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REVIEW

How strong is the relationship between glaucoma, the retinal nerve fibre layer, and neurodegenerative diseases such as Alzheimer's disease and multiple sclerosis?

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Abstract

Glaucoma is a neurodegenerative disorder with established relationships with ocular structures such as the retinal nerve fibre layer (RNFL) and the ganglion cell layer (GCL). Ocular imaging techniques such as optical coherence tomography (OCT) allow for quantitative measurement of these structures. OCT has been used in the monitoring of glaucoma, as well as investigating other neurodegenerative conditions such as Alzheimer's disease (AD) and multiple sclerosis (MS). In this review, we highlight the association between these disorders and ocular structures (RNFL and GCL), examining their usefulness as biomarkers of neurodegeneration. The average RNFL thickness loss in patients with AD is 11 μm, and 7 μm in MS patients. Most of the studies investigating these changes are cross-sectional. Further longitudinal studies are required to assess sensitivity and specificity of these potential ocular biomarkers to neurodegenerative disease progression.
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Introduction

Glaucoma is a neurodegenerative optic neuropathy manifesting with progressive retinal ganglion cell (RGC) and axonal loss, which can result in visual field defects and blindness because of permanent cellular and neuronal damage. Subjective clinical examination of the optic nerve head (ONH) allows for assessment and monitoring of the structural changes associated with glaucoma. However, objective detection of defects in the peripapillary retinal nerve fibre layer (RNFL) by ophthalmoscopy is difficult. The relationship between glaucoma progression and the structural changes observed at the ONH, RGC layer, and RNFL in glaucoma is well established.[1–4]

Early diagnosis and treatment of glaucoma is vital to maintain visual field, as loss of RGCs is irreversible,[5] and even before any detectable visual field defects, there is a substantial loss of RGCs. Morphological abnormalities can develop 3–5 years before any functional loss is detected.[6]

Since the introduction of ocular imaging techniques such as optical coherence tomography (OCT), peripapillary RNFL thickness measurements have been used for the detection and monitoring of glaucoma.[7,8] However, the use of OCT has extended beyond RNFL measurements for glaucoma, and has also been used in the investigation of other neurodegenerative disorders such as Alzheimer's disease (AD)[9–11] and multiple sclerosis (MS).[12–14] In addition, given the similarities of brain and retinal foetal angiogenesis,[15,16] retinal microvasculature has also been investigated as a biomarker of changes in cerebral vasculature and cognitive decline.[17–19]

In this review, we examine the relationships between glaucoma, neurodegenerative disease, and cognitive impairment, and the use of RNFL thickness and RGC loss as potential ocular biomarkers for neurodegenerative disorders such as AD and MS. We review the strength of associations between RNFL and RGC and

neurodegeneration, and whether they are sufficient to be used in a diagnostic and prognostic capacity.

Glaucoma and the RNFL

As RGCs are lost in glaucoma, there is atrophy of the retinal nerve fibres, causing the RNFL to decrease in thickness.[1–4] Before OCT and other imaging techniques,[20] in order to monitor RNFL changes, clinicians depended on ophthalmoscopy, red-free fundus photographs[21] *in vivo*, or histological examination of the RNFL and ONH from enucleated eyes or post-mortem.[2]

Quigley and Sommer[22] gave a detailed description on 'how to use the nerve fibre layer examination in the management of glaucoma'. The RNFL develops from RGC axons converging on the disc to form bundles seen as bright white lines as they reflect light back toward the examiner's eye. The thickness of the RNFL bundles is greater at the disc and becomes less so peripherally, leading to increased reflections around the ONH. Therefore, the thicker the RNFL bundles, the brighter the reflection, with the brightest reflections seen in the vertical meridian. Using green light for retinal illumination produces a dark background that allows for better visualisation of these bundles. They described an early sign of glaucomatous change in the RNFL: a 'wedge defect'. This occurs as a result of local RNFL atrophy contrasting with adjacent normal bright RNFL.[22]

Defects in the RNFL are difficult to quantify *in vivo* on clinical examination or photography. There are several technologies that have been used for measuring the RNFL *in vivo*. These include scanning laser polarimetry, confocal scanning laser ophthalmoscopy, and OCT.

Scanning laser polarimetry

This method was first used for *in vivo* measurements of RNFL thickness in patients with glaucoma in 1995.[23] Weinreb *et al* used a scanning laser polarimeter (the Nerve Fibre Analyzer, Laser Diagnostic Technologies, San Diego, CA, USA) to measure estimated RNFL thickness in normal patients and patients with glaucoma. This instrument utilises the optical properties of the parallel bundled birefringent retinal nerve fibres to measure the phase shift or retardation of reflected polarised light. These retardation measurements correspond to the amount of tissue through which the polarised light has passed, and therefore estimate RNFL thickness. A more recent device using this imaging technique is the GDx (Carl Zeiss Meditec, Vista, CA, USA), which, in various guises, was examined as a diagnostic and monitoring tool for glaucoma.[24–28]

