Scleral Thinning After Repeated Intravitreal Injections of Antivascular Endothelial Growth Factor Agents in the Same Quadrant

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PURPOSE. We assessed the effects of intravitreal anti-vascular endothelial growth factor (anti-VEGF) therapy on scleral architecture using spectral domain anterior segment optical coherence tomography (OCT).

METHODS. A total of 35 eyes of 35 patients treated with at least 30 intravitreal injections in one eye in the inferotemporal quadrant with ranibizumab or aflibercept and 10 or less intravitreal injections in the fellow eye attending the intravitreal injection clinic were included. Enhanced depth imaging anterior segment OCT was used to measure scleral thickness. For each eye the sclera was measured in four quadrants at 3 mm from the limbus. In addition axial eye length was measured in all subjects using partial coherence interferometry.

RESULTS. The mean number of intravitreal injections was 42 (range, 30–73) and 1.6 (range, 0–9) in the fellow eyes. In the study eyes with more than 30 injections the average scleral thickness in the inferotemporal quadrant was 568.4 μm (SD ± 66 μm) and 590.6 μm (SD ± 75 μm) in the fellow eyes with 10 or less injections (P = 0.003). The mean average scleral thickness in the other three quadrants (inferonasal, superotemporal, and superonasal) was 536.6 μm in the study eyes (SD ± 100 μm) and 545.2 μm (SD ± 109 μm) in the fellow eyes (P = 0.22). There was a borderline association of the total number of injections with scleral thickness change in the inferotemporal quadrant (r = 0.3, P = 0.052).

CONCLUSIONS. Intravitreal injections may lead to scleral changes when applied repeatedly in the same quadrant. Thus, alternating the injection site should be considered in patients requiring multiple intravitreal injections.

Keywords: retina, anti-VEGF, intravitreal injections, sclera, anterior segment OCT

The introduction of drugs inhibiting vascular endothelial growth factor (VEGF), such as ranibizumab (Lucentis), aflibercept (Eylea), or bevacizumab (Avastin), has revolutionized the treatment of VEGF driven macular disease, such as age-related macular degeneration (AMD), diabetic macular edema, or macular edema secondary to retinal vein occlusion. Targeting VEGF is an effective treatment for the preservation and improvement of visual acuity in these diseases with the best outcome achieved by monthly intravitreal injection of anti-VEGF drugs.1–4 However, as successful treatment mandates regular intravitreal injections many patients may receive monthly injections over the course of several years. Although the standard 30-gauge needle for intravitreal injections is small and atraumatic, the injections process occurs in a well-defined area, usually the infero- or superotemporal quadrant and 3 mm from the limbus and, thus, repeated injections may lead to structural changes of the sclera. Although it has been voiced that the intravitreal injection site should be alternated to avoid scleral damage, the literature lacks studies that have assessed the effect of repeated intravitreal injections on scleral architecture in the same quadrant. Recent advances in imaging techniques, such as averaging of multiple B-scans and enhanced depth imaging (EDI) acquisition, have enabled the capture of high quality images of dense tissues, such as the sclera,5 and several studies have used this technique for investigation of scleral changes after small gauge vitreoretinal surgery.6–7 The present retrospective controlled study was designed to determine changes in scleral architecture in patients having received in excess of 30 intravitreal ranibizumab injections in the inferior temporal quadrant using enhanced depth imaging anterior segment spectral domain optical coherence tomography (AS SD-OCT).

METHODS

The study design fulfilled the tenets of the Declaration of Helsinki and was approved by the institution’s review board of Bern, Switzerland. Written informed consent was obtained from each patient.

Participants

This was a retrospective interventional cross-sectional study to assess scleral thickness in patients with AMD. We included patients with a difference of more than 30 intravitreal anti-VEGF injections in the inferior temporal quadrant (0.05 mL injectable solution between both eyes and a maximum number of nine injections in one eye. The eye with the higher number of injections was selected as study eye, the fellow eye served as a control eye. The treatment regimen was three monthly 0.05-mL injections for 3 months following diagnosis and further injections...
if there was lesion reactivation on the retina on OCT examination following the Bern treatment algorithm. Subjects were excluded if the image quality of the scleral OCT scan was insufficient or if they had an eye condition that might interfere with the study results, such as a history of filtrating glaucoma surgery, scleral buckling or uveitis with use of topical steroids or an axial eye-length of more than 26 mm using partial coherence interferometry (IOL Master; Carl Zeiss, Jena, Germany).

