2 is located exclusively in the somatodendritic compartment, whereas MAP1B and MAP1A are located in both compartments (43). Likewise, MAP2, MAP1B and MAP1A undergo hyperphosphorylation but fail to form filaments, as does the tau protein (44). In turn, a decrease in MAP2 levels or an increase in the levels of the soluble protein due to abnormal phosphorylation have been suggested to trigger neurotoxic processes (22). These changes are related to the loss of neuronal connectivity induced by amyloid β, tauopathy and the excitotoxic microenvironment that compromises the dynamics of dendritic spines and postsynaptic compartments (22,45).

Neurofilaments in Alzheimer's disease. NFs are the other type of essential filament involved in the neurodegeneration process; they are the largest component of the neuronal cytoskeleton, and together with the tau protein, they predominate in the

nervous system (40). These filaments are composed of a family of 5 intermediate filaments termed NF heavy (NFH), medium (NFM) and light (NFL) chains, and α-internexin and peripherin (46). The assembly of NFs is essential for the growth and stability of axons in both the central and peripheral nervous systems (47). Its functions are broad and mainly mediate the stabilization of the microtubule content and interactions with organelles such as the mitochondria (48). In AD and other neurodegenerative processes, NFs have been described as a common biomarker that reflects the changes in the neuronal cytoskeleton and the progression of the neurodegenerative process that underlies the pathology (48).

NFs have been detected in both cerebrospinal fluid (CSF) and peripheral blood (SP) (49). In a cohort of 1,070 PSEN1 E280A mutation carriers and 1,074 noncarriers with baseline assessments and 242 mutation carriers and 262 noncarriers with longitudinal (6±3 years) measures, ranging in age from 8 to 75 years, plasma NFL levels increased with age in both groups and began to differentiate carriers from noncarriers at age 22 (22 years before the estimated median age of mild cognitive impairment) (50,51). This biomarker is released in to the CSF and SP as the inflammatory process and gliosis progress in the white matter, and has been correlated with cortical and hippocampal atrophy, widening of the ventricles, memory impairment and mild cognitive impairment (52,53). Although NFs have proline-directed phosphorylation sites and are identified in NFTs as one of the proteins that accumulate with PHFs, their contribution to aggregate formation has not been clarified (24). Axonal damage associated with MTs and synaptic compromise are common pathologies such as AD, Down syndrome, frontotemporal dementia, amyotrophic lateral sclerosis, dementia with Lewy bodies, progressive supra-nuclear palsy, and cortico-basal syndrome; along with the aging process, they convert NFs into biomarkers of CNS and peripheral involvement (54-56).

4. Cerebral ischemia

Cerebral ischemia is a disorder that affects the brain tissue and is characterized mainly by the disrupted supply of oxygen and other nutrients, which leads to tissue death (57). This pathology triggers cognitive impairment or dementia, conditions that include alterations in learning, memory and functions needed to perform basic activities of daily life (58). Within the pathophysiology of the disease, changes in the cytoskeleton that reflect neuronal damage have been identified (59).

MAP2 and tau in cerebral ischemia. Numerous antibodies have been used in experimental models to identify the damage and chronological changes in cytoskeletal proteins, including antibodies against microtubule proteins such as MAP2, a marker of cytoskeletal disruption; in fact, studies have documented a correlation of MAP2 expression with prolonged postischemic periods (60-62). In animal models, immunohistochemistry and immunofluorescence techniques revealed the loss or discontinuity of MAP2 immunoreactivity in lesions. The discontinuity reflects the fragmentation of dendrites. The translocation of this protein to neuronal cells has also been reported (63-65) (Fig. 1). Taken together, these data reveal the changes in dendrites that lead to cell death in areas of both the ischemic focus and in regions of the ischemic penumbra, as well as the loss of synaptic plasticity in regions further from the ischemic focus (65). The loss of MAP2 may contribute to the initial phase of neuronal dysfunction, and dendritic degradation may be the first sign of neurodegeneration 1 h after cerebral ischemia (66). In patients who have suffered cerebral ischemia, a decrease in MAP2 immunoreactivity has been observed in the motor (area 4), temporal (area 21), frontal (area 10) and visual (area 17) cortices in the left hemisphere; additionally, in all the areas studied, the most significant decrease in MAP2 expression was detected in cortical layers II-III compared to cortical layers V-VII. The maximum reduction in MAP2-positive pyramidal neurons was observed in cortical layers II-III of the motor cortex after 1 year of survival after cerebral infarction (67). Based on this finding, markers of the cytoskeleton are sensitive to the deterioration that occurs long after cerebral infarction.

Another protein involved in microtubule disruption is the tau protein. The hyperphosphorylation of the tau protein is associated with the development of dementia in the late postischemia phase. Its roles in initiating synaptic and cognitive dysfunction, in addition to neuronal toxicity and neurodegeneration, have been described (68). Experimental animals subjected to cerebral ischemia present disruptions in memory and learning processes accompanied by tau hyperphosphorylation, the formation of PHFs and changes in the immunoreactivity of the MAP2 protein (69).

