Retinal Nerve Fiber Layer Thickness Changes in Patients with Age-Related Macular Degeneration Treated with Intravitreal Ranibizumab

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Purpose. To assess the effects of intravitreal ranibizumab therapy on intraocular pressure (IOP) and retinal nerve fiber (RNFL) thickness.

Methods. Forty-nine eyes of 49 patients with neovascular age-related macular degeneration (AMD) treated with intravitreal ranibizumab injections and 27 fellow eyes not requiring treatment were followed for 1 year. RNFL thickness, as measured by Fourier domain optical coherence tomography, and IOP were determined pre- and postinjection.

Results. After 12 months, the mean number of injections received was 4.8 ± 1.6. The incidence of IOP elevations (>5 mm Hg over baseline) observed at the time of injection was 0.4%. Baseline RNFL thickness was 105.7 ± 12.2 μm in the treatment group compared with 101.8 ± 11.6 μm in the control group ($P = 0.176$). At the end of follow-up, significant RNFL thinning was noted in the treatment group (100.2 ± 11.0 μm, $P < 0.001$), whereas no differences were found in the control group (100.5 ± 10.8 μm, $P = 0.477$).

Conclusions. Intravitreal ranibizumab injections used to treat AMD caused a significant change in RNFL thickness after 12 months of follow-up. (*Invest Ophthalmol Vis Sci.* 2012; 53:6214–6218) DOI:10.1167/iovs.12-9875

Glaucoma and age-related macular degeneration (AMD) are the two leading causes of irreversible blindness in developed countries.[1,2] In glaucoma, the retinal nerve fiber layer (RNFL) becomes gradually thinner and this leads to visual field loss, which in advanced stages can produce blindness.[3] In neovascular or exudative AMD, the wet form of AMD, abnormal blood vessel growth leads to blood and protein leakage below the macula causing irreversible damage to the photoreceptors and rapid central vision loss if left untreated.[2,3]

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The recent introduction of drugs that inhibit vascular endothelial growth factor (anti-VEGF) like ranibizumab (Lucentis), bevacizumab (Avastin), or pegaptanib (Macugen) have revolutionized the treatment of neovascular AMD. When directly injected into the vitreous humor, these drugs prevent the advance of neovascular AMD in an elevated number of patients.[4–6] However, exposure to an antiangiogenic drug, usually given as monthly or 6-weekly injections, often induces intraocular pressure (IOP) elevations, which are usually self-limiting but sometimes may require treatment.[7–10] So far, the literature lacks studies that have assessed the effect of repeated ranibizumab injections on the RNFL.[11,12]

The present prospective controlled study was designed to determine RNFL modifications induced by intravitreal ranibizumab treatment of neovascular AMD by Fourier domain optical coherence tomography (Spectralis SD-OCT, Heidelberg Engineering, Heidelberg, Germany), as well as any IOP effects of this drug.

Methods

We designed a prospective longitudinal cohort study of 1 year's duration to assess RNFL thickness in patients with AMD treated with intravitreal ranibizumab (injectable solution of 10 mg/mL, 0.5 mg, or 0.05 mL).

Consecutive patients with neovascular AMD who were to undergo first-time treatment with intravitreal ranibizumab were enrolled. The diagnosis of neovascular AMD was made on the basis of an abnormal appearance of choroid neovessels or of a choroid vascular membrane that could manifest as pigment epithelium or neuroepithelium, both serous or hemorrhagic, with exudation toward the retinal tissue. The treatment regimen followed the guidelines of the European Medication Agency (EMA). The study design fulfilled the tenets of the Declaration of Helsinki and was approved by our institution's review board. Written informed consent was obtained from each patient. The dosing regimen of ranibizumab was monthly 0.05-mL injections for 3 months following diagnosis and a further injection if there was exudative lesion reactivation on the retina. As controls, fellow eyes that did not fulfill the exclusion criteria and showed no AMD requiring treatment were selected.

Patients were excluded if they had poorly controlled AMD, were unable to cooperate, had received prior treatment for AMD, or had optic nerve disease, including glaucoma, elevated IOP, factors associated with secondary glaucoma, any active eye disease (eg, uveitis, infection, or dry eye syndrome), an eye condition or disease that might contraindicate the programmed tests, or patients whose disease or health state in the opinion of the investigator could significantly increase the risks of treatment, interfere with the study results, or considerably impair their participation in the study.

The following variables were recorded for each eye: type of neovascular membrane (classic, predominantly classic, minimally

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classic, occult, retinal angiomatous proliferation, or polypoidal choroidal vasculopathy); age; sex; number of injections received at 1, 3, 6, and 12 months; and best-corrected visual acuity (BCVA) at baseline and 1, 3, 6, and 12 months after treatment onset.

