Getting Beyond the Ganglion Cell

Morphometric Adjustments for Retinal Optical Coherence Tomography in Multiple Sclerosis

THE TREATMENT OF RELAPSING MULTIPLE sclerosis (MS) remains a rare beacon of success in the long campaign against neurological disease. The ready availability of a phase II clinical outcome (eg, gadolinium-enhancing lesion) that tracks a relevant phase III clinical outcome (eg, annualized relapse rate) is one of the major reasons for this success. Eight therapies have been approved by the US Food and Drug Administration in less than 20 years, and 3 more are under current consideration at the Food and Drug Administration following successful phase III clinical programs. However, before feeling triumphant, we must recognize that our current therapies are principally effective in the prevention of relapses and new brain lesions detected using magnetic resonance imaging (MRI) and that their therapeutic benefit over the long term remains a matter of controversy.[1,2] In fact, it is widely agreed that we lack robust, potent therapies to overcome the fearsome long-term progressive neurological decline that characterizes MS. Furthermore, this progressive neurological decline manifests itself in the later stages of the disease and appears irreversible once it appears—facts that make it

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difficult to adequately time therapeutic intervention and assess its effects. As a result, progressive MS is an arena in desperate need of compelling biomarkers and predictors of disease course. Precisely for this reason, retinal optical coherence tomography (OCT) has garnered great interest as a potential phase II clinical outcome in neuroprotective trials in MS. Nearly 200 papers have been published on the topic in the last 8 years (as of July 2012 in MEDLINE).

In science, advancement often comes at the cost of our cherished assumptions. We open up new paths to discovery by recognizing that our conceptualizations are too simple or that our models lack an essential measurement. Philosophers of science sometimes refer to complete disruptions in our scientific models as a "paradigm shift." However, many smaller gains are about reorientation rather than revolution. The study by Saidha and colleagues[3] in this issue of *Archives in Neurology* contains such a reorientation regarding the application of OCT in MS. To understand their contribution, we must first consider the field.

The major technical reason that retinal OCT has gained attention and interest in clinical neurology is because of the ease with which we measure the thickness of the retinal nerve fiber layer (RNFL) with this method. The RNFL is composed primarily of ganglion cell axons before they coalesce in the optic nerve. From the perspective of OCT imaging, axonal microtubules manifest the attractive optical property that they scatter light. This scatter is the basis of the interference pattern that constitutes the OCT signal.[4] Therefore, the RNFL is easy to distinguish and quantify on OCT scans. The thickness measurement along a single circularized line (B-scan) centered around the nerve head provides a snapshot of the anterior visual pathway at one point along its entire path length. It was because of its ready quantification (and because of increasing interest in axonal injury as the basis of permanent neurological disability) that peripapillary retinal OCT was suggested to offer a useful assessment of axonal health.

The early assumptions regarding the application of OCT to MS were elegant in their simplicity but potentially naive. The RNFL is more than just axons, especially in disease state.[5] The pathobiological basis of OCT-detected RNFL thinning in MS remains unexplored: it is unclear if it reflects axon loss, axon thinning, or other changes in the tissue. However, inner retinal atrophy detected on OCT shows reasonable correlations with other measures of disease, such as disability scores and MRI measures of whole-brain atrophy, and good correlation with measures of visual dysfunction.[6,7] Still, in isolation, peripapillary RNFL OCT is not our holy grail. Its best application may be for monitoring axonal survival after an acute demyelinating optic neuritis. However, despite less scattering in other layers, OCT may offer us additional insights and opportunities given its outstanding spatial resolution (especially in the z plane) and the unique anatomy of the retina.

It has been shown that all the cardinal pathological features of MS (other than demyelination) can be found in the retina.[5] These include perivascular cuffing cells of immune lineage, innate immune activation, and gliosis/fibrosis. Therefore, OCT and retinal imaging do not just provide a simple potential surrogate for global central nervous system axonal loss. The information provided may in fact be far richer than we have previously recognized, especially when considered in context with the psychophysical and electrophysiological tests available for the quantitative and anatomically specific assessment of visual dysfunction.

The study by Saidha and colleagues[3] provides an important methodological and conceptual advance in the application of retinal OCT. The authors establish that intracranial volumes (ICVs) are associated with most OCT

parameters in both patients with MS and healthy controls; that some OCT parameters are not associated with ICV in patients with MS may be because the effect of the disease has overwhelmed the impact of other biological factors. Because ICV measures are also associated with brain volume measures, they have the potential to confound associations between retinal and brain structures in the disease. Saidha and colleagues[3] then go on to show that certain associations are only evident once the correction for ICV has been performed.

