Potential New Diagnostic Tool for Alzheimer's Disease Using a Linear Discriminant Function for Fourier Domain Optical Coherence Tomography

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Submitted: November 17, 2013
Accepted: April 7, 2014

Citation: Larrosa JM, Garcia-Martin E, Bambo MP, et al. Potential new diagnostic tool for Alzheimer's disease using a linear discriminant function for Fourier domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2014;55:3043–3051. DOI: 10.1167/iovs.13-13629

Purpose. We calculated and validated a linear discriminant function (LDF) for Fourier domain optical coherence tomography (OCT) to improve the diagnostic ability of retinal and retinal nerve fiber layer (RNFL) thickness parameters in the detection of Alzheimer's disease (AD).

Methods. We enrolled AD patients ($n = 151$) and age-matched, healthy subjects ($n = 61$). The Cirrus and Spectralis OCT systems were used to obtain retinal measurements and circumpapillary RNFL thickness for each participant. An LDF was calculated using all retinal and RNFL OCT measurements. Receiver operating characteristic (ROC) curves were plotted and compared among the LDF and the standard parameters provided by OCT devices. Sensitivity and specificity were used to evaluate diagnostic performance. A validating set was used in an independent population to test the performance of the LDF.

Results. The optimal function was calculated using the RNFL thickness provided by Spectralis OCT, using the 768 points registered during peripapillary scan acquisition (grouped to obtain 24 uniformly divided locations): $18.325 + 0.056 \times (315°–330°) - 0.122 \times (300°–315°) - 0.041 \times (285°–300°) + 0.091 \times (255°–270°) + 0.041 \times (225°–240°) + 0.183 \times (195°–210°) - 0.108 \times (150°–165°) - 0.092 \times (75°–90°) + 0.051 \times (30°–45°)$. The largest area under the ROC curve was 0.967 for the LDF. At 95% fixed specificity, the LDF yielded the highest sensitivity values.

Conclusions. Measurements of RNFL thickness obtained with the Spectralis OCT device differentiated between healthy and AD individuals. Based on the area under the ROC curve, the LDF was a better predictor than any single parameter.

Keywords: logistic regression, Alzheimer's disease, optical coherence tomography, OCT, retinal nerve fiber layer, retinal thickness

Alzheimer's disease (AD) is a common cause of dementia in the elderly. The worldwide prevalence, estimated to be 35.6 million in 2010, is expected to double every 20 years, increasing to 115.4 million in 2050. This disease typically is characterized by a progressive loss of memory and other cognitive functions, and has a substantial impact on the patient, as well as on the patient's family and on society.[1] The highest incidence of AD occurs in individuals 65 years of age and older. The disease affects behavior and functional ability, and is a leading cause of disability in older people living in developed countries.[2] Biomarkers and tools are needed to diagnose the disease in its early phases, especially in high-risk families, and to monitor disease progression.

Visual impairment often is one the earliest complaints of patients with AD.[3] Although the symptoms of AD historically have been attributed to cerebral cortex damage,[4,5] recent studies have revealed degenerative changes in optic nerve fibers, causing retinal nerve fiber layer (RNFL) thinning in patients with AD[6,7] with mild cognitive impairment.[8] The RNFL comprises axons originating in retinal ganglion cells and can be measured using ocular imaging technologies, such as optical coherence tomography (OCT),[9] which provide noninvasive, rapid, objective, and reliable measurements. Numerous studies have analyzed the ability of OCT to detect RNFL thickness abnormalities and changes in the macula of patients with neurodegenerative diseases.[10–13] Retrograde loss of nerve fibers in the retina and optic nerve may be an early biomarker of neurodegeneration in AD, even before hippocampal damage, which leads to memory impairment.[14] Fourier/spectral domain-OCT devices resolve the retina to 5 μm or less, acquire images quickly, and even distinguish retinal layers. Fast acquisition is an important feature for examining patients with cognitive impairment or fixation difficulties, such as individuals with AD, and may improve the reliability of the technique. Recent studies have revealed the value of RNFL and macular measurements provided by OCT for detecting neurodegenerative disease progression and facilitating the diagnosis of diseases, such as multiple sclerosis[10,15,16] and Parkinson's disease.[17,18]

The aim of the present study was to analyze whether a selective combination of retinal or RNFL OCT parameters could optimize AD diagnosis further when AD is suspected clinically. We evaluated the parameters provided by the two most commonly available spectral domain OCT devices, the Cirrus

(Carl Zeiss Meditec, Inc., Dublin, CA, USA) and the Spectralis (Heidelberg Engineering, Inc., Heidelberg, Germany) OCT instruments. The design of the study followed the 25 items in the guidelines suggested by the Standards for Reporting of Diagnostic Accuracy initiative, which was designed to increase the quality of reporting in diagnostic accuracy studies.[19] The main aim of this study was to assess the diagnostic ability of a linear discriminant function (LDF) designed for OCT, based exclusively on ophthalmologic parameters, for AD.

The diagnosis of idiopathic AD is based on medical history, collateral history from relatives, and clinical observations that are based on the presence of characteristic neurologic and neuropsychologic features in the absence of alternative conditions.[20] Advanced medical imaging with computed tomography, magnetic resonance imaging, single-photon emission computed tomography, or positron emission tomography can exclude other cerebral pathologies or subtypes of dementia. Moreover, imaging may predict conversion from prodromal stages (mild cognitive impairment) to AD.[21] The diagnosis can be confirmed only with postmortem histologic analysis. It sometimes takes several years to obtain a definitive diagnosis; thus, new technologies and accurate tests are needed to improve and accelerate the diagnostic procedure in early stages of the disease.

