RESEARCH

TITLE: Longitudinal changes in outer nuclear layer thickness in soft drusen and reticular pseudodrusen

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RUNNING TITLE: Outer nuclear layer by type of drusen

KEY WORDS: Age-related macular degeneration; spectral domain optical coherence tomography; outer nuclear layer; reticular pseudodrusen; soft drusen

Background: Drusen are seen in the early and intermediate stages of age-related macular degeneration. A retrospective, 2-year observational study at a tertiary centre was designed to assess outer nuclear layer thickness in different types of drusen.

Methods: Patients over 50 years with predominant soft drusen or reticular pseudodrusen were included in the study. Fundus photography, infrared, fundus autofluorescence and spectral domain optical coherence tomography were performed at baseline, years 1 and 2. Outer nuclear layer thickness was measured in the nine Early Treatment Diabetic Retinopathy Study subfields, and the rate of thinning was determined using generalized estimating equations models.

Results: Data were analysed from 17 eyes with soft drusen and 9 eyes with reticular pseudodrusen. Greater outer nuclear layer thinning was seen overall and in all subfields in reticular pseudodrusen as compared to soft drusen, with statistically significant differences found mostly in superior and nasal subfields of ring 2. The outer nuclear layer was 5-12 μm thinner in eyes with reticular pseudodrusen, and the rate of thinning was greater in eyes with reticular pseudodrusen in the outer superior subfield.

Conclusions: In the present sample, outer nuclear layer thickness was consistently lower in patients with reticular pseudodrusen compared with soft drusen, irrespective of subfield location. These structural findings may contribute to explain the functional abnormalities observed in patients with reticular pseudodrusen.

Age-related macular degeneration (AMD) is the leading cause of blindness in people over 50 years of age in developed countries.[1,2] The pathogenesis of AMD is not entirely clear, but drusen are the hallmark signs in the early and intermediate stages of the disease.[3] In recent years, there has been an improved characterisation of the impact of drusen on vision,[4-6] and it has been recognized that the degree of functional impairment depends on the drusen phenotype[7] and its associated features.[8]

Drusen are known to be deposits of trapped extracellular material lying between the basement membrane of the retinal pigment epithelium (RPE) and the inner collagenous layer of Bruch's membrane. They are seen as yellowish, deep lesions into the retina of variable size, ranging from 63 µm to more than 250 µm (lesions <63 µm are termed *drupelets* and are not considered to increase the risk of progression to AMD).[9] There are several types of drusen that are distinguished from each other according to their size, topographic distribution in the fundus, location within the retina, and ultrastructural composition, which is reflected in the varied appearance on multimodal imaging.[10]

More relevant from the perspective of conferring an increase in the risk of progression towards late AMD are soft drusen and reticular pseudodrusen (RPD, also known as subretinal drusenoid deposits,[11] reticular macular disease[12] or reticular drusen,[13] amongst others). Soft drusen are viewed as bright-round yellowish spots with ill-defined margins located under the RPE, and most commonly found at the central macula. In contrast, RPD are yellowish white lesions with discrete, interlacing or confluent shape located above the RPE in the subretinal space, and with a predilection to involve the perifoveal area, mostly in the superior region.[14]

From a functional point of view, dark adaptometry seems to be worse in eyes with RPD than in eyes with soft drusen, with a possible explanation for this being the change in photoreceptor function that occurs in the presence of RPD.[7] Some authors speculate

there is a correlation between the presence of RPD and damage to rods, with cones being more resilient than rods to this sort of injury.[8]

From a structural point of view, RPD evolve through different stages that seem to end in outer nuclear layer (ONL) thinning (that is, photoreceptor atrophy) and subsequent RPE atrophy.[15] The thinning of the ONL is not clinically visible on fundus photography or fundus autofluorescence (FAF), but it is identifiable on spectral domain optical coherence tomography (SD OCT).[15] Also, previous studies have showed ONL thickness is significantly reduced in eyes with soft drusen and there is a linear relationship between photoreceptor thinning and drusen height.[16] Therefore, the thickness of the ONL is an important biomarker of progression in retinal degenerations, but little is known regarding the longitudinal rate of ONL changes according to drusen type.

The purpose of this study was to compare the longitudinal changes in the ONL in patients with soft drusen and RPD, in order to gain insights into the structural causes which may underpin the functional deficits seen in these patients.

METHODS

Study design

This was a retrospective, observational study. Patients were selected from those attending a tertiary retinal clinic (Institut de la Màcula; Barcelona, Spain) between May 2009 and February 2017. The study was conducted according to the tenets of the Declaration of Helsinki, was approved by the Hospital Quirón Teknon Ethics Committee and all patients signed an informed consent.