Confocal scanning laser ophthalmoscopy

This imaging technique has mainly been used for retinal surface mapping and estimation of RNFL thickness from retardation of light that has passed through the birefringent RNFL. A laser scans the retina and obtains a two-dimensional map reflected from the retinal surface, while a detector measures the intensity of the reflected light to allow three-dimensional imaging of the ONH and peripapillary structures. A reference plane automatically placed along the contour line of the optic disc is then used to calculate RNFL thickness. An example of an instrument using this imaging modality is the Heidelberg retinal tomograph (Heidelberg Engineering, GmBH, Heidelberg, Germany), initially designed for disc imaging and for recognition of early glaucomatous structural changes.[29,30]

Optical coherence tomography

OCT is now the most commonly used optical imaging technique for the measurement of the RNFL, and seems likely to supersede the other technologies, given it is ubiquitous in eye departments, with a vital role in monitoring retinal disease. Since its development 24 years ago, OCT has allowed for non-invasive optical imaging and *in vivo* quantitative measurements of the ONH and RNFL. OCT uses low-coherence interferometry to calculate RNFL thickness by measuring the interval of light backscattered from the retina and a reference mirror. Many studies have used OCT measurements of the RNFL to investigate early signs and patterns of glaucomatous damage.[7,31,32]

All methods consistently and quantitatively assess RNFL in glaucoma, a neurodegenerative disease. Given this scenario, there has been interest as to whether RNFL measurements may be used as proxy measures of global axonal loss in neurodegenerative diseases of the brain, and indeed whether the same pathological processes may be affecting RGCs as in brain cortex.

Glaucoma, AD, and the RNFL

AD is the most common form of age-related progressive dementia, affecting approximately over 26 million people worldwide.[33] AD leads to irreversible cognitive decline, memory loss, and results in functional and behavioural problems, which interfere with a person's independence and ability to perform daily activities. This condition places a substantial financial strain on society, costing approximately 210 billion US dollars per year in the United States alone.[34] Therefore, great effort has been expended to explore inexpensive, non-invasive diagnostic tests that can identify early signs of AD before any manifestation of the associated symptoms. Several biomarkers have already been investigated for detecting

AD and its intermediate endophenotype of mild cognitive impairment (MCI), however, these methods are largely invasive and expensive. These methods include analyses of blood and CSF, neuroimaging, and genetic testing.[35,36]

Pathophysiology of AD

AD results in neuronal cell death in the brain as a result of accumulation of extracellular beta amyloid (AB) protein and intracellular hyperphosphorylated tau protein and neurofibrillary tangles. The accumulation of the AB protein interferes with inter-neuronal communication via synapses, whereas the build-up of tau protein affects the transport of essential nutrients within the neurone itself. These processes are believed to contribute to neuronal cell death, which in turn leads to widespread cerebral atrophy and in turn cognitive and functional impairment.[34]

It has been suggested, however, that the deposition of amyloid protein is not solely responsible for the rate of cognitive decline in AD. Research has shown that neurodegeneration, depicted as atrophy on magnetic resonance imaging (MRI) occurs before cognitive decline and also reflects the rate of cognitive deterioration.[37] It is believed that a combination of neurodegeneration and amyloid dysmetablolism is responsible for the major pathological changes associated with AD.

Significant permanent neuronal damage may be present up to 20 years before any signs of cognitive decline become apparent.[36–38] Therefore, there is an urgent need for early detection of AD and screening methods that can identify cognitively normal individuals with structural changes and a greater risk of developing AD for early intervention.

Non-ocular biomarkers for AD that have been investigated include genetic markers such as mutations in the Presenilin genes 1 and 2, and the amyloid precursor gene, which have been linked to familial early onset AD, and the apolipoprotein E gene, which has long been recognised as a major risk factor for the sporadic form of later onset AD.[35] Other biomarkers such as parietal grey matter atrophy, greater right hippocampal, and parahippocampal activation have been identified through MRI brain imaging. Patients carrying the Presenilin 1 gene have been reported to have significantly higher concentrations of plasma and CSF AB 1–42,[36] although these findings have not always been replicated.[39] These biomarkers, although informative, are invasive, time consuming, and expensive. A non-invasive ocular biomarker that has been investigated is the RNFL.

Glaucoma and AD

Given both glaucoma and AD are chronic, neurodegenerative, and age-related diseases, scientists have explored whether they share common pathophysiological mechanisms and therefore are associated. These mechanisms include neuro-inflammation,[40] high levels of tumour necrosis factor alpha,[41,42] and upregulation of complement component 1q.[43,44] In addition, AB 1–42 and tau protein have been implicated in the pathogenesis of both AD and glaucoma. AB 1–42 levels have been shown to be significantly decreased and tau protein levels to be increased in the CSF of patients with AD when compared with controls.[45] Yoneda *et al*[46] have reported similar findings obtained from vitreous fluid analysis of patients with glaucoma. There appears to be similar apoptotic processes in RGC death in patients with glaucoma and neuronal cell death in AD.[47] Furthermore, histopathological studies conducted by Sadun and Bassi[48] have provided evidence that the largest RGCs, the M cells, may be involved in the primary neurodegenerative process in the eyes of AD patients.