The relationship between the CA1 region of the hippocampus and the expression of the tau gene has been established after transient global cerebral ischemia in rats with a survival period of 2, 7 and 30 days (70). In the hippocampal CA1 region, the expression of the tau gene increased to a maximum 3.3-fold change on the second day after cerebral ischemia. A total of 7 days after ischemic episodes, the expression ranged from 0.2 to 0.5 times the base value (70). On the 30th day of survival after ischemic injury, the expression of the tau protein gene decreased to 0.4 times that of the base value (70). Studies have shown that tau protein phosphorylation patterns differ depending on the models of cerebral ischemia. After ischemia and global brain recirculation, the tau protein is phosphorylated and slowly accumulates (71). Transient focal cerebral ischemia with 1 day of reperfusion induces local hyperphosphorylation (regions of injury) of the tau protein (72,73). Current research indicates that after ischemia, hyperphosphorylated tau protein accumulates in cortical neuronal cells and is accompanied by apoptosis (72,74). Additionally, hyperphosphorylation of tau leads to the formation of NFTs 24 h postinfarction in regions such as the motor, sensory and hippocampal cortex. These tangles persist in these same regions for up to 1 month after infarction, and lead to learning and memory deficits in the affected animals (69).

In clinical studies, an increase in total tau levels in human CSF and blood has been reported after brain injury, including ischemic stroke (75-78). Measurable tau has been detected in serum within 6 h after the onset of ischemic symptoms (78). The concentration may peak after 3-5 days (78) or later (79). Furthermore, a significant correlation between serum tau levels and the severity of the clinical deficit or disability evaluated using the Barthel index (BI) was not observed. However, serum tau levels correlate with infarct volumes (7-48 ml) and

functional results at 90 days postischemia (78). These findings are consistent with other studies indicating that the absence of serum tau during the acute phase (<24 h) of ischemia might predict good clinical outcomes 90 days after stroke (80). Patients in whom tau was detected in serum had more severe neurological deficits and poorer functional outcomes than patients without tau (81). However, other researchers found that tau protein levels are correlated with neurological deficit (BI) scores after 48 h. Additionally, serum tau levels did not have a significant correlation with the etiology of stroke, as represented by the TOAST criteria (82). A prospective study revealed that tau levels in both plasma and CSF were closely related not only to stroke severity assessed using the National Institutes of Health Stroke Scale but also to long-term outcomes (83). In particular, the study of autopsy specimens of the brains from patients with cerebral infarction showed an increase in tau immunoreactivity and tau deposition in the ischemic area (73,84). However, the tau protein has been detected in the serum of ~40% of patients with stroke (78,79). Some researchers propose that tau appears in the blood due to disruption of the blood-brain barrier (BBB). Some factors, such as matrix metalloproteinase 9 (MMP9), may play a key role in the release of tau into the circulation (79) (Fig. 1).

In addition to BBB damage after stroke, another major cause of persistent disability after stroke is neuroaxonal damage, which is crucial for the functional outcomes and long-term survival of patients with stroke. Predicting functional outcomes after ischemic stroke is very important for patients and clinicians in terms of allocating healthcare resources and optimizing patient care. Furthermore, the amount of acute neuroaxonal damage reflected by the infarct nucleus guides patient selection for stroke therapies, e.g., endovascular thrombectomy beyond the 6-h time window. To date, only diffusion magnetic resonance imaging (MRI) and computed tomography (CT) perfusion approaches serve to assess the core of the infarct; therefore, blood biomarkers are urgently needed to guide individualized treatments of patients. NFs may be a suitable candidate for this purpose as they are part of the neuronal cytoskeleton, are exclusively expressed in neurons (85), and show encouraging results, as described further below.

Neurofilaments in cerebral ischemia. After the occlusion of a cerebral artery and subsequent neuroaxonal damage, the NF protein is released into the CSF and to a lesser extent in SP. Ultrasensitive assays with the single molecule matrix method facilitate the highly sensitive quantification of NF levels in blood (86). Due to the ease and wide applicability, research on NFs in various diseases, such as MS and other neurodegenerative diseases, including cerebral infarction, is emerging (16). In a study conducted by Peters *et al* (87), where they evaluated 503 patients with small vessel disease (SVD), NFs were associated with the presence of lacunae and microbleeds and with MRI imaging markers related to SVD. In addition, NF was associated with gap incidents during patient follow-up, as well as with future cognitive decline after adjustment for age, sex, education and depression. The risk of dementia increased with higher NF levels. Therefore, NFs were proposed as an emerging blood biomarker for neuroaxonal damage in various neurological diseases affecting the elderly, including

small vessel neurodegenerative and cerebral disease (88,89). Thus, cerebrovascular diseases appear to be a major vascular contributor to dementia (88-90).

In 2019, Timo Uphaus and colleagues were the first to show that NF is a valuable biomarker for functional independence at 90 days after ischemic stroke and predicts long-term cardiovascular outcomes (85). Accordingly, NF may be useful in selecting patients at high risk of future cardiovascular events. The superiority of NF over other existing biomarkers was also shown with respect to its predictive value for functional outcomes and cardiovascular survival after ischemic stroke (85). However, studies examining larger patient cohorts are required to confirm the results and to change current clinical practice, since NF may be used in clinical practice as a screening biomarker to select patients at high risk of accident occurrence, recurrent cerebrovascular disease and those more susceptible to death following cerebral ischemia.

5. Multiple sclerosis

MS is a chronic demyelinating disease of the central nervous system (CNS). Although its etiology remains unknown, immune cells are generally accepted to invade the CNS, where demyelination occurs. This process is attributed to the migration of autoreactive T lymphocytes from the periphery toward the CNS with the capacity to cross the BBB and progressively compromise brain function (91) (Fig. 1). The clinical manifestation of MS represents the final stage of a process that involves inflammation, demyelination, remyelination and the depletion of oligodendrocytes, astrocytes and neurons along with axonal degeneration (91).

