Optic neuritis (ON) is an inflammatory injury of the optic nerve, which represents with first demyelinating event in approximately 20% of patients with multiple sclerosis (MS). The purpose of this study was to determine whether RNFL measurements obtained within two years of an optic neuritis (ON) event distinguish patients at increased risk of developing clinically-definite MS (CDMS). Methods: Fifty consecutively sampled patients who experienced a single ON event were followed prospectively for a mean period of 34 months with OCT testing. Values of RNFL in clinically-affected and non-affected eyes were compared between patients who developed CDMS and those that did not develop MS after ON. Findings: Twenty-one patients (42%) developed CDMS during the course of the study, with a mean conversion time of 27 months. Mean RNFL values were thinner in the clinically - affected eyes of non - MS patients than CDMS eyes after one year (p = 0.0462) due to more severe ON events in the former. By year two, CDMS patients manifested more recurrent ON events and RNFL thinning than non - MS patients. Temporal RNFL values were thinner in the non-affected eyes of CDMS patients with a trend towards significance (p = 0.1302). Interpretation: Our results indicate that RNFL thickness does not reliably distinguish patients at higher risk of converting to CDMS after ON. The severity of ON has a greater effect on RNFL thickness than risk of CDMS at one year. The CDMS patients demonstrate progressive RNFL thinning likely due to recurrent sub-clinical ON events, which may help differentiate them from non - MS patients over time.
patients with ON and other clinically-isolated syndromes, if initiated as early as the first demyelinating event. Accordingly, efforts are ongoing to identify new clinical tools, which capture the effects of sub-clinical disease activity and distinguish patients at increased risk of MS-related disability.

One technology, which has generated recent interest in MS research is a non-invasive ocular imaging device called optical coherence tomography (OCT). Optical coherence tomography uses low-coherence interferometry to generate high resolution (≤10 microns (μm)), cross-sectional images of the retinal nerve fiber layer (RNFL) by measuring backscatter of infrared light. Optical coherence tomography imaging is analogous to ultrasound, except that the time-of-flight delay is measured for light instead of sound. The RNFL contains the retinal ganglion cell axons that comprise the optic nerve; and because it lacks myelin, represents a unique region of the central nervous system. Retinal nerve fiber layer defects are caused by retrograde degeneration of axons damaged by optic nerve inflammation in ON and MS patients. The OCT-measured RNFL values are diminished among ON and MS patients. Furthermore, reduced RNFL values in ON and MS patients have been shown to correlate with: diminished visual and neurological function; reduced optic nerve magnetization transfer ratios; MRI-measured optic nerve and brain atrophy; and decreased cerebral brain matter volumes. In MS patients, OCT-measured RNFL values have also been correlated with disease progression and relapses.

The evidence supporting OCT as a structural biomarker of optic nerve integrity in ON and MS patients continues to mount. Yet, the value of OCT-measured RNFL values in capturing sub-clinical disease activity, and thus identifying clinically isolated syndrome (CIS) patients at risk for CDMS after ON is not known, because prospective data are lacking. In this study, we compared RNFL values between ON patients who developed CDMS to patients who did not develop MS after ON. Our primary objective was to determine whether RNFL values could be used to distinguish patients at greater risk for CDMS after ON. We aimed to use this data to model expected RNFL changes and events over the two-years that follow ON in CIS patients, in order to design future intervention studies.

METHODS

Study design and Sampling. This was a prospective cohort of consecutively sampled patients assessed in the Neuro-Ophthalmology Clinics at the Ottawa Hospital (FC) between January 2003 and June 2007. The study received approval from the ethics board at the Ottawa Hospital, and participating patients provided informed and written consent.

Inclusion and Exclusion Criteria. Fifty consecutive patients (100 eyes) who experienced a single clinical unilateral ON event were included in the study. Patients were diagnosed with ON if they demonstrated the following clinical features: decreased visual acuity, a visual field defect which followed the topography of the retinal nerve fiber layer, color vision loss, a relative afferent pupil defect, and a compatible fundus examination (mild or no optic disc edema, and the absence of pallor at the time of the acute event). Exclusion criteria included other established causes of vision loss in the affected eye (including amblyopia, glaucoma, and dense cataracts), a known diagnosis of MS, and inability to undergo reliable OCT testing.

Outcome measures. The primary outcome measure in this study was the comparison of RNFL values in clinically-affected eyes and non-affected eyes between patients who converted to CDMS (CDMS patients) and patients who did not develop CDMS (non-MS patients) after ON. For the purposes of this study, CDMS was defined according to Poser criteria and included patients who experienced two clinical attacks, separated by a period of at least one month, with documented neurological signs of lesions in more than one area. Visual function scores after ON were compared between CDMS and non-MS patients, because differences in visual recovery could affect RNFL comparisons between the two groups.