**Anterior Segment EDI OCT; Image Acquisition and Analysis**

For OCT imaging a spectral domain anterior segment OCT (Heidelberg Engineering, Dossenheim, Germany) was used and the scleral mode with EDI was chosen. The number of B-Scans was 11 with a pattern size of $15^\circ \times 5^\circ$ (8.3 × 2.8 mm) and the distance between B-Scans was 277 $\mu$m. The OCT images were averaged with 8 to 10 scans for better resolution. For image acquisition of the four quadrants of the sclera patients were asked to fixate on a fixation light and care was taken to include the limbus on all images. For each eye four regions were measured: Inferotemporal, inferonasal, superonasal and superotemporal (Fig. 1). Scleral thickness was measured independently by two masked observers.

The Heidelberg Eye explorer software (version 1.7.1.0) was used to measure a 3-mm distance from the scleral spur (SS) using the caliper tool, corresponding to the scleral area used for intravitreal injections. Here, the scleral thickness was measured on three sections per quadrant and averaged (Fig. 1A). The sclera was discerned from the conjunctiva and the episclera by identifying the deep episcleral vascular plexus (Figs. 1B, 2A).

**Statistical Analysis**

Study data were collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools.
hosted at the Department of Ophthalmology Inselspital, Bern University Hospital, and University of Bern, Switzerland. The REDCap is a secure, web-based application designed to support data capture for research studies.\textsuperscript{9} Continuous quantitative variables were expressed as their corresponding means and standard deviation (SDs). The d’Agostino and Pearson omnibus normality test was used to check for a normal distribution of quantitative data. Scleral thickness parameters did follow a Gaussian distribution. Within-group changes within the scleral thickness were analyzed using paired \( t \)-tests. The level of significance was 0.05 (2-sided) in all statistical tests. To correlate the changes of scleral thickness with the number of injections Spearman correlation was applied. Bland–Altman plotting was performed to assess the clinically relevant magnitude of the variance between the scleral thickness of the study eye versus the fellow eye. For comparison of statistical data, analysis was performed using GraphPad Prism version 6 for Windows (GraphPad Software, Inc., La Jolla, CA, USA). The intraclass correlation coefficient was calculated with SPSS for windows (IBM SPSS Statistics, Chicago, IL, USA).

RESULTS

Patient Characteristics

We included 35 subjects in this study. The mean age was 76 years (range, 61–87). The male-to-female ratio was 3:4. A total of 27 patients received intravitreal anti-VEGF treatment in one eye for exudative AMD, seven for cystoid macular edema due to central retinal vein occlusion, and one patient had diabetic macular edema. Number of injections, medication used, IOP at time of baseline, and follow up OCT and axial length measurements are summarized in Table 1.

Metrics of Scleral Thickness Measurements

Both eyes displayed characteristic intraindividual variation in scleral thickness with the inferonasal quadrant having the
thickest sclera followed by the inferotemporal, and superonasal and superotemporal quadrants, respectively (Figs. 2B1, 2B2, Table 2; \( P < 0.0001 \), 1-way ANOVA). Interobserver agreement for all quadrants was assessed using the interclass correlation coefficient (ICC). The ICC was 0.86 for measurements of scleral thickness.

**Ocular Surface Changes at the Injection Site**

Many patients who had received intravitreal injections displayed episcleral cystoid cavities as seen on AS SD-OCT (Fig. 3). These cystoid changes were found approximately 3 mm from the limbus and corresponded well with the injection site.

**Scleral Changes at the Injection Site**

In some patients the intravitreal injection site was clearly visible (Fig. 4A) with up to 19 injection marks counted on the infrared image (Fig. 4A). Furthermore these injection marks correlated with scleral tunnels seen in OCT (Fig. 4A). We investigated next whether multiple injections could lead to localized scleral changes, such as thinning.

For this we measured the sclera in the inferotemporal quadrant in the area of repeated intravitreal injections and compared this to the same area in the fellow eye. The mean scleral thickness in the study eyes was 568.4 \( \mu m \) (SD \( \pm \) 66 \( \mu m \)) and 590.6 \( \mu m \) (SD \( \pm \) 75 \( \mu m \)) in the fellow eyes with nine or less injections (\( P = 0.003 \), Fig. 4B).

Next, we analyzed whether the scleral changes found at the injection site were localized or whether repeated injections lead to a generalized thinning of the sclera. For this we averaged the other three quadrants and compared these to the fellow eye using a paired \( t \)-test. Although the scleral thickness averaged over the other three quadrants was 536.6 \( \mu m \) (SD \( \pm \) 100 \( \mu m \)) and, therefore, slightly thinner than that found in the fellow eyes with 545.2 \( \mu m \) (SD \( \pm \) 110 \( \mu m \)) this was not statistically significant (\( P = 0.22 \), Fig. 5A1). The agreement between scleral thickness measurements between the fellow eyes is shown in the Bland Altman plots in Figure 5A2.

**Correlation of Number of Injections With Scleral Thinning**

Next, we analyzed whether the difference in number of injections between both eyes could be correlated with the observed difference in scleral thickness between study eye and fellow eye in the inferotemporal quadrant. Plotting the difference of injections against difference in scleral thickness did show a borderline correlation (\( r = 0.3 \), \( P = 0.052 \), Fig. 5B).