Tau and MAP2 in multiple sclerosis. In the affected part of the CNS, constitutively expressed proteins in the axon, such as tau protein and NFs, can be detected using antibodies (Fig. 1). CSF tau protein levels are associated with axonal injury, are elevated in patients with MS and correlate with the progression of disability (75,92). In serum samples from patients with MS collected before and after autologous hematopoietic stem cell transplantation, tau protein levels were increased 3 months after transplantation; this observation may reflect brain atrophy induced by chemotherapy-mediated toxicity (88). In a case-controlled study of 30 healthy women and 30 women with MS, the levels of the total tau protein in serum and saliva were analyzed. Total tau protein was level in the serum of patients with MS compared with the control group; therefore, tau represents a potential biomarker for MS. However, the levels of tau protein in saliva was not a suitable biomarker for the detection of MS (93). Some MS studies have sought to investigate the mechanisms of remyelination by analyzing the MAP2 protein levels in brain lesions affected by MS using immunocytochemistry and a series of specific monoclonal antibodies. MAP2 expression was increased in the brains of patients with MS (94). MAP2 was expressed in the regenerating oligodendrocytes associated with demyelinated lesions, with the highest levels detected in regions with extensive remyelination. Using electron microscopy, MAP2 was shown to be located in oligodendrocytes involved in remyelination, as evidenced by the extension of its process and association with finely myelinated (remyelinated) axons (94).

Neurofilaments in multiple sclerosis. NF, a cytoskeletal protein that has been most closely associated with axonal deterioration in MS, has been used as a biomarker for axonal degeneration and may be used to predict the neurological outcomes of patients (48,86). Following axonal damage in the CNS, NF proteins released into the CSF reflect the degree of axonal damage and neuronal death. Since neurofilaments are located in the cytoplasm of neurons, all diseases that lead to neuronal and axonal damage potentially increase the levels of these proteins in the CSF (86). In fact, NFs have been evaluated both in clinical studies and in *in vivo* models that simulate the degenerative processes of MS, where the most commonly used model is the experimental autoimmune encephalitis (EAE) model. In this model, the mean concentration of NFL is higher in the supernatant and brain pellet of rats with all EAE subtypes compared to samples collected from controls (95). Furthermore, NFM and NFH levels change in the later phases of the disease. Therefore, the NFL biomarker may reflect acute axonal damage mediated by inflammatory mechanisms, and may have prognostic value for the evaluation of MS, while NFM and NFH may be better indicators of nerve regeneration after acute inflammation (95).

In humans, NFs were used for the first time as markers of neuronal damage in a study of 12 patients with amyotrophic lateral sclerosis and 11 patients with AD (96). Subsequently, CSF NF levels were higher in 60 patients with relapsing-remitting multiple sclerosis than in the control subjects (97), suggesting that these proteins may serve as a biomarker of the disease activity of MS.

NFs have been associated with the progression of disability in patients with MS (74), and according to studies comparing the serum and CSF levels of NFs, they are strongly correlated (83). An ultrasensitive technique called single molecule arrays (SIMOA) has been developed recently to detect NF levels in blood, enabling, for the first time, the detection of NF levels in serum. Compared to detection using ELISA or ECL-based assays, SIMOA has >25-fold higher analytical sensitivity (SIMOA: 0.62 pg/ml, ECL: 15.6 pg/ml, ELISA: 78, 0 pg/ml) (98).

NFL is associated with axonal damage, while NFH is associated with the progression of disability (87,99). In some of the population based studies, NFL levels were shown to be associated with an increased risk of progressive phenotypes (16), and they have also been useful in diagnosing the pathology; when NFL levels were compared between patients with MS and controls, where the values between the two groups were different, they were higher in patients with MS (100).

A recent meta-analysis found that patients with MS present with axonal injury (101), which may explain the neurodegeneration caused by the disease and its effect on NF levels, especially in the early stages of the disease. The cause of axonal loss is not yet known, but a destructive process directed against components of the axonal cytoskeleton appears to contribute to the progression of the disability (99). Serum NF levels also correlate with MRI activity, degree of disability and rate of brain atrophy (102,103). Furthermore, NFs are also suitable as a prognostic biomarker for the conversion of clinically isolated syndrome (CIS) to MS (104,105). A recent study showed the prognostic importance of serum NF levels in the conversion of isolated radiological syndrome to CIS (106).

The serum concentration of NFL, which does not require a lumbar puncture, appears to correlate with several clinical and magnetic tomographic features of MS (107,108). Therefore, it may conceivably be established as a prognostic biomarker in clinical practice in the future.

6. Conclusions

Cytoskeletal damage, including disruptions to microtubule stability, NFs and axonal transport, have been characterized in several unrelated neurodegenerative conditions. Although no direct link has been established between the different pathologies addressed in this review, common cytoskeletal players have been shown to contribute to deterioration, suggesting that defects in the organization of the cytoskeleton are a common feature that contributes to neurodegeneration. Findings from the *in vitro* and *in vivo* experimental models discussed in this review show that the tau protein, MAP2 and NFs are essential for maintaining the stability of the cytoskeleton, and that their dysregulation triggered by anoxia (cerebral ischemia), the Aβ peptide (AD) and demyelination (MS) leads to the disassembly of MAP2 and NFs, hyperphosphorylation and tau accumulation. These proteins are subsequently released into the SP after the disruption of the BBB through the actions of metalloproteases such as MMP9 or the post-traumatic inflammatory response (Fig. 1). The latter suggests that cytoskeletal components detected in the SP are promising protein targets that may facilitate the diagnosis and progression of cerebral ischemia, AD and MS in a practical and safe manner, as reported in several clinical studies discussed in the review. However, more studies are required to correlate the clinical manifestations presented by patients with these pathologies.

