The retinal nerve fiber layer as a window to the glymphatic system

Wostyn, Peter; De Deyn, Peter Paul

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Full names:
Peter Wostyn, MD[a,*], Peter Paul De Deyn, MD, PhD[b,c,d]

Affiliations:
[a]Department of Psychiatry, PC Sint-Amandus, Reigerlostraat 10, 8730 Beernem, Belgium
[b]Department of Biomedical Sciences, Laboratory of Neurochemistry and Behavior, Institute Born-Bunge, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium
[c]Department of Neurology and Alzheimer Research Center, University of Groningen and University Medical Center Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands
[d]Department of Neurology and Memory Clinic, Middelheim General Hospital (ZNA), Lindendreef 1, 2020 Antwerp, Belgium

***Corresponding author:**
Peter Wostyn
Department of Psychiatry, PC Sint-Amandus, Reigerlostraat 10, 8730 Beernem, Belgium
Phone: 32-472713719
Fax: 32-50-819720
E-mail address: wostyn.peter@skynet.be

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Dear Editor,

The retinal nerve fiber layer (RNFL), the innermost layer of the retina, is comprised of unmyelinated axons originating from the retinal ganglion cells (RGCs) that converge to the optic disc, cross the lamina cribrosa at the optic nerve head, and form the optic nerve [1]. In a previous article published in *Clinical Neurology and Neurosurgery*, Kesler et al. [2], using optical coherence tomography (OCT), demonstrated a significant thinning of the RNFL both in patients with mild cognitive impairment and in those with Alzheimer's disease (AD) compared with control subjects. These findings have been confirmed by other studies [3], and it seems that the RNFL loss in AD patients may be localized preferentially to the superior and inferior quadrants, mimicking the pattern described in glaucoma [1]. Interestingly, RNFL thinning has also been demonstrated in other neurodegenerative disorders such as Parkinson's disease, Huntington's disease, frontotemporal dementia, and amyotrophic lateral sclerosis [1,4,5]. Such neurodegenerative proteinopathies are characterized by the accumulation of aberrantly processed and misfolded proteins, such as beta-amyloid (Aβ), tau, alpha-synuclein, transactive response DNA-binding protein 43 and huntingtin, that lose their physiological roles, aggregate and acquire neurotoxic properties [6]. Defective protein clearance plays a crucial role in their accumulation and spread [6].

The frequent and consistent finding of RNFL thinning in several neurodegenerative diseases emphasizes the close relationship between this retinal layer and the brain. Embryologically, the retina and optic nerve extend from the diencephalon, and share many features with the brain in terms of structural and pathogenic pathways [7,8]. Therefore, pathological changes in the retina and optic nerve may shed light on the mechanisms underlying neurodegenerative

diseases. On the basis of the evidence described below, we propose that RNFL thinning in neurodegenerative disorders, especially those associated with protein accumulation, may be explained, at least in part, by the increasing role attributed to the glymphatic system in the pathogenesis of these diseases.

In 2012, a team of researchers headed by Iliff and Nedergaard [9] demonstrated the existence of a brain-wide paravascular pathway along which a large proportion of subarachnoid cerebrospinal fluid (CSF) recirculates through the brain parenchyma, facilitating the clearance of interstitial solutes, including Aβ, from the brain. Within this so-called "glymphatic system", CSF enters the brain along para-arterial channels to exchange with interstitial fluid (ISF), which is in turn cleared from the brain along paravenous pathways [9]. Glymphatic pathway function is mediated by aquaporin-4 (AQP4) water channels, which are localized to perivascular astrocytic endfeet ensheathing the cerebral vasculature [9]. AQP4 gene deletion in mice has been shown to result in markedly impaired Aβ clearance [9]. Glymphatic activity decreases sharply during aging [10,11]. In the aging rodent brain, widespread loss of perivascular AQP4 polarization along the penetrating arteries accompanied the decline in CSF-ISF exchange [11]. Furthermore, impairment of the glymphatic system has been shown in animal models of AD and in AD patients [12,13]. Glymphatic system dysfunction has also been proposed to play a role in other neurodegenerative disorders such as Parkinson's disease, Huntington's disease, frontotemporal dementia, and amyotrophic lateral sclerosis [6,14,15]. Intriguingly, a recent study revealed that dysfunctions of the glymphatic clearance are involved in the early pathological processes of the A53T alpha-synuclein mouse model of Parkinson's disease [15]. The decrease of the glymphatic clearance was mainly due to AQP4 mislocalization [15]. This study further demonstrated that AQP4 deletion impairs clearance of interstitial alpha-synuclein from the brain parenchyma [15].

