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Evaluación de la función visual en enfermedades neurodegenerativas: Alzheimer, Parkinson y Esclerosis Múltiple

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EVALUACIÓN DE LA FUNCIÓN VISUAL EN ENFERMEDADES NEURODEGENERATIVAS: ALZHEIMER, PARKINSON Y ESCLEROSIS MÚLTIPLE

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Visual function evaluation in neurodegenerative diseases: ALZHEIMER, PARKINSON AND MULTIPLE SCLEROSIS

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Trabajo de investigación presentado por la Licenciada en

Medicina y Cirugía

María Jesús Rodrigo Sanjuán

Para optar al grado de doctora con mención internacional

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La presente tesis doctoral ha sido estructurada siguiendo la normativa para las tesis por compendio de publicaciones con mención internacional. Los artículos incluidos en la tesis pertenecen a la misma línea de investigación y han sido publicados previamente.

A continuación, se detallan los cuatro artículos que constituyen el cuerpo de la tesis:

1. **Polo V, Rodrigo MJ, Garcia-Martin E, Otin S, Larrosa JM, Fuertes MI, Bambo MP, Pablo LE, Satue M. Visual dysfunction and its correlation with retinal changes in patients with Alzheimer´s disease. Eye (Lond) 2017 (en prensa).**

2. **Polo V, Satue M, Rodrigo MJ, Otin S, Alarcia R, Bambo MP, Fuertes MI, Larrosa JM, Pablo LE, Garcia-Martin E. Visual dysfunction and its correlation with retinal changes in patients with Parkinson's disease: an observational cross-sectional study. BMJ Open 2016;6:e009658.**

3. **Satue M, Rodrigo MJ, Otin S, Bambo MP, Fuertes MI, Ara JR, Martin J, Polo V, Larrosa JM, Pablo L, Garcia-Martin E. Relationship between visual dysfunction and retinal changes in patients with multiple sclerosis. PLoS ONE 2016;11(6):e0157293.**

4. **Satue M, Obis J, Rodrigo MJ, Otin S, Fuertes MI, Vilades E, Gracia H, Ara JR, Alarcia R, Polo V, Larrosa JM, Pablo LE, Garcia-Martin E. Optical coherence tomography as a biomarker for diagnosis, progression, and prognosis of neurodegenerative diseases. J Ophthalmol. 2016;2016:8503859. Review.**

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Certifica:

Que el trabajo de investigación titulado "Evaluación de la función visual en enfermedades neurodegenerativas: Alzheimer, Parkinson y Esclerosis Múltiple" que presenta, MARÍA JESÚS RODRIGO SANJUÁN, Licenciada en Medicina y Cirugía, para optar al GRADO DE DOCTOR, fue realizado bajo mi dirección, se ajusta al proyecto de tesis presentado con anterioridad y doy mi autorización para su defensa como tesis por compendio de publicaciones y con mención internacional.

Y para que conste a los efectos oportunos, firmo el presente en Zaragoza, a 31 de Enero de 2017.

Fdo. Dra. Elena García-Martín

Dª. MARÍA ISABEL FUERTES LÁZARO, Licenciada en Ciencias Físicas, Doctora en Medicina y profesora ayudante doctor de la Universidad de Zaragoza.

Certifica:

Que el trabajo de investigación titulado "Evaluación de la función visual en enfermedades neurodegenerativas: Alzheimer, Parkinson y Esclerosis Múltiple" que presenta, MARÍA JESÚS RODRIGO SANJUÁN, Licenciada en Medicina y Cirugía, para optar al GRADO DE DOCTOR, fue realizado bajo mi dirección, se ajusta al proyecto de tesis presentado con anterioridad y doy mi autorización para su defensa como tesis por compendio de publicaciones y con mención internacional.

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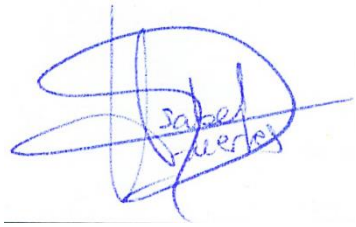

Fdo. Dra. María Isabel Fuertes Lázaro

ÍNDICE

1.ABREVIATURAS

-AV: Agudeza visual.

-VA: Visual Acuity.

-EA: Enfermedad de Alzheimer.

-AD: Alzheimer's Disease.

-EP: Enfermedad de Parkinson.

-PD: Parkinson's Disease.

-EM: Esclerosis Múltiple.

-MS: Multiple Sclerosis.

-OCT: Tomografía de Coherencia Óptica // Optic Coherence Tomography.

-CFNR: Capa de Fibras Nerviosas de la Retina.

-RNFL: Retina Nerve Fiber Layer.

-CCG: Capa de Células Ganglionares.

-GCL: Ganglion Cell Layer.

-MAVC: Mejor Agudeza Visual Corregida.

-DSM-IV: Manual diagnóstico y estadístico de los desórdenes mentales 4ª edición // Diagnostic and Statistical manual of Mental Disorders 4th edition.

-MMSE: Mini examen del estado mental // Mini Mental State Examination.

-ETDRS: Estudio del tratamiento precoz de la retinopatía diabética // Early Treatment Diabetic Retinopathy Study.

-EDSS: Escala ampliada del estado de discapacidad // Expanded Disability Status Scale.

-UPDRS: Escala unificada de clasificación de la enfermedad de Parkinson // Unified Parkinson Disease Rating Scale.

-C-Index: Índice de confusión // Confusion Index.

-CCI: Índice de confusion del color // Color Confusion Index.

-AC CCI: Índice de confusión del color corregido por edad // Age Corrected Color Confusion Index.

-Conf angle: Ángulo de confusión // Confusion angle.

-S-index: Índice de dispersión // Scatter index.

-CSV: Contrast Sensitivity vision.

-SC: Sensibilidad al contraste.

-HD: Alta definición // High Definition.

-PEV: Potenciales Evocados Visuales.

-GCIPL: Capa plexiforme interna células ganglionares // Ganglion cell Inner Plexiform Layer.

-CSF: Función de sensibilidad al contraste.

2. APARTADO INTERNACIONAL: SUMMARY OF THE THESIS

The main purpose of the four international publications that compose this doctoral thesis is the study of the structural parameters (macular, retinal nerve fiber layer [RNFL], and ganglion cell layer [GCL] thicknesses), and the functional parameters (visual acuity [VA], contrast sensitivity vision [CSV] and color perception) of the retina and optic nerve, in patients with three neurodegenerative diseases: Alzheimer's disease (AD), Parkinson's disease (PD) and Multiple Sclerosis (MS).

All these neurodegenerative diseases are characterized by neuronal death (by amyloid deposits in AD, by alteration in dopaminergic circuits in PD or by immune-mediated in MS), clinical symptoms and functional disability, as a consequence of the neuronal damage. We postulate that the neuronal degeneration cause RNFL thinning and reduction in visual function.

The retina is a part of the central nervous system that is easily accessible to clinical examination. The RNFL comprises mainly nonmyelinated axons of retinal ganglion cells, so thickness measurements of the different retinal layers may provide a relatively direct assessment of the axons and neural damage. Large scientific evidence already demonstrated retinal thinning (macular and RNFL), examined with the optic coherence tomography (OCT) technology, in AD, PD and MS; and this retinal thinning was correlated with progression and even with worse quality of life. The newest OCT software provides measurements of the ganglion cell layer, where parvocellular, magnocellular and koniocellular ganglion cells are located. These specialized cells are responsible of identifying color and spatial contrast at different frequencies, therefore general loss of these retinal cells is likely to be an important contributory mechanism for visual impairment.

Vision has a high impact on quality of life of people. Visual function evaluation using visual acuity (ETDRS charts at 100%, 2.5%, 1.25%), contrast sensitivity (CSV 1000-E and Pelli-Robson charts) and chromatic vision (Farnsworth-Munsell D15 and L'Anthony D15 tests), can help us to a better understanding of the visual impairment in patients with AD, PD and MS. It is already known that these diseases have visual function alterations such as difficulty in face recognition, reading and/or driving, related to contrast sensitivity; as well as acquired alterations in color vision because of macular or optic nerve pathologies, as it happens in optic neuritis in patients with MS. These disturbances were initially attributed to cortical causes, but alterations in the anterior visual pathway, as decreased retinal thickness due to neuronal death (secondary to amyloid deposition in AD or α-synuclein in PD) have recently been suggested. Ganglion cells death is postulated to be responsible for the alterations in contrast sensitivity and color from the earliest stages, even before the detection of decreased RNFL of the optic nerve and especially of the papillary pallor.

The articles published in this doctoral thesis showed that visual function parameters are altered and that correlate with structural parameters, even at early stages. These results suggest that visual function analysis could be considered as a biomarker for the neurodegenerative diseases: AD, PD, and MS.

Below includes a brief presentation of each international article and its thematic unity is justified:

1. **Polo V, Rodrigo MJ, Garcia-Martin E, Otin S, Larrosa JM, Fuertes MI, Bambo MP, Pablo LE, Satue M. Visual dysfunction and its correlation with retinal changes in patients with Alzheimer´s disease. Eye (Lond) 2017 (in press).**

Twenty-four mild-moderate AD patients and 24 age and sex-matched healthy subjects were included in this prospective cross-sectional study. The aim was to evaluate visual dysfunction and its correlation with retinal structural changes in patients with AD.

The following functional parameters were analyzed: VA was evaluated with Early Treatment Diabetic Retinopathy Study (ETDRS) charts at high (100%) and low (2.5% and 1.25%) contrast levels, contrast sensitivity was evaluated using CSV 1000-E and Pelli-Robson charts and color vision was evaluated with specific parameters (Confusion Index [C-Index], Color Confusion Index [CCI], Age Corrected Color Confusion Index [AC-CCI], Confusion angle [Conf Ang] and Scatter index [S-Index]) from Farnsworth-Munsell D15 and L'Anthony D15 tests.

Structural parameters were explored with Cirrus high definition (HD) OCT device. Macular cube and optic disc cube protocols were performed.

Several previous studies demonstrated disturbances in contrast sensitivity and color vision. These alterations were suggested as predictors of cognitive dysfunction. However, on one hand, there are conflicting studies about RNFL defects: one study did not find significant differences between AD patients and healthy controls, but other study postulated RNFL alterations as the earliest sign of AD, even before hippocampal damage. On the other hand, several studies found important macular alteration that was correlated with cognitive impairment. But we did not find any article that correlate

structural retinal parameters with functional parameters of contrast sensitivity and color vision.

In our study, functional and structural parameters were compared between healthy controls and patients with AD, resulting in a statistically significant decrease in contrast sensitivity and color vision, as well as a significant decrease in 8 of the 9 analyzed macular sectors, and in the superior quadrant and average thickness of the RNFL.

Contrast sensitivity was the functional parameter most strongly correlated with structural measurements (specifically with macular thickness), although color vision (Farnsworth's test) and visual acuity associations were also found.

Our results conclude that visual dysfunction in patients with AD correlate with morphologic parameters, being the changes in the macular area those that are more closely associated with visual function alterations.

2. **Polo V, Satue M, Rodrigo MJ, Otin S, Alarcia R, Bambo MP, Fuertes MI, Larrosa JM, Pablo LE, Garcia-Martin E. Visual dysfunction and its correlation with retinal changes in patients with Parkinson's disease: an observational cross-sectional study. BMJ Open 2016;6:e009658.**

Thirty-seven patients and 37 age and sex-matched healthy subjects were included in this prospective cross-sectional study. The aim was to evaluate visual dysfunction and its correlation with the retinal structural changes in patients with PD.