Availability of data and materials

Not applicable.

Authors' contributions

JAGV, JFCA, JFZB, KAR and AAN wrote and revised the current review. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Fletcher DA and Mullins RD: Cell mechanics and the cytoskeleton. Nature 463: 485-492, 2010.
2. Rottner K, Faix J, Bogdan S, Linder S and Kerkhoff E: Actin assembly mechanisms at a glance. J Cell Sci 130: 3427-3435, 2017.
3. Jay D, García EJ, Lara JE, Medina MA and de la Luz Ibarra M: Determination of a cAMP-dependent protein kinase phosphorylation site in the C-terminal region of human endothelial actin-binding protein. Arch Biochem Biophys 377: 80-84, 2000.
4. Liem RKH and Messing A: Dysfunctions of neuronal and glial intermediate filaments in disease. J Clin Invest 119: 1814-1824, 2009.
5. Da Silva JS and Dotti CG: Breaking the neuronal sphere: Regulation of the actin cytoskeleton in neuritogenesis. Nat Rev Neurosci 3: 694-704, 2002.
6. Medana IM and Esiri MM: Axonal damage: A key predictor of outcome in human CNS diseases. Brain 126: 515-530, 2003.
7. Goodson HV and Jonasson EM: Microtubules and microtubule-associated proteins. Cold Spring Harb Perspect Biol 10: a022608, 2018.
8. Jellinger KA: Cell death mechanisms in neurodegeneration. J Cell Mol Med 5: 1-17, 2001.
9. Muñoz-Lasso DC, Romá-Mateo C, Pallardó FV and Gonzalez-Cabo P: Much more than a scaffold: Cytoskeletal proteins in neurological disorders. Cells 9: E358, 2020.
10. Henriques AG, Müller T, Oliveira JM, Cova M, da Cruz e Silva CB and da Cruz E Silva OA: Altered protein phosphorylation as a resource for potential AD biomarkers. Sci Rep 6: 30319, 2016.
11. Mietelska-Porowska A, Wasik U, Goras M, Filipek A and Niewiadomska G: Tau protein modifications and interactions: Their role in function and dysfunction. Int J Mol Sci 15: 4671-4713, 2014.
12. McMurray CT: Neurodegeneration: Diseases of the cytoskeleton? Cell Death Differ 7: 861-865, 2000.
13. Guo T, Noble W and Hanger DP: Roles of tau protein in health and disease. Acta Neuropathol 133: 665-704, 2017.
14. Kosik KS, Joachim CL and Selkoe DJ: Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Acta Neuropathol 83: 4044-4048, 1986.
15. Islas-Hernandez A, Aguilar-Talamantes HS, Bertado-Cortes B, Mejia-delCastillo GJ, Carrera-Pineda R, Cuevas-Garcia CF and Garcia-delaTorre P: BDNF and Tau as biomarkers of severity in multiple sclerosis. Biomark Med 12: 717-726, 2018.
16. Siller N, Kuhle J, Muthuraman M, Barro C, Uphaus T, Groppa S, Kappos L, Zipp F and Bittner S: Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. Mult Scler 25: 678-686, 2019.
17. Zetterberg H: Plasma Neurofilament light in progressive multiple sclerosis. Acta Neurol Scand 141: 14-15, 2020.
18. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators: Global, Regional, and national incidence, prevalence, and years lived with disability for 354 Diseases and Injuries for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. Lancet 392: 1789-1858, 2018.
19. Sanchez JS, Hanseeuw BJ, Lopera F, Sperling RA, Baena A, Bocanegra Y, Aguillon D, Guzmán-Vélez E, Pardilla-Delgado E, Ramirez-Gomez L, et al: Longitudinal amyloid and tau accumulation in autosomal dominant Alzheimer's disease: Findings from the Colombia-Boston (COLBOS) biomarker study. Alzheimers Res Ther 13: 27, 2021.
20. Busche MA and Hyman BT: Synergy between amyloid-β and tau in Alzheimer's disease. Nat Neurosci 23: 1183-1193, 2020.
21. Drummond E, Pires G, MacMurray C, Askenazi M, Nayak S, Bourdon M, Safar J, Ueberheide B and Wisniewski T: Phosphorylated tau interactome in the human Alzheimer's disease brain. Brain 143: 2803-2817, 2020.
22. Kandimalla R, Manczak M, Yin X, Wang R and Reddy PH: Hippocampal phosphorylated tau induced cognitive decline, dendritic spine loss and mitochondrial abnormalities in a mouse model of Alzheimer's disease. Hum Mol Genet 27: 30-40, 2018.
23. Castro-Alvarez JF, Uribe-Arias A, Raigoza DM and Cardona-Gómez GP: Cyclin-dependent kinase 5, a node protein in diminished tauopathy: A systems biology approach. Front Aging Neurosci 6: 232, 2014.
