This scientific commentary refers to ‘Retinal inner nuclear layer volume reflects response to immunotherapy in multiple sclerosis’, by Knier et al. (doi:10.1093/brain/aww219).

A recent article in Brain by Gelfand et al. (2012) on the inner nuclear layer (INL) of the retina in multiple sclerosis triggered a lively exchange of Letters to the Editor. With the advent of optical coherence tomography (OCT), a forgotten autopsy observation was restored to the top of the research agenda in multiple sclerosis. Over 150 years ago, severe atrophy of the retinal nerve fibre layer (RNFL) and ganglion cell layer (GCL) was shown to be associated with only subtle changes in the INL whilst the outer retinal layers were completely preserved (Müller, 1857). Although Heinrich Müller was cautious in interpreting potential INL changes, he was more convinced about the existence of secondary (retrograde) axonal degeneration. ‘Die sogenannten Körner erschienen etwas körnig, doch war mir sehr zweifelhaft, ob dies als pathologische Veränderung anzusprechen sei, da man Ähnliches auch sonst zu Gesicht bekommt.’ This case was discussed on pages 92–98 and the citation taken from page 93 (Müller, 1857). For the contemporary translation it is important to refer back to the terminology of the time. There were three ‘Körnerschichten’ that correspond to the present day: ‘ganglion cell layer’, ‘inner nuclear layer’ and ‘outer nuclear layer’.

Müller was not able to see the GCL and his comment is on the next nuclear layer, the INL: ‘The so-called nuclei seemed more grainy, but I am rather doubtful if this should be interpreted as a (specific) pathological finding as one can see similar features in other situations’.

Subsequent histological studies of the macaque monkey eye following experimental lesions to the afferent visual pathway provided evidence for retrograde trans-synaptic degeneration reaching the GCL with associated changes in the INL (van Buren, 1963). Lesions of the optic chiasm led to thinning of the RNFL and GCL and to formation of cystic spaces in the INL, which ‘were irregularly rounded [the larger of which] contained fine strands of tissue and debris’. Occipital cortex lesions, by contrast, did not affect the INL or any of the outer retinal layers (van Buren, 1963). INL changes following optic nerve or chiasm lesions are the likely histological equivalent of what has been called ‘microcystic macular oedema’ (MMO) (Gelfand et al., 2012). I will later return to the debate surrounding MMO in an attempt to explain the next relevant observation, the frequent increase in INL thickness in multiple sclerosis patients in the absence of obvious MMO. Increased thickening of the pooled outer plexiform and INLs was found to be associated with signs of inflammation in multiple sclerosis (Saidha et al., 2012). Together these studies raised the possibility that MMO and INL thickening might be comparable to an MRI ‘T₂ lesion’ and could be useful as a surrogate outcome for treatment trials in multiple sclerosis (Petzold, 2012). In this issue of Brain, Knier et al. report that effective disease-modifying treatment in patients with multiple sclerosis is associated with volume reduction of the INL (Knier et al., 2016).

Recruitment for the present study started at the time of publication of the two landmark papers that reported a positive correlation between INL thickness and multiple sclerosis disease activity (Gelfand et al., 2012; Saidha et al., 2012). Three years later 121 patients suffering from relapsing-remitting multiple sclerosis and 40 healthy control subjects had been enrolled. Patients were grouped into those who had received no disease-modifying therapy (no DMT, n = 36), a first line drug (1st DMT, n = 47) or a second line drug (2nd DMT, n = 25). Not surprisingly the latter subgroup consisted of patients who had significantly more disease activity based on clinical and radiological criteria.

A potential confound is multiple sclerosis-associated optic neuritis (MSON), which occurs at some stage in ~80% of patients with multiple sclerosis. Such patients have more severe damage to the inner retinal layers and are more likely to show MMO. A frequent criticism of OCT studies is the potential bias caused by inclusion of MSON. In fact, in the
present study MMO was observed only in three patients, all of whom experienced MSON and were excluded from subsequent analysis. The frequency (2.5%) is comparable to previously reported values of 1% (Balk et al., 2012), 4.7% (Gelfand et al., 2012) and 6% (Saidha et al., 2012). Knier et al. are therefore to be commended for having excluded patients with MSON prior to enrolment or during the study period before moving on to the exciting statistical analysis of INL volume changes.

Consistent with the literature, there was more severe atrophy in the inner retinal layer in patients with multiple sclerosis compared to controls. Moreover, a larger INL volume was predictive of a greater number of new contrast-enhancing and T2 MRI lesions. The striking and novel finding is that a decrease in INL volume over time was related to clinical and radiological evidence of reduced inflammatory disease activity. A potential bias owing to the presence of new optic tract T2 lesions was carefully excluded.

In patients in whom inflammatory disease activity completely ceased under treatment, a reduction of the INL volume could be detected within 6 months. This trend continues for the entire observation period up to 18 months. In contrast, no changes of the INL volume were observed in those patients in whom inflammatory disease activity completely ceased.
disease activity could not be controlled. So is INL volume reduction a response marker for successful treatment of inflammation in multiple sclerosis?

