In-vivo detection of deep retinal neuronal layer changes following acute optic neuritis

Omar Al-Louzi, MD; Pavan Bhargava, MD; Scott Newsome, DO; Peter Calabresi, MD; Shiv Saidha, MD

Department of Neuroimmunology and Neuroinfectious disorders
Johns Hopkins University

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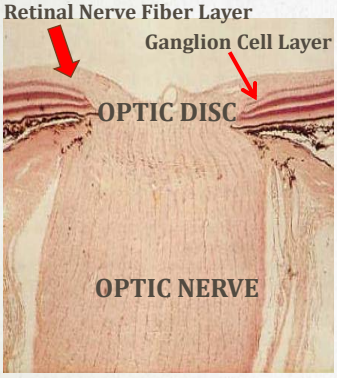
Multiple sclerosis (MS)

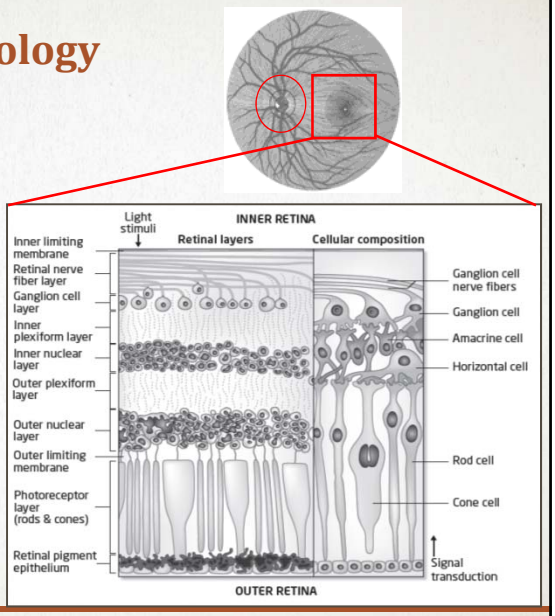
- MS is an immune-mediated demyelinating disorder of the Central Nervous System (CNS) with both inflammatory and degenerative components.
- MS commonly involves the optic nerves; acute optic neuritis (AON) is the presenting feature in ~20% of patients, while 50% experience it at some point during the course of their disease[1].
- Autopsy studies demonstrate that optic nerve pathology is present in the majority of MS patients even in the absence of overt clinical involvement[2].

1. Balcer, L. J. Optic Neuritis. *N Engl J Med* **354,** 1273–1280 (2006)
2. Toussaint, D., Périer, O., Verstappen, A. & Bervoets. *J. Clin. Neuroophthalmol.* **3,** 211–20 (1983).

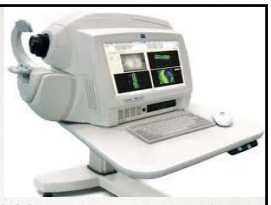
Retinal histology



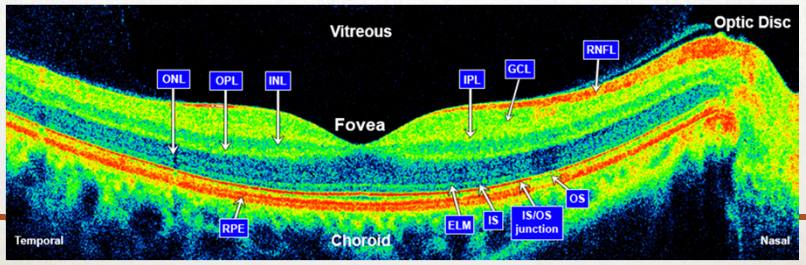
Microscopic cross-sectional view through the optic nerve including the retinal layers
http://hubel.med.harvard.edu