24. Rudrabhatla P, Jaffe H and Pant HC: Direct evidence of phosphorylated neuronal intermediate filament proteins in neurofibrillary tangles (NFTs): Phosphoproteomics of Alzheimer's NFTs. FASEB J 25: 3896-3905, 2011.
25. Richetin K, Steullet P, Pachoud M, Perbet R, Parietti E, Maheswaran M, Eddarkaoui S, Bégard S, Pythoud C, Rey M, et al: Tau accumulation in astrocytes of the dentate gyrus induces neuronal dysfunction and memory deficits in Alzheimer's disease. Nat Neurosci 23: 1567-1579, 2020.
26. Ma QL, Zuo X, Yang F, Ubeda OJ, Gant DJ, Alaverdyan M, Kiosea NC, Nazari S, Chen PP, Nothias F, et al: Loss of MAP function leads to hippocampal synapse loss and deficits in the Morris water maze with aging. J Neurosci 34: 7124-7136, 2014.
27. Lopez-Tobón A, Cepeda-Prado E and Cardona-Gómez GP: Decrease of tau hyperphosphorylation by 17β estradiol requires sphingosine kinase in a glutamate toxicity model. Neurochem Res 34: 2206-2214, 2009.
28. Posada-Duque RA, Ramirez O, Härtel S, Inestrosa NC, Bodaleo F, González-Billault C, Kirkwood A and Cardona-Gómez GP: CDK5 downregulation enhances synaptic plasticity. Cell Mol Life Sci 74: 153-172, 2017.
29. Uribe-Arias A, Posada-Duque RA, González-Billault C, Villegas A, Lopera F and Cardona-Gómez GP: p120-catenin is necessary for neuroprotection induced by CDK5 silencing in models of Alzheimer's disease. J Neurochem 138: 624-639, 2016.
30. Martin L, Latypova X, Wilson CM, Magnaudeix A, Perrin ML, Yardin C and Terro F: Tau protein kinases: Involvement in Alzheimer's disease. Ageing Res Rev 12: 289-309, 2013.
31. Reimer L, Betzer C, Kofoed RH, Volbracht C, Fog K, Kurhade C, Nilsson E, Överby AK and Jensen PH: PKR kinase directly regulates tau expression and Alzheimer's disease-related tau phosphorylation. Brain Pathol 31: 103-119, 2021.
32. Posada-Duque RA, López-Tobón A, Piedrahita D, González-Billault C and Cardona-Gómez GP: p35 and Rac1 underlie the neuroprotection and cognitive improvement induced by CDK5 silencing. J Neurochem 134: 354-370, 2015.
33. Zheng YL, Li BS, Kanungo J, Kesavapany S, Amin N, Grant P and Pant HC: Cdk5 modulation of mitogen-activated protein kinase signaling regulates neuronal survival. Mol Biol Cell 18: 404-413, 2007.
34. Cicero S and Herrup K: Cyclin-dependent kinase 5 is essential for neuronal cell cycle arrest and differentiation. J Neurosci 25: 9658-9668, 2005.
35. Piedrahita D, Castro-Alvarez JF, Boudreau RL, Villegas-Lanau A, Kosik KS, Gallego-Gomez JC and Cardona-Gómez GP: β-Secretase 1's targeting reduces hyperphosphorilated tau, implying autophagy actors in 3xTg-AD mice. Front Cell Neurosci 9: 498, 2016.
36. Choi SH, Kim YH, Hebisch M, Sliwinski C, Lee S, D'Avanzo C, Chen H, Hooli B, Asselin C, Muffat J, et al: A three-dimensional human neural cell culture model of Alzheimer's disease. Nature 515: 274-278, 2014.
37. Choi AMK, Ryter SW and Levine B: Autophagy in human health and disease. N Engl J Med 368: 651-662, 2013.
38. Komatsu M, Qing JW, Holstein GR, Friedrich VL Jr, Iwata J, Kominami E, Chait BT, Tanaka K and Yue Z: Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. Proc Natl Acad Sci USA 104: 14489-14494, 2007.
39. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E and Tanaka K: Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 441: 880-884, 2006.
40. Kast DJ and Dominguez R: The cytoskeleton-autophagy connection. Curr Biol 27: R318-R326, 2017.
41. Villamil Ortiz JG and Cardona Gomez GP: Comparative analysis of autophagy and tauopathy related markers in cerebral ischemia and Alzheimer's disease animal models. Front Aging Neurosci 7: 84, 2015.
42. Mohan R and John A: Microtubule-associated proteins as direct crosslinkers of actin filaments and microtubules. IUBMB Life 67: 395-403, 2015.
43. Xie C, Soeda Y, Shinzaki Y, In Y, Tomoo K, Ihara Y and Miyasaka T: Identification of key amino acids responsible for the distinct aggregation properties of microtubule-associated protein 2 and tau. J Neurochem 135: 19-26, 2015.