Patient population and eligibility criteria

This study defined soft drusen as yellowish retinal lesions >63 µm in diameter[9] that induced an elevation in the RPE on SD OCT. In contrast, RPD were considered present when they were seen on infrared or FAF covering an area of at least two disc sizes,[17] or as elevated subretinal lesions deforming or invading the ellipsoid zone on SD OCT. All RPD types (dot, ribbon or peripheral pseudodrusen)[18] were eligible. As both soft drusen and RPD are commonly present in the same eye,[19] eyes were only included if one phenotype was clearly predominant over the other according to the opinion of two retinal specialists (LF, JM). The predominant drusen type had to involve at least three contiguous or non-contiguous Early Treatment Diabetic Retinopathy Study (ETDRS) subfields, while the least predominant type could not span more than one.

Patients over 50 years old of both genders were included in the study. All patients had data for baseline (visit 1), 12±2 months (visit 2) and 24±2 months (visit 3), and either predominantly soft drusen or RPD in the study eye (Figure 1).

Patients were excluded from this study if they had, throughout the study period, any retinal disease beside soft drusen or RPD that could affect ONL thickness. This included specifically prevalent or incident neovascular AMD or geographic atrophy (GA), but also retinal dystrophies, a spherical equivalent beyond ±6.00 D, if they received intravitreal injections or laser photocoagulation, or if they had undergone any type of intraocular surgery, with the exception of phacoemulsification with intraocular lens implantation at least 3 months before the study visit. In addition, patients taking drugs known to be toxic to the retina (for example, hydroxicloroquine, tamoxifen, vigabatrin) and those with poor SD OCT image quality for any reason (media opacities, poor cooperation) were also excluded, as eyes with tilted B-scans that allowed

visualization of Henle's fibre layer on one side of the foveola but not the other. Only one eye per patient was included; if both eyes were potentially eligible, the study eye was randomly chosen.

ONL thickness evaluation

The ONL was defined on SD OCT as the hyporeflective structure between the moderately hypereflective outer plexiform layer and external limiting membrane (ELM) in each B-scan of a volumetric scan (Figure 2), and therefore it also included Henle's fibre layer (macular photoreceptor axons) in the thickness measurements. Anatomically, the ONL represents the location of the nuclei of photoreceptors.

The thickness of the ONL was measured with the SD OCT Spectralis® (Heidelberg Engineering, Heidelberg, Germany), which allows semi-automatic segmentation of several retinal layers, included the ONL. Manual correction of segmentation errors was performed when required by an experienced observer (CR). Measurements of ONL thickness were conducted in each of the nine subfields defined by the ETDRS.[20] This included one inner circle of 1.0 mm in diameter centred in the foveola, an inner ring of 3 mm in diameter and an outer ring of 6 mm in diameter. In turn, each ring was further divided into four subfields (superior, inferior, nasal, and temporal), resulting in nine ETDRS subfields. An average value was provided for each of the nine subfields (Figure 2) and for the ETDRS circle overall. Eye-tracking software was used to minimise motion artefacts during image acquisition and to facilitate rescanning of the macula at the same location on follow-up measurements[21] taken at years 1 (visit 2) and 2 (visit 3).

The SD OCT protocols used in this study combined FAF or infrared visualisation of the fundus with high resolution (1536 x 1536 pixels) B-scans. This was either a 20º (horizontal) x 15º (vertical) pattern size (roughly equivalent to 5.8 x 4.3 mm in the

fundus) consisting in 19 B-scans equally spaced 240 µm between B-scans, or a 30º x 15º pattern size (8.7 x 4.3 mm) consisting in 37 B-scans equally spaced 120 µm between B-scans. The minimum Automated Real Time rate (averaging) was 9 B-scans for all exams.

Data analysis

Descriptive statistics were provided for the whole sample and by drusen subgroup (soft drusen and RPD) using the mean (standard deviation, SD), median (interquartile range, IQR) or percentage as appropriate. Graphical and statistical (Shapiro-Wilk) tests for normality of ONL thickness by group and ETDRS subfield were evaluated. Since normality could not be assumed, results by group were summarised as median (IQR) and non-parametric tests were used (Mann-Whitney or Wilcoxon, as required).

Firstly, differences in ONL thickness between drusen type, by each ETDRS subfield (central and nasal, temporal, superior and inferior of inner ring -N1, T1, S1 and I1, respectively, and nasal, temporal, superior and inferior of outer ring -N2, T2, S2 and I2, respectively) and overall, were determined. This was performed in each period (baseline, year 1 and 2). Secondly, longitudinal differences from baseline to year 2 within drusen subgroup in each subfield and overall were investigated using a paired Wilcoxon test. Finally, we tested if there were longitudinal changes in ONL thickness between drusen type by period (baseline, years 1 and 2) in each ETDRS subfield and overall with an interaction test. For that purpose, we used repeated measures generalised estimating equations (GEE) models with an exchangeable working correlation and robust standard errors (SEs)[22,23], and we estimated the effect of drusen type on ONL thickness considering the soft drusen group as the reference. These models were adjusted for age.

To test the intraobserver agreement in manual measurements, five patients were randomly selected and measurements of ONL thickness in all B-scans of a given volume at a given visit were repeated following the procedure outlined above. The results were analysed using Bland-Altman plots.