The following functional parameters were analyzed: visual acuity was evaluated with ETDRS charts at high (100%) and low (2.5% and 1.25%) contrast levels, contrast sensitivity was evaluated using CSV 1000-E and Pelli-Robson charts and color vision

was evaluated with specific parameters (C-Index, CCI, AC-CCI, Conf Ang y S-Index) from Farnsworth-Munsell D15 and L'Anthony D15 tests.

Structural parameters were explored with Cirrus HD OCT device. Macular cube, optic disc cube and ganglion cell protocols were performed.

In our study, functional and structural parameters were compared between healthy controls and patients with PD, resulting in a statistically significant decrease in visual acuity, contrast sensitivity and color vision (L'Anthony test), as well as a significant decrease in 5 of the 9 analyzed macular sectors, in the superior quadrant of the RNFL in the superior and superonasal sectors and minimum thickness of the GCL.

Contrast sensitivity was the functional parameter most strongly correlated with structural measurements. Regarding the structural parameters, thickness of the ganglion cell layer was associated with most of functional parameters, and mainly with color. Finally, we found a strong correlation between thickness and macular volume with functional parameters.

Previously published studies demonstrated diminished retinal structures and association between macular thinning and progression or severity of PD. Only two studies described correlation between visual function and retinal structure, but strong correlations were not demonstrated.

According to our results, we can affirm that visual dysfunction significantly correlates with morphologic parameters in patients with PD. Changes in macular area and GCL measurements are more closely associated with visual dysfunction and could be reliable indicators of visual impairment in PD patients. Our study is the first showing this strong correlation.

3. **Satue M, Rodrigo MJ, Otin S, Bambo MP, Fuertes MI, Ara JR, Martin J, Polo V, Larrosa JM, Pablo L, Garcia-Martin E. Relationship between visual dysfunction and retinal changes in patients with multiple sclerosis. PLoS ONE 2016;11(6):e0157293.**

Eighty-four relapse-remiting MS patients, with and without previous episodes of optic neuritis, and 84 age and sex-matched healthy subjects were included in this prospective cross-sectional study. The aim was to evaluate visual dysfunction and its correlation with retinal structural changes in MS patients.

The following functional parameters were analyzed: visual acuity with ETDRS charts at high (100%) and low (2.5% and 1.25%) contrast levels, contrast sensitivity with CSV 1000-E and Pelli-Robson charts and color vision with specific parameters (C-Index, CCI, AC-CCI, Conf Ang y S-Index) from Farnsworth-Munsell D15 and L'Anthony D15 tests.

Structural parameters were explored with Cirrus HD OCT device. Macular cube, optic disc cube and ganglion cell protocols were performed.

In our study, functional and structural parameters were compared between healthy controls and patients with MS, finding a statistically significant decrease in visual acuity, contrast sensitivity and color vision (L'Anthony test), as well as a statistically significant decrease in 7 of the 9 analyzed macular sectors, in the temporal quadrant and average thickness of the RNFL, as well as in the inferotemporal and superotemporal sectors and minimum thickness of GCL. Worse data were recorded in patients with previous history of optic neuritis.

Contrast sensitivity was the functional parameter most strongly correlated with structural measurements (macular and RNFL thickness, but mainly with GCL

thickness). Regarding the structural parameters, mean macular thickness showed the highest correlation with color vision (L'Anthony test).

Our results support those of previous publications, suggesting the utility of macular and ganglion cell layer analysis as good indicators of visual impairment in MS patients.

4. **Satue M, Obis J, Rodrigo MJ, Otin S, Fuertes MI, Vilades E, Gracia H, Ara JR, Alarcia R, Polo V, Larrosa JM, Pablo LE, Garcia-Martin E. Optical coherence tomography as a biomarker for diagnosis, progression, and prognosis of neurodegenerative diseases. J Ophthalmol. 2016;2016:8503859. Review.**

In this paper a literature review and analysis on the utility of OCT as a biomarker for the diagnosis and prognosis of the neurodegenerative diseases (AD, PD and MS) was carried out. In addition, it helps to understand the pathophysiology of axonal degeneration.

We analyze the utility of macular, RNFL and ganglion cell protocols of the OCT technology in all the three neurodegenerative studied diseases in this doctoral thesis (AD, PD and MS). Segmentation analysis with ganglion cell measurements is suggested as the latest and most sensible biomarker measured with OCT.

Developing biomarkers that allow an early detection of the disease or predict the evolution would improve the clinical management and monitoring of the treatments effectiveness and, consequently, the quality of life of these patients.

RESUMEN

El tema principal de las cuatro publicaciones internacionales que componen esta tesis doctoral, se basa en el estudio de los parámetros estructurales (espesor macular, capa de fibra nerviosa de la retina [CFNR] y capa de células ganglionares [CCG]) y de los parámetros funcionales (agudeza visual [AV], sensibilidad al contraste [SC] y percepción del color) de la retina y del nervio óptico, en pacientes con enfermedades neurodegenerativas (Alzheimer [EA], Parkinson [EP] y Esclerosis Múltiple [EM]).

Estas tres enfermedades neurodegenerativas se caracterizan por una muerte neuronal (secundaria a depósitos amiloides en la EA, por alteración de los circuitos dopaminérgicos en la EP o inmunomediada en la EM), cuyos síntomas clínicos y discapacidad funcional son consecuencia de este daño o pérdida neuronal.

La retina es una parte del sistema nervioso central, fácilmente accesible para el examen clínico. La CFNR comprende principalmente axones no mielinizados de las células ganglionares de la retina, por lo que las mediciones del espesor de las diferentes capas retinianas, pueden proporcionan una evaluación relativamente directa de los axones y/o del daño neuronal.

Amplias evidencias científicas ya han demostrado un adelgazamiento de la retina (macular y de la CFNR), en la EA, EP y EM, examinado con la tecnología de tomografía de coherencia óptica (OCT). Este adelgazamiento de la retina se ha correlacionado con la progresión y el empeoramiento de la calidad de vida. Recientemente, los nuevos softwares de los OCT nos permiten medir específicamente la capa de células ganglionares (CCG), donde se localizan las células parvocelulares, magnocelulares y koniocelulares. Estas células especializadas son las responsables de

identificar el color y el contraste espacial en diferentes frecuencias: por lo tanto, la pérdida general de estas células de la retina es probable que sea un importante mecanismo contributivo para la discapacidad visual.

La visión constituye uno de los sentidos con mayor impacto en la calidad de vida de las personas. La evaluación de la función visual mediante los test de agudeza visual (test ETDRS 100%, 2,5%, 1,25%), sensibilidad al contraste (test CSV 1000-E y test de Pelli-Robson) y percepción de la visión cromática (tests Farnsworth-Munsell D15 y L'Anthony D15) nos pueden ayudar a conocer mejor la discapacidad visual de pacientes afectos de EA, EP y EM. Ya se tiene constancia de que dichas enfermedades causan alteraciones en la función visual; como puede ser la dificultad para el reconocimiento de caras, la lectura y/o conducción, relacionadas éstas con la sensibilidad al contraste; así como alteraciones adquiridas de la visión cromática por patología macular o del nervio óptico, como ocurre en las neuritis ópticas de la EM. Estas alteraciones fueron al principio atribuidas a causas corticales, pero recientemente también se han sugerido alteraciones en la vía visual anterior por disminución del espesor retiniano debido a una muerte neuronal secundaria a depósito amiloide en la EA o de α-sinucleína en la EP. Esta muerte de células ganglionares se postula como la responsable de las alteraciones en la sensibilidad al contraste y color desde las etapas más precoces, incluso antes de la detección de la alteración de la CFNR del nervio óptico y sobre todo de la clásica palidez papilar.

Los artículos publicados en esta tesis doctoral demuestran que en los pacientes con dichas enfermedades neurodegenerativas (EA, EP y EM) los parámetros de la función visual se encuentran alterados y se correlacionan, incluso en las primeras etapas, con parámetros estructurales disminuidos. Estos resultados nos permiten

concluir que el análisis de la función visual, podría considerarse como un posible biomarcador para la EA, la EP y la EM.

3. RELACIÓN DE PUBLICACIONES QUE COMPONEN LA TESIS

A continuación se incluye una breve presentación de cada trabajo justificando su unidad temática:

1. **Polo V, Rodrigo MJ, Garcia-Martin E, Otin S, Larrosa JM, Fuertes MI, Bambo MP, Pablo LE, Satue M. Visual dysfunction and its correlation with retinal changes in patients with Alzheimer´s disease. Eye (Lond) 2017 (en prensa).**

Se trata de un estudio transversal observacional en el que se incluyeron un total de 24 pacientes con EA leve o moderada y 24 sujetos sanos. El objetivo fue evaluar la disfunción visual y su correlación con los cambios estructurales de la retina en pacientes con EA.

Los parámetros funcionales analizados fueron: la AV, que se evaluó con cartas ETDRS de alto (100%) y bajo contraste (2,5% y 1,25%); la SC, que fue evaluada con el test CSV 1000-E y el test de Pelli-Robson; y el color, evaluado mediante los protocolos Farnsworth-Munsell D15 y L'Anthony D15, concretamente con los parámetros: índice de confusión (C-Index), índice de confusión del color (CCI), índice de confusión del color corregido por edad (AC-CCI), ángulo de confusión (Conf Ang) e índice de dispersión (S-Index).

Los parámetros estructurales se exploraron con el dispositivo Cirrus HD, utilizando los protocolos de cubo macular y de disco óptico.

Ya se conoce que la SC y el color están frecuentemente alterados en estos pacientes y se ha sugerido que pueden ser predictores de disfunción cognitiva. Sin

embargo, hay estudios contradictorios sobre los defectos de la CFNR: ya que un estudio no encontró diferencias significativas entre pacientes con EA y controles sanos, pero sin embargo, otro estudio lo consideró como el signo más precoz de EA, incluso antes del daño hipocampal. Por otro lado, distintos estudios han encontrado una importante afectación macular, la cual ha sido correlacionada con el daño cognitivo. Sin embargo no encontramos ningún artículo que correlacionara los cambios estructurales de la retina con las alteraciones funcionales de SC y color.

En nuestro estudio se compararon los parámetros funcionales y estructurales entre controles sanos y pacientes con EA, demostrado una disminución estadísticamente significativa de la SC y de la visión cromática en los pacientes, así como una disminución estadísticamente significativa en 8 de los 9 sectores maculares analizados, y en el espesor medio y cuadrante superior de la CFNR.

El parámetro funcional que más frecuentemente se asoció a las medidas estructurales (especialmente en el espesor macular) fue la SC, aunque también se encontraron asociaciones en el color (test de Farnsworth) y en la AV.

Según nuestros resultados podemos afirmar que la disfunción visual en los pacientes con EA se correlaciona significativamente con los parámetros morfológicos, siendo los cambios en el área macular los que se asocian más estrechamente con la alteración de la función visual.

2. **Polo V, Satue M, Rodrigo MJ, Otin S, Alarcia R, Bambo MP, Fuertes MI, Larrosa JM, Pablo LE, Garcia-Martin E. Visual dysfunction and its correlation with retinal changes in patients with Parkinson's disease: an observational cross-sectional study. BMJ Open 2016;6:e009658.**

Se trata de un estudio transversal observacional en el que se incluyeron un total de 37 pacientes con EP y 37 sujetos sanos. El objetivo fue evaluar la disfunción visual y su correlación con los cambios estructurales de la retina en pacientes con EP.

Los parámetros funcionales analizados fueron: la AV, que se evaluó con cartas ETDRS de alto (100%) y bajo contraste (2,5% y 1,25%); la SC, que fue evaluada con el test CSV 1000-E y el test de Pelli-Robson; y el color, evaluado mediante los protocolos Farnsworth-Munsell D15 y L'Anthony D15, concretamente con los parámetros C-Index, CCI, AC-CCI, Conf Ang y S-Index.