Currently, no clear guidelines are available on whether one, several, or all of the retinal or RNFL parameters provided by OCT can be used to diagnose AD. Based on the area under the receiver operating characteristic (ROC) curve, overall RNFL mean thickness is the best diagnostic parameter provided by OCT to detect various inner retinal or optic nerve pathologies, such as glaucoma,[22,23] and it is the most sensitive parameter for detecting neurodegenerative disease.[24] Optimal neurodegenerative disease detection, however, likely depends on a combination of several parameters. Garcia-Martin et al.[15] designed an LDF to detect multiple sclerosis using parameters provided by Fourier domain OCT with a sensitivity of 83%. The LDF performed better than any single OCT parameter in the ability to differentiate between the eyes of healthy subjects and eyes of patients with multiple sclerosis.[15]

Materials and Methods

This cross-sectional study 150 patients with AD, and 61 sex- and age-matched healthy subjects. All subjects underwent neuro-ophthalmologic and neurologic examinations. The AD diagnosis was determined by neurologists according to the National Institute of Neurologic and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association,[25] and the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) criteria.[26] Patients with early diagnosis (less than 3 years) and low-to-moderate dementia (Mini Mental State Examination [MMSE] $\geq$ 20) were included, because the objective of this study was to evaluate the diagnostic ability of OCT in incipient phases of AD (advanced phases of the disease are more easily diagnosed).

Inclusion criteria were confirmed AD diagnosis of less than 3 years, best-corrected visual acuity (BCVA) of 0.1 or higher (using a Snellen chart) in each eye to allow for performance of the protocol, and IOP less than 21 mm Hg to exclude the possibility of RNFL thinning due to other processes, such as open-angle chronic glaucoma.[15] Exclusion criteria were presence of significant refractive errors (>5 diopters of spherical equivalent refraction or 3 diopters of astigmatism); MMSE $<$ 20; systemic conditions that could affect the visual system; history of ocular trauma or concomitant ocular diseases, including a previous history of retinal pathology, glaucoma (defined by IOP $\geq$ 21 mm Hg, cup-to-disc ratio of 0.5 or higher, or arcuate nerve fiber bundle visual field defects), or laser therapy; and ocular pathologies affecting the cornea, lens, retina, or optic nerve. Healthy controls had no evidence of disease of any nature, including neurologic disorders. All procedures adhered to the tenets of the Declaration of Helsinki, and the experimental protocol was approved by the local ethics committee.

All subjects provided informed consent to participate in the study, and underwent a complete neurologic examination that included measurement of disease duration since diagnosis; an MMSE evaluation; and a complete neuro-ophthalmologic evaluation that included visual acuity, biomicroscopy of the anterior segment using a slit-lamp, Goldmann applanation tonometry, and ophthalmoscopy of the posterior segment. At least one reliable standard automated perimetry test per eye was performed using a Humphrey Field analyzer, model 750i (Zeiss Humphrey Systems, Dublin, CA, USA), with the Swedish Interactive Threshold Algorithm Standard 30-2 strategy. If fixation loss was greater than 15% and false-positive or false-negative rates were greater than 20%, the test was repeated.[27] The perimetry test was used to detect patients with neurologic alterations that affect vision, such as cerebrovascular accidents or hypophyseal tumors, or patients with glaucoma. These patients were excluded from the study.

OCT Evaluation

The OCT tests were performed to measure the retinal thickness and peripapillary RNFL with the Cirrus and the Spectralis OCT devices, which were used in random order to prevent fatigue bias. Each eye was considered separately and one eye from each subject was selected randomly to be included in the analyses, except when one of the eyes was excluded. All scans were performed by the same experienced operator. No manual correction was applied to the OCT output. An internal fixation target was used because it provides the highest reproducibility.[11] Scan quality was assessed before analysis and poor-quality scans were rejected. The Cirrus OCT device determines the quality of images using a signal strength measurement that combines the signal-to-noise ratio with the uniformity of the signal within a scan. Quality is measured on a scale of 1 to 10, where 1 is categorized as poor and 10 as excellent. Images with a score of at least 7 were analyzed. The Spectralis OCT device uses a blue quality bar in the image to indicate signal strength. The quality score range is 0 (poor quality) to 40 (excellent quality). Images that scored greater than 25 were analyzed. A total of 19 images with artifacts, missing parts, or seemingly distorted anatomy was excluded, and the OCT scan was repeated in those subjects.[28]

The OCT protocols for the Cirrus device (software version 6.0) were the macular cube 200 $\times$ 200 scan and the optic disc cube 200 $\times$ 200 scan. The OCT protocols for the Spectralis device (software version 5.4b) were the fast retinal scan and the RNFL scan of the classic glaucoma application using TruTrack eye-tracking technology. Nine scans produced each circular B scan.

The Cirrus macular cube and the Spectralis fast retinal protocols provide thickness measurements for each of the nine subfields defined by the Early Treatment Diabetic Retinopathy Study (ETDRS).[29] Three concentric circles defined nine macular sectors. The mean of all points within the inner circle (1-mm radius) was defined as the central foveal subfield thickness. The intermediate (inner) and outer rings each were subdivided into superior, nasal, inferior, and temporal areas. The Cirrus optic disc cube 200 $\times$ 200 protocol analyzed mean, quadrant (superior, inferior, temporal, and nasal), and 12-clock hour sector RNFL thickness. The Spectralis RNFL scan generates a thickness map with mean thickness, thickness of

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the four quadrants (superior, nasal, inferior, and temporal), and thickness of the six sectors (superonasal, nasal, inferonasal, inferotemporal, temporal, and superotemporal). The device also generates a database with RNFL thickness measurements at all 768 points registered during circular peripapillary scan acquisition.