It should be noted, however, that several aspects of the glymphatic hypothesis are still controversial, including whether fluid transport in brain parenchyma is propagated by convective flow or diffusion [16]. The results of a recent study by Smith et al. [17] did not support the glymphatic clearance mechanism proposed by Iliff and colleagues in which transfer of solutes from CSF to ISF requires AQP4-dependent convection in brain parenchyma. Instead, their data suggested that fluid movement occurs exclusively via diffusion in the extracellular space, with a component of convective flow present only in the paravascular spaces [17]. In humans, intrathecal contrast agent flows deep into the brain parenchyma achieving distances that exceed simple diffusion, suggesting that convective flow is also an important driver of fluid movement within the human brain [18]. In conclusion, there seems to be agreement that transport in grey matter is best described by non-directional, parenchymal diffusion coupled to fast solute transport in the paravascular spaces [16].

Importantly, a rapidly evolving literature also suggests the existence of an "ocular glymphatic system" that extends to the optic nerve and retina [19-24]. The presence of a glymphatic pathway in the optic nerve was first proposed in our hypothesis paper published in 2015 [19]. To investigate the possibility of a paravascular circulation in the human optic nerve, we examined cross-sections of human optic nerves by light microscopy after postmortem administration of India ink into the subarachnoid space of the optic nerve [21,22]. The study demonstrated a very striking accumulation of India ink in paravascular spaces of the optic nerve [21,22]. More recently, Mathieu et al. [23] provided the first evidence to support the existence of a glymphatic pathway in the optic nerve following tracer injection into the CSF of live mice. Their findings built on early research in which tracers injected into the CSF were found diffusely throughout the optic nerve [25-28]. These studies were conducted in rabbits, cats, dogs, guinea pigs, and rhesus monkeys. The route of entry was either not described or assumed to be free diffusion from the subarachnoid space. The findings of the study by

Mathieu et al. [23] indicated that CSF enters the optic nerve via spaces surrounding blood vessels, bordered by AQP4-positive astrocytic endfeet. Jacobsen et al. [24] very recently performed a magnetic resonance imaging study of human visual pathway structures following intrathecal administration of gadobutrol serving as a CSF tracer. CSF tracer enrichment was found within the optic nerve, optic chiasm, optic tract, and primary visual cortex. Based on their observations, the authors hypothesized the existence of a glymphatic system in the human visual pathway. However, as visual pathway structures lie in close proximity to the CSF, the authors could not rule out diffusion of gadobutrol from CSF. Mathieu et al. [29] further demonstrated that CSF entry into the optic nerve subarachnoid space and optic nerve paravascular spaces is impeded in a mouse model of glaucoma. The results of this study seem to support the glymphatic hypothesis of glaucoma, which was initially postulated by our group [19].

As the ocular glymphatic system may be critical for the maintenance of normal optic nerve and eye functioning, it is reasonable to suggest that a deficient passage of fluids through these pathways may induce several kinds of ocular dysfunction, such as RGC loss and RNFL thinning. This may be even more striking in neurodegenerative proteinopathies, in which the RNFL thickness may reflect the degree of neurotoxic protein burden. From this point of view, RNFL thinning might be of diagnostic value to detect a disturbance of CSF and glymphatic circulation associated with neurodegenerative diseases. Given that a possible CSF outflow route along the optic nerve into lymphatic vessels of the dura mater or orbit has long been known [29-31], a decline in this CSF lymphatic outflow might also contribute to RNFL thinning. Obviously, an increased protein burden may also result from several other clearance pathways that may be compromised in neurodegenerative disorders.

In favor of the above hypothesis, a new study by Song et al. [32] demonstrated the importance of AQP4 water channels for retinal and optic nerve health. This study revealed that deletion of

liver X receptor β from the mouse genome resulted in loss of RGCs, reduced RNFL, and accumulation of Aβ in the retina, which was preceded by loss of AQP4 expression and microglial activation in the optic nerve. The authors concluded that the loss of RGCs was secondary to optic nerve degeneration and that optic neuritis in these mice was caused by loss of AQP4 expression. Given that the AQP4 water channel is a characteristic feature of the glymphatic system, we believe reduced AQP4-mediated glymphatic system clearance function could be one contributing factor in explaining the findings of this study.

In conclusion, based on the above findings, we propose that RNFL thinning in neurodegenerative proteinopathies might serve as an ocular biomarker of glymphatic system dysfunction. If confirmed, non-invasive ocular imaging technologies, such as OCT, could be used to assess glymphatic pathway function.

Sincerely yours,

Peter Wostyn, MD

Peter Paul De Deyn, MD, PhD

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