Data analysis was conducted with Stata/IC 13.1 (StataCorp LP, College Station, TX). A two-tailed *P*-value of <.05 was considered statistically significant. No correction for multiple comparisons was made.[24]

RESULTS

A total of 73 eyes from 73 patients were initially identified with a predominant drusen type who did not develop late AMD in the study period (45 with soft drusen and 28 with RPD), but only 26 of these patients met the full eligibility criteria with regular yearly examinations for two years, and were finally included in the study. There were 17 eyes (17/26, 65.4%) with soft drusen and nine eyes (9/26, 34.6%) with RPD. The mean age was 72.5 years (SD 8.1 years), 22 were female (22/26, 84.6%), and all were Caucasian. Seven of the 26 eyes were right eyes (26.9%). Median best-corrected visual acuity (BCVA) was 1.0 (20/20) at baseline (IQR 0) and at year 2 (IQR 0.1). A comparison of baseline features between groups is provided in Table 1. Measures of ONL thickness showed a high degree of agreement, with an overall mean difference between the first and second measurements of less than 1 µm (Figure 3).

The differences in ONL thickness between soft drusen and RPD are shown in Table 2. These results are split according to subfield and time. Median values in Table 2 show that ONL thickness was lower in all subfields in RPD compared with soft drusen, with

statistically significant results found mostly in subfields of ring 2 (outer sector), where RPD are usually located. This is shown graphically in Figure 4. The ONL thickness in year 2 was compared with baseline measurements to evaluate the longitudinal trend in ONL thinning in soft drusen and RPD (Table 3). Thicknesses in the soft drusen group varied slightly, except in the inner superior subfield where they reached statistical significance (62 µm vs 56 µm, p=0.03). Progressive ONL thinning seen the RPD group, particularly in the nasal and superior subfields (all p≤003).

The longitudinal changes in ONL thickness in the 2-year study period in patients with RPD compared with soft drusen (reference group) are shown in Table 4. The results are stratified by ETDRS subfield and overall, and are adjusted for age. The values of the GEE model show ONL thickness values consistently thinner in the RPD group as compared with the soft drusen group (negative coefficient of variable “RPD” in the table) in a range between -4.6 µm and -9.7 µm, with an approximate overall decrease of 8 µm in eyes of patients with RPD as compared with those with soft drusen. The “Visit” variable shows if the change in ONL thickness is more marked in year 1 or 2 of follow-up as compared with baseline (regardless of drusen group); overall, it seemed slightly more marked in the second year. Of more interest is the “Drusen x visit” interaction term, which shows differences in the rate on ONL thickness loss by group (soft drusen, RPD) and time (years 1 or 2) that are statistically significant only in the second year for the S2 subfield (p<0.001); nonetheless, the negative coefficient for most periods (14/18) suggests that the rate of decline is faster for the RPD than for the soft drusen group. All these effects were age-independent.

DISCUSSION

The results of the present study revealed that the average ONL thickness across all subfields in the ETDRS grid and overall was lower in patients with RPD compared with soft drusen. Also, after 2 years of follow-up, the ONL thickness decreased slightly in the soft drusen group, but there was an obvious thinning in all regions in eyes with RPD, which was statistically significant overall and in the superior, nasal and in the foveal region.

Spaide[15] reported that areas in which RPD regressed showed a thinning of the ONL and coined the term “outer retinal atrophy”, suggesting that this represented an unrecognized form of late AMD. The presence of these lesions is a significant fundus finding, which not only increases the risk to progression to the classical advanced forms of AMD (neovascular AMD and GA),[25-27] but also impairs visual function. The effects of RPD on vision have been measured using dark adaptometry, where delays in rod intercept time were observed (which imply a delayed dark adaptation)[7], and by microperimetry, where frank[28] or modest[6] reductions in macular sensitivity were encountered. More contradictory results have been found on multifocal electroretinography, ranging from no abnormalities[29] to delays in implicit time.[6] The generalised macular ONL thinning found herein could help to explain the structural causes underlying the wide range of functional abnormalities found in patients who present with drusen.

The damage to photoreceptors (observed by ONL thinning) caused by RPD may be explained by either mechanical, toxic or metabolic causes. Physical displacement, disruption of the normal photoreceptor or RPE function (possibly related to ocular perfusion pressure)[30] and/or blockage of the normal diffusion of metabolic activity between photoreceptors and the RPE may contribute to photoreceptor degeneration.

On the other hand, photoreceptor thinning overlying soft drusen has also been reported previously,[31] but the lack of a control group in this study precludes comparison of the degree of ONL thinning caused by soft drusen with that experienced by age-matched healthy individuals. Of note, the changes in SD OCT reflectivity overlying soft drusen, initially attributed to a degenerative photoreceptor process, can be explained by optical changes in the reflectivity of Henle's fibre layer due to an increased tilted position caused by the elevated drusen.[32]

Two-year changes in unadjusted analyses (Table 3) in the soft drusen group showed a trend towards minor thinning, while in the RPD sample consistent small ONL loss was seen in all subfields, reaching statistically significant results in the foveal, and nasal and superior hemi-fields. While nasal and superior sectors are regions known to harbour RPD, the involvement of the fovea is unexpected and suggests a disease that spreads beyond the focal location of the lesions.