Neurologic Evaluation

The stage and severity of AD were determined using the MMSE scale, a 10-min bedside measure of impaired thinking in underdeveloped, uneducated, diseased, or very old populations. The summed score of the individual items indicates the severity of cognitive impairment. Deterioration in cognition is indicated by decreasing scores of repeated tests over time. The items of the MMSE include tests of orientation, registration, recall, calculation, attention, naming, repetition, comprehension, reading, writing, and drawing. A score of 30 indicates 100% correct. The mean score for a community-dwelling population over 65 years old is approximately 27.[30] The AD patients lose 3 to 4 points per year of illness after the onset of memory disturbance, although there is wide variability.[31] The MMSE scale was performed by one trained neurologist who was blind to the OCT results. Disease duration since AD diagnosis was recorded.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (version 20.0; SPSS, Inc., Chicago, IL, USA) and MedCalc statistical software (version 9.6.4.0; MedCalc Software, Mariakerke, Belgium). A binary logistic regression analysis was used to design an LDF with retinal thicknesses and RNFL parameters using both OCT devices to detect the presence of disease (AD). The dependent variable was AD diagnosis (positive or negative). For Cirrus Retinal LDF, the nine ETDRS area thicknesses were included in the statistical analysis, and for Cirrus RNFL LDF, the mean, four quadrants, and 12-clock hour sectors thicknesses were included. For Spectralis OCT, two analyses were performed. The first analysis included the nine ETDRS area thicknesses and provided the Spectralis Retinal LDF. The second analysis included the 768 points registered during circular peripapillary scan acquisition, grouped to obtain 24 uniformly divided locations (each location representing 15°); the mean; and 6-sector RNFL thicknesses, to calculate the Spectralis RNFL LDF.

For the logistic regression, the predicted dependent variable was a function of the probability that a particular subject would be in one of the categories (e.g., the probability that one subject has AD given the set of scores of the predictor variables). The relative importance of each independent variable was assessed by a stepwise binary logistic regression analysis using the forward Wald method. The Wald w2-statistic tests the unique contribution of each predictor in the context of the other predictors (holding constant the other predictors), eliminating any overlap between predictors.

Hence, the parameters with higher sensitivity or specificity values are not necessarily the selected variables in the logistic regression method. The stepwise probability test determined the criteria by which the variables were entered into and removed from the model. The LDF was calculated by taking the weighted sum of the predictor variables. Significant OCT parameters were combined to generate a new variable (LDF) in such a way that the measurable differences between healthy and AD eyes were maximized. The ROC curves were plotted for all of the parameters and compared to the proposed LDFs.

Differences among ROC curves were compared using the Hanley-McNeil method.[32] The cutoff points were calculated with MedCalc software (available in the public domain at http://www.medcalc.com) as the points with the best sensitivity–specificity balance. Sensitivities at 85% and 95% (15% and 5% false-positive rate, respectively) fixed specificities, and positive and negative likelihood ratios also were calculated.

A new population was selected randomly from among 50 different AD patients and 50 different healthy subjects to test the performance of the LDF. The test was based on 768 RNFL measurements provided by the Spectralis OCT device in this independent population, referred to as the "validating set." The ROC curve was plotted for the proposed LDF.

The predicted residual sums of squares (PRESS) statistic was used to cross-validate the model, calculating it as the sums of squares of the prediction residuals for observations. The lowest PRESS value indicates the best structure. Models that are overparameterized tend to give small residuals for observations included in the model-fitting, but large residuals for observations that are excluded.[33]

In the AD group, the comparison between men and women was performed using Student's *t*-test, and the correlation between age and thickness measurements was evaluated using the Pearson analysis.

Results

We examined 151 eyes from 151 AD patients and 61 eyes from 61 healthy subjects. Epidemiologic and disease characteristics of patients with AD and healthy subjects are shown in Table 1. Age, sex, and IOP did not differ significantly between the two groups. The duration of AD since diagnosis ranged from 6 months to 3 years and the mean age was 75.3 years.

All registered parameters tended to be more affected in AD patients than in healthy controls. Structural (retinal and RNFL thickness measurements provided by the Cirrus and Spectralis OCT devices) and visual functional parameters (BCVA, mean deviation, and pattern standard deviation of visual field) were significantly different between groups (Tables 1, 2; Figs. 1, 2).

A stepwise procedure identified retinal and RNFL parameters of each OCT that accounted for the greatest amount of error, which then was included in the model. The next best variable then was identified and included, and so on. We calculated four LDFs: Cirrus Retinal LDF (using the nine retinal ETDRS area thicknesses provided by the Cirrus OCT device), Cirrus RNFL LDF (including mean, four RNFL quadrant thicknesses, and 12-clock hour sectors thicknesses provided by the Cirrus OCT device), Spectralis Retinal LDF (using the nine retinal ETDRS area thicknesses provided by the Spectralis OCT device), and Spectralis RFNL LDF (including mean, six RNFL sectors thickness, and the 768 RNFL thickness provided during Spectralis circular peripapillary scan acquisition, grouped in 24 uniformly divided locations of 15° per location).

In the Cirrus Retinal LDF, only the superior inner thickness was selected at the logistic regression statistical analysis and the formula obtained was $11.975 - 0.038 \times$ superior inner thickness. The area under the ROC curve was 0.697 (Table 3; Fig. 3) when Cirrus Retinal LDF was used to detect AD versus healthy controls.