Other Variables. Demographic and clinical variables including age; gender; the presence of pain; lesion burden on the baseline MRI scan; mono-focal ON (ON without other neurological symptoms) versus multi-focal ON (ON plus neurological symptoms referable to a region of the central nervous system different from the afferent visual pathway); presentation; treatment with high dose corticosteroids; and the initiation of disease-modifying agents (Interferon beta - 1a, interferon beta - 1b, or glatiramer acetate) were recorded. The time to baseline MRI acquisition and MRI protocols varied between patients. For this reason, we were not able to correlate specific MRI parameters with RNFL values in this study; nor, were we able to employ radiological criteria to define conversion to MS after ON. Instead, we documented whether patients had white matter lesions or a normal MRI scan at the time of the ON event. The treating physician employed individualized discretion concerning the decision to administer corticosteroid therapy for acute ON. Patients who received corticosteroid therapy were treated within two weeks of the ON event with the equivalent of 1000mg intravenous methyl-prednisolone daily for three days. Disease-modifying drugs were administered to a minority (24%) of patients in this study. No patients initiated disease-modifying agents earlier than six-months after ON. The limited number (12/50) of patients who received these therapies precluded efforts to compare the effects of disease-modifying drugs on RNFL values after ON.

Clinical Assessment. Patients underwent repeat neuro-ophthalmic assessment and OCT testing at approximately three-six month intervals for a minimum period of 24 months. The RNFL values between CDMS and non-MS patients were compared at year one and year two after ON. Neuro-Ophthalmic Assessment: With each visit, patients were questioned regarding new and residual subjective symptoms of vision loss, and underwent a complete neuro-ophthalmic evaluation (FC). The assessment included best-corrected Snellen visual acuity (converted to log MAR [log (minimal scale of resolution)] visual field analysis, and dilated ophthalmoscopy. Visual field mean deviation (VFMD) measured in decibels (dB)] was determined with Humphrey perimetry 30-2 full threshold SITA algorithm (Zeiss Meditech-Dublin, California). Humphrey test results were used if the false positive, false negative and fixation loss scores measured less than one third. Neuro-ophthalmic testing: Patients underwent clinical evaluations at six-month intervals by a neurologist in the MS Clinic at the Ottawa Hospital. Patients aged greater than 45 years, or those with atypical features (including incomplete recovery after ON) underwent visual electro-diagnostic testing (including pattern visual evoked potential, and multi-focal electroretinogram studies) to exclude
RESULTS

Demographics and Clinical Presentation. Fifty patients (mean age 34 years) were followed for a mean period of 34 months (range 24-44 months). Twenty-one patients (42%) developed CDMS during the course of the study, with a mean conversion time of 27 months. The baseline demographics and clinical data for all (n = 50) patients are included in Table 1. In the CDMS patient group, 18/21 (86%) were female; 18/21 (86%) reported pain at the onset of the ON event; 19/21 (91%) had an abnormal baseline MRI scan; 14/21 (67%) had a multi-focal presentation; and 12/21 (57%) received disease-modifying drug therapy.

The RNFL Values and Future Risk of CDMS. Year one: Mean overall and quadrant RNFL values tended to be thinner in non-affected eyes of non-MS patients; except for the temporal RNFL quadrant, which was thinner in CDMS patients, with a trend towards significance (p = 0.1733) (Table 2). In clinically-affected eyes, mean RNFL values were significantly thinner in non-MS eyes than CDMS eyes (p = 0.0462). Year two: Differences between RNFL values in non-affected eyes were not significant between non-MS and CDMS patients, albeit temporal RNFL values were again thinner in CDMS patients, with a trend towards significance (p = 0.1302). At this point in follow-up, the relationship between CDMS and non-MS patients reversed, such that RNFL values were thinner in the clinically-affected eyes of CDMS patients as compared to non-MS patients.

Table 1: Demographic and clinical characteristics among ON patients

<table>
<thead>
<tr>
<th>Demographics/Characteristics (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>Mean age (range)</td>
</tr>
<tr>
<td>Male: Female</td>
</tr>
<tr>
<td>CIS*: CDMS†</td>
</tr>
<tr>
<td>Pain (%)</td>
</tr>
<tr>
<td>RON‡: AON¶</td>
</tr>
<tr>
<td>MONO§: MULTI</td>
</tr>
<tr>
<td>Abnormal MRI# (%)</td>
</tr>
<tr>
<td>Abnormal CSF** (%)</td>
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<tr>
<td>Steroids^ (%)</td>
</tr>
</tbody>
</table>

* Clinically isolated syndrome patients; † Clinically definite MS patients; ‡ Retrobulbar optic neuritis patients; § Anterior optic neuritis (or papillitis) patients with mild disc hyperemia at presentation; ¶ Monofocal ON patients; || Multi-focal ON patients; # Percentage abnormal magnetic resonance imaging scans at presentation; ** Percentage of patients with positive oligoclonal bands in their cerebrospinal fluid (n = 20 patients); ^ Percentage of patients who received treatment with the equivalent of 1000mg intravenous methylprednisolone at presentation for optic neuritis.
patients. Within groups, CDMS patients manifested RNFL thinning in both eyes from year one to year two after ON; whereas RNFL values in non-MS patients marginally increased or stabilized by year two (Table 2).

