

# 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  $\alpha$  and  $\beta$ -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- $\mu$ m 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 anterograde 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 A $\beta$  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  $\beta$  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  $\beta$  amyloid, such

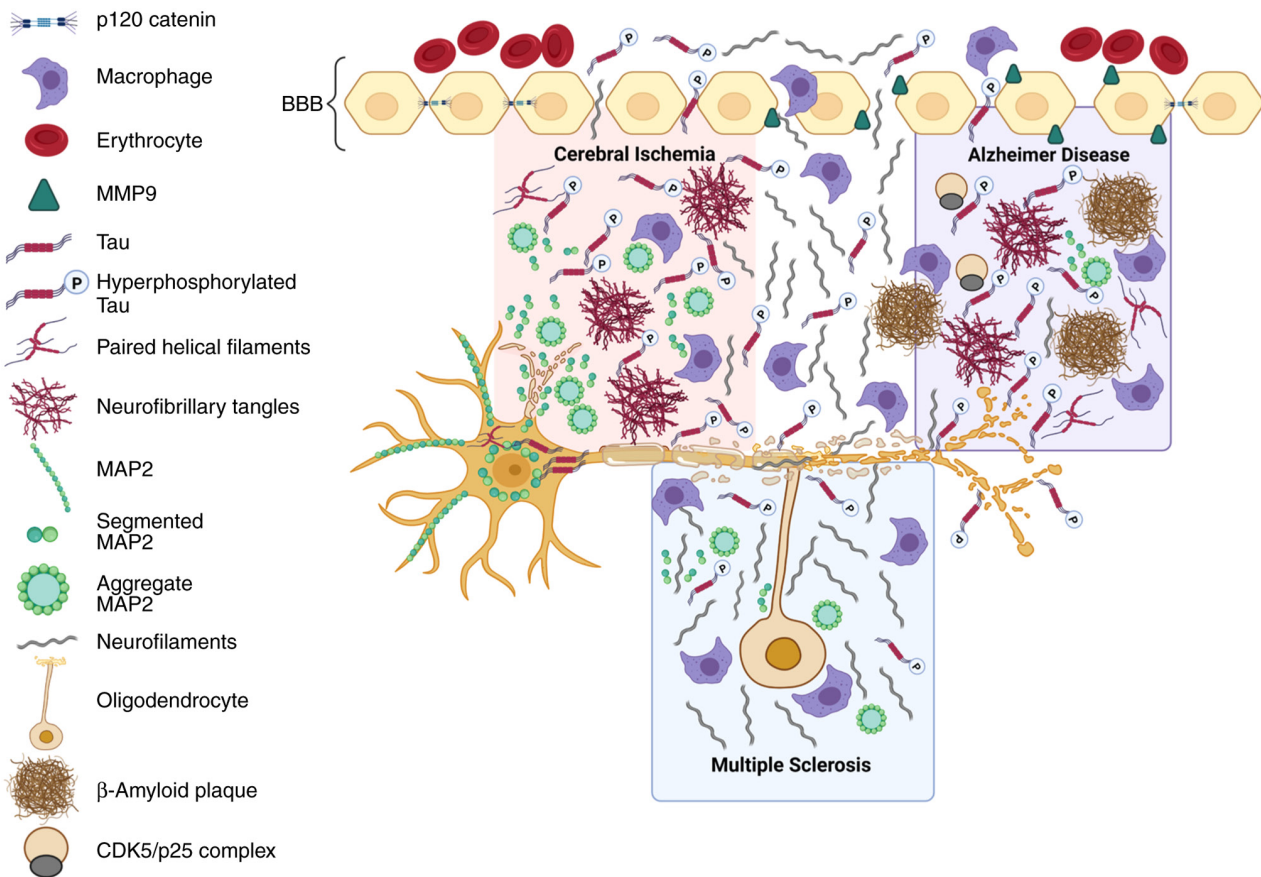


Figure 1. Cytoskeletal alterations in AD, cerebral ischemia and MS. In patients with AD, cerebral ischemia and multiple sclerosis, the tau protein is hyperphosphorylated by different kinases, such as CDK5, which leads to the formation of NFTs in patients with AD and cerebral ischemia. In individuals with AD, these tangles are accompanied by the formation of  $\beta$  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  $\beta$ , 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  $\alpha$ -interneixin 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 ( $6\pm 3$  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 A $\beta$  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.

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## 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.

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