44. Xie C, Miyasaka T, Yoshimura S, Hatsuta H, Yoshina S, Kage-Nakadai E, Mitani S, Murayama S and Ihara Y: The homologous carboxyl-terminal domains of microtubule-associated protein 2 and Tau induce neuronal dysfunction and have differential fates in the evolution of neurofibrillary tangles. PLoS One 9: e89796, 2014.

45. Takahashi RH, Capetillo-Zarate E, Lin MT, Milner TA and Gouras GK: Accumulation of Intraneuronal β-Amyloid 42 peptides is associated with early changes in microtubule-associated protein 2 in neurites and synapses. PLoS One 8: e51965, 2013.

46. Yuan A, Sasaki T, Kumar A, Peterhoff CM, Rao MV, Liem RK, Julien JP and Nixon RA: Peripherin is a subunit of peripheral nerve neurofilaments: Implications for differential vulnerability of CNS and peripheral nervous system axons. J Neurosci 32: 8501-8508, 2012.

47. Yuan A, Sershen H, Veeranna, Basavarajappa BS, Kumar A, Hashim A, Berg M, Lee JH, Sato Y, Rao MV, *et al*: Neurofilament subunits are integral components of synapses and modulate neurotransmission and behavior in vivo. Mol Psychiatry 20: 986-994, 2015.

48. Gafson AR, Barthélemy NR, Bomont P, Carare RO, Durham HD, Julien JP, Kuhle J, Leppert D, Nixon RA, Weller RO, *et al*: Neurofilaments: Neurobiological foundations for biomarker applications. Brain 143: 1975-1998, 2020.

49. Barry DM, Stevenson W, Bober BG, Wiese PJ, Dale JM, Barry GS, Byers NS, Strope JD, Chang R, Schulz DJ, *et al*: Expansion of Neurofilament Medium C terminus increases axonal diameter independent of increases in conduction velocity or myelin thickness. J Neurosci 32: 6209-6219, 2012.

50. Guzmán-Vélez E, Zetterberg H, Fox-Fuller JT, Vila-Castelar C, Sanchez JS, Baena A, Garcia-Ospina G, Aguillon D, Pardilla-Delgado E, Gatchel JR, *et al*: Associations between plasma neurofilament light, in vivo brain pathology, and cognition in non-demented individuals with autosomal-dominant Alzheimer's disease. Alzheimers Dement 17: 813-821, 2021.

51. Quiroz YT, Zetterberg H, Reiman EM, Chen Y, Su Y, Fox-Fuller JT, Garcia G, Villegas A, Sepulveda-Falla D, Villada M, *et al*: Plasma neurofilament light chain in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: A cross-sectional and longitudinal cohort study. Lancet Neurol 19: 513-521, 2020.

52. Rajan KB, Aggarwal NT, McAninch EA, Weuve J, Barnes LL, Wilson RS, DeCarli C and Evans DA: Remote blood biomarkers of longitudinal cognitive outcomes in a population study. Ann Neurol 88: 1065-1076, 2020.

53. Walsh P, Sudre CH, Fiford CM, Ryan NS, Lashley T, Frost C and Barnes J; ADNI Investigators: The age-dependent associations of white matter hyperintensities and neurofilament light in early- and late-stage Alzheimer's disease. Neurobiol Aging 97: 10-17, 2021.

54. Delaby C, Alcolea D, Carmona-Iragui M, Illán-Gala I, Morenas-Rodríguez E, Barroeta I, Altuna M, Estellés T, Santos-Santos M, Turon-Sans J, *et al*: Differential levels of Neurofilament Light protein in cerebrospinal fluid in patients with a wide range of neurodegenerative disorders. Sci Rep 10: 9161, 2020.

55. Idland AV, Sala-Llonch R, Borza T, Watne LO, Wyller TB, Bræhus A, Zetterberg H, Blennow K, Walhovd KB and Fjell AM: CSF neurofilament light levels predict hippocampal atrophy in cognitively healthy older adults. Neurobiol Aging 49: 138-144, 2017.

56. Henson RL, Doran E, Christian BT, Handen BL, Klunk WE, Lai F, Lee JH, Rosas HD, Schupf N, Zaman SH, *et al*: Cerebrospinal fluid biomarkers of Alzheimer's disease in a cohort of adults with Down syndrome. Alzheimers Dement (Amst) 12: e12057, 2020.

57. Sveinsson OA, Kjartansson O and Valdimarsson EM: Cerebral ischemia/infarction-epidemiology, causes and symptoms. Laeknabladid 100: 271-279, 2014 (In Icelandic).

58. Cao L, Tan L, Wang HF, Jiang T, Zhu XC and Yu JT: Cerebral Microinfarcts and dementia: A systematic review and meta-analysis. Curr Alzheimer Res 14: 802-808, 2017.

59. Pluta R, Januszewski S and Czuczwar SJ: Brain ischemia as a prelude to Alzheimer's disease. Front Aging Neurosci 13: 636653, 2021.

60. Yoshimi K, Takeda M, Nishimura T, Kudo T, Nakamura Y, Tada K and Iwata N: An immunohistochemical study of MAP2 and clathrin in gerbil hippocampus after cerebral ischemia. Brain Res 560: 149-158, 1991.

61. Vanicky I, Balchen T and Diemer NH: Alterations in MAP2 immunostainability after prolonged complete brain ischaemia in the rat. Neuroreport 7: 161-164, 1995.

62. Mages B, Fuhs T, Aleithe S, Blietz A, Hobusch C, Härtig W, Schob S, Krueger M and Michalski D: The Cytoskeletal Elements MAP2 and NF-L show substantial alterations in different stroke models while elevated serum levels highlight especially MAP2 as a sensitive biomarker in stroke patients. Mol Neurobiol 58: 4051-4069, 2021.

63. Johanna GV, Fredy CA, David VC, Natalia MV, Angel CR and Patricia CG: Rac1 activity changes are associated with neuronal pathology and spatial memory long-term recovery after global cerebral ischemia. Neurochem Int 57: 762-773, 2010.

64. Gutiérrez-Vargas JA, Moreno H and Cardona-Gómez GP: Targeting CDK5 post-stroke provides long-term neuroprotection and rescues synaptic plasticity. J Cereb Blood Flow Metab 37: 2208-2223, 2017.