Over the course of follow-up, RNFL thickness and macular thickness were measured by spectral domain OCT (Spectralis SD-OCT, Heidelberg Engineering, Heidelberg, Germany). A pupil diameter of at least 4 mm was required for scanning. RNFL thickness measurements (diameter 3.5 mm, 768 A-scans) were obtained at baseline and at 3, 6, and 12 months of follow-up. An online tracking system was used to compensate for eye movement. RNFL thickness (from the inner margin of the internal limiting membrane to the outer margin of the RNFL layer) was automatically segmented using the Spectralis software. Quality criteria included sharp scan beam and definition of vessels, scan beam centered on optic disc, even illumination, automatic real-time (ART) score of 16, and signal-to-noise ratio of 15 dB or higher. Macular thickness measurements were obtained at baseline and at the 12-month visit by acquiring 25 frames in an area of 30 × 30 degrees at 1536 × 1536 pixels. We selected the retinal thickness map analysis to display numeric averages of the measurements for each of the nine subfields as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS) (Fig.). The inner, intermediate, and outer rings of diameters 1, 3, and 6 mm, respectively, were considered for the analyses. The average of all points within the inner 1-mm radius circle was defined as central foveal thickness (CFT). The intermediate ring is divided into four zones designated as inner superior (IS), inner nasal (IN), inner inferior (II), and inner temporal (IT); and the outer ring designated into outer superior (OS), outer nasal (ON), outer inferior (OI), and outer temporal (OT). The numerical values recorded for each of the nine zones were used in the analyses. In both analyses, the autorescan function was activated to minimize variation in allocating the acquisition protocols to the follow-up sessions.

IOP was measured in each patient before each injection and 1 hour after the injection procedure. Any pressure elevation above 5 mm Hg was recorded. If IOP was higher than 30 mm Hg, topical IOP-lowering medication treatment was prescribed and the patient reassessed after 24 hours.

All statistical tests were performed using the SPSS package version 18.0 for Windows (SPSS, Inc., Chicago, IL). The Kolmogorov-Smirnov test was used to check for a normal distribution of quantitative data. Quantitative variables were expressed as their corresponding means and SDs. Medians and interquartile ranges were used to describe variables showing a non-normal distribution. Within-group changes from baseline were analyzed using paired *t*-tests. Within-group measurements during follow-up were analyzed using an ANOVA test for repeated measures. Correlation between macular thickness and RNFL thickness was assessed using the Pearson correlation coefficient. The level of significance was set at P less than 0.05. The effect of multiple comparisons was corrected by the Bonferroni test.

Results

Forty-nine patients who fulfilled the inclusion and exclusion criteria were included in the study; 49 eyes were deemed valid as cases and 27 fellow eyes met the requirements as controls.

Mean age was 78.5 ± 6.9 years; 33 (67.3%) of the neovascular membranes were classified as occult, whereas the remaining 16 (32.7%) were assigned to the other categories (Table 1). The overall number of injections given over the 12 months of follow-up was 236 (range 3–8), with a mean of 4.8 ± 1.6 injections per eye. The incidence of pressure spikes (IOP elevation greater than 5 mm Hg) following the injection procedure was 0.4%. No patient required IOP-lowering treatment for spikes greater than 10 mm Hg. BCVAs during follow-up are provided in Table 1.

Baseline RNFL thickness means recorded over the 12 months were 105.7 ± 12.2 μm for the cases and 101.8 ± 11.6 μm for the controls ($P = 0.176$), and means at 3, 6, and 12 months were 101.4 ± 10.4, 101.1 ± 10.7, and 100.1 ± 11.0 μm for the cases and 100.4 ± 11.7, 99.3 ± 11.1, and 100.5 ± 10.8 μm for the controls, respectively. Compared with baseline, thickness reductions were 5.6 ± 9.1 μm in the case group ($P < 0.001$) and 1.3 ± 9.6 μm in the control group ($P = 0.477$). Differences in these measurements during follow-up between the two groups were not significant ($P = 0.065$).

Table 2 provides the mean RNFL thicknesses recorded by quadrant (superior, nasal, inferior, and temporal). At the end of follow-up, significant differences compared with baseline were observed in superior, inferior, and temporal quadrants in the case group, whereas no differences were found in the control group in any quadrant.