In the Cirrus RNFL LDF, the clock-hour sector number 7 thickness was selected at the first logistic regression iteration and the temporal quadrant thickness was selected at the second logistic regression iteration. Using this procedure, the Cirrus RNFL LDF was defined as follows: $2.822 - 0.097 \times$ clock hour sector 7 thickness $+ 0.138 \times$ temporal quadrant thickness. The area under the ROC curve with Cirrus RNFL LDF was 0.830 (Table 3; Fig. 3).

In the Spectralis Retinal LDF, only the inferior inner thickness was selected at the logistic regression statistical

Table 1. Epidemiologic and Disease Characteristics of Patients With AD and Healthy Subjects, and Statistical Significance (*P*)

	Alzheimer's Disease Eyes	Healthy Eyes	*P* Value
Eyes, *n*	151	61	–
Age, y (range)	75.29 (56–90)	74.87 (55–91)	0.211
Men:women (% women)	56:95 (62.9%)	23:38 (62.3%)	0.459
Mean IOP (SD)	14.1 (1.7)	14.3 (1.8)	0.609
Mean BCVA, Snellen scale (SD)	0.76 (0.17)	0.91 (0.13)	<0.001
Mean MD visual field (SD)	−4.89 (2.34)	−0.65 (1.85)	<0.001
Mean PSD visual field, dB (SD)	4.31 (1.78)	1.5 (0.91)	<0.001
Mean Mini Mental State examination (SD)	18.31 (3.28)	–	–
Mean disease duration, y (SD)	2.01 (1.21)	–	–

MD, mean deviation of visual field; PSD, pattern standard deviation of visual field.

analysis and the formula obtained was 27.546 − 0.081 × inferior inner thickness. The area under the ROC curve was 0.827 (Table 4; Fig. 4) in AD detection.

The Spectralis RNFL LDF was defined as follows: 18.325 + 0.056 × (mean thickness from 315°–330°) − 0.122 × (mean thickness from 300°–315°) − 0.041 × (mean thickness from 285°–300°) + 0.091 × (mean thickness from 255°–270°) + 0.041 × (mean thickness from 225°–240°) + 0.183 × (mean thickness from 195°–210°) − 0.108 × (mean thickness from 150°–165°) − 0.092 × (mean thickness from 75°–90°) + 0.051 × (mean thickness from 30°–45°). The area under the ROC curve was 0.967 for the Spectralis RNFL LDF (Table 4; Fig. 4).

Finally, all retinal and RNFL measurements provided by the Cirrus and the Spectralis OCT devices were included in the

Table 2. Retinal and RNFL of Patients With AD and Healthy Subjects, and Statistical Significance (*P*)

	Alzheimer Patients, *n* = 151		Healthy Subjects, *n* = 61		
	Mean	SD	Mean	SD	*P*
Cirrus retinal thickness					
Foveal	260.32	22.11	262.34	18.99	0.076
Superior inner	321.78	18.12	329.19	18.00	<0.001*
Nasal inner	318.34	17.66	324.56	18.98	<0.001*
Inferior inner	312.09	18.08	333.33	17.67	<0.001*
Temporal inner	308.90	18.90	317.53	16.89	<0.001*
Superior outer	270.37	16.90	287.46	15.11	<0.001*
Nasal outer	288.91	18.80	298.04	16.77	<0.001*
Inferior outer	263.10	18.96	280.22	17.39	<0.001*
Temporal outer	257.43	18.04	271.72	16.22	<0.001*
Spectralis retinal thickness					
Foveal	277.31	20.78	279.99	18.55	0.096
Superior inner	332.82	19.00	349.38	17.23	<0.001*
Nasal inner	341.87	20.00	347.11	17.88	0.006*
Inferior inner	326.25	20.44	343.17	16.65	<0.001*
Temporal inner	322.44	17.98	335.32	16.04	<0.001*
Superior outer	292.11	17.67	302.46	17.91	<0.001*
Nasal outer	289.33	19.73	310.00	17.23	0.003*
Inferior outer	277.63	19.26	315.91	18.92	<0.001*
Temporal outer	273.01	18.34	287.61	16.09	<0.001*
Cirrus RNFL					
Average	97.55	14.12	100.55	12.99	0.067
Superior	113.22	18.67	117.81	19.00	0.010*
Nasal	72.67	17.31	74.55	17.26	0.090
Inferior	120.44	20.98	127.38	20.99	<0.001*
Temporal	64.47	21.76	67.83	20.01	0.023*
Spectralis RNFL					
Average	98.21	17.07	102.70	6.67	0.049*
Nasal superior	108.05	29.62	104.98	14.37	0.319
Nasal	88.94	47.02	77.53	7.51	0.005*
Nasal inferior	112.32	14.67	117.43	15.11	0.020*
Temporal inferior	132.64	26.62	156.23	14.98	<0.001*
Temporal	67.54	15.46	69.52	9.99	0.288
Temporal superior	146.67	13.17	128.93	32.51	<0.001*

Thickness measurements are in micrometers (μm).
* Significant difference ($P < 0.05$) in Student's *t*-test between normal and AD groups.