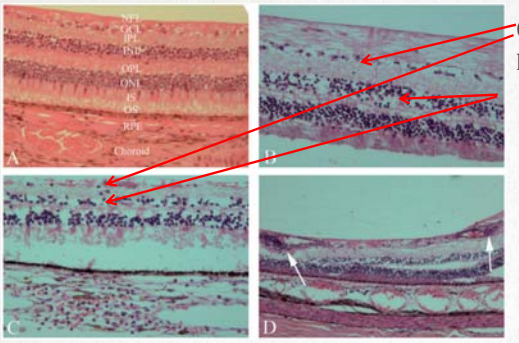
Optical coherence tomography (OCT)



- OCT is a technique that employs low coherence interferometry of near-infrared light.
- It is used to generate *in-vivo* high-resolution (< 5 μm), cross-sectional images of the retina.
- Because of the depth-resolving capacity of OCT, it enables visualization of retinal tissue structures similar to tissue sections under a microscope.



Evidence that retinal neuronal loss occurs in MS



Ganglion cell dropout (79% of MS patient eyeballs)

Inner nuclear layer neuron dropout (40% of MS patient eyeballs)

Green et al. Brain 2010; 133: 1591-601

- Retrograde neurodegeneration is thought to culminate in drop out of retinal ganglion cells.
- Our group has previously shown using macular segmentation that thinning of the composite ganglion cell + inner plexiform (GCIP) layers occurs following AON[1].
- However, comprehensive longitudinal *in-vivo* assessment of deep retinal neuronal layers following ON remains largely unexplored.

1. Syc, S. B. *et al.*. *Brain* **135,** 521–33 (2012).

Objectives

- To determine whether objective changes in INL and ONL thicknesses occur following AON.
- To explore whether these changes may be temporally related to thickness changes of the composite ganglion cell + inner plexiform layer thickness (GCIP).

Methods - Participants

- 34 patients diagnosed with acute unilateral demyelinating ON.
- Baseline evaluation was performed with a mean delay of 14 days from onset (SD 8.8, range: 1-33 days).
- A comparison cohort of 34 MS patients, who did not develop AON, were matched 1:1 based on age, sex, and duration of OCT follow-up.

Demographic and clinical characteristics

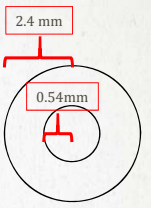
	Patients presenting with AON at baseline	Patients with MS who did not develop AON at baseline or during follow-up	*P*-value
Age, y, mean (SD)	36.4 (9.4)	35.9 (9.1)	0.83[a]
Female, n (%)	30 (88)	30 (88)	1.00[b]
Diagnosis, n (%)			0.01[b]
CIS	7 (20.6)	0 (0.0)	
RRMS	26 (76.5)	33 (97.1)	
SPMS	1 (2.9)	1 (2.9)	
Eyes with a previous history of AON, n (%)	12 (17.6)	19 (27.9)	0.15[d]
Follow-up duration, months, median (IQR; range)	22.5 (12.6-34.2)	22.9 (14.2-36.4)	0.65[c]

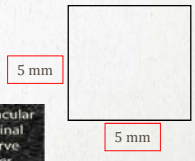
Abbreviations: AON = Acute optic neuritis; MS = multiple sclerosis; CIS = clinically isolated syndrome; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; IQR = inter-quartile range.

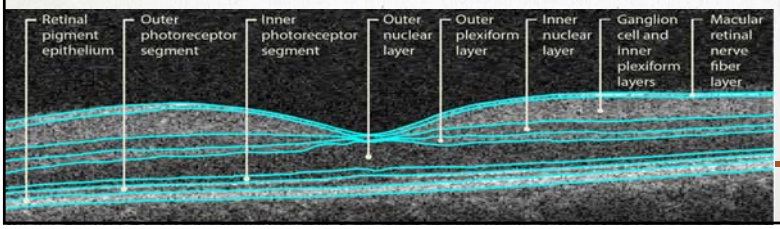
[a] Two-sample Student's *t*-test. [b] Fisher's exact test. [c] Mann–Whitney U test. [d] Chi-squared test.