Los parámetros estructurales se exploraron con el dispositivo Cirrus HD, utilizando los protocolos macular, de la CFNR y de células ganglionares.

En nuestro estudio se compararon los parámetros funcionales y estructurales entre controles sanos y pacientes con EP, encontrando una disminución estadísticamente significativa de la AV, de la SC y de la visión cromática (test de L'Anthony), así como una disminución estadísticamente significativa en 5 de los 9 sectores maculares analizados, en el cuadrante superior de la CFNR y en los sectores superior, superonasal y espesor mínimo de la CCG.

El parámetro funcional que más frecuentemente se asoció a las medidas estructurales fue la SC. Respecto a los parámetros estructurales, el espesor de la CCG se asoció con la mayoría de los parámetros funcionales, y principalmente con el color. Finalmente encontramos una fuerte correlación del espesor y volumen maculares con los parámetros funcionales.

Estudios anteriormente publicados han demostrado disminución de las estructuras retinianas y asociación entre el adelgazamiento macular y la progresión y gravedad de la EP. Únicamente se han publicado dos trabajos que correlacionan función visual y

estructura retiniana, pero ninguno demostró correlaciones fuertes, por lo que podemos afirmar que nuestro trabajo es el primer estudio que lo demuestra.

Según nuestros resultados, podemos confirmar que la disfunción visual en los pacientes con EP se correlaciona significativamente con los parámetros morfológicos, siendo los cambios en el área macular y segmentación retiniana con medición del espesor de la CCG los que se asocian más estrechamente con la alteración de la función visual; lo que sugiere que pueden ser utilizados como indicadores de la discapacidad visual en los pacientes con EP.

3. **Satue M, Rodrigo MJ, Otin S, Bambo MP, Fuertes MI, Ara JR, Martin J, Polo V, Larrosa JM, Pablo L, Garcia-Martin E. Relationship between visual dysfunction and retinal changes in patients with multiple sclerosis. PLoS ONE 2016;11(6):e0157293.**

Se trata de un estudio transversal observacional en el que se incluyeron un total de 84 pacientes con EM remitente–recurrente con y sin episodios previos de neuritis óptica y 84 sujetos sanos. El objetivo fue evaluar la disfunción visual y su correlación con los cambios estructurales de la retina en pacientes con EM.

Los parámetros funcionales analizados fueron: la AV, que se evaluó con cartas ETDRS de alto (100%) y bajo contraste (2,5% y 1,25%); la SC, que fue evaluada con el test CSV 1000-E y el test de Pelli-Robson; y el color, evaluado mediante los protocolos Farnsworth-Munsell D15 y L'Anthony D15, concretamente con los parámetros C-Index, CCI, AC-CCI, Conf Ang y S-Index.

Los parámetros estructurales se exploraron con el dispositivo Cirrus HD, utilizando los protocolos: macular, de la CFNR y de células ganglionares.

En nuestro estudio se compararon los parámetros funcionales y estructurales entre controles sanos y pacientes con EM, encontrando una disminución estadísticamente significativa de la AV, de la SC y de la visión cromática (test de L'Anthony), así como una disminución estadísticamente significativa en 7 de los 9 sectores maculares analizados, en el espesor medio y cuadrante temporal de la CFNR y en los sectores ínferotemporal, superotemporal y espesor mínimo de la CCG, siendo significativamente peor en pacientes con historia de neuritis óptica.

La SC fue el parámetro funcional que más frecuentemente se asoció a las medidas estructurales (espesor macular, CFNR, pero sobre todo con el espesor de la CCG). Respecto a los parámetros estructurales, el espesor medio macular mostró la mayor correlación con el color (test de L'Anthony).

Nuestros resultados corroboran los de artículos previamente publicados, sugiriendo la utilidad del análisis macular y de la CCG como buenos indicadores de la discapacidad visual en los pacientes de EM.

4. **Satue M, Obis J, Rodrigo MJ, Otin S, Fuertes MI, Vilades E, Gracia H, Ara JR, Alarcia R, Polo V, Larrosa JM, Pablo LE, Garcia-Martin E. Optical coherence tomography as a biomarker for diagnosis, progression, and prognosis of neurodegenerative diseases. J Ophthalmol. 2016;2016:8503859. Review.**

En este estudio se realizó una revisión bibliográfica y un análisis profundo sobre la utilidad de la OCT como biomarcador para el diagnóstico y pronóstico de las enfermedades neurodegenerativas sobre la que versa esta tesis doctoral (EA, EP y EM); además de ayudar en la comprensión de fisiopatología de la degeneración axonal.

El trabajo analiza para cada una de las enfermedades a estudio (EA, EP y EM) la utilidad de los protocolos de análisis del espesor macular, de la CFNR y de la segmentación retiniana con el espesor de la CCG; sugiriendo éste último como el biomarcador neurodegenerativo más sensible medible con OCT. El desarrollo de biomarcadores que permitan una detección temprana de la enfermedad o predigan su evolución, mejorarían el manejo clínico y monitorización de la efectividad de los tratamientos y en consecuencia, la calidad de vida de dichos pacientes.

4. INTRODUCCIÓN

El tema principal de las publicaciones que componen esta tesis doctoral se centra en el estudio de la afectación subclínica de la función visual en etapas precoces (analizando AV, SC y percepción del color), así como el análisis estructural de la retina y del nervio óptico (espesores macular, de la CFNR y de la CCG) en pacientes con enfermedades neurodegenerativas (EA, EP y EM), como nueva perspectiva para mejorar y acelerar el proceso diagnóstico, seguimiento y monitorización de estas patologías.

4.1 Enfermedad de Alzheimer

La EA es la enfermedad neurodegenerativa más frecuente y la forma más común de demencia, con una prevalencia mundial de 47 millones, estimándose que la cifra sea duplicada antes del 2050[1].

Se caracteriza por una pérdida progresiva de la memoria reciente y de otras funciones cognitivas. En etapas intermedias-avanzadas son frecuentes los síntomas neuropsiquiátricos.

También pueden estar presentes, aunque con menor frecuencia, síntomas como la apraxia, disfunción olfativa, trastorno del sueño, epilepsia y signos motores piramidales y extrapiramidales; que nos pueden hacer plantear el diagnóstico diferencial con otros tipos de demencias, como enfermedad cerebrovascular de pequeño vaso y otras enfermedades neurodegenerativas como la demencia de cuerpos de Lewy (parkinsonismos, alucinaciones visuales…) y la demencia frontotemporal (alteración de la personalidad y el comportamiento, afasia…).

Aunque la patogenia no está totalmente esclarecida, se han emitido numerosas hipótesis, fundamentalmente basadas en los hallazgos neuropatológicos y en genes que se han relacionado con la enfermedad.

A nivel neuropatológico, se caracteriza por el acúmulo extracelular de placas seniles formadas por proteína β-amiloide y marañas neurofibrilares intracelulares formadas por proteína tau hiperfosforilada predominantemente en los lóbulos frontal y temporal. Junto a la presencia de microgliosis reactiva y neuritas atróficas, originan muerte neuronal y pérdida de conexiones sinápticas en regiones selectivas del cerebro, produciendo neurodegeneración progresiva y atrofia cerebral, localizada principalmente en el hipocampo y la corteza[2].

Se han aislado tres genes estrechamente relacionados con la forma familiar de inicio temprano: *APP* (gen de la proteína precursora de amiloide), *PSEN1* (gen de la presenilina 1) y *PSEN2* (gen de la presenilina 2). En la variante más frecuente de la enfermedad, la esporádica, y en la familiar de inicio tardío, el genotipo APOE4 aparece con frecuencia (casi en el 50%) y se considera un factor de riesgo[3].

La EA progresa inexorablemente. Cuanto más tardío es el inicio de la enfermedad, más lento es el deterioro cognitivo. En cambio, la sintomatología no amnésica precoz como, por ejemplo, los síntomas neuropsiquiátricos, determinan un deterioro más veloz [4]. La supervivencia media desde el diagnóstico es de unos 8 a 10 años.

Actualmente, no existe un tratamiento modificador de la enfermedad o curativo. Las terapias actuales se centran en ralentizar la progresión de la enfermedad, con resultados dispares. Los fármacos más utilizados son los inhibidores de la colinesterasa

denominados antidemenciales como el donepezilo (Aricept®) y la rivastigmina (Exelon® y Prometax®)[5] y la memantina un antagonista del receptor de NMDA (N-metil-D-aspartato)[6].

4.1.1 Alzheimer y visión

Los síntomas visuales son frecuentes en los comienzos de la EA[7-10] con alteraciones en la sensopercepción y en el procesamiento visual[11-13].

Los pacientes refieren quejas a nivel de la lectura, la conducción o el reconocimiento de objetos. En la exploración de la función visual se han descrito alteraciones en los movimientos oculares, en la SC, los potenciales evocados visuales y la percepción de colores entre otras. La AV y el campo visual rara vez se encuentran afectados al inicio de la enfermedad, aunque si se pueden presentar déficits moderados respecto a la población general.

Recientes estudios han demostrado disminución del espesor retiniano macular[14-16] y de la CFNR, sobre todo en los sectores temporales medidos con OCT[15], siendo un buen marcador para el diagnóstico y seguimiento evolutivo de la patología, ya que defectos en la CFNR puede ser incluso un signo más precoz que el daño hipocampal[17]. Las pruebas estructurales del espesor macular y CFNR y de función visual, especialmente las de visión cromática, se correlacionan con la gravedad de la enfermedad[18].

Habitualmente, las alteraciones en la función visual se han atribuido al daño en el córtex de asociación visual producido por la enfermedad[19-20] ya que estudios necrópsicos han encontrado reducciones, sobre todo a nivel del lóbulo occipital, pero no a nivel del nervio óptico o núcleos geniculados. Por el contrario, se encontraron

reducciones a nivel de los núcleos troncoencefálicos, aun cuando no son frecuentes las quejas de diplopía en estos pacientes.

4.2 Enfermedad de Parkinson

La EP es la segunda enfermedad neurodegenerativa más frecuente, después de la EA.

Entre las alteraciones que puede producir esta enfermedad se encuentran los trastornos del movimiento y síntomas no motores tales como demencia, depresión y disfunción del sistema nervioso autónomo[21].

Se produce una pérdida selectiva de neuronas dopaminérgicas, en la sustancia negra a nivel de los ganglios basales cerebrales.

Las células nerviosas y circuitos neuronales fuera de los ganglios basales también pueden verse dañados, de forma simultánea o incluso antes de la afectación del sistema nigroestriatal[22]. Uno de los sistemas no motores afectado en la EP es la visión, particularmente el campo visual correspondiente al área foveal[23].

4.2.1 Parkinson y visión

La EP se asocia no sólo con la muerte de neuronas dopaminérgicas pigmentadas en la sustancia negra, sino también con una pérdida de neuronas en otras áreas, tales como células amacrinas dopaminérgicas, células ganglionares de la retina y células pertenecientes a áreas de la vía visual (por ejemplo, el cuerpo geniculado lateral, núcleo colinérgico basal de Meynert, y la corteza visual)[24]. Esta reducción de las células ganglionares de la retina conduce a una disminución de los espesores de la retina y de la CFNR, que pueden ser detectados utilizando dispositivos de OCT. Algunos autores como Satue et al. y Rohani et al. demostraron que los OCT de dominio espectral son dispositivos capaces de detectar la atrofia de la CFNR en pacientes con EP. Bambó et

al. detectaron una disminución en los espesores de la CFNR con los dispositivos OCT Cirrus y Spectralis, especialmente en el cuadrante inferior[25-26].