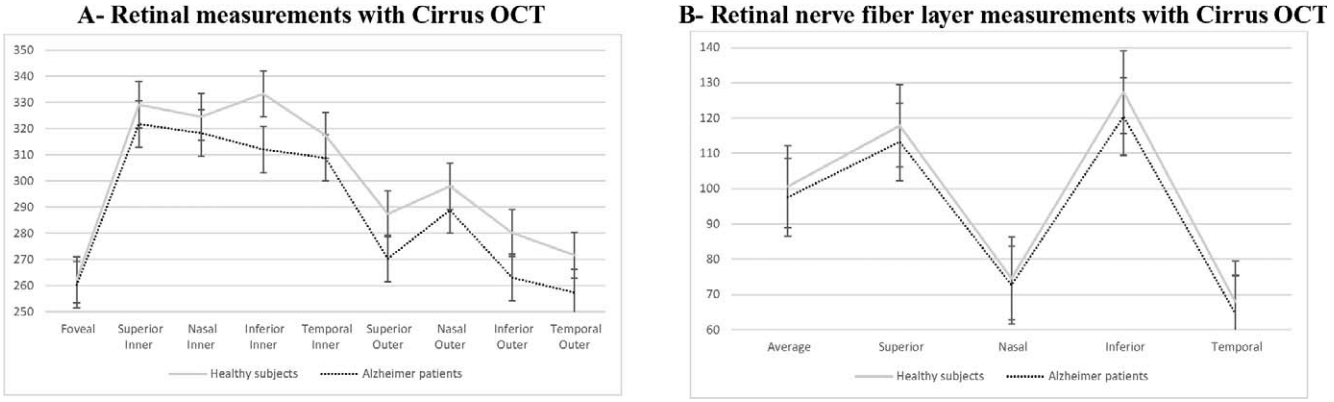

Figure 1. (**A**) Representation of retinal measurements provided by the Cirrus OCT device in AD patients and healthy controls. The retinal thickness map analysis provides measurements for each of the nine subfields, as defined by the ETDRS areas. In the AD patient group, retinal thickness was reduced significantly in all retinal areas except the fovea. (**B**) Representation of RNFL measurements provided by the Cirrus OCT device in AD patients and healthy controls. In the AD patient group, retinal thickness was reduced significantly in the superior, inferior, and temporal quadrants.

statistical analysis to calculate the definitive LDF combining all OCT measurements. The optimal function was the Spectralis RNFL LDF (the regression analysis provided the same formula).

The RNFL LDFs provided by the Cirrus and Spectralis devices had the best sensitivity–specificity pairs, while retinal parameters had the poorest diagnostic ability for detecting AD. The inferior inner thickness and the Retinal LDF provided by the Spectralis OCT device had the highest positive likelihood ratios, and the Spectralis RNFL LDF had the lowest negative likelihood ratio. The largest areas under the ROC curves were 0.967 for the Spectralis RNFL LDF and 0.830 for the Cirrus RNFL LDF (Figs. 3, 4). Compared to the OCT-provided parameters, both LDFs had larger areas under the ROC curves ($P < 0.05$), and a comparison between Cirrus and Spectralis RNFL LDFs revealed significant differences ($P = 0.009$). The best parameter for distinguishing AD patients from healthy controls using OCT measurements was the Spectralis RNFL LDF.

We used a validation set that included 50 healthy eyes and 50 AD eyes that were not part of the study set to validate our Spectralis RNFL LDF. Mean age of the normal group was 74.99 ± 8.1 years and mean age of the AD group was 74.93 ± 8.8 years. Mean IOP was 14.2 mm Hg in the AD group and 14.4 mm Hg in the control group. Sex, age, and IOP did not differ significantly between the groups in either sample. Both groups (AD and controls) included 31 men (62%) and 19 women (38%). Mean disease duration in the validating set was 1.97 years (range, 0.5–3 years) and the mean MMSE score was 18.29 ± 3.88. In the validating set, the area under the ROC curve was 0.965 for the Spectralis RNFL LDF, higher than that for classic OCT parameters. The area under the ROC curve for nasal RNFL thickness was 0.635 using the Spectralis OCT and 0.598 using the Cirrus OCT, and that for the superior inner retinal thickness was 0.600 using the Spectralis OCT and 0.566 using the Cirrus OCT.

The PRESS value was 0.0198 for the Spectralis RNFL LDF. This low value indicated that our logistic regression statistical analysis is a good model for predicting AD diagnosis in the study population.

The comparison between men and women in the AD group revealed that sex did not affect the RNFL or retinal thickness reduction: the mean foveal thickness using Spectralis OCT was 278.16 ± 25.91 μm in men and 278.13 ± 24.76 μm in women ($P = 0.879$). The mean RNFL average thickness using Spectralis OCT was 94.80 ± 10.10 μm in men and 95.13 ± 10.51 μm in women ($P = 0.451$). In addition, the correlation analysis revealed that the age of AD patients did not correlate with RNFL or retinal thickness measurements ($P > 0.05$).