When the analyses were adjusted for age and interaction terms were added in the model to determine if the rate of ONL thinning differed between drusen types (Table 4), the only region showing a statistically significant greater ONL loss was the outer superior subfield, were RPD are very common. In general, eyes with RPD tended to show faster thinning and this tended to increase with time (more marked in year 2 than year 1). Nonetheless, results did not reach statistical significance, suggesting sampling variability or (more probably) limited power and/or follow-up time to properly address this finding.

Of note, there was a marked thinning of the ONL in the fovea despite the fact that RPD tend to be rarely seen in this location.[17] Similarly, ONL was thinner in RPD compared with soft drusen throughout the macular area regardless of the direct presence of RPD

lesions in each subfield of the ETDRS grid. These findings suggest RPD may be a diffuse disease, not necessarily involving just the areas where RPD is present.[4] In addition, the magnitude of ONL thinning seen in soft drusen after 2 years was approximately 1-2 µm (Table 3), while the difference in ONL thickness between soft drusen and RPD was between 5 and 10 µm. This highlights the impact of these different lesions on retinal morphology.

ONL thinning progressed despite a stable visual acuity, which suggests this functional parameter should not be used to detect ongoing retinal degeneration, especially if RPD are present. A multimodal imaging approach that includes SD OCT (and, ideally, FAF and/or infrared)[33] is therefore required to detect the presence of RPD, and more comprehensive psychophysical testing using dark adaptometry and microperimetry[34] is necessary to monitor the natural course of RPD and its impact on vision and patient quality of life.

The main limitations of this study are its retrospective nature, the lack of a healthy control group to assess the impact of the presence of each drusen type on ONL thickness and the small sample size due to the strict eligibility criteria. As such, this should be regarded as an exploratory study. In addition, the SD OCT protocols used in this study (19 or 37 B-scans) covered all central, inner, and outer nasal and temporal locations in the ETDRS subfields, but only approximately half of the superior and inferior subfields in the outer ring. Given this is a more common location for RPD compared with soft drusen, the effect (if any) this may have caused is an underestimation of the thinning of the ONL caused by RPD in this region, and hence our substantive results would have not changed. We also examined global (mean subfield ONL thickness) rather than local (changes overlying individual drusen) effects, and axial length was not measured, which could have introduced some error in ONL thickness measurements. In addition, all ONL measurements include Henle's fibre

layer thickness and therefore they cannot be directly compared with results from histology; on the other hand, all included B-scans were acquired horizontally to minimize the directional OCT phenomenon,[35] and thus comparisons between groups and longitudinal differences seem appropriate. For future studies, a prospective design will allow capturing this information and allow further exploration of the relationship between structural changes and functional performance; also, the inclusion of a group with both, soft drusen and RPD, as frequently seen clinically, would allow to draw conclusions which are more relevant to patients. A confirmatory study should establish the robustness of these results.

In summary, we found the presence of RPD is consistently associated with a thinner ONL compared with soft drusen, irrespective of location within the macular area. These findings are also observed in the fovea, where soft drusen are common but RPD are rarely seen. The rate of thinning was also faster for RPD than for soft drusen in the superior outer ring of the ETDRS, a location where RPD are commonly found.

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Table 1. Comparison of features between groups according to drusen type

	Soft drusen	**RPD**	***P***
n	17	9	-
Female (n, %)	14 (82.4)	8 (88.9)	1.00
Age (SD), years	72.5 (8.6)	72.7 (7.6)	0.95
BCVA change (IQR), decimal	0 (0.1)	0 (0)	0.37

Quantitative variables are expressed as mean (SD) or median (IQR) and categorical variables as number (percentage).

BCVA: best-corrected visual acuity; IQR: interquartile range; RPD: reticular pseudodrusen; SD: standard deviation.

Table 2. Comparison of ONL thickness by drusen group. Results are reported as median (interquartile range) or mean (SD). All thickness values are in µms.

	Baseline (visit 1)			**Year 1 (visit 2)**			**Year 2 (visit 3)**		
Location	**Soft drusen**	**RPD**	*P*	**Soft drusen**	**RPD**	*P*	**Soft drusen**	**RPD**	*P*
Foveal	91 (16)	88 (7)	0.07	93 (18)	87 (16)	0.08	90 (19)	86 (12)	0.08
N1	71 (11)	68 (19)	0.23	73 (11)	67 (22)	**0.03**	72 (12)	61 (31)	**0.046**
S1	62 (11)	54 (18)	0.21	60 (7)	50 (12)	**0.03**	56 (20)	44 (10)	**0.03**
T1	70 (8)	66 (11)	0.29	67 (8)	59 (10)	0.23	68 (10)	59 (12)	0.12
I1	64 (13)	57 (7)	0.09	66 (13)	56 (8)	**0.04**	63 (17)	55 (13)	0.29
N2	58 (9)	50 (12)	**0.008**	59 (5)	50 (12)	**0.008**	59 (8)	48 (16)	**0.01**
S2	61 (12)	48 (12)	**0.005**	61 (6)	51 (12)	**0.002**	60 (11)	46 (10)	**0.002**
T2	58 (6)	52 (9)	**0.01**	58 (5)	51 (10)	0.07	59 (7)	51 (8)	**0.01**
I2	57 (8.5)	49 (10)	**0.02**	57.5 (7.5)	47.5 (9.5)	**0.02**	56.5 (7)	46.5 (11.5)	0.07
Overall	65.3 (7.1)	57.6 (7.3)	**0.016**	64.7 (7.6)	55.7 (7.5)	**0.009**	63.5 (7.9)	53.4 (9.7)	**0.009**