La retina de los mamíferos contiene neuronas dopaminérgicas, las células amacrinas, que están situadas a nivel de la capa interna de la retina, suponiendo hasta el 20% de su espesor. Estas células modulan el campo receptivo de las células ganglionares y se les atribuye una función importante en las interacciones laterales a larga distancia en la capa plexiforme interna de la retina[23]. Las células ganglionares a su vez se encargan de la percepción de la SC y de la visión en color. Por lo tanto la visión, la SC, así como la sensibilidad absoluta y temporal, la visión en color y los potenciales evocados visuales (PEV), están al menos parcialmente, controladas por dopamina[23]. Estudios previos han demostrado que la extensión de la dopamina en células retinianas es menor en pacientes con EP que en sujetos sanos[27].

En lo que a clínica oftalmológica se refiere, las alteraciones más prevalentes encontradas en los pacientes con EP son ojo seco patológico (exacerbado por los fármacos anticolinérgicos), glaucoma y hasta un 25% de los pacientes sufre alucinaciones de tipo visual (pudiendo ser consecuencia de los fármacos agonistas dopaminérgicos o un mal diagnóstico de enfermedad de Lewy)[28]; también es frecuente encontrar diplopia por exotropia secundaria a una insuficiencia de convergencia e incomitancia cerca – lejos, siendo esto último muy dependiente de los niveles de dopamina, o periodos off.

Se han encontrado alteraciones histológicas en retina y nervio óptico que pueden producir una afectación subclínica de la función visual al inicio del proceso y llevar a una pérdida progresiva de la visión en etapas posteriores de la enfermedad. Diversos estudios han demostrado alteración en la transmisión de los impulsos nerviosos a nivel de capas externas de la retina[28] y pérdida de fibras nerviosas a nivel del nervio óptico[29].

4.3 Esclerosis Múltiple

La EM es una compleja enfermedad neurodegenerativa que afecta principalmente a pacientes jóvenes. Se caracteriza por lesiones axonales en el sistema nervioso central que conducen a déficits neurológicos progresivos, causantes de una importante discapacidad funcional y empobrecimiento de la calidad de vida.

Clásicamente se ha considerado una enfermedad desmielinizante en la que la mielina que recubre los nervios es destruida por procesos de inflamación y cicatrización; sin embargo en los últimos años se ha demostrado que se asocian otros de daño axonal[30-31].

Existen evidencias de que el daño axonal aparece desde fases precoces de la enfermedad, sin relación con episodios inflamatorios o autoinmunes contra la mielina, y de que esta degeneración axonal está directamente relacionada con la discapacidad funcional permanente[30-31]. Estudios previos han demostrado que el deterioro axonal de los pacientes con EM puede ser observado y cuantificado a nivel de la CFNR mediante técnicas de análisis digital de la imagen[32-37].

Algunos autores han sugerido que la evaluación de la CFNR podría ser más útil que la resonancia magnética para medir la atrofia neuronal[38] y un número creciente de neurólogos utiliza la evaluación de la CFNR para monitorizar la progresión de la enfermedad[39-40].

El tratamiento consistirá en el control de los brotes agudos mediante fármacos corticoideos y fármacos patogénicos, o modificadores de la enfermedad, para prevenir la progresión y las recaídas (ver tabla 1).

Principio Activo	Nombre comercial	Laboratorio titular	Año de aprobación (EMA)	Modo de administración	Frecuencia de administración
Acetato de glatirámero	Copaxone® 20 o 40	Teva Pharmaceuticals Ltd	2002	Subcutáneo	Cada día o 3 veces por semana
Alemtuzumab	Lemtrada®	Genzyme Therapeutics Ltd	2013	Intravenoso	Ciclos de 5 o 3 días anuales
Dimetilfumarato	Tecfidera®	Biogen	2014	Oral	Dos veces/día
Interferón beta-1a	Avonex ®	Biogen	1997	Intramuscular	Una vez/semana
Interferón beta-1a	Rebif®	Merck Serono Europe Ltd	1998	Subcutáneo	3 veces/semana
Interferón beta-1b	Betaferon®	Bayer Pharma Ag	1995	Subcutáneo	Cada dos días
Interferón beta-1b	Extavia®	Novartis Europharm Ltd	2008	Subcutáneo	Cada dos días
Interferón beta-1a pegilado	Plegridy®	Biogen	2014	Subcutáneo	Cada dos semanas
Fingolimod	Gilenya®	Novartis Europharm Ltd	2011	Oral	Cada día
Mitoxantrona (en genérico desde 2006)	Novantrone®	Meda Pharma, S.A.U.	1998 (proc. nacional)	Intravenoso	Frecuencia variable. Dosis máxima acumulada: 140 mg/m2
Natalizumab	Tysabri®	Biogen	2006	Intravenoso	Cada 4 semanas
Teriflunomida	Aubagio®	Sanofi-Aventis Groupe	2013	Oral	Una vez/día

Tabla 1: Fármacos modificadores de la enfermedad de Esclerosis Múltiple.

4.3.1 Esclerosis Múltiple y visión

La retina es una parte del sistema nervioso central de fácil acceso para el examen clínico. La CFNR se compone fundamentalmente de axones no mielinizados de las células ganglionares de la retina; por lo que las mediciones de su espesor aportan una valoración relativamente directa de los fibras nerviosas y por lo tanto del daño axonal.

La atrofia en el nervio óptico y el adelgazamiento de la CFNR son dos hallazgos típicos en los pacientes con EM habiendo o no sufrido episodios de neuritis óptica previamente[37,41,42]. Estudios recientes han demostrado adelgazamiento de la capa de células ganglionares más plexiforme interna (GCIPL) entre los 3 y 6 meses posteriores a un episodio de neuritis óptica. También se ha detectado atrofia de la CCG, incluso con un número axonal intacto, en estudios postmorten[43].

La clínica típica de la enfermedad será de disminución de AV progresiva o en brotes (la disfunción visual puede ocurrir en más del 80% de los pacientes en el curso de la enfermedad)[44]. También se han descrito alteraciones en la sensibilidad a bajo contraste y el color[45-46], lo cual produce alteraciones en las tareas cotidianas como la lectura, conducción o reconocimiento facial[47]. Bajas AV a bajos contrastes se han correlacionado con pérdida neuronal en la vía visual anterior[48-50], y esta pérdida neuronal se relaciona con la progresión, la discapacidad y el empobrecimiento de la calidad de vida en los pacientes afectos de EM[51-52].

5. ESTADO ACTUAL DEL TEMA

La pérdida de axones en el nervio óptico que se produce en pacientes con EA, EP y EM, se ha podido observar tradicionalmente como una palidez progresiva de la cabeza del nervio óptico mediante el examen del fondo de ojo con un oftalmoscopio. El ojo humano, sin embargo, no es capaz de cuantificar o detectar la pérdida axonal de manera temprana (es decir, sólo vemos palidez cuando se han perdido más de un 50% de las fibras nerviosas). La pérdida de células ganglionares de la retina puede detectarse, incluso en fases subclínicas, utilizando tecnologías de análisis digital de la imagen, como por ejemplo la OCT[53] que proporcionan de manera no invasiva, rápida y objetiva, mediciones reproducibles del espesor de la CFNR.

En estudios previos llevados a cabo en pacientes con EA se ha encontrado una disminución en la CFNR y en el espesor macular, sobre todo en sectores temporales; así como alteración funcional cromática, que se relaciona con la gravedad de la enfermedad. En pacientes con EP, la OCT de dominio espectral ha demostrado la existencia de espesores reducidos a nivel macular[25, 54-56] y en la CFNR[25,57-58] así como alteración en la percepción del color y en la SC respecto a controles sanos. Por último, diversos estudios han encontrado una correlación entre la pérdida axonal en el nervio óptico de pacientes con EM y su disfunción visual, con el grado de incapacidad funcional[37, 59-60] y la afectación de su calidad de vida[61].

6. HIPÓTESIS

Las enfermedades neurodegenerativas causan una reducción subclínica de la función visual en relación a la agudeza visual, la sensibilidad al contraste y la visión cromática, que se relacionan con la disminución de las células nerviosas de la retina (espesor macular, espesor de la capa de fibras nerviosas de la retina y espesor de la capa de células ganglionares).

HYPOTHESIS

Neurodegenerative diseases cause subclinical reduction in visual function (visual acuity, contrast sensitivity, and color vision) that correlates with decreased retinal cells (macular thickness, retinal nerve fiber layer thickness and ganglion cell layer thickness).

7. OBJETIVOS

I. Analizar si los sujetos afectos de las enfermedades neurodegenerativas de Alzheimer, Parkinson y esclerosis múltiple presentan cambios estructurales en la retina y en el nervio óptico, y si éstos son detectables mediante tomografía de coherencia óptica tipo Cirrus.

II. Evaluar si los espesores retinianos disminuyen más a medida que aumenta la duración de la patología.

III. Evaluar si los pacientes con las enfermedades neurodegenerativas de Alzheimer, Parkinson y esclerosis múltiple presentan alteraciones en su capacidad visual, detectables con los test de agudeza visual, sensibilidad al contraste y percepción del color.

IV. Analizar las correlaciones entre las pruebas funcionales y estructurales de la visión.

V. Evaluar la asociación entre las mediciones de los espesores maculares y de la capa de fibras nerviosas de la retina, y los parámetros funcionales en la enfermedad de Alzheimer.

VI. Estudiar la correlación entre las mediciones estructurales y los test de función visual en la enfermedad de Parkinson.

VII. Evaluar la asociación entre las mediciones de los espesores maculares, de la capa de fibras nerviosas de la retina y de las células ganglionares, y los parámetros de función visual en la esclerosis múltiple.

VIII. Analizar si la función visual es peor en los pacientes con grados más severos de enfermedad.

IX. Descubrir si la evaluación neurooftalmológica funcional y estructural de los pacientes afectos de las enfermedades neurodegenerativas de Alzheimer, Parkinson y esclerosis múltiple es un biomarcador útil en el estudio de estas patologías.

OBJECTIVES

I. To analyze if subjects affected with neurodegenerative diseases of Alzheimer, Parkinson and multiple sclerosis present structural changes in the retina and optic nerve, and if these changes are detectable with Cirrus OCT..

II. Evaluate if the retinal thickness decreases more as the duration of the disease increases.

III. To evaluate if patients with neurodegenerative diseases of Alzheimer's, Parkinson's and multiple sclerosis present alterations in their visual function, detectable with tests of visual acuity, contrast sensitivity and color perception.

IV. To analyze the correlations between functional and structural tests of vision.

V. To evaluate the association between measurements of macular thickness and retinal nerve fiber layer, with functional parameters in Alzheimer's disease.

VI. To study the correlation between structural measurements and visual function tests in Parkinson's disease.

VII. To evaluate the association between measurements of macular thickness, retina nerve fiber layer thickness and ganglion cells layer thickness, with visual function parameters in multiple sclerosis.

VIII. To analyze if patients with more severe degrees of disease show worse visual function parameters.

IX. To find out if the functional and structural neurophthalmological evaluation of the patients affected by the neurodegenerative diseases of Alzheimer, Parkinson and multiple sclerosis is a useful biomarker in the study of these pathologies.

8. METODOLOGÍA

Los artículos que forman parte de esta tesis doctoral son el resultado de varios estudios transversales que se llevaron a cabo en las Consultas Externas y en la Unidad de Función Visual del Servicio de Oftalmología, con la participación del Servicio de Neurología del Hospital Universitario Miguel Servet de Zaragoza. El diseño de estos estudios siguió los principios de la Declaración de Helsinki, y el protocolo fue aprobado por el Comité Ético de Investigaciones Clínicas de Aragón (CEICA).