McKinnon *et al*[49] have also suggested that RGC death in glaucoma mimics AD at a molecular level because of chronic neurotoxicity from AB deposition. Using an experimental glaucoma rat model with chronic ocular hypertension, the researchers demonstrated increased production of AB in the RGCs in rats, which was attributed to abnormal amyloid precursor protein (APP) processing and activation of caspases 8 and 3. The authors suggested that these abnormalities lead to a build-up of APP, which in turn activates caspases and upregulation of AB production. This 'vicious cycle' of gradual AB build-up in RGCs leading to axonal loss and neuronal cell death, could possibly explain the progressive nature and association between AD and glaucoma.

Patients with AD may have a significantly higher risk of glaucoma and vice versa.[50–52] Bayer *et al*[53] attributed a five times greater risk of visual field defects and optic disc cupping in patients with AD to a higher prevalence of glaucoma. However, given no AD patients had ocular hypertension (in comparison with 7.5% of controls) and had no family history of glaucoma, it may be visual field abnormalities were not due to glaucoma and reflect difficulties in performing psychophysical visual field tests in AD patients. A subsequent study by the same authors[54] showed an accelerated progression of visual field defects in patients with open-angle glaucoma and AD when compared with patients with open-angle glaucoma but without AD. However, given possible selection bias, a small sample size, non-blinded observers, and use of fundus photographs rather than more objective RNFL measures, these results need to be considered with caution. Visual field and optic nerve changes may not be specific to AD but have also been demonstrated in patients with Parkinson's disease.[55]

A recent systematic review by Tsilis *et al*[56] identified eight pertinent studies exploring the relationship between glaucoma and AD (Table 1). The studies were conducted over a wide geographical area, often with small sample sizes: the largest study[57] of 63 325 participants showed an inverse association between AD and glaucoma. Meta-analysis suggested that patients with AD had a decreased risk of glaucoma compared with controls (RR, 0.92; 95% CI, 0.89–0.94; I^2, 89%; $P_{\text{heterogeneity}}$, <0.001). Results were skewed by the largest study, but even when excluded, there was still high heterogeneity. The two largest studies[57,58] in this review showed a protective association between AD and glaucoma, whereas the smaller studies suggested greater risk. Methodology varied; half of the studies evaluated both diseases cross-sectionally,[51,53,55,59,60] limiting ability to make conclusions about causality and progression. Smaller case–control studies tended to adjust for age and sex only, whereas the larger studies adjusted for other confounding variables. The largest study by Ou *et al*[57] collected retrospective diagnostic codes from Medicare claims records to identify 63 325 individuals with glaucoma and matched these participants with controls without glaucoma, AD, or dementia at recruitment. There may be misclassification bias as significant proportions of patients with glaucoma (and AD) in the population are undiagnosed.

These results suggest that the relationship between glaucoma and AD is complex, and many studies are flawed because of sample size, possible selection bias, and misattribution of visual field defects assessed by psychophysical testing to glaucoma. Therefore, it is important that well designed, longitudinal studies with adequate follow-up are conducted to establish the relationship between these two neurodegenerative processes.

Table 1 Summary table of the associations between glaucoma and AD

Study (reference)	*Number of cases*	*Relative risk (95% confidence interval)*
Studies investigating risk of developing POAG in patients with AD		
Tamura *et al*[51]	172	3.13 (1.67–5.85)
Bayer *et al*[53]	112	6.41 (2.56–16.1)
Bayer *et al*[55]	49	4.70 (1.95–11.4)
Chandra *et al*[59]	7195	2.60 (1.06–6.43)
Studies investigating risk of developing AD in patients with POAG		
Ekstrom and Kilander[126]	174	1.09 (0.69–1.74)
Cumurcu *et al*[60]	21	5.44 (1.88–15.6)
Ou *et al*[57]	63 325	0.91 (0.88–0.93)
Kessing *et al*[58]	11 721	0.76 (0.56–1.05)

Abbreviation: POAG, primary open-angle glaucoma. Results table adapted from Tsilis *et al*.[56]

The eye in AD

Visual disturbance can be an early symptom in AD reflecting neuronal damage of cerebral visual pathways[61] and insufficiency of acetylcholine, an essential neurotransmitter in the visual system.[62,63] Histopathological evidence shows neuritic plaques and neurofibrillary tangles in visual cortex of patients with AD, even occurring before hippocampal involvement in cognitively intact patients with preclinical AD.[61] Visual problems may therefore precede memory impairment, particularly in the posterior cortical atrophy variant of AD. Visual deficits include abnormalities in visual field, colour vision, contrast sensitivity, motion perception, visuo-spatial construction, visual attention, visual memory, and fixation.[64] However, none of these deficits are pathognomonic or diagnostic of AD.