Retinal imaging

- Patients underwent Cirrus-HD OCT imaging, with automated intra-retinal layer segmentation, at each study visit.
- Two macular segmentation methods were used to obtain measures of retinal layer thickness:
  1. Manufacturer's algorithm
  2. Graph-based, open-access method







Statistical analysis

- Time was taken as a continuous variable starting at the onset of AON symptoms.
- Comparisons between clinically affected and fellow eyes, at set time intervals, were done using mixed-effects linear regression accounting for within-subject inter-eye correlation.
- Multilevel linear spline models were used to analyze the course of OCT measure changes over time.
- Breakpoints (allowing for changes in slope to occur) were positioned, according to the best fit to the data.

Abbreviations: GCIP = ganglion cell+innerplexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = outer nuclear layer; PRL = photoreceptor layer

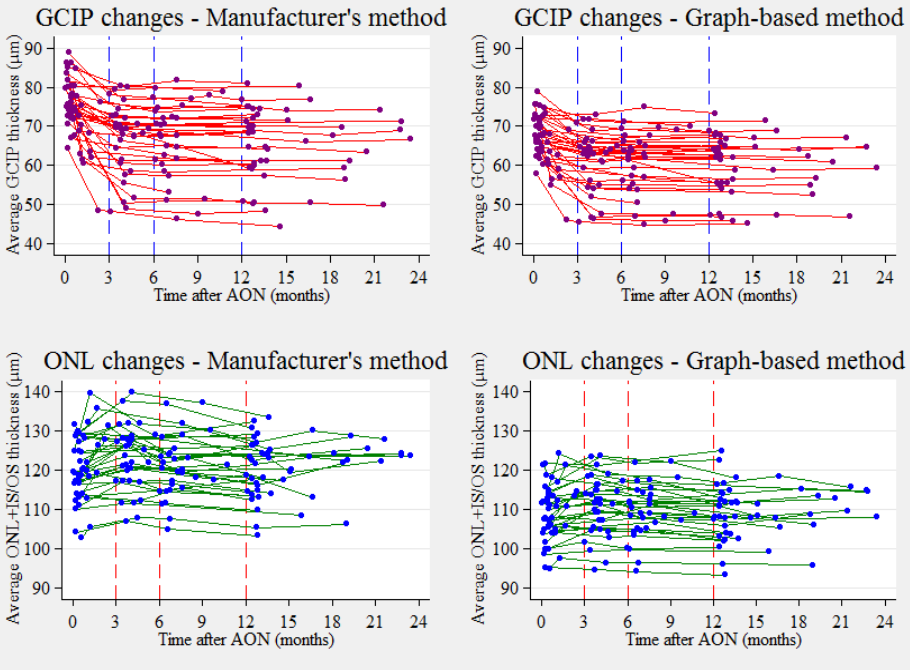
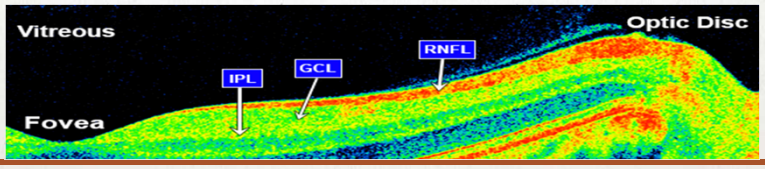


Table 2: Estimated rates of change in average retinal layer thicknesses in clinically-affected eyes after ON

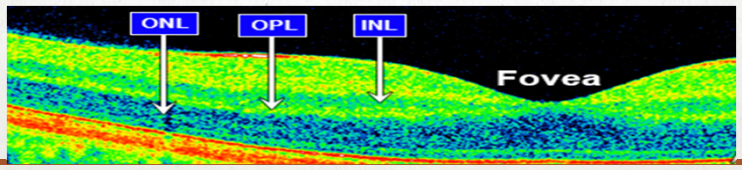
OCT measure		Baseline to 3 months		3 to 6 months		6 to 12 months	
		Rate of change (µm/month)	*P*-value	Rate of change (µm/month)	*P*-value	Rate of change (µm/month)	*P*-value
RNFL		**-9.85**	**<0.001**	-0.91	0.713	-0.36	0.695
GCL+IPL	Manufacturer	**-3.68**	**<0.001**	0.17	0.668	-0.16	0.281
	Graph-based	**-2.70**	**<0.001**	0.11	0.731	-0.14	0.220