De acuerdo a la Ley Orgánica 15/1999, de 13 de diciembre, de *Protección de Datos de Carácter Personal*, utilizamos datos disociados, en el que a cada ojo de cada paciente se le asignó un número específico único, para proteger su identidad en los análisis estadísticos; así mismo, la base de datos se protegió con una clave para restringir el acceso únicamente a los investigadores del estudio.

8.1. Sujetos de estudio

Se propuso la participación en el estudio a aquellos pacientes afectos de EA, EP y/o EM que acudan a seguimiento de su patología en el servicio de neurología del Hospital Miguel Servet.

Para la realización de estos trabajos se contó con diferentes poblaciones:

-Para el estudio "*Visual dysfunction and its correlation with retinal changes in patients with Alzheimer´s disease"* (Eye), se analizaron 24 pacientes con EA leve o moderada (que acudieron al seguimiento de su patología en el servicio de Neurología del Hospital Miguel Servet) pareados con 24 controles sanos (reclutados entre el personal sanitario del Hospital Miguel Servet y familiares o amigos, que aceptaron voluntariamente participar en el estudio) por edad y sexo. El diagnóstico de la enfermedad fue determinado por un neurólogo según los criterios del National Institute

of Neurologic and Communicative Disorders and Stroke-Alzheimer´s Disease and Related Disorders Association y el Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), y su estadio con la escala Minimental (MMSE)[62-63]. Se recogieron los datos de duración de la enfermedad, la edad al diagnóstico y el tipo de tratamiento.

-Para el estudio "*Visual dysfunction and its correlation with retinal changes in patients with Parkinson's disease: an observational cross-sectional study"* (BMJ Open) se reclutaron 37 pacientes con EP (que acudieron al seguimiento de su patología en el servicio de Neurología del Hospital Miguel Servet, así como aquellos pacientes con EP miembros de la Asociación de Parkinson de Aragón) y 37 sujetos sanos (reclutados entre familiares o amigos), que aceptaron voluntariamente participar en el estudio. El diagnóstico de la EP se basó en los criterios del "*UK Brain Bank Criteria*", que incluyen la bradicinesia y un síntoma adicional; es decir, rigidez, 4-6 Hz de temblor en reposo, o inestabilidad postural[64-65]. Así pues, para el diagnóstico de EP "definitiva", se requieren tres o más de los siguientes criterios en combinación con los mencionados anteriormente: inicio unilateral, carácter progresivo, asimetría, excelente respuesta (70% -100%) a la levodopa, corea severa inducida por levodopa, respuesta a levodopa durante 5 años o más, y el curso clínico de 10 años o más[65-66].

-Para el estudio "*Relationship between visual dysfunction and retinal changes in patients with multiple sclerosis"* (PLoS ONE); se incluyeron, dos muestras independientes de 84 individuos sanos (reclutados entre el personal sanitario del Hospital Miguel Servet y familiares o amigos, que aceptaron voluntariamente participar en el estudio) y 84 pacientes con EM (que asistieron a nuestro Servicio referidos del Neurólogo para su examen oftalmológico anual). El diagnóstico de EM se basó en los criterios clínicos y de neuroimagen de Polman[67]. Los registros médicos del grupo de pacientes fueron revisados cuidadosamente, incluyendo la duración de la enfermedad, la

escala de disfunción neurológica (EDSS: Expanded Disability Status Scale), los tratamientos modificadores de la enfermedad, y los antecedente de ataques agudos de neuritis óptica.

Todos los sujetos incluidos en los 3 estudios cumplieron los siguientes criterios de inclusión: AV igual o superior a 0,1 con la escala de Snellen en cada ojo para permitir el desarrollo correcto del protocolo exploratorio, y valores de presión intraocular de aplanamiento inferiores a 20 mm Hg, ya que valores superiores podrían provocar afectación de la CFNR por mecanismos diferentes a la enfermedad neurodegenerativa a estudio (como ocurriría en el glaucoma crónico simple). Los sujetos incluidos en el estudio no presentaban patología oftalmológica previa que alterara en cualquier forma la estructura o función del nervio óptico o de la retina.

Se incluyeron en el estudio voluntarios sanos, procedentes de personal trabajador del hospital, familiares de los mismos, y familiares de los pacientes que participaron en el estudio, que no presentaran ninguno de los criterios de exclusión anteriormente mencionados. Todos ellos fueron pareados a los casos por edad y sexo.

A todos los sujetos del estudio se les solicitó la firma de un consentimiento informado en el que se detallaban las características del estudio y sus objetivos. Dicho consentimiento informado fue imprescindible para su inclusión en la investigación.

Fueron excluidos del estudio aquellos sujetos con defectos refractivos importantes (más de 5 dioptrías de equivalente esférico o 3 dioptrías de astigmatismo), opacificación de medios, enfermedades neurooftalmológicas o sistémicas que puedan afectar a la visión o procesos oculares concomitantes incluyendo historia previa de patología retiniana, glaucoma, terapia con láser, o alteraciones importantes en córnea, cristalino, retina o nervio óptico.

Los pacientes con antecedente de neuritis óptica en los 6 meses previos al estudio fueron excluidos (para estudiar únicamente el proceso neurodegenerativo, y no el secundario al daño neuronal tras los brotes inflamatorios); así como los pacientes con EA, EP o EM muy severa, dada la incapacidad de completar el protocolo exploratorio en estos casos.

8.2. Protocolo exploratorio

El protocolo exploratorio que se llevó a acabo constó de una valoración neurológica y oftalmológica, que se describen a continuación.

8.2.1 Evaluación neurológica

La valoración neurológica fue realizada por el Servicio de Neurología y proporcionaba la siguiente información:

-Tiempo de evolución de la enfermedad desde su diagnóstico.

-Severidad y grado de afectación neurológica, medidos mediante la escala Mini Mental State Examination (MMSE)[68] para EA, la escala de valoración funcional Hoehn Yahr[69] y la escala unificada para la clasificación de la EP (Unified Parkinson Disease Rating Scale [UPDRS])[69] y la escala Expanded Disability Status Scale (EDSS)[70] para pacientes afectos de EM.

-Tipo (recidivante-remitente, primaria progresiva, y secundaria progresiva) o presencia o no de brotes de neuritis óptica, en el caso de EM.

-Edad a la que se diagnosticó la enfermedad.

-Tratamiento que habían llevado o llevaban.

8.2.1.1 Evaluación neurológica (escalas)

-La puntuación MMSE[68] es un método muy utilizado para detectar el deterioro cognitivo y vigilar su evolución en pacientes con alteraciones neurológicas, especialmente en ancianos. Consta de 30 preguntas agrupadas en 10 secciones que evalúan: orientación espacio temporal, capacidad de atención, concentración y memoria, capacidad de abstracción (cálculo), capacidad de lenguaje y percepción viso-espacial, capacidad para seguir instrucciones básicas. Los resultados dependen de la puntuación alcanzada una vez terminada la prueba. Puntuaciones entre 30 y 27 se clasifican como "sin deterioro", entre 26 y 25 se considera "dudoso o posible deterioro", entre 24 y 10 se clasifica como "demencia leve a moderada", entre 9 y 6 es considerado "demencia moderada a severa" y menos de 6 es valorado como "demencia severa".

- La escala Hoehn Yahr es una prueba validada de uso habitual para cuantificar la progresión de los síntomas de la EP[69]. Su puntuación va desde 0 (sin síntomas de la enfermedad) hasta 5 (postrado o en silla de ruedas). En esta escala, la discapacidad motora progresiva de la enfermedad se clasifica como unilateral (estadio 1), enfermedad bilateral sin dificultades en el equilibrio (estadio 2), presencia de inestabilidad postural (estadio 3), pérdida de independencia física (estadio 4) y postrado en cama/silla de ruedas (estadio 5). La afectación de la visión comienza a ser notable a partir del estadio 2, comenzando con la disminución del parpadeo.

-La UPDRS es la escala más utilizada en investigación y en la práctica clínica diaria para realizar el seguimiento longitudinal de los pacientes con EP[69]. La escala tiene 3 secciones que evalúan la discapacidad, y una cuarta sección que

valora las complicaciones relacionadas con el tratamiento. La puntuación de la UPDRS será calculada utilizando las 3 secciones que analizan las áreas de discapacidad, junto con la sección que analiza las complicaciones del tratamiento.

- La EDSS es un método de cuantificación de la discapacidad en los pacientes con EM[70]. La EDSS cuantifica la discapacidad en ocho sistemas funcionales y permite a los neurólogos asignar un nivel de discapacidad para cada uno de ellos. Los sistemas funcionales son los siguientes: piramidal, cerebeloso, tronco cerebral, sensorial, del intestino y la vejiga, visual y cerebral. Va desde puntuación 0 (examen neurológico normal) a 10 (muerte por EM) (ver ilustración 1).

Función piramidal

0. Normal.
1. Presencia únicamente de signos sin afectación funcional.
2. Mínima paresia.
3. Paresia o hemiparesia moderada. Monoparesia grave.
4. Marcada paresia. Monoplejia, tetraparesia moderada.
5. Paraplejia, hemiplejia o marcada tetraparesia.
6. Tetraplejia

Función cerebelosa

0. Normal.
1. Presencia únicamente de signos sin afectación funcional.
2. Ataxia leve.
3. Ataxia moderada de tronco o extremidades.
4. Ataxia grave de las cuatro extremidades.
5. Incapacidad de realizar movimientos coordinados.
X Desconocido o no valorable por afectación piramidal.

Función de tronco cerebral

0. Normal.
1. Presencia únicamente de signos sin afectación funcional.
2. Nistagmo moderado u otra afectación leve de los cuales el paciente es consciente.
3. Nistagmo intenso, paresia extraocular, afectación moderada de otro par craneal.
4. Disartria, disfagia u otra alteración marcada.
5. Anartria o incapacidad de tragar.

Función mental

0. Normal.
1. Alteración del humor (no afecta al EDSS).
2. Leve afectación en funciones superiores
3. Moderada afectación de funciones superiores.
4. Síndrome cerebral orgánico moderado o disminución mental marcada.
5. Demencia o síndrome cerebral orgánico crónico grave.

Función sensitiva

0. Normal.
1. Disminución de la sensibilidad vibratoria leve en una o dos extremidades.
2. Disminución leve de la sensibilidad táctil o algésica o artrocinética, o moderada de la vibratoria en dos extremida-des, o leve de la vibratoria en tres o cuatro extremidades
3. Disminución moderada de la sensibilidad táctil, algésica o artrocinética, o pérdida de la sensibilidad vibratoria en 1-2 extremidades, o disminución moderada de la vibratoria en 3-4 extremidades, o disminución ligera de la tactoalgésica en 3-4 extremidades.
4. Disminución marcada de la sensibilidad táctil, algésica o pérdida de la artrocinética en 1-2 extremidades, o moderada pérdida de sensibilidad artrocinética táctil o algésica, en 3-4 extremidades.
5. Pérdida de la sensibilidad en uno o más miembros, o disminución moderada de las sensibilidades por debajo de la cabeza.
6. Sensibilidad perdida por debajo de la cabeza.

Función visual

0. Normal.
1. Escotoma con agudeza visual mejor de 20/30
2. Agudeza visual entre 20/30 y 20/59 o escotoma importante
3. Gran escotoma o afectación moderada de campos visuales, pero con agudeza entre 20/60 y 20/99.
4. Afectación grave de campos visuales o agudeza visual entre 20/100 y 20/200, o grado 3 con agudeza del ojo mejor menor de 20/60.
5. Agudeza visual máxima menor de 20/200 o grado 4 con ojo mejor con agudeza menor de 20/60
6. Grado 5 con agudeza visual del ojo mejor menor de 20/60.