As mentioned above, the discovery of MMO and volume changes of the INL prompted an extensive correspondence in Brain. Abegg et al. (2012) pointed out that MMO may be caused by retrograde axonal degeneration as a result of any type of lesion in the anterior optic pathways, and proposed the term ‘retrograde maculopathy’ to describe this phenomenon. At the same time we (Balk et al., 2012) also showed that MMO was not specific to multiple sclerosis, and suggested that an impaired capacity of Müller cells to maintain retinal fluid homeostasis might be relevant. The Müller cell hypothesis is consistent with the finding that the INL volume can be reduced by acetazolamide, which improves the water-pumping function of Müller cells (Abegg, 2016). However, it has also been suggested that MMO results from neither inflammatory nor trans-synaptic degeneration (Barboni et al., 2013). An alternative hypothesis implicates traction at the vitreoretinal interface (Petzold, 2012; Lujan and Horton, 2013). Among observations that call for explanation is that MMO is transient in 84% of cases studied longitudinally (Burggraaff et al., 2014).

In summary, the Knier et al. study takes the discovery of MMO and INL volume changes (Gelfand et al., 2012; Saidha et al., 2012) to the next level. But because it is essentially correlative, it further emphasizes the need for a comprehensive understanding of the underlying mechanisms in the retina. Taken together, the observations imply that dynamic fluid shifts, likely related to inflammation, vascular permeability and Müller cell function, need to be considered, as well as non-inflammation related changes such as traction and a retrograde maculopathy. The location of MMO suggests a close relationship to Müller cells and the retinal vasculature (Balk et al., 2012). Müller cell somata reside in the INL and their processes encompass the capillary plexus of the retina (Fig. 1). Above the INL lies the superficial vascular plexus; below the INL lies the deep capillary plexus. Both are readily visualized by OCT angiography and are connected vertically by small vessels. Understanding fluid trafficking through the retina and Müller cells will now be important to better explain volume changes of the INL: Fig. 1 summarizes the potential mechanisms involved.

Borrowing from the introductory allegory of a ‘$T_2$ lesion’ for INL oedema, an increase of volume from normal (Fig. 1A) could be caused by extracellular fluid accumulation (Fig. 1B and C). Müller cells were proposed to play a central role in this process (Balk et al., 2012). Müller cells manage retinal fluid homeostasis by water and ion buffering. Therefore, Müller cells express water and ion channels. The aquaporin 4 (AQP4) and potassium (Kir 4.1) channels expressed on Müller cells have been shown to be a target of autoimmune disease (Petzold et al., 2016). There is new evidence for loss of AQP4 on Müller cell sidebranches following experimental transfer of AQP4-specific T cells (Zeka et al., 2016). Moving from the retina to the brain, the glial water channel AQP4, located in the astrocytic perivascular endfeet, facilitates water movement in the CNS. Conventional understanding is that AQP4 governs the rate of osmotic astrocytic volume change in the brain. Osmotic pressure gradients were understood to be more relevant than hydrostatic pressure gradients. More recently the ‘glymphatic’ hypothesis of the brain proposed that hydrostatic pressure also determines trans-astrocytic water flow through AQP4 channels with blood vessel pulsation as an important contributing factor (Iliff et al., 2012). There is a considerable degree of variation of brain interstitial space with physiological/diurnal changes of flow through the brain glymphatic system. Moving back from the brain to the retina, it is
therefore instructive to note that hyperaemia but not hydration is relevant to a transient, physiological change of retinal layer thicknesses (Balk et al., 2013, 2014). In disease, with two notable exceptions, traction and trans-synaptic axonal degeneration (Fig. 1D), INL volume changes might therefore be explained by extending the glymphatic hypothesis from the brain to the retina. This concept might appeal to both the neurological and ophthalmological communities as findings are likely to encompass a broad disease spectrum (Müller, 1857; Balk et al., 2012; Bugggraaff et al., 2014).

To conclude, patient numbers in the exciting longitudinal data presented by Knier et al. were small, but this throws down the gauntlet to everyone who can get their hands on existing multiple sclerosis OCT trial data. Can reduction of INL volume be validated as a biomarker for sustained control of CNS inflammation by therapeutic interventions? Publication of both positive and negative data will be highly informative for answering this question.

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Defining retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations

This scientific commentary refers to ‘Retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations’, by Stam et al. (doi:10.1093/brain/aww217).

Cerebral small vessel diseases refer to pathological processes that affect the structure or function of small vessels of the brain, including arteries, arterioles, capillaries, and occasionally venules (Pantoni, 2010). While the majority of small vessel diseases are sporadic age-related conditions driven by a complex interaction between genetic and cardiovascular risk factors or cerebrovascular amyloid deposition, inherited (familial) cerebral small vessel diseases have been increasingly reported in recent years (Joutel and Faraci, 2014). Of these inherited diseases, CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and