65. Pérez-Corredor PA, Gutiérrez-Vargas JA, Ciro-Ramírez L, Balcazar N and Cardona-Gómez GP: High fructose diet-induced obesity worsens post-ischemic brain injury in the hippocampus of female rats. Nutr Neurosci: Mar 2, 2020 (Epub ahead of print).

66. Dawson DA and Hallenbeck JM: Acute focal ischemia-induced alterations in MAP2 immunostaining: Description of temporal changes and utilization as a marker for volumetric assessment of acute brain injury. J Cereb Blood Flow Metab 16: 170-174, 1996.

67. Akulinin VA and Dahlstrom A: Quantitative analysis of MAP2 immunoreactivity in human neocortex of three patients surviving after brain ischemia. Neurochem Res 28: 373-378, 2003.

68. Pluta R, Ułamek-Kozioł M, Januszewski S and Czuczwar SJ: Tau protein dysfunction after brain ischemia. J Alzheimers Dis 66: 429-437, 2018.

69. Gutiérrez-Vargas JA, Múnera A and Cardona-Gómez GP: CDK5 knockdown prevents hippocampal degeneration and cognitive dysfunction produced by cerebral ischemia. J Cereb Blood Flow Metab 35: 1937-1949, 2015.

70. Pluta R, Bogucka-Kocka A, Ułamek-Kozioł M, Bogucki J, Januszewski S, Kocki J and Czuczwar SJ: Ischemic tau protein gene induction as an additional key factor driving development of Alzheimer's phenotype changes in CA1 area of hippocampus in an ischemic model of Alzheimer's disease. Pharmacol Rep 70: 881-884, 2018.

71. Mailliot C, Podevin-Dimster V, Rosenthal RE, Sergeant N, Delacourte A, Fiskum G and Buée L: Rapid tau protein dephosphorylation and differential rephosphorylation during cardiac arrest-induced cerebral ischemia and reperfusion. J Cereb Blood Flow Metab 20: 543-549, 2000.

72. Wen Y, Yang S, Liu R and Simpkins JW: Transient cerebral ischemia induces site-specific hyperphosphorylation of tau protein. Brain Res 1022: 30-38, 2004.

73. Uchihara T, Nakamura A, Arai T, Ikeda K and Tsuchiya K: Microglial tau undergoes phosphorylation-independent modification after ischemia. Glia 45: 180-187, 2004.

74. Fujii H, Takahashi T, Mukai T, Tanaka S, Hosomi N, Maruyama H, Sakai N and Matsumoto M: Modifications of tau protein after cerebral ischemia and reperfusion in rats are similar to those occurring in Alzheimer's disease-Hyperphosphorylation and cleavage of 4- and 3-repeat tau. J Cereb Blood Flow Metab 37: 2441-2457, 2017.

75. Shiiya N, Kunihara T, Miyatake T, Matsuzaki K and Yasuda K: Tau protein in the cerebrospinal fluid is a marker of brain injury after aortic surgery. Ann Thorac Surg 77: 2034-2038, 2004.

76. Hesse C, Rosengren L, Andreasen N, Davidsson P, Vanderstichele H, Vanmechelen E and Blennow K: Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. Neurosci Lett 297: 187-190, 2001.

77. Onatsu J, Vanninen R, JÄkÄlÄ P, Mustonen P, Pulkki K, Korhonen M, Hedman M, HÖglund K, Blennow K, Zetterberg H, *et al*: Tau, S100B and NSE as blood biomarkers in acute cerebrovascular events. In Vivo 34: 2577-2586, 2020.

78. Bitsch A, Horn C, Kemmling Y, Seipelt M, Hellenbrand U, Stiefel M, Ciesielczyk B, Cepek L, Bahn E, Ratzka P, *et al*: Serum tau protein level as a marker of axonal damage in acute ischemic stroke. Eur Neurol 47: 45-51, 2002.

79. Kurzepa J, Bielewicz J, Grabarska A, Stelmasiak Z, Stryjecka-Zimmer M and Bartosik-Psujek H: Matrix metalloproteinase-9 contributes to the increase of tau protein in serum during acute ischemic stroke. J Clin Neurosci 17: 997-999, 2010.

80. Lasek-Bal A, Jedrzejowska-Szypulka H, Rozycka J, Bal W, Kowalczyk A, Holecki M, Dulawa J and Lewin-Kowalik J: The presence of Tau protein in blood as a potential prognostic factor in stroke patients. J Physiol Pharmacol 67: 691-696, 2016.