Función intestinal y vesical

1. Ligera disfunción (urgencia o retención) urinaria.
2. Moderada urgencia o retención o escasa incontinencia urinaria.
3. Frecuente incontinencia.
4. Precisa sondaje urinario.
5. Pérdida de la función vesical.
6. Pérdida de la función vesical e intestinal.

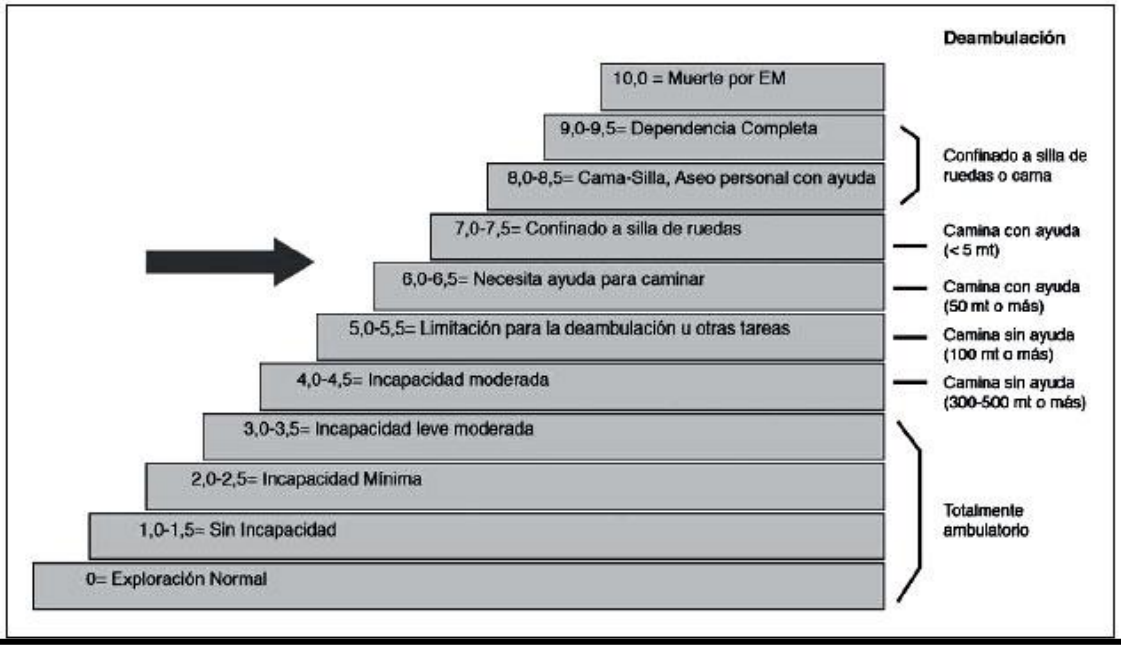

Ilustración 1: Escala de discapacidad para Esclerosis Múltiple

8.2.2 Evaluación oftalmológica

La exploración oftalmológica fue realizada por el Servicio de Oftalmología y constó de las siguientes exploraciones:

- Mejor agudeza visual corregida (MAVC), medida con optotipo ETDRS a tres diferentes contrastes: 100%, 2,50% y 1,25%.

- Medición de la SC utilizando los test de Pelli-Robson y CSV-1000E.

- Evaluación de la visión cromática utilizando el programa Vision color recorder: tests de Farnsworth 15D y Lanthony 15D.

- Examen del segmento anterior.

- Pupilometría

- Tonometría de aplanación.

- Fondo de ojo (evaluación del aspecto de la cabeza del nervio óptico).

-Tomografía de coherencia óptica (OCT) con el dispositivo de dominio espectral Cirrus HD (Carl Zeiss Meditec Inc, Dublin, CA). Se realizaron evaluaciones de los espesores maculares, de la CFNR y de células ganglionares. Estas pruebas fueron realizadas por el mismo operador, en tres momentos dentro de la misma visita, utilizando una fijación interna determinada por el aparato.

En el estudio referente a EA se aplicó el análisis de espesor macular mediante la aplicación de cubo macular 512x128 y el análisis de la CFNR mediante la aplicación de cubo de disco óptico 200x200.

En los estudios de EP y EM, además de estas dos aplicaciones, se utilizó la referente a análisis de células ganglionares.

Todas las pruebas, tanto funcionales como estructurales, se realizaron en la Unidad de Función Visual del Hospital Universitario Miguel Servet.

8.2.2.1. Estudio estructural de la CFNR

El estudio estructural de la CFNR se llevó a cabo mediante técnicas de análisis digital de la imagen de última generación: OCT de alta definición Cirrus HD.

La OCT, ideada en 1991 por Huang et al, nos permite obtener imágenes de cortes histológicos de la retina y medir cuantitativamente in vivo el espesor de la CFNR y de la mácula. Este dispositivo se basa en hacer recorrer a un mismo rayo de luz dos caminos diferentes, separándolo previamente en dos haces de luz. Uno de los caminos es conocido (brazo de referencia) y el otro se dirige hacia el medio de estudio (brazo de prueba), de manera que al volver a ser reflejados los dos haces en un mismo punto se lleva a cabo la comparación de las interferencias que ha sufrido el haz que ha recorrido el camino desconocido con las que ha sufrido el que ha recorrido el camino conocido. Mediante el análisis de este patrón, podemos inferir las modificaciones que el medio recorrido ha producido en nuestro haz de luz, permitiéndonos calcular la intensidad y el retardo de la luz reflejada. Con todo ello se genera una imagen final con una escala de colores donde las zonas de alta reflectividad, que corresponden a áreas de bloqueo parcial o total al paso de la luz (sangre, fibrosis…), se muestran en tonos rojo-blanco, mientras que las imágenes de zonas de baja reflectividad, que implican baja o nula resistencia al paso de la luz (quistes, edema…), se muestran en el espectro azul-negro.

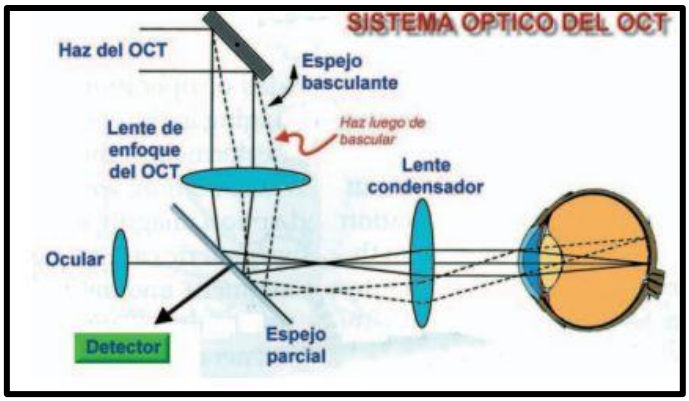

Ilustración 2: Ejemplo del sistema óptico de la tomografía de coherencia óptica.

Hasta el año 2006 los modelos de OCT se basaban en el sistema *On Time Domain*, en el que la información profunda se adquiría secuencialmente moviendo el espejo de referencia y se observaba el cambio en el tiempo del patrón de interferencia, necesitándose 1,28 segundos para la adquisición de las imágenes. Posteriormente apareció el sistema *Fourier Domain o Spectral Domain*, que utiliza en esencia el mismo sistema que el *On Time Domain*, pero el espejo de referencia permanece estacionario y la información profunda se obtiene con el análisis de los patrones de interferencia en un espectro de luces reflejadas y mezcladas. En vez de emplear un fotorreceptor, utiliza un espectrómetro de alta velocidad, lo que permite una detección mucho más eficiente de señales débiles, adquisición de datos con mayor rapidez y mejor resolución (que alcanza axialmente un rango entre 6 y 2 µm).

El Cirrus HD OCT es capaz de realizar mediciones con una resolución de 5/1000 milímetros y más de 67.000.000 puntos de información diferentes mediante el uso de la tecnología de infrarrojos (ilustración 3).

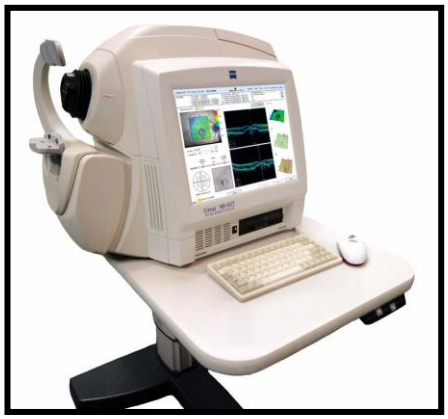

Ilustración 3: Dispositivo de tomografía de coherencia óptica de dominio Fourier de última generación, *Cirrus HD, Carl Zeiss.*

Los protocolos empleados en este estudio para la medición de los diferentes parámetros fueron los siguientes:

OCT Cirrus	Protocolo Optic disc cube 200x200 para CFNR
	Protocolo macular 512x128 para espesores y volumen macular
	Protocolo Análisis células Ganglionares

- El protocolo de **nervio óptico** de la OCT Cirrus genera imágenes de un cubo de 200 por 200 μm (ilustración 4). Analiza 6 mm^3 de CFNR alrededor del nervio óptico. Los parámetros analizados en nuestro estudio fueron el espesor medio de la CFNR, el espesor en cada uno de los cuadrantes de la CFNR (superior, inferior, temporal y nasal), y el espesor en los doce sectores horarios alrededor del nervio óptico. A los sectores horarios se les asignó un número de posición (C1 a C12) siguiendo el sentido horario para el ojo derecho y el sentido anti horario para el izquierdo.

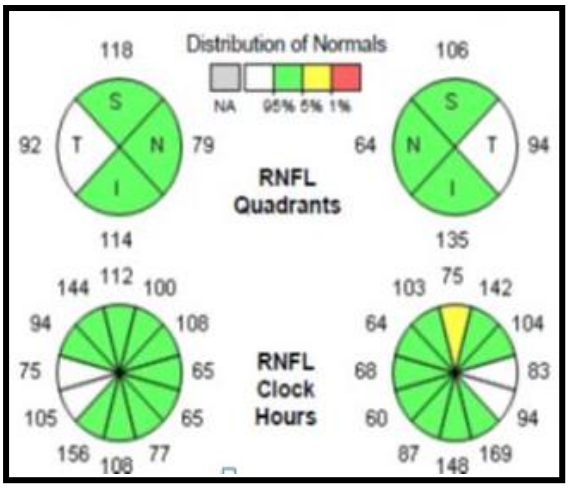

Ilustración 4: Cuadrantes (arriba) y sectores horarios (abajo) en los que divide el OCT Cirrus la CFNR.

- El protocolo **macular** de la OCT Cirrus incluye un cubo macular de 512x128 μm que proporciona los valores de los espesores de la retina en las nueve áreas descritas en el Early Treatment Diabetic Retinopathy Study (ETDRS) (ilustración 5). Este estudio divide la mácula en 9 áreas definidas por tres círculos ubicados a 1, 3, y 6 mm; estos dos últimos a su vez están divididos en cuatro cuadrantes: superior, inferior, nasal y temporal. El área 1 representa la fóvea. Las áreas 2, 3, 4 y 5 forman el anillo interno y las áreas 6, 7, 8 y 9 forman el anillo externo. Los parámetros recogidos en nuestro estudio fueron el espesor medio y el volumen macular total calculado en el anillo de 6 mm de diámetro.