It has been generally believed visual disturbance in AD is cortical in origin, rather than from pathology of the retina or optic nerve.[65] The distinctive features found in the brains of patients with AD (ie, neuritic plaques and neurofibrillary tangles) had not been found in the human retina,[66] until a recent study identifying amyloid plaques in the retinas of AD patients.[67] AB proteins, tau, and APPs have also been found in 'aged' human retinas.[68] Furthermore, AB plaques, hyperphosphorylated tau, and retinal microvascular neuro-inflammation have been identified in the retinas of AD transgenic mice.[69,70]

The main ocular biomarker investigated for AD has been thinning of the RNFL, as this may reflect both generalised neurodegeneration and local involvement, and this is reviewed below. Other ocular biomarkers investigated in AD include pupillary abnormalities: hypersensitivity to pupillary dilatation with cholinergic antagonist eye drops,[71–73] supersensitive pupillary response to cholinergic agonists,[74] and altered pupil flash responses.[75,76] AB deposition (AB 1–40 and AB 1–42) has been found in the lens with comparable concentrations to those in the brain.[77] AB 1–40 has also been found in aqueous humour with comparable concentrations in the CSF,[77] and AB 1–42 in the vitreous humour.[46] Specific equatorial supranuclear cataracts have been found in patients with AD with colocalisation with AB deposits in the lens cytosolic proteins.[77] Further research is required to determine the precise link between the presence of these proteins and cataracts to AD.

Retinal vasculature may also be a biomarker for AD. AD patients had significantly reduced venous blood column diameter and venous blood flow rate (measured by laser Doppler) compared with age-matched controls.[78] Wong *et al*[19] also demonstrated changes in retinal microvasculature were independently associated with poorer cognitive function. Glaucoma, particularly normal tension glaucoma, has a vascular component, and there is

evidence of microvascular abnormalities in AD and multi-infarct dementia;[79,80] the advent of improved vascular imaging with OCT angiography opens up the possibilities of future retinal biomarkers of the brain's microvascular structure.

The RNFL in AD

Histological studies have found a reduction in RNFL thickness in patients with AD in comparison with normal controls.[66] Early studies with retinal photography showed a higher proportion of AD patients with RNFL abnormalities when compared with controls.[52,81] However, photography is difficult in patients with advanced AD, which led to inter-observer disagreement in almost a quarter of subjects in one study.[81] Given technical difficulties, and a lack of quantitative analysis, retinal photography is limited as a biomarker for diagnosis and progression of AD.

Parisi *et al*,[11] using OCT, showed thinning of the RNFL in all quadrants in AD patients compared with age-matched normal control subjects. The thinning correlated with abnormal retinal function tested with pattern electroretinogram (PERG).[11] Although statistical significance was achieved, the sample size was very small (17 subjects and 14 controls) limiting the generalisability of these results. Other relatively small OCT case–control studies have demonstrated similar findings.[10,82] Paquet *et al*,[83] including subjects with MCI and mild AD, as well as moderate-to-severe AD, showed that RNFL thickness in all groups of patients was significantly reduced, replicated by Kesler *et al*.[84] These data suggest that the RNFL may be affected in early AD (ie, in MCI), and therefore may be a useful biomarker of probable development of cognitive impairment in the future. Studies have shown consistent RNFL loss, summarised in Table 2, and this loss seems to be particularly apparent in the superior, and inferior, quadrants of patients with cognitive impairment (Table 2).

The superior quadrant seems to be particularly affected in AD when compared with controls, reported by Berisha *et al*[78] in a study of only nine patients, and Kesler *et al*[84] in a study of 30 AD patients. In addition, the latter study found inferior RNFL thickness to be significantly decreased in the AD group and also 24 subjects with MCI when compared with controls (110.1 ± 19.1 and 111.9 ± 16.1 (mean $\pm$ SD) μm *versus* 127.0 ± 15.5 μm). The specificity of superior retinal neurodegeneration is supported by Trick *et al*[85] who observed pronounced visual field loss in the inferior visual field of patients with AD. There was also a decreased global visual sensitivity in patients with severe dementia, which may suggest progressive loss of retinal sensitivity, in addition to the cerebral neurodegenerative processes.

Peripapillary RNFL loss in all four quadrants has been reported by other investigators using OCT.[11,82] However, the subjects in these studies had more advanced AD when compared with those in the earlier studies. The range of MMSE scores in the Berisha *et al* group was 17–30, whereas those reported by Iseri *et al*[82] were 8–28 and 11–19 in the study by Parisi *et al*.[11] This further supports the notion that early retinal changes in AD may be confined to inferior and superior retina. Other studies have shown a significant correlation between RNFL thickness and MMSE scores in patients with MCI and AD[86] and RNFL thickness has been significantly associated with other cognitive domains including reaction times.[87] However, the findings are not universal: both an inverse association[88] and no association[89] between RNFL and cognitive test scores have been reported. A large-scale population-based study[90] showed that better cognitive performance was significantly associated with a thicker RNFL ($r^2 = 0.028$, $P = 0.03$) in healthy young individuals without any cognitive impairment, but there were no significant associations found in older participants. This raises the question of ascertainment bias in clinic-based studies. However, a majority of the evidence demonstrates reduced RNFL thickness in patients with cognitive dysfunction.