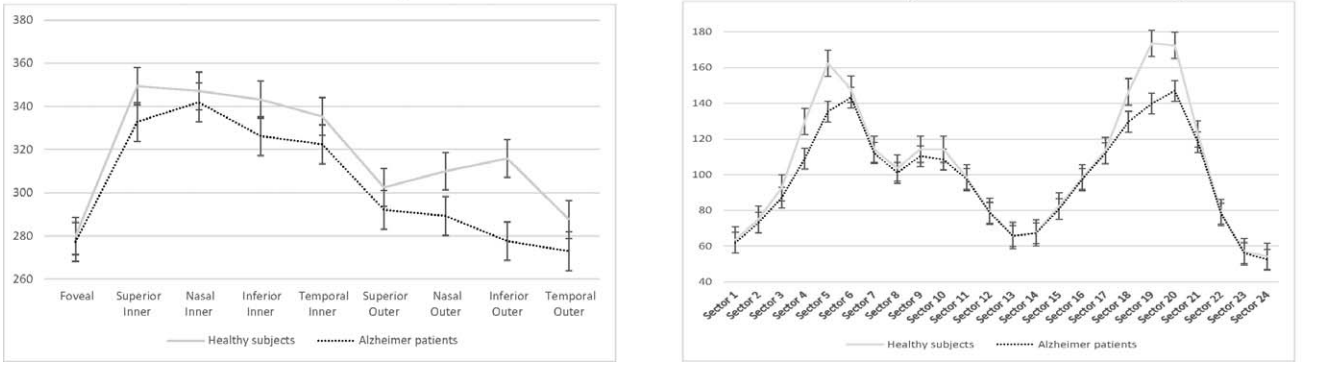

Figure 2. (**A**) Representation of retinal measurements provided by the Spectralis OCT device in AD patients and healthy controls. The retinal thickness map analysis provides measurements for each of the nine subfields, as defined by the ETDRS areas. In the AD patient group, retinal thickness was decreased significantly in all retinal areas except the fovea. (**B**) Representation of RNFL measurements provided by the Spectralis OCT in AD patients and healthy controls. In the AD patient group, retinal thickness was reduced significantly in all sectors, except in nasal superior sector and temporal quadrant RNFL thickness.

Investigative Ophthalmology & Visual Science

Table 3. Areas Under the ROC Curves, Best Sensitivity–Specificity Balance, and Likelihood Ratios to Discriminate Between Normal Subjects and AD Patients for Retinal and RNFL Parameters Provided by the Cirrus OCT Device and for the LDF Calculated in the Study

									Sensitivity	
Cirrus OCT Parameters	**AUC**	**95% CI**	**AUC *P* Value**	**Cutoff Point**	**Sens, %**	**Spec, %**	**+LR**	**−LR**	**Spec 85%**	**Spec 95%**
Foveal thickness	0.545	0.438–0.648	0.456	≤238	24.4	88.5	2.11	0.85	24.5	14.6
Superior inner thickness	0.630	0.524–0.728	0.023*	≤314	53.7	73.1	1.99	0.63	39.0	28.3
Cirrus retinal LDF	0.697	0.593–0.788	<0.001*	>0.01	65.9	80.8	3.42	0.42	51.2	34.1
Mean RNFL thickness	0.693	0.540–0.821	<0.001*	≤92	69.8	67.9	2.18	0.45	46.5	30.2
Temporal RNFL thickness	0.565	0.411–0.711	0.481	≤69	84.6	39.4	1.40	0.39	7.7	0.2
Hour sector 7 thickness	0.768	0.620–0.879	<0.001*	≤136	76.7	60.4	1.94	0.39	44.2	16.3
Cirrus RNFL LDF	0.830	0.690–0.924	<0.001*	>−0.77	76.9	87.9	6.35	0.26	77.0	61.5

The cutoff points were calculated using the MedCalc software as the points with the best sensitivity–specificity balance. Sensitivities at 85% and 95% fixed specificities are shown. AUC, area under the receiver operating characteristic curve; Sens, sensitivity; Spec, specificity; +LR, positive likelihood ratio; −LR, negative likelihood ratio.

* Significant difference.

Discussion

The RNFL thickness in patients with AD compared to healthy subjects using time-domain OCT reveals significant thinning in AD patients,[6,8,12,13] similar to our results. These findings demonstrated that AD progression is associated with optic nerve degeneration, but we also demonstrated retinal thinning in AD patients. Although Iseri et al.[7] reported a reduction in macular volume in AD patients that correlated with the severity of the disease, they did not evaluate all ETDRS sectors using Fourier domain OCT devices. We found that foveal thickness is the less useful retinal parameter for detecting atrophy in AD, but the inner and outer ETDRS areas show retinal thinning in AD patients.

Due to the distribution of ganglionic cell fibers in the optic nerve head, the temporal RNFL quadrant is the sector most affected in early neurodegenerative diseases,[34] as the fibers of the temporal quadrant follow the papillomacular bundle. Our results are consistent with these findings and we found that temporal RNFL parameters were selected in the regression analysis to configure the LDF with both OCT devices (Cirrus and Spectralis).

Histopathologic studies have revealed retinal ganglion cell loss and optic nerve degeneration in patients with AD.[35,36] Neuroimaging reveals alterations in AD patients, even in early stages of the disease.[37] Thus, we postulated that axonal loss secondary to other pathologic changes that occur in the brain can be detected by scanning the RNFL and the optic nerve, as these axons form the optic path that culminates in the occipital cortex. Many instruments recently have been introduced to quantify retinal ganglion cells. Changes in the RNFL may reflect similar pathologic changes occurring elsewhere in the brain.[38,39] Ocular imaging technologies, such as OCT, scanning laser polarimetry, or confocal scanning laser ophthalmoscopy, allow the axonal constituents of the anterior visual pathway to be observed, thereby allowing direct visualization of part of the central nervous system. Studies evaluating the correlation between RNFL thickness and magnetic resonance imaging measurements of the brain, however, such as the parenchymal fraction and brain volumes in AD patients, are needed.[40]

No previous studies have evaluated the capability of OCT to detect AD or have tested new diagnostic tools based on ophthalmologic evaluation for this pathology. This is the main scientific contribution of the present study.