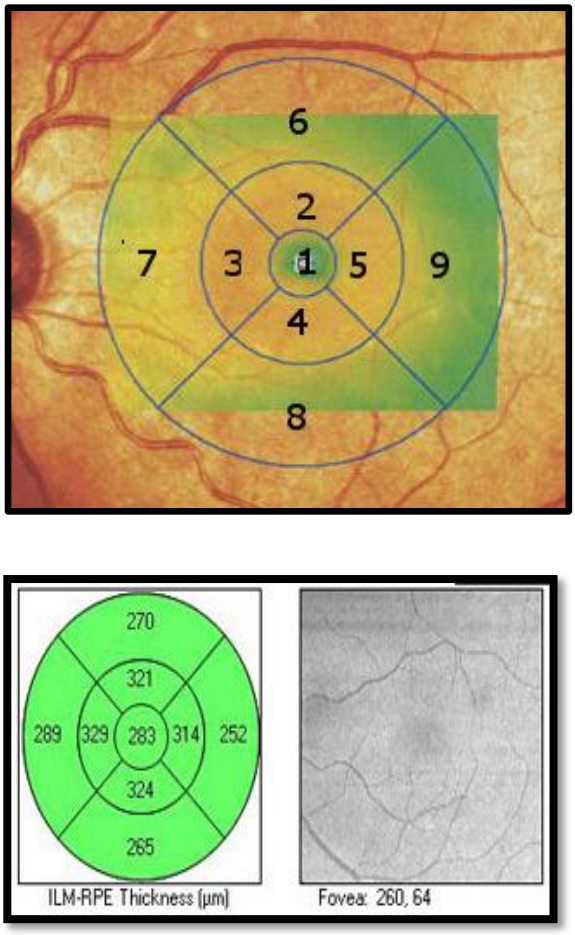

Ilustración 5: Arriba: cubo macular donde se observa la numeración de las 9 áreas según el ETDRS. Abajo: Representación del espesor macular en los diferentes sectores con OCT *Cirrus*.

- El análisis de segmentación de las capas retinianas también ofrece medidas del espesor de la **capa de células ganglionares** (ilustración 6) evaluando 6 áreas de un cubo macular (sectores superior, superonasal, inferonasal, inferior, inferotemporal y superotemporal) y medidas de la media y el mínimo de CCG más la capa plexiforme interna (IPL: Inner Plexiform Layer). Se selecciona el mínimo porque la porción más fina de GCL+IPL en la región perifoveal se considera indicativa de daño de células ganglionares.

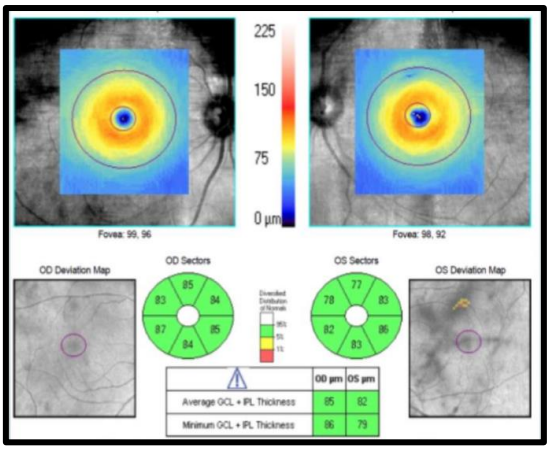

Ilustración 6: Representación del espesor en sectores de la capa de células ganglionares de la retina con OCT *Cirrus.*

8.2.2.2 Estudio funcional

El estudio funcional realizado incluyó la medición de la AV, SC y los test de visión cromática.

- La medición de la **agudeza visual** puede realizarse mediante diferentes escalas, siendo la prueba más utilizada para la evaluación de la función visual. La AV se define como la capacidad para distinguir dos elementos separados de un objeto e identificarlos como un todo, y se cuantifica como el ángulo mínimo de separación entre dos objetos que permite distinguirlos como objetos separados. Evalúa la función macular e informa de la precisión del enfoque retiniano, de la integridad de los elementos neurológicos del ojo y de la capacidad interpretativa del cerebro.

En este estudio se empleó el optotipo de ETDRS (Early Treatment Diabetic Retinopathy Study) (ilustración 7) con la finalidad de evaluar la función visual de forma más precisa, estandarizada y reproducible. Se trata del optotipo más usado en investigación, y es el test estándar mundial para medir la baja visión. El optotipo ETDRS está basado en la carta logarítmica de Bailey-Lovie[71]. Cada fila, tiene el mismo número de letras, concretamente, cinco. Además, el espacio entre ellas es proporcional

al tamaño de las letras y la separación entre filas, también. Cambiar de fila equivale a aumentar o disminuir 0.1 unidades logarítmicas de AV. Los optotipos deben ser igualmente legibles en cada nivel de AV.

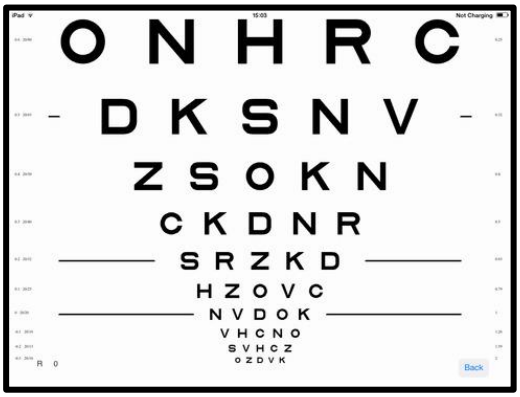

Ilustración 7: Optotipo ETDRS de contraste 100%

Se evaluó la AV LogMAR a tres niveles de contraste diferente: 100% (usando la carta ETDRS), 2,50% y 1,25% (AV a bajo contraste, usando las cartas de bajo contraste Letter Charts-Precision Vision. LaSalle, IL-) (ilustración 8). El porcentaje indica el nivel de contraste, por ejemplo 100% representa letras negras sobre fondo blanco y 1,25% letras gris claras sobre fondo blanco. Las medidas se obtuvieron en visión monocular bajo condiciones de luz controladas (fotópica: luminancia media de 85cd/m^2, mesópica alta: 5cd/m^2, y mesópica baja 3cd/m^2), con la mejor corrección.

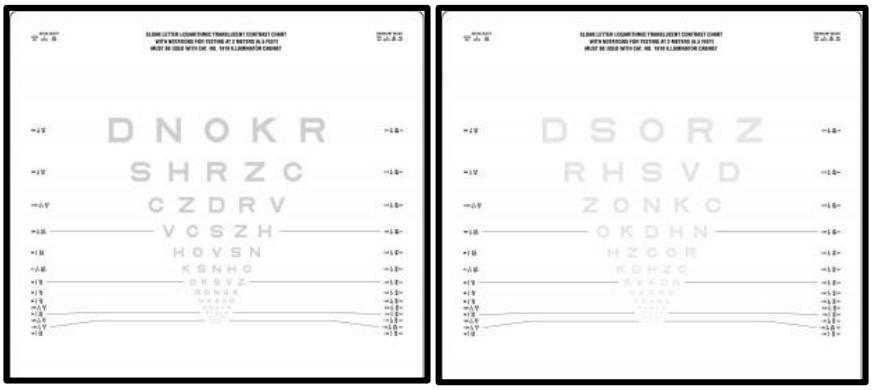

Ilustración 8: Cartas ETDRS de contraste 2,5% (izquierda) y de contraste 1,25% (derecha) de contraste.

- La **sensibilidad al contraste (SC)** es un indicador mucho más sólido de la función visual que la AV[72]. La función de sensibilidad al contraste (CSF), en función de la frecuencia espacial, establece de una forma muy fiable los límites de la percepción visual y está relacionada con la capacidad del sistema visual para distinguir entre un objeto y su fondo.

La SC es una técnica útil y eficaz para evaluar la calidad de la visión. Para determinar la SC de un sujeto, se utilizan como test redes sinusoidales caracterizadas por una frecuencia espacial (que se mide en ciclos de red por grado de ángulo visual subtendido) y un contraste en luminancia, que adopta la definición estándar:

$$C = \frac{L_{\max} - L_{\min}}{L_{\max} + L_{\min}} .$$

donde Lmax y Lmin son la luminancia máxima y mínima respectivamente de la red en cada ciclo. En la curva de SC se representa la visibilidad umbral (inversa del contraste umbral) en función de la frecuencia espacial del estímulo. La visibilidad umbral es la inversa del mínimo valor de contraste que es capaz de percibir un sujeto para una determinada frecuencia espacial. Así, cuanto mayor sea la visibilidad menor es el contraste mínimo percibido y mayor, por tanto, la capacidad o sensibilidad del sujeto a dicho estímulo.

Es una de las cualidades del sistema visual que primero se ve afectada en las enfermedades neurodegenerativas como pueden ser la EA, la EP o la EM[73].

Para evaluar esta característica, en nuestro estudio hemos utilizado la carta de letras de Pelli-Robson y el modelo CSV-1000E de Vector Vision.

El test de Pelli-Robson (ilustración 9), consta de 8 líneas de letras. Cada línea tiene 6 letras mayúsculas con un tamaño de 4,9 ×4,9 cm. Las letras de cada línea se agrupan en dos grupos de tres, de tal manera que en cada triplete las letras tienen el mismo contraste. El contraste decrece de un triplete al siguiente, incluso en la misma línea. Las letras con mayor contraste son las superiores izquierdas, estableciéndose un valor de 1 o 100%, y las de menor contraste son las inferiores derechas cuyo valor es de 0,006 o 0,6%. Se pasa de un triplete al siguiente cuando el paciente ve por lo menos dos de las letras de dicho contraste, y se concluye la prueba cuando el paciente no ve ninguna o solo una de las letras de cada trío. Se evalúa de manera monocular y binocular, a una distancia de 1 metro, con su mejor corrección, y bajo condiciones fotópicas controladas (85cd/m^2).

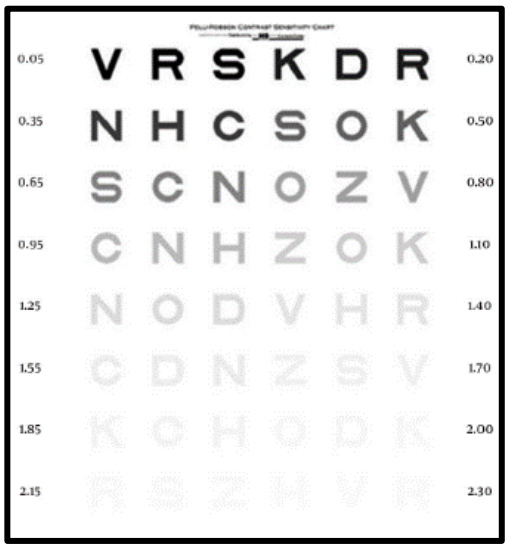

Ilustración 9: Test de Pelli-Robson.

El test CSV-1000E se utiliza para la estandarización de la SC y el deslumbramiento (ilustración 10). El test CSV-1000E explora cuatro frecuencias espaciales (3, 6, 12 y 18 ciclos por grado). La carta se compone de 4 filas con 17 parches circulares en cada una. Los estímulos se organizan en una doble hilera de círculos, uno de los cuales es uniforme y el otro contiene al estímulo de un valor de contraste determinado decreciente de izquierda a derecha en la misma línea. La tarea del sujeto es determinar dónde está situado el test dentro de cada pareja de círculos. El examinador, que conoce la respuesta correcta gracias al panel de control de respuestas, deberá anotar el número correspondiente al último estímulo identificado correctamente, para cada una de las cuatro frecuencias que componen el test. Cada valor de contraste para cada frecuencia se transforma en una escala logarítmica de acuerdo a valores estandarizados. Las pruebas se realizan de manera monocular a una distancia de 2,4 metros con su mejor AV corregida.