The ganglion cell layer in AD

The ganglion cell complex is made up of the RNFL (the axons of the ganglion cells), the ganglion cell layer (GCL), which comprises the cell bodies, and the inner plexiform layer (the RGC dendrites). These are the three innermost layers of the retina. The macula contains a significant proportion of RGCs[91] and therefore macular volume and thickness measurements via OCT can be useful ocular biomarkers. With advances in OCT development, segmentation of distinct layers of the retina is now possible, allowing for quantitative measurements of both neuronal (RGC) and axonal (RNFL) degeneration. Iseri *et al*[82] found total macular thickness and volume of AD patients were significantly reduced and related to the degree of cognitive impairment. Morphologic deficits, such as a reduction in the number of RGCs and optic nerve axons, have been linked to AD and identified via histopathological studies.[66] A study by Blanks *et al*[92] demonstrated significant RGC degeneration in 14 of 16 AD patients. The degree of RGC pathology was consistent with the degree of ONH degeneration and marked RGC loss was associated with RNFL atrophy. The study found no neurofibrillary tangles, neuritic plaques or amyloid deposits in the retinas or optic nerves, suggesting that the loss of ganglion cells and their fibres was secondary to the neuronal degenerative processes in AD. Further work from the same group corroborated the findings, reporting

Table 2 Summary table of relationship between RNFL thickness and AD

Study (reference)	*Sample*			*Device*	*Global RNFL thickness (mean, SD) in μm*				*Quadrant(s) thinned*
	Diagnosis	N *cases*	N *controls*		*Cases*	*Controls*	P-*value*	*Cognitive assessment*	
Liu *et al*[127]	MCI, mild, moderate and severe AD	93	39	OCT	MCI 95.37 ± 17.11 Mild 91.61 ± 10.10 Moderate 91.68 ± 12.37 Severe 87.13 ± 17.05	100.12 ± 15.01	$P<0.05$	NINCDS-ADRDA, DSM-IV	Superior and inferior
Oktem *et al*[86]	MCI, AD	70	35	OCT	MCI 82.5 ± 7.3 AD 80.6 ± 9.6	91.5 ± 7.1	$P<0.001$	MMSE	Global
Gao *et al*[89]	MCI, AD	51	21	OCT	MCI 92.38 ± 1.94 AD 85.99 ± 1.90	98.60 ± 1.67	$P<0.05$	MMSE	Inferior, superior, and temporal
Shi *et al*[128]	MCI	[a] 18	[a] 60	OCT	[a] −11.0 ± 12.8	[a] 0.4 ± 15.7	$P=0.009$	DSM-IV	
Kesler *et al*[84]	MCI, AD	54	24	OCT	MCI 85.8 ± 10.0 AD 84.7 ± 10.6	94.3 ± 11.3	$P<0.05$	MMSE	Inferior and superior
Paquet *et al*[83]	MCI, mild, and severe AD	49	15	OCT	MCI 89.3 ± 2.7 Mild 89.2 ± 2.9 Severe 76.6 ± 3.8	102.2 ± 1.8	$P<0.01$	MMSE	Global
Iseri *et al*[82]	AD	14	15	OCT	AD 87.46 ± 23.78	113.16 ± 6.72	$P<0.05$	MMSE	Superior, inferior, and nasal

Study (reference)	*Sample*			*Device*	*RNFL Measure*	*Correlated variable*	*Correlation*	P-*value*
	Diagnosis	N *cases*	N *controls*					
Shen *et al*[88]	MCI	52	23	OCT	Inferior quadrant	Word list learning	$r=-0.652$	$P=0.001$
						Story memory	$r=-0.429$	$P=0.041$
						Story recall	$r=-0.502$	$P=0.015$
Shen *et al*[129]	MCI	[a] 18	[a] 60	OCT	Inferior quadrant	List recall	$r=-0.493$	$P=0.031$
						Delayed memory IS	$r=-0.589$	$P=0.033$
						Story recall	$r=-0.472$	$P=0.048$
Laude *et al*[130]	Unknown	96	0	OCT	Global	g-factor g-speed	$r=-0.229$ to -0.243	$P<0.05$
Van Koolwijk *et al*[90]		0	1485	SLP	Global	DART, RAVMT, SF, TMT, BD	Variance, $R^2=0.028$	$P<0.03$

Abbreviations: BD, block design; CI, cognitive impairment; DART, Dutch adult reading test; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; g-factor, general cognitive ability; g-speed, general processing; IS, index score; MMSE, mini mental state examination; MOCA, Montreal cognitive assessment; NINCDS-ADRDA, National Institute of Neurologic and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association; RAVMT, Rey auditory verbal memory test; SF, semantic fluency; SLP, scanning laser polarimetry; TMT, trail making test.[a] Subjects with normal cognition or MCI at baseline, 'cases' = progression from normal cognition to MCI, or from MCI to dementia, 'controls' = cognitively stable, values represent longitudinal change.

a 25% total reduction in the GCL in the foveal and parafoveal retina, with the greatest reduction of 52% being observed in the temporal region of the fovea.[93] Neuronal loss appeared most prominent in the superior and inferior quadrants.[94] These findings are consistent with the clinical case–control series reported above of greater loss in the superior and inferior quadrants in AD patients compared with controls. Not all studies,

however, have found significant RGC loss or optic neuropathy in AD.[95,96] A study using the RTVue-100 (Optovue, Inc., Fremont, CA, USA) for global RNFL+GCL thickness measurements showed significant differences between AD and controls.[97] Studies using OCT have demonstrated a significant reduction in ganglion cell-inner plexiform layer in AD and MCI patients[98] and have further established its association with decreasing grey matter volume in the occipital and temporal lobes.[99]