81. Bielewicz J, Kurzepa J, Czekajska-Chehab E, Stelmasiak Z and Bartosik-Psujek H: Does serum Tau protein predict the outcome of patients with ischemic stroke? J Mol Neurosci 43: 241-245, 2011.
82. Wunderlich MT, Lins H, Skalej M, Wallesch CW and Goertler M: Neuron-specific enolase and tau protein as neurobiochemical markers of neuronal damage are related to early clinical course and long-term outcome in acute ischemic stroke. Clin Neurol Neurosurg 108: 558-563, 2006.
83. De Vos A, Bjerke M, Brouns R, De Roeck N, Jacobs D, Van den Abbeele L, Guldolf K, Zetterberg H, Blennow K, Engelborghs S and Vanmechelen E: Neurogranin and tau in cerebrospinal fluid and plasma of patients with acute ischemic stroke. BMC Neurol 17: 170, 2017.
84. Irving EA, Nicoll J, Graham DI and Dewar D: Increased tau immunoreactivity in oligodendrocytes following human stroke and head injury. Neurosci Lett 213: 189-192, 1996.
85. Uphaus T, Bittner S, Gröschel S, Steffen F, Muthuraman M, Wasser K, Weber-Krüger M, Zipp F, Wachter R and Gröschel K: NfL (Neurofilament Light Chain) levels as a predictive marker for long-term outcome after ischemic stroke. Stroke 50: 3077-3084, 2019.
86. Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, Gattringer T, Barro C, Kappos L, Comabella M, Fazekas F, et al: Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol 14: 577-589, 2018.
87. Peters N, van Leijsen E, Tuladhar AM, Barro C, Konieczny MJ, Ewers M, Lyrer P, Engelter ST, Kuhle J, Duering M and de Leeuw FE: Serum Neurofilament light Chain is associated with incident Lacunes in progressive cerebral small vessel disease. J Stroke 22: 369-376, 2020.
88. Duering M, Konieczny MJ, Tiedt S, Baykara E, Tuladhar AM, Leijsen EV, Lyrer P, Engelter ST, Gesierich B, Achmüller M, et al: Serum Neurofilament Light Chain levels are related to small vessel disease burden. J Stroke 20: 228-238, 2018.
89. Paolini Paoletti F, Simoni S, Parnetti L and Gaetani L: The contribution of small vessel disease to neurodegeneration: Focus on Alzheimer's disease, Parkinson's disease and multiple sclerosis. Int J Mol Sci 22: 4958, 2021.
90. Knopman DS: Cerebrovascular disease and dementia. Br J Radiol 80 (Suppl 2): S121-S127, 2007.
91. Dendrou CA, Fugger L and Friese MA: Immunopathology of multiple sclerosis. Nat Rev Immunol 15: 545-558, 2015.
92. Virgilio E, Vecchio D, Crespi I, Serino R, Cantello R, Dianzani U and Comi C: Cerebrospinal Tau levels as a predictor of early disability in multiple sclerosis. Mult Scler Relat Disord 56: 103231, 2021.
93. Mirzaii-Dizgah MH, Mirzaii-Dizgah MR and Mirzaii-Dizgah I: Serum and saliva total tau protein as a marker for relapsing-remitting multiple sclerosis. Med Hypotheses 135: 109476, 2020.
94. Shafit-Zagardo B, Kress Y, Zhao ML and Lee SC: A novel microtubule-associated protein-2 expressed in oligodendrocytes in multiple sclerosis lesions. J Neurochem 73: 2531-2537, 1999.
95. Wang P, Jiang LL, Wang C, Zhu Z and Lai C: Neurofilament protein as a potential biomarker of axonal degeneration in experimental autoimmune encephalomyelitis. Neurol India 68: 364-367, 2020.

96. Rosengren LE, Karlsson JE, Karlsson JO, Persson LI and Wikkelsø C: Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J Neurochem 67: 2013-2018, 1996.
97. Lycke JN, Karlsson JE, Andersen O and Rosengren LE: Neurofilament protein in cerebrospinal fluid: A potential marker of activity in multiple sclerosis. J Neurol Neurosurg Psychiatry 64: 402-404, 1998.
98. Kuhle J, Barro C, Andreasson U, Derfuss T, Lindberg R, Sandelius Å, Liman V, Norgren N, Blennow K and Zetterberg H: Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electro-chemiluminescence immunoassay and Simoa. Clin Chem Lab Med 54: 1655-1661, 2016.
99. Varhaug KN, Torkildsen Ø, Myhr KM and Vedeler CA: Neurofilament light Chain as a biomarker in multiple sclerosis. Front Neurol 10: 338, 2019.
100. Norgren N, Rosengren L and Stigbrand T: Elevated neurofilament levels in neurological diseases. Brain Res 987: 25-31, 2003.
101. Cai L and Huang J: Neurofilament light chain as a biological marker for multiple sclerosis: A meta-analysis study. Neuropsychiatr Dis Treat 14: 2241-2254, 2018.
102. Disanto G, Barro C, Benkert P, Naegelin Y, Schädelin S, Giardiello A, Zecca C, Blennow K, Zetterberg H, Leppert D, et al: Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol 81: 857-870, 2017.
103. Barro C, Benkert P, Disanto G, Tsagkas C, Amann M, Naegelin Y, Leppert D, Gobbi C, Granziera C, Yaldizli Ö, et al: Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain 141: 2382-2391, 2018.
104. Arrambide G, Espejo C, Eixarch H, Villar LM, Alvarez-Cermeño JC, Picón C, Kuhle J, Disanto G, Kappos L, Sastre-Garriga J, et al: Neurofilament light chain level is a weak risk factor for the development of MS. Neurology 87: 1076-1084, 2016.
105. Comabella M and Montalban X: Body fluid biomarkers in multiple sclerosis. Lancet Neurol 13: 113-126, 2014.
106. Matute-Blanch C, Villar LM, Álvarez-Cermeño JC, Rejdak K, Evdoshenko E, Makshakov G, Nazarov V, Lapin S, Midaglia L, Vidal-Jordana A, et al: Neurofilament light chain and oligo-clonal bands are prognostic biomarkers in radiologically isolated syndrome. Brain 141: 1085-1093, 2018.
107. Khalil M: Are neurofilaments valuable biomarkers for long-term disease prognostication in MS? Mult Scler 24: 1270-1271, 2018.
108. Giovannoni G: Peripheral blood neurofilament light chain levels: The neurologist's C-reactive protein? Brain 141: 2235-2237, 2018.