To assess the effects of changes in macular thickness induced by ranibizumab on RNFL thickness, macular thickness was determined during follow-up. Table 3 shows the thickness of each macular zone at the start and end of follow-up. Significant differences during the study were detected in the central (CFT) and two of the four zones of the inner ring (nasal and inferior). In contrast, the thicknesses recorded for the four outer ring zones failed to vary at the end of follow-up from the baseline visit. Correlations were examined between thickness changes (thickness at 12 months − baseline thickness) produced in the nasal region of the outer macular ring and those produced in the superior, inferior, and temporal quadrants of the RNFL. No significant correlations were detected for any of the quadrants (superior $r = -0.026$, $P = 0.884$; inferior $r = 0.170$, $P = 0.334$; temporal $r = -0.312$, $P = 0.077$).

Neither did we observe significant correlations between thickness changes produced during follow-up in the superior zone of the outer macular ring and those produced in the superior RNFL quadrant ($r = -0.200$, $P = 0.264$), nor between the inferior zone of the outer macular ring and the inferior RNFL quadrant ($r = 0.156$, $P = 0.385$).

Discussion

The use of intravitreal injections to treat diseases of the retina, especially neovascular AMD, is becoming common practice. The results of our study indicate that several monthly 0.05-mL doses of ranibizumab lead to significant RNFL thinning.

To the best of our knowledge, no prior controlled randomized study has addressed the possible effects of repeated intravitreal injections of anti-VEGF agents on the RNFL. In theory, RNFL modifications could be induced by the intraocular volume increase produced by the injection or through a direct toxic effect of the drug on the RNFL.

Several authors have assessed the relationship between anti-VEGF agents and the intraocular pressure spikes observed in response to their administration.[8,9,13] The general consensus is that IOP spikes are limited to within the first few minutes of the injection procedure, prophylactic IOP-lowering treatment is not efficient to prevent their appearance,[14] and that in practically all cases, pressures return to normal values without the need for additional treatment. Hollands et al.[9] examined the short-term effects of intravitreal bevacizumab on IOP and detected a significant IOP increase in the first minutes posttreatment, passing from a baseline IOP of 14.0 (95% confidence interval [CI] 13.4–14.7) mm Hg to 36.1 (95% CI 33.5–38.6) mm Hg 2 minutes after the injection. By 30 minutes postinjection, IOP had returned to its baseline value. Only in 3 eyes (2.9%) of the 104 examined, did pressure remain over 25 mm Hg for more than 30 minutes. In two cases, pressure normalized 2 hours later without medication and in the remaining eye, pressure-lowering