Results are shown by subfield in three periods: at baseline, year 1 and year 2. Values are median (IQR). 1: inner ring; 2: outer ring; I: inferior; IQR: interquartile range; N: nasal; ONL: outer nuclear layer; RPD: reticular pseudodrusen; S: superior; T: temporal. *P*-values in bold denote statistical significance.

Table 3. Outer nuclear layer (ONL) thickness at baseline compared with year 2. Results are reported as median (interquartile range) or mean (SD). All thickness values are in µm.

	Soft drusen			Reticular pseudodrusen		
Location	*Baseline*	*Year 2*	*P*	*Baseline*	*Year 2*	*P*
Foveal	91 (16)	90 (19)	0.05	88 (7)	86 (12)	**0.01**
N1	71 (11)	72 (12)	0.16	68 (19)	61 (31)	**0.02**
S1	62 (11)	56 (20)	**0.03**	54 (18)	44 (10)	**0.02**
T1	70 (8)	68 (10)	0.57	66 (11)	59 (12)	0.26
I1	64 (13)	63 (17)	0.20	57 (7)	55 (13)	0.63
N2	58 (9)	59 (8)	0.31	50 (12)	48 (16)	**0.03**
S2	61 (12)	60 (11)	0.64	48 (12)	46 (10)	**0.01**
T2	58 (6)	59 (7)	0.88	52 (9)	51 (8)	0.51
I2	57 (8.5)	56.5 (7)	0.45	49 (10)	46.5 (11.5)	0.48
Overall	66.9 (7.7)	63.7 (7.2)	0.07	57.8 (7.3)	55.8 (5.6)	**0.008**

Comparisons were made by subfield and overall, separately for patients with soft drusen and reticular pseudodrusen. Values represent median (interquartile range). 1: inner ring; 2: outer ring; I: inferior; N: nasal; ONL: outer nuclear layer; S: superior; T: temporal. *P*-values in bold denote statistical significance.

Table 4. Effect of drusen type on outer nuclear layer thickness by subfield and overall throughout the 2-year period. The significant interaction term “drusen x visit” ($p<0.05$) indicate regions were the rate of change in outer nuclear layer in the study period (in years 1 and 2) differs between soft drusen and RPD.

Subfield	*Variables*	*Coefficient*	*Rost SE*	*P*	*95% CI*
Foveal	*Drusen x visit:*				
	RPD x Y1	0.3	2.5	0.91	-4.6 to 5.2
	RPD x Y2	-0.8	3.2	0.80	-7.1 to 5.5
	RPD	-9.7	5.3	0.07	-20.1 to 0.7
	Visit:				
	Y1	-1.3	1.4	0.34	-4.0 to 1.4
	Y2	-4.8	2.0	**0.01**	-8.7 to -1.0
	Age	-0.02	0.05	0.57	-0.13 to 0.07
	Constant	95.2	4.5	**<0.001**	86.5 to 104.0
N1	*Drusen x visit:*				
	RPD x Y1	-4.0	2.5	0.11	-9.0 to 0.9
	RPD x Y2	-4.5	2.9	0.13	-10.2 to 1.3
	RPD	-7.6	5.5	0.17	-18.3 to 3.2
	Visit:				
	Y1	-0.3	1.0	0.77	-2.3 to 1.7
	Y2	-2.2	1.3	0.10	-4.9 to 0.4
	Age	0.23	0.05	**<0.001**	0.12 to 0.33
	Constant	53.9	4.5	**<0.001**	45.0 to 62.8
S1	*Drusen x visit:*				
	RPD x Y1	-2.6	2.9	0.38	-8.3 to 3.2
	RPD x Y2	-3.7	2.7	0.17	-9.1 to 1.6
	RPD	-6.9	5.0	0.17	-16.6 to 2.9
	Visit:				
	Y1	-1.5	1.7	0.38	-5.0 to 1.9
	Y2	-3.8	1.6	**0.02**	-7.0 to -0.7
	Age	0.05	0.05	0.27	-0.04 to 0.15
	Constant	56.7	4.4	**<0.001**	48.1 to 65.4
T1	*Drusen x visit:*				
	RPD x Y1	1.1	2.2	0.63	-3.2 to 5.3
	RPD x Y2	-2.5	1.9	0.20	-6.3 to 1.3
	RPD	-4.6	4.0	0.25	-12.4 to 3.3
	Visit:				
	Y1	-0.9	0.9	0.30	-2.7 to 0.8
	Y2	0.3	1.4	0.83	-2.4 to 3.0
	Age	-0.10	0.03	**<0.001**	-0.16 to -0.05
	Constant	75.0	2.8	**<0.001**	69.6 to 80.5