Logistic regression was performed, using CDMS conversion as the dependent variable, and overall RNFL value in clinically-affected eyes to predict the odds of conversion to CDMS after ON. The simple logistics regression model yielded an odds ratio of 1.033 (p=0.0427) for year one and 0.9962 for year two (p=0.8071). These results indicate that overall RNFL thickness had limited use in predicting CDMS conversion one year after ON, and its predictive value diminished thereafter. Given that all other clinical conditions remained unchanged, a 5μm increase in mean the RNFL thickness of a clinically-affected eye increased the odds of converting to CDMS 1.18 fold one year after ON. Otherwise stated, for every 1μm increase in RNFL thickness in clinically-affected eyes, ON patients increased their chance of converting to CDMS at one year by 3.3%.

Visual recovery after ON: Visual field mean deviation and log MAR visual acuity scores were compared between the two groups at year one and year two after ON. Non-MS patients manifested less complete visual recovery than CDMS patients in this study. More specifically, six non-MS patients and two CDMS patients had visual acuity scores worse than 20/40 in AE; and seven non-MS patients and two CDMS patients manifested VFMD scores worse than -5.00 dB in clinically-affected eyes after ON. Median log MAR visual acuity and VFMD scores were worse in both eyes of non-MS patients as compared to CDMS patients at year one and year two after ON, albeit differences between the two groups were not significant (Table 3).

Recurrent ON events: Recurrent ON events occurred more frequently in CDMS patients than non-MS patients. Sub-clinical ON Events: Five patients (four CDMS patients and one non-MS patient) developed progressive RNFL thinning (>10μm) and associated changes in visual function in the original non-affected eye during year two of the study. These patients did not report awareness of pain or vision loss with their sub-clinical ON events. Three CDMS patients developed objective worsening of visual function scores and RNFL thinning during the second year of the study, consistent with recurrent sub-clinical ON in the original clinically-affected eye. Clinical ON Events: Two CDMS patients underwent assessment for recurrent clinical ON in their original non-affected eye in year two of the study. In both cases progressive RNFL thinning (>10μm) was observed.

Table 2: Comparing RNFL values between CDMS patients and non-MS patients at year one and year two after ON

<table>
<thead>
<tr>
<th>RNFL* NE† (CI)#</th>
<th>Year 1</th>
<th>Year 2</th>
<th>p-value</th>
<th>Year 1</th>
<th>Year 2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDMS (n=21)</td>
<td>Non-MS (n=29)</td>
<td>p-value</td>
<td>CDMS (n=21)</td>
<td>Non-MS (n=29)</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>105.1 (101.5,108.7)</td>
<td>102.0 (97.7,106.3)</td>
<td>0.2887</td>
<td>104.8 (99.2,110.3)</td>
<td>103.7 (99.5,107.9)</td>
<td>0.7548</td>
</tr>
<tr>
<td>Superior</td>
<td>133.7 (127.2,140.2)</td>
<td>130.3 (124.7,136.0)</td>
<td>0.4338</td>
<td>133.1 (123.4,142.9)</td>
<td>135.9 (129.9,141.9)</td>
<td>0.6031</td>
</tr>
<tr>
<td>Inferior</td>
<td>134.9 (129.7,140.0)</td>
<td>128.1 (121.2,135.0)</td>
<td>0.1409</td>
<td>132.9 (124.9,140.8)</td>
<td>127.2 (121.0,133.4)</td>
<td>0.2463</td>
</tr>
<tr>
<td>Nasal</td>
<td>86.6 (79.0,94.2)</td>
<td>80.2 (73.6,86.8)</td>
<td>0.1995</td>
<td>88.1 (79.8,96.5)</td>
<td>81.4 (74.0,88.8)</td>
<td>0.2216</td>
</tr>
<tr>
<td>Temporal</td>
<td>64.9 (59.3,70.4)</td>
<td>69.6 (65.1,74.1)</td>
<td>0.1733</td>
<td>64.8 (59.4,70.2)</td>
<td>70.8 (65.2,76.3)</td>
<td>0.1302</td>
</tr>
</tbody>
</table>

*Retinal nerve fiber layer thickness (μm); †Clinically non-affected eyes; ‡Clinically affected eyes; §Optic neuritis patients who did not develop CDMS; ‖Mean overall RNFL value, and mean RNFL values in the superior, inferior, nasal and temporal quadrants; # 95% confidence interval.
Two CDMS patients experienced recurrent clinical ON in their original clinically - affected eye during the second year of follow-up. One patient manifested progressive RNFL thinning (>10 μm), whereas the second patient maintained stable RNFL values in the clinically - affected eye.