Animal disease models of AD and the GCL

Liu *et al*[69] used the single transgenic mouse model Tg2576 containing an APP mutant gene, which results in age-dependent cognitive deficits along with deposits of extracellular AB and amyloid plaques in the cortex, cerebellum, and hippocampus.[100] AB deposits were mainly found in the GCL and outer nuclear layer, but also in some cases the photoreceptor layer and the ONH. Double transgenic mouse models have supported these findings, with RGCs undergoing apoptosis and APP deposition occurring in the GCL and inner nuclear layer.[101] Other studies of human tau transgenic mice[79] have reported accumulations of hyperphosphorylated transgenic tau in the RNFL and RGCs. Other transgenic mice models have suggested that the inner plexiform layer may be a more sensitive biomarker for detecting AD-related changes compared with RGCs,[70,80] making it difficult to draw firm conclusions.[102]

However, both human and mouse studies suggest that AD-related pathology is seen in the retina. RGC loss may be a useful biomarker for assessing the neurodegenerative processes in AD, whether it reflects local pathology or reflects global loss of cortical neurons. Further investigation into the use of RNFL measurement to reflect AD is required, particularly as studies to date have been cross-sectional and its use as a tool for monitoring disease progression requires further larger, longitudinal studies.

The RNFL in MS

MS with ON

MS is an autoimmune disorder affecting the central nervous system (CNS). The characteristic pathophysiological features of inflammation and demyelination lead to axonal and neuronal degeneration that can affect the anterior visual pathway,[103] with 20–50% of MS cases initially presenting with optic neuritis (ON).[104] Parisi *et al*[105] first investigated patients with MS who had a previous history of ON using OCT. Results showed a 46% reduction in RNFL thickness in eyes affected by ON in comparison with control subjects and a 28% reduction in RNFL in affected eyes when compared with unaffected eyes of the same patient. Macular volume is also lost in eyes affected with ON when compared with disease-free control eyes.[14]

Significant RNFL thinning was found after ON in a study by Henderson *et al*,[106] evident within 3 months with 99% of the total loss occurring within 5 months of the episode of ON. Eyes with greater RNFL loss had a worse visual prognosis at 3 months. Recurrent attacks of ON significantly reduce RNFL thickness compared with eyes affected by only one episode of ON (64.2 *vs* 86.3 μm, $P<0.0001$).[107]

Further studies have compared an annual loss of 0.017% of the total RNFL thickness (105 μm) in healthy control eyes[108] to RNFL losses between 10 and 40 μm in eyes affected with ON, which have been reported in approximately 75% of patients with MS within a 3- to 6-month period following the acute attack of ON.[107,108] These researchers[107] also noted a cutoff point of 75 μm for RNFL thickness, below which a notable decline in visual field sensitivity corresponded to RNFL loss. A recent review of OCT data for patients with MS showed significant differences in RNFL thickness when compared with normal controls (95.5 ± 14.5 μm *vs* 104.5 ± 10.7 μm)[108] with more substantial degrees of thinning in MS patients with a history of ON (85.7 ± 19.0 μm).

MS without ON

Parisi's 1999 study[105] demonstrated that RNFL thinning still occurs in patients with MS without any previous history of ocular involvement. The aetiology of axonal atrophy and progressive RGC loss in MS (in the absence of ON) is unknown, but is presumed to be due to retrograde trans-synaptic degeneration. The RNFL is therefore a useful tool for studying axonal damage because of its unique unmyelinated structure. Gundogan *et al*[109] investigated 39 MS patients with no previous visual symptoms. Results revealed a significant reduction in RNFL thickness ($P=0.011$) only in the temporal quadrant. Other studies have found RNFL thinning in all quadrants,[110] temporal, and inferior quadrants[111] and in the superior and inferior quadrants,[112] which correlated to their corresponding visual field regions.[14] Gundogan *et al*[109] also assessed the retinal functional status of these patients using pattern VEP, which showed significant delays in P100 latency in 60 and 15-min arc checks ($P<0.001$) in patients with MS, however, P100 amplitude was only significantly reduced in 60-min arc checks. These findings are further supported by Sriram *et al*[113] who demonstrated a significant latency delay and amplitude reduction in multifocal VEPs of 62 MS patients with no previous history of ON, and an inverse correlation of GCL, total and temporal RNFL thickness with multifocal VEP latency.