The approach used helps extend and advance the use of OCT in 2 important and ultimately converging ways. One problem with peripapillary RNFL OCT is that its use as a measure at a single time point may be limited by significant interindividual variation. This suggests that OCT measures at a single time point could be made informative by normalizing values based on ICV or some other morphometric parameter; in fact, it may best be normalized based on a number of morphometric parameters (including axial eye length and height). Associations between morphometric measures in an individual may help to account for variations in overall size; otherwise, associations between brain volume and retinal volume in a disease population could incorrectly be assumed to be driven by disease-related factors rather than by physical size alone. No one would think that an association between foot size and ICV had any relation to a disease process. Saidha and colleagues[3] overcome this problem by normalizing values using ICV. This could allow single measurements to have greater clinical utility. This is not unprecedented. Similar corrections for head size with visual evoked potential suggest that sex-related differences in latency on visual evoked potential may be a consequence of physical size, although it has not been evaluated whether using such a normalization procedure with visual evoked potentials would make them more sensitive.[8] Second, Saidha and colleagues[3] were able to use this correction for size to reduce the interindividual noise in their measures and to identify potential associations between deeper retinal layers and MRI measures. This shows us that retinal segmentation may be used as an in vivo method to detect changes in deeper retinal layers (beyond the ganglion cell).

By accounting for size-related morphometric differences, cross-sectional data have the potential to be meaningful. Saidha and colleagues[3] used ICV as assessed from MRI; however, a simple assessment of head circumference or even height may suffice. Additional adjustments for eye length may also help with data normalization. Their work demonstrates some utility with regard to this approach in cross-sectional data interpretation and analysis, although it may also have some use with regard to interpreting the change in values seen in a longitudinal study.

Their study[3] uses the insight about data normalization to expand our ability to look at deeper layers of the retina using segmentation methods. Segmenting the layers of the retina is not a technically trivial endeavor.[9] The retina is less than 0.5 mm thick, and the adjacent layers do not always evidence a significantly different OCT signal. For the purpose of segmentation, a line of demarcation must be drawn along a complex biological interface that does not have any true natural boundaries. It

has also been argued that retinal segmentation is both scientifically and clinically unimportant (ie, a technical feat without application) because whole retinal thickness can easily be assessed (as the boundary at the internal limiting membrane and those in the outer retinal layers, such as the Bruch membrane, provide sharp borders) and fully informative. If retinal atrophy is all of a piece, then atrophy in one layer could be presumed to be consistent across all layers concurrently.

However, there are a few reasons why segmented OCT data provide us with new avenues for investigation. First, given the complexity of the biological processes in the MS retina, as well as their differential impact on different retinal layers, one layer may show evidence of volume reduction, while another may show evidence of an increase in volume. From a quantitative perspective, these important biological effects would be lost in measuring whole retinal thickness. Furthermore, given the differences in the anatomy and physiology of different retinal neurons, observing and distinguishing differing pathology in various layers may help reveal important pathophysiologic features about the disease. Segmentation is also a means of assessing possible transsynaptic processes. Whole retinal thickness will never tell us when the unmyelinated neurons in the inner nuclear layer begin their atrophy or whether injury extends to the photoreceptor, and segmenting these layers promises to open a new window into in vivo monitoring of these parameters.

Saidha and colleagues[3] find a number of intriguing associations after making an adjustment for ICV. First, they find that standard inner retinal thickness measures (peripapillary RNFL and macular ganglion cell–inner plexiform complex) are correlated with gray matter thickness across the cortex. This suggests that inner retinal thickness measures may reflect some of the same biological processes that drive loss of gray matter, which, incidentally, is the MRI metric most strongly associated with overall disability. Once these results are confirmed, it would be important to know whether these associations are uniform across the cortex or more pronounced in the visual-associated cortex. Second, Saidha and colleagues[3] found an inverse association between inner nuclear layer thickness and both white matter lesions and white matter atrophy in patients with relapsing-remitting MS. These findings are particularly intriguing in light of the recent identification of inner nuclear layer microcysts associated with disability and visual dysfunction in MS.[10] Lastly, Saidha and colleagues[3] found trends associating most gray matter structures and outer nuclear layer thickness in subjects with a history of optic neuritis. The exact meaning of this finding cannot be ascertained within the limitations of this study. However, it suggests that (1) injury to the retina may extend all the way to the photoreceptors after optic neuritis and that (2) the presence or degree of this injury may be associated with the amount of overall gray matter lost due to the disease. All these findings require replication and further investigation using independent data sets and longitudinal monitoring. However, they begin to hint that OCT, as a method, may offer us a number of potential biomarkers for monitoring or predicting disease progression, and they indicate that the data are richer than we

previously imagined. Perhaps one of these methods (or a number of them in conjunction) will serve as the basis for the predictive phase II outcome that we so desperately need.

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