**Discussion**

The sclera provides mechanical strength to the globe and protects the eye from external trauma. The human sclera is composed of dense, primarily collagenous tissue consisting mainly of types I, III, V, and VI collagen. Furthermore the sclera is composed of proteoglycans containing approximately 70% water. Measurements of scleral thickness have been reported mostly in fixed specimens of enucleated eyes yielding on average 430 \( \mu m \) at the ora serrata. Another report using ultrasound biomicroscopy has shown the average scleral thickness to be approximately 550 \( \mu m \) at 3 \( mm \) from the limbus. These reports are in keeping with our measurements. Furthermore, the distinct distribution of scleral thickness according to the individual quadrants in this report supports the reproducibility of the quantification of scleral thickness.

Recent advances in OCT imaging techniques, such as enhanced depth imaging allow obtaining a detailed view of this vital structure. With most intraocular surgical techniques access to the eye occurs via the sclera and with the increased use of intravitreal injections, mainly with anti-VEGF agents, a growing number of patients receive multiple intravitreal injections. To deliver medication into the vitreous the sclera has to be penetrated with a needle. Here, we show that multiple intravitreal injections lead to localized scleral changes at the injection site when compared to the fellow eye. Recent publications have shown that axial length is significantly correlated to scleral thickness. Given the similarity of axial length in the fellow eyes (Table 1) this is not likely to be a confounding factor in our study.

We can only speculate about the pathomechanisms leading to scleral thinning in these patients. Using a 30-gauge needle the size of penetration is 0.05 \( mm^2 \) which would, with an average injection number of 42, yield a total scleral area to be affected to be 2.1 \( mm^2 \) if one injection would be placed just next to the other. Thus, it is unlikely that mechanical factors...
alone would lead to the scleral changes observed in our report. Scar formation within the injection path may lead to fibrosis and impaction of the collagenous structure. Two possible additional etiologies merit further discussion. In addition to mechanical stress, there may be a direct effect of anti-VEGF antibodies on scleral hydration. Several reports have provided data on reflux through the injection site.\textsuperscript{16–18} This reflux may lead to a high concentration of anti-VEGF antibodies in the sclera at the injection site. Previous studies have shown that sclera is permeable to molecules with molecular weights up to 150 kDa.\textsuperscript{19,20} Therefore, ranibizumab with a molecular weight of 48 kDa or aflibercept with a molecular weight of 97 kDa may be able to diffuse into the sclera through the injection tunnel and may cause dehydration of the sclera by altering the permeability of scleral vessels leading to a localized scleral thinning after repeated injection of these agents. Reflux also may cause incarceration of vitreous in the wound, a finding that has been reported in small gauge vitreoretinal surgery,\textsuperscript{21} leading to changes within the scleral architecture.

Another possible additional etiological factor may be IOP elevation associated with intraocular injections of anti-VEGF agents.\textsuperscript{22–24} A recent investigation of scleral architecture in monkeys with experimentally induced high pressure glaucoma has shown significant scleral thinning\textsuperscript{25} and a study investigating the influence of high IOP on excised sclera has found that sclera thinned significantly when pressurized to IOP levels of 60 mm Hg or higher.\textsuperscript{26} This could explain that the inferonasal and the superotemporal quadrant were slightly thinner in the injected eye compared to the fellow eye. However, as there was no significant generalized thinning of the sclera in the other quadrants in our study, this may only be an additional factor leading to the observed scleral changes in the quadrant used for intravitreal injections.

To our knowledge, this report constitutes the only study in the literature evaluating the effect on repeated intravitreal injections on the sclera. The study has limitations due to its retrospective nature and the relatively small number of patients included. In addition, as the effect size of our observations is relatively small, and as such this study has limited power. Larger numbers of patients or a longer follow-up time will be needed to confirm these preliminary findings. In our study, we found thinning of the sclera in the inferotemporal quadrant suggesting a mechanical injury after multiple intravitreal injections. As such, we suggest to alternate the quadrant used for intravitreal injections, especially in

Figure 4. Infrared and AS OCT images of the inferotemporal quadrant of the sclera. Scale bar: 200 µm. (A1, A2) Representative infrared image of the inferotemporal quadrant of the sclera showing intravitreal injection marks (circles) and magnified inlays showing the injection marks (black arrows, correspond to green circles). Some injection marks correspond with scleral tunnels seen in OCT (white arrows). (B) Scatter plot showing mean ± SD inferotemporal scleral thickness for eyes having received more than 30 intravitreal injections (>30 IVT) versus the contralateral eye (fellow eye, difference of injections >30; n = 35; P < 0.005, paired t-test).
patients requiring more than 30 intravitreal injections for treatment of macular disease.

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**References**