Table 3 Summary table of relationship between RNFL thickness and MS

Study (reference)	*Sample*			*Device*	*Global RNFL thickness (mean, SD) in μm*				*Comparison*	*Comments*
	Healthy controls n = *eyes*	*MSON* n = *eyes*	*MS NON* n = *eyes*		*Cases*	*Controls*	P-*value*	*Correlation*		
Huang *et al*[111]	13	22	16	OCT	MSON 83.68 ± 29.91 MS NON 108.83 ± 15.33	111.60 ± 14.90	$P<0.05$			Global, temporal, and inferior
Sriram *et al*[113]	25		58	OCT	MS NON 93.6 ± 9.9	99.2 ± 7.5	$P<0.002$	Average RNFL ($r=-0.53$, $P<0.0001$) Temporal RNFL ($r=-0.59$, $P<0.0001$) GCL thickness ($r=-0.48$, $P<0.0001$)	mfVEP mfVEP mfVEP	
Abalo-Lojo *et al*[131]	118	123	53	OCT	MS eyes 84.51 ± 14.27	98.44 ± 6.83	$P<0.001$	Average RNFL MS NON ($r=-0.48$, $P=0.002$)	BCR	
Feng *et al*[112]	26	16	12	OCT	MS eyes 81.9 ± 17.8	102.1 ± 18.1	$P=0.00$			Global, superior, inferior, and temporal
Costello *et al*[107]		154	* 232	OCT	RON 64.2 ± 12.0 SON 86.3 ± 19.8 MS NON 97 ± 14.3	100.1 ± 14.0	$P<0.0001$	($r=-0.48$, $P<0.0001$) ($r=0.61$, $P<0.0001$)	VA VF	*Used as controls
Bock *et al*[132]	406	73	189	OCT	MSON 86.2 ± 16.2 MS NON 97.0 ± 13.1	105.2 ± 9.4	$P<0.0001$			Temporal quadrant
Burkholder *et al*[133]	219	328	730	OCT	MSON 85.7 ± 19 MS NON 95.6 ± 14.5	104.5 ± 10.7	$P<0.001$	MSON ($r=0.68$, $P<0.001$) MS NON ($r=0.62$, $P<0.001$)	TMV TMV	
Ratchford *et al*[134]	77	157	338	OCT	MSON 88.3 ± 16.5 MS NON 97.4 ± 13.9	102.4 ± 11	$P<0.01$	* MSON 6.5 ± 8, $P=0.003$	LCLA	*Mean no. of correct letters ± SD
Toledo *et al*[121]	* 18	~ 52		HRT	254.5 ± 100.6	275.0 ± 49.8	$P<0.05$	OCT RNFL values and cognitive tests. Average RNFL ($r=0.463$, $P<0.05$) Temporal RNFL ($r=0.754$, $P<0.001$)	SDMT	Global and temporal RNFL *No. of subjects ~Total no. of MS subjects

Table 3. (Continued)

Study (reference)	*Sample*			*Device*	*Global RNFL thickness (mean, SD) in μm*				*Comparison*	*Comments*
	Healthy controls n = *eyes*	*MSON* n = *eyes*	*MS NON* n = *eyes*		*Cases*	*Controls*	P-*value*	*Correlation*		
Zaveri *et al*[135]	85	68	87	OCT	MSON 81.8 ± 19.3 MS NON 95.6 ± 15	104.6 ± 10.3		Average RNFL ($r = 0.54$, $P < 0.001$) ($r = 0.44$, $P < 0.001$)	LCLA HCVA	
Siger *et al*[136]	24	40	62	OCT	MSON 83.9 ± 17.3 MS NON 94.38 ± 15	100.3 ± 12.1	$P = 0.01$	MS NON RNFL and MRI lesion volume ($P = 0.03$) ($P = 0.0001$)	TI T2	
Pueyo *et al*[137]	25	25	75	OCT	MSON 84.46 MS NON 94.2	104.97	MSON $P < 0.0005$ MS NON $P = 0.002$	Temporal RNFL ($r = -0.262$, $P = 0.011$)	Disease duration	
Gundogan *et al*[109]	76		78	OCT	* 68 ± 12.7	* 78 ± 15	$P = 0.011$	* ($r = -0.314$, $P = 0.034$)	* PVEP	*Temporal RNFL, P100 latency
Pulicken *et al*[114]	94	82	202	OCT	MSON 84.2 ± 14.7 MS NON 95.9 ± 14	102.7 ± 11.5	MSON $P < 0.0001$ MS NON $P = 0.04$	($r = 0.35$, $P < 0.001$) ($r = 0.39$, $P < 0.001$)	HCVA LCLA	
Fisher *et al*[12]	72	63	108	OCT	MSON 85 ± 17 MS NON 96 ± 14	105 ± 12	$P < 0.001$	($r = 0.33$, $P < 0.0001$) ($r = 0.31$, $P < 0.0001$)	LCLA CS	For every 1-line decrease in LCLA or CS score, mean RNFL decreased by 4 μm
Parisi *et al*[105]	14	14		OCT	59.79 ± 10.80	111.11 ± 11.42	$P < 0.05$	Average RNFL ($r = -0.744$, $P < 0.01$) Temporal RNFL ($r = -0.635$, $P < 0.01$)	 PERG PERG	Global and temporal

Abbreviations: BCR, bicaudate ratio (MRI brain atrophy estimate); CS, contrast sensitivity; CV, colour vision; HCVA, high contrast visual acuity; HRT, Heidelberg retinal tomography; LCLA, low-contrast letter acuity; mfVEP, multifocal visual evoked potentials; MS NON, multiple sclerosis without optic neuritis; MSON, multiple sclerosis with optic neuritis; PERG, pattern electroretinogram; (P)VEP, (pattern) visual evoked potentials; RON, recurrent optic neuritis; SDMT, symbol digit modality test; SON, single episode optic neuritis; TMV, total macular volume; VA, visual acuity; VF, visual function.