The diagnosis of AD currently is based on medical history, neurologic examination, and neuropsychologic screening tests, such as the MMSE, because to our knowledge there is no laboratory test that clearly identifies the disease. The AD is difficult to diagnose in its early stages and differential diagnosis with other diseases often is complicated. Supplemental testing, such as blood, thyroid function, B12, or syphilis tests, provides additional information that is useful for ruling out other

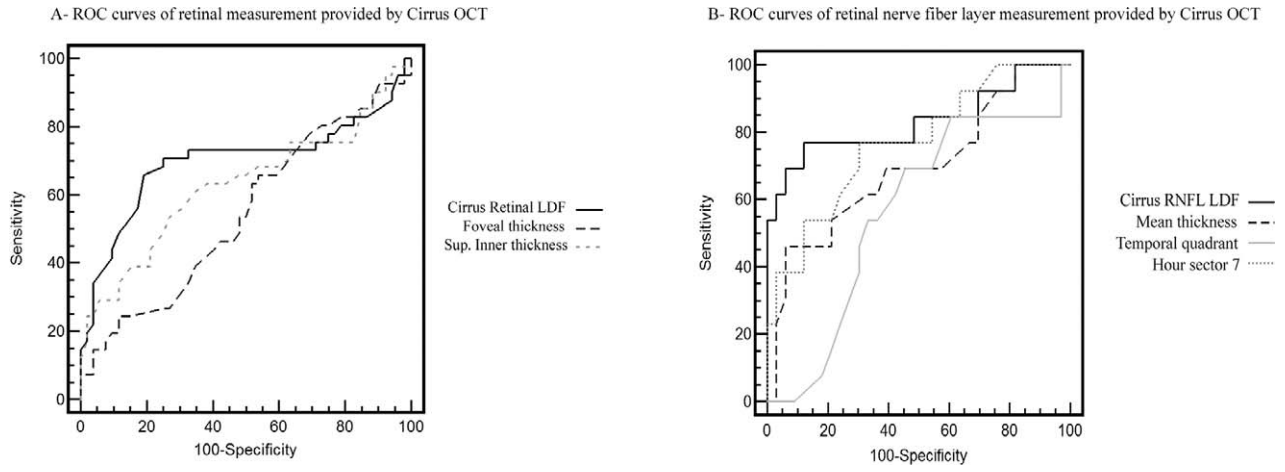

Figure 3. (**A**) Representation of ROC curves obtained with the retinal LDF, foveal thickness, and superior inner retinal thickness parameters provided by the Cirrus OCT device. (**B**) Representation of ROC curves obtained with the RNFL LDF, mean RNFL thickness, temporal quadrant RNFL thickness, and clock-hour sector number 7 RNFL thickness provided by the Cirrus OCT. The largest areas under the ROC curves were for the Retinal LDF (0.687; 95% confidence interval [CI], 0.593–0.788) and for the RNFL LDF (0.830; 95% CI, 0.690–0.924). Sup, superior.

Table 4. Areas Under the ROC Curves, Best Sensitivity–Specificity Balance, and Likelihood Ratios to Discriminate Between Normal Subjects and AD Patients for Retinal and RNFL Parameters Provided by the Spectralis OCT Device and for the LDF Calculated in the Study

									Sensitivity	
Spectralis OCT Parameters	**AUC**	**95% CI**	**AUC *P* Value**	**Cutoff Point**	**Sens, %**	**Spec, %**	**+LR**	**−LR**	**Spec 85%**	**Spec 95%**
Foveal thickness	0.501	0.379–0.623	0.990	>266	73.8	42.9	1.31	0.61	7.1	2.4
Inferior inner thickness	0.770	0.654–0.862	<0.001*	≤328	61.9	96.4	17.31	0.40	69.2	63.0
Spectralis retinal LDF	0.827	0.719–0.907	<0.001*	>0.897	71.4	92.9	10.00	0.31	72.9	67.2
Mean RNFL thickness	0.677	0.607–0.742	<0.001*	≤97	58.1	80.0	2.90	0.52	44.9	34.6
Temporal inferior RNFL thickness	0.820	0.759–0.860	<0.001*	≤141	64.3	88.3	5.51	0.40	65.2	30.1
Temporal superior RNFL thickness	0.780	0.715–0.836	<0.001*	≤143	77.5	68.3	2.45	0.33	54.9	34.0
Spectralis RNFL LDF	0.967	0.930–0.986	<0.001*	>0.08	94.3	88.3	8.09	0.06	94.3	83.7

The cut-off points were calculated using the MedCalc software as the points with the best sensitivity–specificity balance. Sensitivities at 85% and 95% fixed specificities are shown.

* Significant difference.

diagnoses. The analysis of cerebrospinal fluid for beta-amyloid or tau protein concentrations is a recent objective marker of AD that can predict the onset of disease with high sensitivity (94%–100%).[41] Our LDFs had comparable accuracy, but more studies evaluating the ability of OCT to detect AD in its early stages are needed. Improvements in AD diagnosis may be important to justify initiating therapies in patients suspected of having AD.

This study also compared the diagnostic ability of standard OCT parameters to LDFs obtained using logistic regression statistical analysis. Although the retina and RNFL were affected in AD patients, the retinal parameters demonstrated only moderate diagnostic accuracy, while the RNFL measurements (especially by the 768 RNFL thickness provided during Spectralis circular peripapillary scan acquisition, grouped into 24 sectors) were a very useful and precise tool for AD diagnosis. The LDFs were at least as sensitive and specific as the methods currently used for AD diagnosis. The OCT has advantages over other diagnostic methods in AD: it is noninvasive, inexpensive, and does not cause damage or inconvenience to the patients during the test.