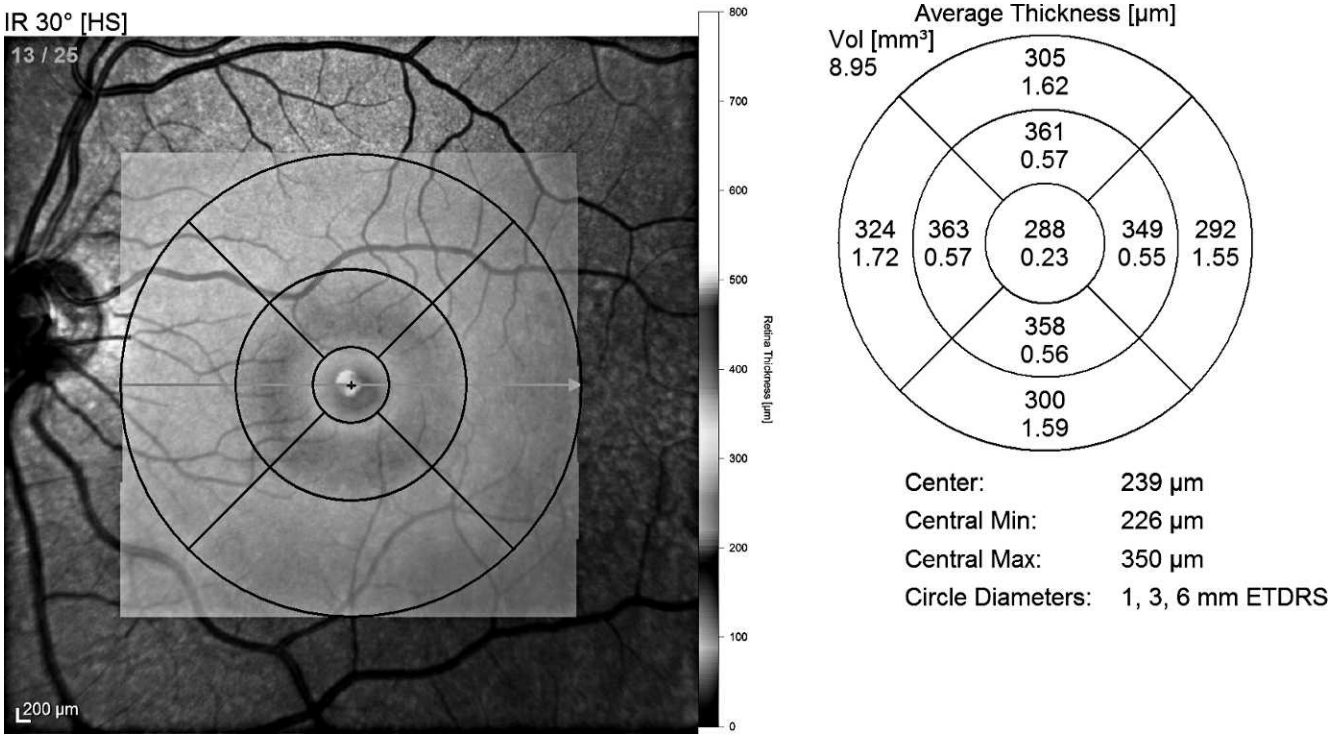

Figure. Retinal thickness (RT) analysis protocol showing the mean thickness in each of the 9 ETDRS subfields.

treatment was required for a week until baseline IOP was recovered. Falkenstein et al.[13] reported similar results with mean increases of 21 mm Hg observed 3 minutes after injection. All cases showed pressures under 30 mm Hg at 15 minutes postinjection without the need for treatment. Using the same anti-VEGF drug and dosing regimen as that used here, Sharei et al.[15] described mean IOP increases of 25 mm Hg immediately after the injection procedure in 45 eyes: 71.1% of treated eyes showed IOPs above 40 mm Hg and 42.2% above 50 mm Hg. A return to normal IOP was observed without the need for treatment and the difference at 10 minutes was only $+4.6 \pm 7.0$ mm Hg from baseline IOP.

In our cohort, only a single case showed a significant IOP spike; however, the fact that IOP was determined 1 hour after injection rather than after the first few minutes may have prevented the observation of greater IOP peaks. Such peaks may have even been the cause of RNFL thinning during follow-up when treated eyes had received several injections.

A recent study has revealed that patients treated with multiple anti-VEGF injections may, over time, show sustained IOP elevation with no as-yet known origin requiring IOP-lowering treatment.[16] It has also been determined that the incidence of such increased IOP is more frequent among patients with preexisting glaucoma.[17]

A second hypothesis, although less plausible, to explain the RNFL thinning observed here would be a direct toxic effect of the drug on this nerve fiber layer. Thus, Zayit-Soudry et al.[11] assessed in rabbits the toxicity of nine intravitreal injections of bevacizumab or ranibizumab given on a 2-weekly basis. Follow-up included electrophysiological tests and acquiring morphological data but no toxic effects on the retina were detected. To date, no study in humans has addressed this hypothesis.

The main objective of our study was to quantify possible RNFL damage induced by IOP spikes or the action of the drug itself, given that no prior study has analyzed this possible adverse effect of intravitreal anti-VEGF therapy in a prospective and controlled manner. Our findings indicate that whether because of the pressure elevations produced immediately following the injection procedure or because of the drug's toxicity, significant RNFL thinning occurred in the treated eyes (5.6 µm), whereas no significant changes were detected in the control group. The lack of significance of the ANOVA test for repeated measures could be a consequence of nonsignificant differences between the pairs of measures compared but a statistically significant difference did emerge in the treatment group between baseline and 12 months. In a recent study, Horsley et al.[12] retrospectively examined the effect on the RNFL of a series of injections of bevacizumab, ranibizumab, or pegaptanib in patients with AMD. Of 41 eyes receiving a minimum of 10 injections, no significant differences were detected by temporal domain OCT (Stratus OCT; Carl Zeiss Meditec, Inc., Dublin, CA) after a mean follow-up of 27.0 ± 9.7

Table 1. Subject Demographics and Eye Characteristics

	Cases ($n = 49$)	Controls ($n = 27$)	
Mean age, y	79.2 ± 7.0	77.2 ± 6.5	$P = 0.233$
Sex, M/F	20/29	13/14	
CNV lesion subtype, *n* (%)			
Occult with no classic	33 (67.3%)		
Minimally classic	3 (6.1%)		
Predominantly classic	11 (22.4%)		
Other	2 (4.2%)		
BCVA (letters)			
Baseline	55.1 ± 20.4	62.9 ± 28.9	
3 mo	61.3 ± 18.3	64.8 ± 27.1	
6 mo	61.3 ± 18.8	65.2 ± 28.7	
12 mo	58.6 ± 21.3	64.9 ± 28.4	
Average RNFL at baseline	105.7 ± 12.2	101.8 ± 11.6	$P = 0.176$

Mean ± SD (mm Hg). CNV, choroidal neovascularization.