	Drusen x visit:				
	RPD x Y1	-0.8	2.0	0.67	-4.7 to 3.0
	RPD x Y2	-0.4	4.0	0.91	-8.4 to 7.5
	RPD	-7.9	4.4	0.07	-16.6 to 0.8
I1	*Visit:*				
	Y1	-0.8	1.5	0.59	-3.8 to 2.1
	Y2	-2.5	2.2	0.27	-6.9 to 1.9
	Age	-0.04	0.06	0.52	-0.16 to 0.08
	Constant	67.2	4.6	**0.001**	58.1 to 76.3
	Drusen x visit:				
	RPD x Y1	-1.9	1.1	0.08	-3.9 to 0.2
	RPD x Y2	-2.2	1.4	0.1	-4.9 to 0.4
	RPD	-8.7	3.2	**0.007**	-15.0 to -2.3
N2	*Visit:*				
	Y1	-0.4	0.8	0.68	-2.0 to 1.3
	Y2	-0.6	0.8	0.39	-2.1 to 0.8
	Age	0.07	0.02	**0.001**	0.03 to 0.11
	Constant	51.4	2.2	**<0.001**	47.1 to 55.7
	Drusen x visit:				
	RPD x Y1	-3.1	1.9	0.11	-6.9 to 0.7
	RPD x Y2	-5.1	1.4	**<0.001**	-7.8 to -2.3
	RPD	-9.4	2.9	**0.001**	-15.1 to -3.6
S2	*Visit:*				
	Y1	0.9	1.3	0.51	-1.7 to 3.5
	Y2	-0.6	1.0	0.57	-2.5 to 1.4
	Age	0.04	0.04	0.25	-0.03 to 0.12
	Constant	55.3	3.3	**<0.001**	48.7 to 61.8
	Drusen x visit:				
	RPD x Y1	0.05	0.8	0.57	-1.2 to 2.1
	RPD x Y2	-1.6	1.2	0.18	-4.1 to 0.8
	RPD	-6.2	2.3	**0.007**	-10.6 to -1.7
T2	*Visit:*				
	Y1	-0.5	0.4	0.26	-1.3 to 0.3
	Y2	0.2	0.6	0.72	-1.0 to 1.5
	Age	-0.03	0.02	0.05	-0.06 to 0.00
	Constant	60.6	1.6	**<0.001**	57.5 to 63.7

	Drusen x visit:				
	RPD x Y1	-0.8	1.2	0.50	-3.2 to 1.6
	RPD x Y2	0.0	1.6	1.00	-3.1 to 3.1
	RPD	-7.7	3.0	**0.01**	-13.6 to -1.9
I2	*Visit:*				
	Y1	0.1	1.0	0.95	-1.9 to 2.0
	Y2	-0.8	0.8	0.37	-2.4 to 0.9
	Age	0.00	0.15	0.99	-0.30 to 0.30
	Constant	56.6	10.6	**<0.001**	35.8 to 77.5
	Drusen x visit:				
	RPD x Y1	-1.2	0.8	0.16	-2.9 to 0.5
	RPD x Y2	-6.3	3.6	0.08	-13.3 to 0.8
	RPD	-7.7	2.8	**0.007**	-13.3 to -2.1
Overall	*Visit:*				
	Y1	-0.6	0.6	0.36	-1.8 to 0.7
	Y2	-1.7	0.8	**0.02**	-3.2 to -0.2
	Age	0.04	0.05	0.42	-0.14 to 0.06
	Constant	68.1	3.8	**<0.001**	60.7 to 75.5

The RPD group was compared with the soft drusen group (reference group); age in years. 1: inner ring; 2: outer ring; I: inferior; CI: confidence interval; N: nasal; RPD: reticular pseudodrusen; S: superior; SE: standard error; T: temporal; Y: year.

FIGURES

Figure 1. Examples of predominant drusen types. *Top,* fundus photography, infrared, fundus autofluorescence (FAF) and spectral domain optical coherence tomography (SD OCT) images, respectively, of a patient with predominantly soft drusen. The retinal pigment epithelium (RPE) is elevated on SD OCT due to extracellular deposits located between it and Bruch's membrane. *Bottom,* corresponding images of a patient with predominantly reticular pseudodrusen (RPD). The extracellular deposits are located in the subretinal space between the RPE and the photoreceptors. In this image, the RPD do not break through the ellipsoid band and are therefore classified as stage 2.