Several investigators have attempted to increase the diagnostic ability of OCT for some pathologies using learning classifiers,[42] including neurologic diseases, such as multiple sclerosis,[15] yet the sensitivity and specificity of OCT as a neuroimaging technique to diagnose AD has not improved. The logistic regression analysis performed in our study evaluated the relative importance of each independent variable using the forward Wald method, so the calculated LDF has better diagnostic ability compared to individual OCT parameters. Our Spectralis RNFL LDF yielded the highest sensitivity at a high specificity compared to any single parameter obtained using OCT. The high sensitivity and specificity demonstrated by OCT may be better than some of the accepted criteria in the current AD diagnostic procedure. Depending on the pretest probability, positive or negative likelihood ratios indicate the extent to which a factor (i.e., probability of disease) will increase or decrease, respectively. A likelihood ratio value close to 1 indicates insignificant effects, whereas likelihood ratio values higher than 10 or lower than 0.1 often indicate higher posttest odds of the disease. The Spectralis RNFL LDF had the lowest negative likelihood ratio (0.06); thus, normal results were associated with a high posttest probability of disease for these variables, indicating a better ability to exclude the presence of AD. This is the strength of the Spectralis RNFL LDF, which yielded a very good sensitivity (94.3%). A likelihood ratio value higher than 8.09 for our Spectralis RNFL LDF (cutoff point for 95% specificity) virtually rules out the chance that the patient has AD. The cutoff point for 95% specificity may lead to overestimated performance,[43] however, so analysis of the validating set and PRESS analysis were performed to evaluate the actual diagnostic accuracy of our LDF.

A limitation of the present study might be the inclusion criteria. Subjects with glaucoma, diabetes, or previous ocular

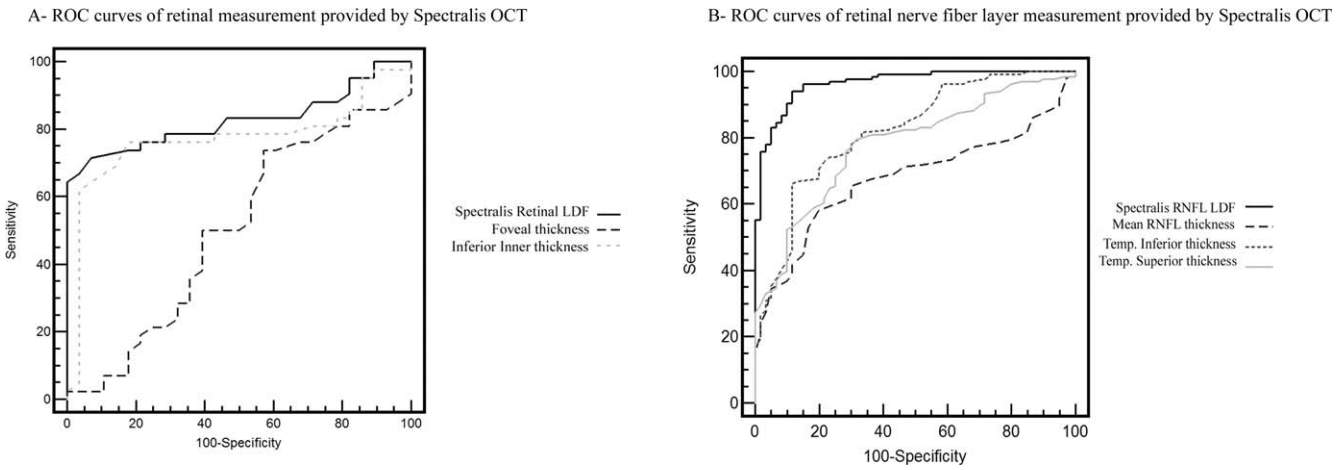

Figure 4. (**A**) Representation of ROC curves obtained with the retinal LDF, foveal thickness, and inferior inner retinal thickness parameters provided by the Spectralis OCT device. (**B**) Representation of ROC curves obtained with the RNFL LDF, mean RNFL thickness, temporal inferior, and temporal superior sectors RNFL thickness provided by the Spectralis OCT device. The largest areas under the ROC curves were for the Retinal LDF (0.827; 95% CI, 0.719–0.907) and for the RNFL LDF (0.967; 95% CI, 0.930–0.986). Temp, temporal.

surgery were excluded to avoid RNFL reduction by mechanisms other than AD. These criteria resulted in the exclusion of older patients. In addition, high IOP, glaucomatous optic nerve head morphology (cup-to-disc ratio of 0.5 or higher), and arcuate nerve fiber bundle visual field defects were the criteria for glaucoma detection in our study population, so some individuals with low-tension preperimetric glaucoma could have been included in both groups (AD and controls), which may have biased the findings. Another limitation of our study is that case-control studies like this one may overstate the diagnostic capabilities of a test due to the inclusion of two well-defined populations.[44] In addition, the regression analysis of our study may overestimate the ability of the LDF to detect AD. Our study revealed the thickness reduction typical for neurodegenerative diseases (such as in the nasal RNFL quadrant or the superior inner retinal sector), but the LDF that we propose combines all RNFL and retinal sectors to obtain a formula in which some parameters have more weight than others. This combination of measurements improves the capability of OCT to detect AD. The inability to diagnose AD until autopsy is another limitation of this study because the identification of controls and patients was not definitive.

Clinical application of the findings of the present study may facilitate AD diagnosis in patients in whom AD is suspected clinically. Although our results suggested that RNFL thinning could be useful for identifying AD patients, longer-term studies using Fourier domain OCT to analyze the ability, sensitivity, and specificity of RNFL thickness measurements to diagnose AD, as well as longitudinal and prospective studies to monitor AD progression using OCT measurements are needed.

Acknowledgment

The authors alone are responsible for the content and writing of the paper.

Disclosure: **J.M. Larrosa**, None; **E. Garcia-Martin**, None; **M.P. Bambo**, None; **J. Pinilla**, None; **V. Polo**, None; **S. Otin**, None; **M. Satue**, None; **R. Herrero**, None; **L.E. Pablo**, None

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