Table 2. RNFL Thickness during Follow-Up

	Cases ($n = 49$)	Controls ($n = 27$)
Average RNFL thickness		
Baseline	105.7 ± 12.2	101.8 ± 11.6
3 mo	101.4 ± 10.4	100.4 ± 11.7
6 mo	101.1 ± 10.7	99.3 ± 11.1
12 mo	100.2 ± 11.0	100.5 ± 10.8
Change from baseline (*P*)	<0.001	0.477
Superior quadrant		
Baseline	126.0 ± 23.4	118.0 ± 17.7
3 mo	118.9 ± 17.1	117.0 ± 16.7
6 mo	117.9 ± 18.8	116.5 ± 16.2
12 mo	118.7 ± 20.0	119.4 ± 17.7
Change from baseline (*P*)	0.030	0.695
Nasal quadrant		
Baseline	82.7 ± 19.9	79.1 ± 12.6
3 mo	79.9 ± 16.3	81.5 ± 18.8
6 mo	80.2 ± 15.8	76.6 ± 13.8
12 mo	78.8 ± 14.3	77.2 ± 12.8
Change from baseline (*P*)	0.064	0.333
Inferior quadrant		
Baseline	130.3 ± 16.6	124.1 ± 22.7
3 mo	128.5 ± 15.9	125.7 ± 20.3
6 mo	126.9 ± 15.1	123.7 ± 21.6
12 mo	126.3 ± 14.6	127.3 ± 29.3
Change from baseline (*P*)	0.005	0.562
Temporal quadrant		
Baseline	87.1 ± 22.5	85.2 ± 17.7
3 mo	76.1 ± 11.9	78.7 ± 14.4
6 mo	76.5 ± 14.5	79.7 ± 16.0
12 mo	76.1 ± 14.5	81.6 ± 19.3
Change from baseline (*P*)	<0.001	0.201

Mean ± SD (microns).

months. The study design, lack of a control group, and the measuring instrument used could explain the different results to ours. Compared with temporal domain OCT, spatial domain OCT offers better resolution and reproducibility of measures because its software is designed to take measurements in a given zone during the course of follow-up. This minimizes variation and improves the power to detect small-thickness changes in the RNFL.[18]

During follow-up, macular thickness was also measured to assess whether the changes produced in the RNFL could be caused by macular edema possibly reaching the peripapillary region. Of the three concentric rings into which the retinal thickness map is divided according to the ETDRS (1, 3, and 6 mm) only the two innermost rings were affected by the changes induced by ranibizumab. None of the 4 quadrants of the outermost zone showed changes at the end of follow-up from baseline. In addition, in no case were correlations between changes detected in the outer macular ring and changes in the RNFL statistically significant. According to these data, it seems unlikely that the RNFL thinning observed in response to treatment was secondary to a change in the extent of macular edema induced by ranibizumab.

The loss of nerve fibers in the study group at the end of the 12 months of follow-up amounts to 5.2% with respect to baseline thickness. Although statistically significant, this difference could be only of relative clinical significance in a patient with an initially normal nerve fiber layer thickness. However, in a patient with an already compromised RNFL, the changes induced by intravitreal anti-VEGF therapy could worsen the patient's clinical situation.

Table 3. Macular Thickness during Follow-Up

	Baseline	12 mo	*P*
Central foveal thickness	353.4 ± 113.4	301.9 ± 58.5	0.011
Inner ring			
Superior	347.6 ± 72.5	337.7 ± 54.7	0.320
Nasal	359.5 ± 84.1	334.6 ± 53.9	0.036
Inferior	351.6 ± 64.7	330.5 ± 43.0	0.039
Temporal	353.0 ± 63.9	334.4 ± 52.7	0.080
Outer ring			
Superior	306.9 ± 38.1	303.8 ± 45.0	0.563
Nasal	309.5 ± 32.6	308.3 ± 48.9	0.833
Inferior	292.4 ± 32.8	285.7 ± 27.7	0.340
Temporal	290.6 ± 40.7	279.1 ± 28.0	0.194

Mean ± SD (microns).

Thus, in patients with glaucoma and considerable prior RNFL thinning scheduled to receive anti-VEGF treatment, it would be prudent to more closely monitor IOP elevations and to record any RNFL modifications by OCT.

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