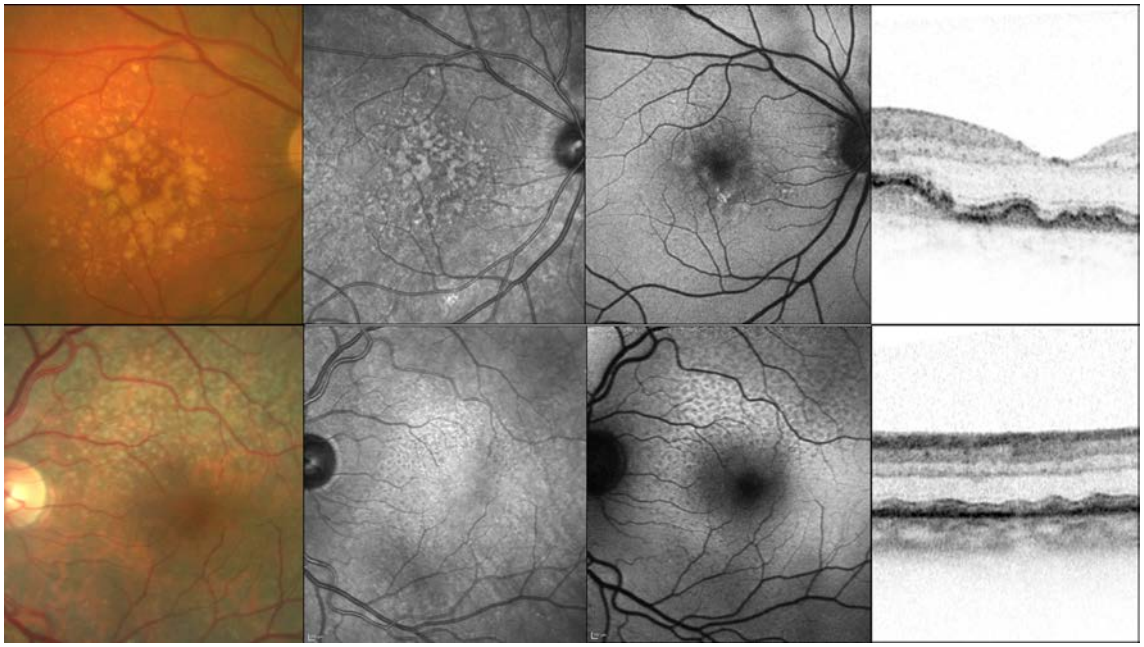

Figure 2. *Top,* example of colour (*left*) and quantitative (*right*) maps of the outer nuclear layer (ONL) thickness on the nine subfields in the Early Treatment Diabetic Retinopathy Study in a patient with reticular pseudodrusen. Notice that part of the superior and inferior subfields of the outer ring are not measured. *Bottom,* B-scan with segmented ONL, delimited superiorly by the outer plexiform layer and inferiorly by the external limiting membrane.