OCT measurements of RNFL thickness have also been shown to vary in the different subtypes of MS. RNFL thickness values in patients with secondary progressive MS (SPMS) were reduced in comparison with the other subtypes particularly with patients with the clinically isolated syndrome of MS (84.7 *vs* 105.7 μm, $P<0.0001$).[107] Pulicken *et al*[114] demonstrated decreased RNFL in relapsing remitting (RRMS 94.4 μm, $P<0.001$), primary progressive (PPMS 88.9 μm, $P<0.01$) and SPMS (81.8 μm, $P<0.0001$) when compared with controls. RNFL thickness and mean macular volume have also been shown to be significantly reduced in SPMS but not in PPMS.[115]

Macular OCT segmentation shows a significant decrease in GCL thickness[113] and volume in eyes of MS patients when compared with disease-free controls. GCL and inner plexiform layer thickness measurements may predict generalised axonal damage in MS more sensitively than RNFL analysis in MS.[116] Histological studies[117] have corroborated OCT findings, identifying involvement of the inner nuclear layer, with significant atrophy noted in 40% of MS eyes, with no atrophy in control eyes. In addition, the degree of retinal atrophy was inversely associated with brain weight, that is, loss of RGCs appears related to loss of cortical volume, potentially reflecting the overall burden of disease. The amount of retinal atrophy also appeared to reflect a longer duration of MS.[117]

A meta-analysis conducted by Petzold *et al*[118] of time domain OCT data from 32 studies showed less but significant RNFL thinning (7.08 μm (5.52–8.65, $n=3154$, $P<0.0001$)) in eyes of MS patients without ON. Overall, these studies (Table 3) demonstrate consistent findings that the neurodegenerative processes of neuro-axonal loss are reflected in the retina and therefore the RNFL and potentially the RGC layer are useful tools for quantification of these changes. These potential biomarkers are important, as RNFL thickness has been shown to correlate with disability in MS,[107] and they are currently being used in clinical trials (ClinicalTrials.gov, ID NCT01838174, ID NCT02273635).

Cognitive impairment in MS

A variety of symptoms, including cognitive, motor, and neuro-psychiatric abnormalities develop in MS because of the pathophysiological features of widespread lesions in the brain and spinal cord.[119] As well as visual symptoms, cognitive impairment is also common in MS with prevalence rates ranging between 40 and 65% depending on study settings[120] and the subtype of MS.[119] Various cognitive domains are affected in MS, particularly processing speed and immediate and delayed visual memory, but also information processing efficiency, attention, executive functioning, and long-term memory. Least affected domains include the main verbal skills such as comprehension and fluency.[119]

Studies have shown significant correlations between RNFL atrophy and cognitive disability, in particular attention and executive deficits, assessed using the symbol digit modality test in patients with MS when compared with healthy controls.[121] These findings may be explained by the reported correlations between RNFL thickness and brain atrophy.[122,123] Not all studies have found the same, however, others have reported an association between cognitive impairment in MS patients and performance in visual function tests such as low contrast sensitivity testing.[124]

Early detection of cognitive dysfunction is important, as cognitive symptoms significantly impact the quality of life in MS patients.[125] Further research is required to confirm whether RNFL or other ocular biomarkers are sensitive or specific in early detection of cognitive impairment in MS, to allow more aggressive management to reduce progression, or if the cognitive dysfunction merely reflects overall disease burden, which in turn is reflected in RNFL measures.

Summary

This review has highlighted the associations between neurodegenerative processes such as glaucoma and AD and ocular structures such as the RNFL and GCL. Evidence from animal and human studies is accumulating of a link between CNS degeneration (seen in AD and MS) and RNFL thinning; reflecting both retrograde transynaptic loss of RGCs and the same primary pathological process affecting the retina as in the brain (in AD). Given that patients with early disease (MCI) have thinning of the RNFL, as well as in later AD, and more severe MS phenotypes have thinner RNFL than those with less severe MS, loss of RNFL may be a useful biomarker of neurodegeneration, and in particular superior RNFL loss seems to reflect early disease in AD.

A limitation of most of the studies investigating ocular changes is their small sample sizes, and they are to date mostly cross-sectional. There is limited longitudinal data to date, and no assessment of sensitivity and specificity to change. Therefore, the challenge remains to find a specific ocular biomarker with sufficient sensitivity to detect preclinical disease and to monitor progression accurately. OCT carries many advantages, as it provides an inexpensive, non-invasive approach for visualising the CNS, and obtaining quantitative measurements of ocular morphologies that have been implicated in neurodegenerative processes. However, these ocular changes are not disease specific, and are complicated by the fact that both glaucoma and AD are strongly age

related, and that therefore the signs overlap and attribution of RNFL thinning to one pathology rather than the other may be difficult. The effect size is generally small: with an average loss of 11 μm, range of 6.2–25.7 μm in AD patients when compared with controls, and 7 μm in MS patients without ON compared with a normal RNFL thickness of ~100 μm.

We find encouraging data to support the retina as a potential ocular biomarker for evaluation of disease progression in AD and other neurodegenerative processes with similar pathophysiological mechanisms. However, further longitudinal studies with larger sample sizes are needed to judge sensitivity and specificity to disease progression. Clinical trials are in progress using OCT to determine whether ocular parameters including RNFL thickness may prove to be useful indicators for detection of early AD and decline in cognitive function.

Conflict of interest

The authors declare no conflict of interest.

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