**DISCUSSION**

In this study, we demonstrated that RNFL values differ between CDMS and non-MS patients after ON. After one year, RNFL values were significantly thinner in the clinically - affected eyes of non-MS patients, which may have been due to less complete visual recovery from ON in this group. In the second year of follow-up, CDMS patients manifested more recurrent ON events, and demonstrated greater thinning of the RNFL than non-MS patients. CDMS patients had greater atrophy in the temporal RNFL region in non - affected eyes than non - MS patients, with a trend toward significance.

Prior studies have shown that RNFL values are reduced in MS patients as compared to healthy control subjects, with the lowest RNFL values reported in eyes previously affected by ON.11,18 Henderson and colleagues19 recently compared RNFL thickness and macular volumes in 23 patients with primary progressive MS, 27 patients with secondary progressive MS, and 20 healthy controls. While their results indicated significant reductions in RNFL thickness and macular volume in the eyes of secondary progressive MS patients not previously affected by ON, the authors concluded that “clinically overt ON has adverse consequences for the RNFL over and above that of secondary progressive MS alone.” There have been no prior studies, which have tracked RNFL changes after ON to determine the future risk of CDMS. Our results are consistent with related reports, and indicate that lower RNFL values manifest in patients with less complete recovery after ON, irrespective of MS status. The findings from our study confirm that RNFL thinning is heavily dependent on the severity of the ON event; and that as a surrogate biomarker for axonal integrity, RNFL thickness has limited use in predicting CDMS conversion in first year of follow up after ON.

We observed that differences in RNFL thickness were not significant between CDMS and non-MS patients two years after ON; but progressive RNFL thinning was more apparent in the clinically - affected eyes of CDMS patients than non-MS patients during the second year of this study. Accordingly, recurrent subclinical ON events occurred with greater frequency in CDMS patients, which correlated to the progressive RNFL thinning observed in this group. This observation suggests that subclinical insults to the afferent visual pathway cause progressive axonal attrition, which is the substrate for reduced RNFL values in MS patients, with and without a history of ON.11,18 Our results indicate that cross-sectional comparisons do not aptly capture disease dynamics, which distinguish patients at greater risk for developing CDMS after ON. Instead, longitudinal studies designed to track RNFL changes and detect recurrent ON events may better identify patients at risk for future MS. More specifically, OCT may have a role in detecting ON patients at risk for CDMS, by revealing trends of change in RNFL thickness over time, which mirror sub-clinical disease activity in the afferent visual pathway.

There were limitations in this study, which likely impacted our results. First, the follow up period of two years may not have been an adequate time interval to detect significant differences in RNFL thickness, which distinguish CDMS from non-MS patients. Secondly, the small size of our patient population hindered the statistical power of comparisons between RNFL values in CDMS and non-MS patients. We observed that temporal RNFL values in non - affected ON eyes were generally thinner in CDMS than non-MS patients; with a trend towards significance. It is possible that in a larger study population, a more significant relationship between temporal RNFL thinning and future CDMS risk would have emerged. In a recent two-year prospective study, Sepulcre et al showed that temporal quadrant RNFL values were reduced in MS patients; and that baseline temporal RNFL atrophy was associated with the presence of new relapses and MS-related disability.16 Furthermore, Henderson and colleagues19 reported that RNFL loss was most evident in the temporal quadrant of progressive MS patients; such that significant reduction was seen in primary progressive MS versus controls and in secondary versus primary progressive MS. Therefore, it is plausible that temporal RNFL atrophy may distinguish patients at increased risk for CDMS after ON, but this remains to be established.
In conclusion, we documented RNFL changes and events in ON patients over a two-year period to aid in the design of future intervention studies. Our results indicate that RNFL values should be followed longitudinally in concert with tests of visual function to fully capture the effects of sub-clinical disease activity on structural RNFL measures in the afferent visual pathway. Trends of change in RNFL thickness, and temporal atrophy may be used to identify patients at increased risk for CDMS after ON, but this awaits further study. Future prospective clinical trials should include a larger patient population than we used in this study, to determine whether RNFL changes after ON may be used to identify patients at risk for future CDMS.

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REFERENCES