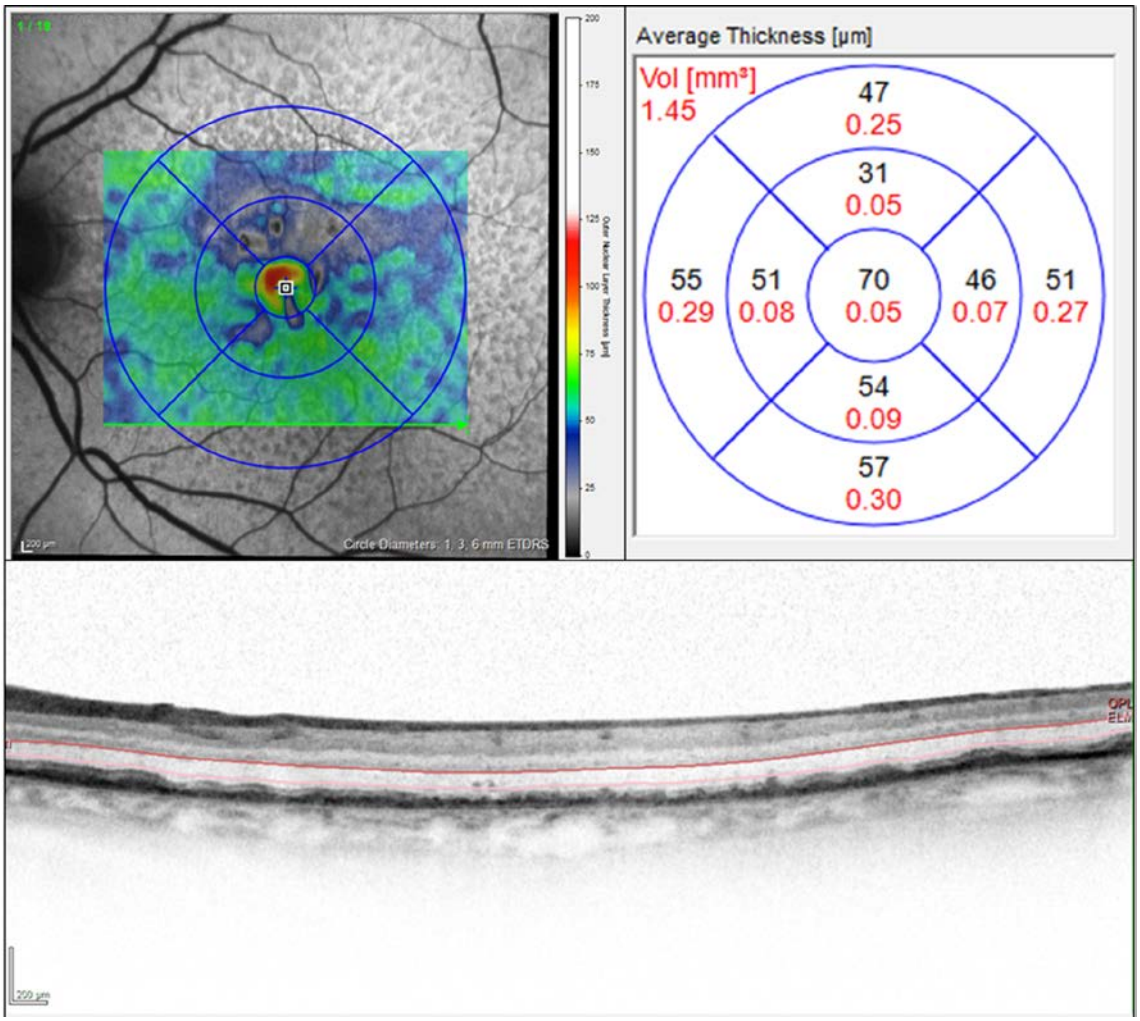

Figure 3. Bland-Altman plot of agreement between repeated first and second ONL thickness measurements in a given volume SD OCT at a given visit. The results show excellent overall agreement, with a slight tendency to obtain thinner thicknesses upon the second measurement. ONL: outer nuclear layer.

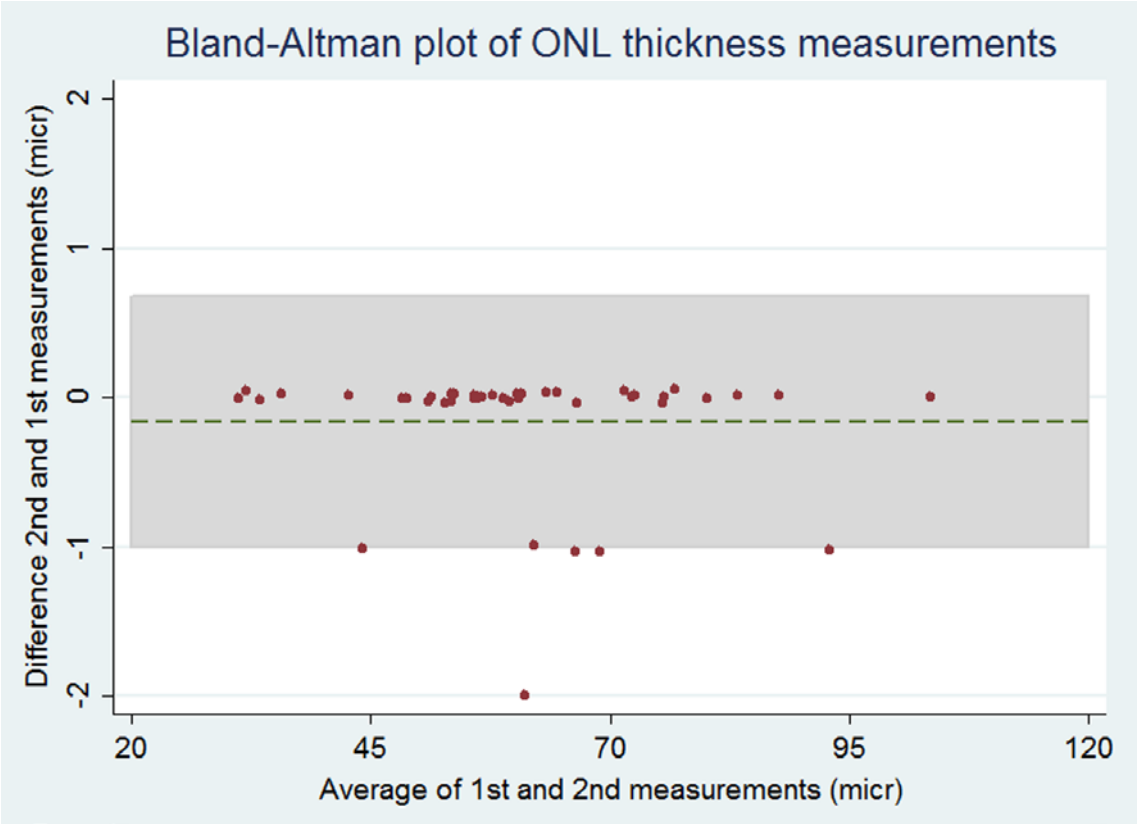

Figure 4. Longitudinal two-year evolution of outer nuclear layer (ONL) thickness by drusen group (solid line for soft drusen, dotted line for reticular pseudodrusen) according to their topographic distribution in the Early Treatment Diabetic Retinopathy Study grid.