Abbreviations: ON = optic neuritis; GCIP = ganglion cell layer + inner plexiform layer; RNFL = retinal nerve fiber layer.

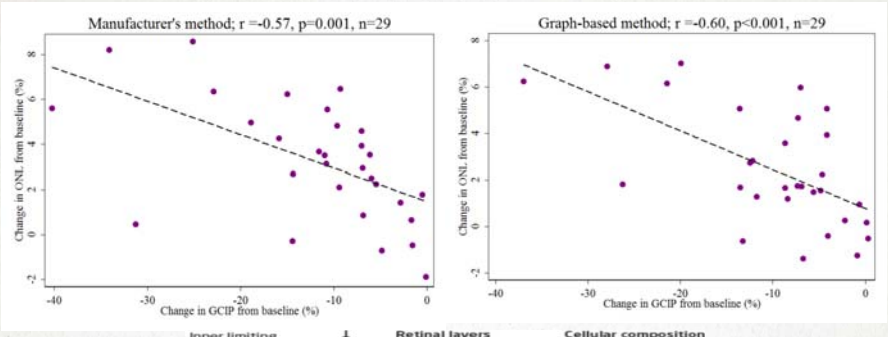
Table 3: Estimated rates of change in average retinal layer thicknesses in clinically-affected eyes after ON

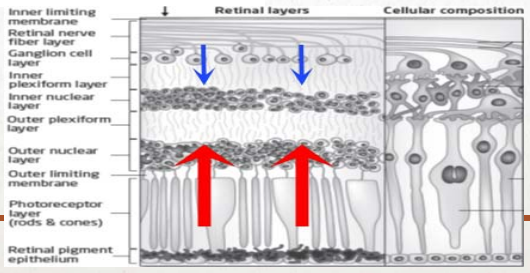
OCT measure	Segmentation method	Baseline to 3 months		3 to 6 months		6 to 12 months	
		Rate of change (µm/month)	*P*-value	Rate of change (µm/month)	*P*-value	Rate of change (µm/month)	*P*-value
INL+OPL	Manufacturer	**0.71**	**<0.001**	-0.31	0.103	0.01	0.897
	Graph-based	0.11	0.417	-0.26	0.061	-0.04	0.506
ONL+PRL	Manufacturer	**2.18**	**<0.001**	**-1.34**	**<0.001**	-0.17	0.095
	Graph-based	**1.37**	**<0.001**	**-0.65**	**0.001**	-0.15	0.038



Abbreviations: ON = acute optic neuritis; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = outer nuclear layer; PRL = photoreceptor segments layer.

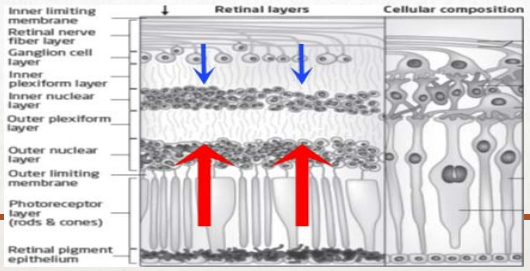
Relationship between GCIP loss and ONL thickening at the 4±1 month visit





Take home messages

- Ganglion cell layer thinning following AON appears to be most rapid in the early months.
- OCT segmentation demonstrates a transient increase in ONL thickness that appears to be proportional to the degree of GCIP loss in affected eyes.
- This raises the possibility of biological trans-synaptic changes occurring in the deep retinal neuronal layers and may help us understand the cellular response to injury in MS.



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