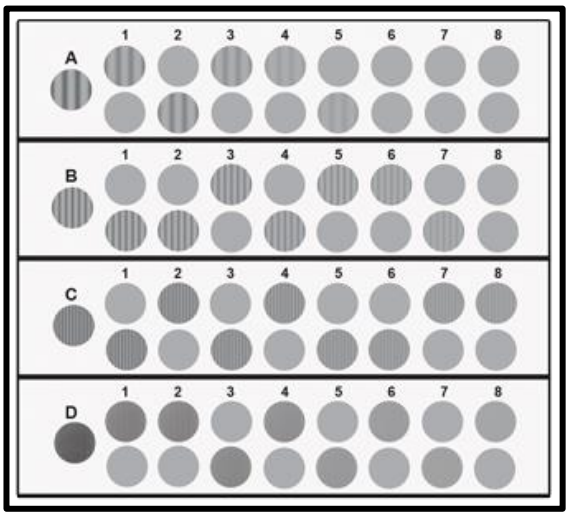

Ilustración 2: Test CSV 1000E con frecuencias espaciales de 3, 6, 12 y 18 cpg.

- La exploración de la **visión cromática** permite valorar y cuantificar las alteraciones en la percepción de los colores. Estas anomalías de la visión del color pueden ser congénitas o adquiridas. Las formas adquiridas aparecen desde en enfermedades maculares hasta ambliopía; sin embargo, son las neuropatías las que muestran una mayor y más temprana afectación de la visión de colores[74]. En alteraciones del nervio óptico, como la neuritis óptica, generalmente se afecta la percepción en el eje rojo-verde del espectro y, con menor frecuencia, en el azul-amarillo, cuando la afectación del nervio es más severa[75]. La discromatopsia (afectación de la visión de los colores) puede detectarse de forma sencilla mediante la visualización de un color monocular, que se obtiene pidiendo al paciente que compare el color de un objeto rojo entre los dos ojos.

El gold standard para identificar y clasificar los defectos de la visión cromática son los anomaloscopios, basados en igualar un espectro amarillo con una mezcla de longitudes de onda rojo-verde. Por su complejidad y coste, su uso queda relegado al ámbito de la investigación.

El procedimiento más empleado para el diagnóstico de discromatopsias son las cartas pseudoisocromáticas de Ishihara, una serie de 38 láminas en las que el sujeto debe identificar un objeto (números) de cierto color sobre un fondo de otro color. Aunque práctico como método de cribado, es limitado a la hora de clasificar el tipo de defecto (protan, deutan y tritan), que sí se consigue con las pruebas de ordenación (Farnsworth D15 y Lanthony D15)[76-77] que consisten en ordenar en secuencia una serie de colores. Además, son especialmente útiles para detectar y monitorizar defectos no específicos, los más frecuentes en trastornos adquiridos.

En nuestro estudio, para la exploración de la visión cromática, se utilizaron las pruebas Farnsworth 15D y Lanthony 15D integradas en el software Vision Color Recorder (CVR, Optical Diagnosis, Inc., Beusichem, The Netherlands).

Se realizó el test de Farnsworth 15 y de Lanthony 15 con el programa Color Vision Recorder, el cual nos permitió realizar un análisis de discriminación cromática por ordenación de los colores, incluyendo de forma simultanea el test de Farnsworth 100-hue (FM-100), Farnsworth - Munsell 15 D y de Lanthony 15 D. Tras la realización de un test de ordenamiento, el procesamiento de los resultados nos llevó a un gráfico en el que valoramos si existía o no un determinado eje de confusión, es decir, una polaridad, que se suele simplificar en los términos «rojo-verde» (para defectos protan y deuteran) o «azul-amarillo» (para defectos tritan) (ilustración 11).

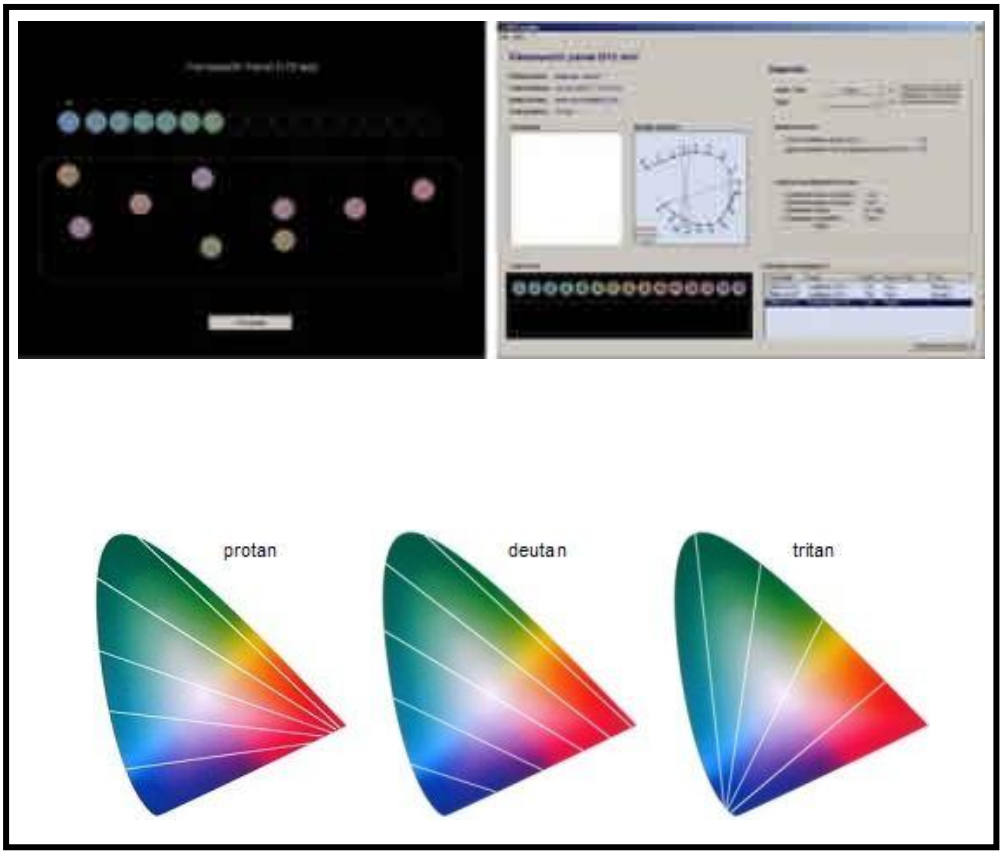

Ilustración 11: Programa informático Color Vision Recorder. Arriba: prueba de ordenación de colores. Abajo; gráfico representando los ejes protan, deutan y tritan.

Los protocolos Farnsworth D15 y L'Anthony D15, se usan a menudo para diferenciar entre sujetos con pérdida severa de la visión de color y aquellos con defectos medios o visión colorimétrica normal. Se analizan los parámetros AC CCI que representa el cálculo del ratio entre el radio y la distancia entre picos, Conf angle que representa el eje de la deficiencia de color, y el S-index que representa el paralelismo de los vectores de confusión con el ángulo de confusión personal[78-79]. Todos estos parámetros evalúan la severidad de la discromatopsia. Por ejemplo, una puntuación AC CCI mayor de 1 indica alteraciones en la visopercepción del color. Cuanta mayor sea la puntuación en AC CCI y S-index, peor la condición.

Los test se realizaron de forma monocular con la mejor visión corregida.

8.3. Variables

Una vez realizado todo el protocolo exploratorio, las variables que se seleccionaron para nuestro estudio fueron:

-Variables de valoración neurológica: edad, sexo, tiempo de evolución desde el diagnóstico, edad al diagnóstico y puntuación en las escalas de valoración neurológica antes descritas.

-Variables de valoración oftalmológica: puntuación de AV (escala ETDRS de contrastes 100%, 2,5% y 1,25%), de SC (en test de Pelli-Robson, y en las 4 frecuencias del test CSV1000E), parámetros de los test de visión cromática Farnsworth 15D y Lanthony 15D (AC CCI, Conf angle, S-index), presión intraocular, color de la papila (normal, palidez difusa, palidez sectorial), tamaño de excavación papilar, espesores de la CFNR, espesores y volumen macular y espesores de CCG medidos con OCT Cirrus HD.

8.4. Recogida y análisis de datos

Todas las variables mencionadas fueron registradas en una base de datos elaborada con el programa Microsoft Excel 2010.

Para los análisis estadísticos se utilizaron los programas SPSS versión 20.0 (SPSS Inc., Chicago, United States).

Previamente al análisis de los datos, se comprobó su ajuste a la normalidad mediante el test de Kolmogorov-Smirnov. Se analizó la media y desviación estándar de cada una de las variables y, mediante un test de T de Student, se compararon las diferentes variables entre el grupo de sanos y de pacientes. Para averiguar una correlación lineal entre los parámetros funcionales y estructurales se utilizó el coeficiente de correlación de Pearson. Se consideró significación estadística a los valores con una P menor a 0.05. La corrección de Bonferroni se aplicó en comparaciones múltiples.

Para una mejor comprensión del análisis de los datos, a continuación se detalla la técnica estadística utilizada en los tres primeros trabajos de investigación. El cuarto artículo no se describe porque se trata de una revisión.

1. **Polo V, Rodrigo MJ, Garcia-Martin E, Otin S, Larrosa JM, Fuertes MI, Bambo MP, Pablo LE, Satue M. Visual dysfunction and its correlation with retinal changes in patients with Alzheimer´s disease. Eye (Lond) 2017 (en prensa).**

Se realizó un estudio transversal en el que se incluyeron un total de 24 pacientes con EA leve o moderada (24 ojos) y 24 sujetos sanos (24 ojos), a los que se les realizó una valoración neuro-oftalmológica completa, se evaluó la función visual (AV, SC y color) y estructura retiniana (espesor macular y de la CFNR) mediante Cirrus HD OCT.

Se comprobó la normalidad de la distribución mediante el test de Kolmogorov-Smirnov.

Los resultados de cada variable obtenidos en la exploración fueron comparados entre ambos grupos (EA y controles sanos), con el objetivo de detectar diferencias en los resultados asociadas a la enfermedad. Se compararon las variables funcionales (AV, SC y color) de pacientes con EA con sujetos sanos mediante la T de Student con corrección de Bonferroni para comparaciones múltiples.

Así mismo, se compararon el volumen, espesor medio y espesores de los nueve sectores maculares, correspondientes a las áreas ETDRS; así como los resultados en los espesores de la CFNR para cada uno de los cuatro cuadrantes del disco óptico y su espesor medio, entre ambos grupos (EA y controles sanos), mediante la T de Student con corrección de Bonferroni para comparaciones múltiples (nivel de significación $p=0.0045$ para el espesor macular, y $p=0.010$ para el espesor de la CFNR).

Se analizaron las correlaciones en el grupo de pacientes con EA entre los parámetros funcionales (AV, SC y color) y estructurales (espesor macular y de la CFNR). El test utilizado para evaluar las correlaciones fue la P de Pearson, (nivel de significación $p<0.05$).

2. **Polo V, Satue M, Rodrigo MJ, Otin S, Alarcia R, Bambo MP, Fuertes MI, Larrosa JM, Pablo LE, Garcia-Martin E. Visual dysfunction and its correlation with retinal changes in patients with Parkinson's disease: an observational cross-sectional study. BMJ Open 2016;6:e009658.**

Se realizó un estudio transversal en el que se incluyeron 37 ojos de 37 pacientes con EP y 37 ojos de 37 sujetos sanos, a los que se realizó una exploración neuro-

oftalmológica completa, con OCT para análisis estructural de la retina y nervio óptico, evaluando el espesor macular, la CFNR y el espesor de la CCG; así como evaluación de los parámetros de función visual utilizando la AV de alto y bajo contraste, la SC y la percepción del color.

Se comprobó la normalidad de la distribución de las variables mediante el test de Kolmogorov-Smirnov.

Los resultados de cada variable obtenidos en la exploración fueron comparados entre ambos grupos (EP y controles sanos) mediante un test de T de Student, con el objetivo de detectar diferencias en los resultados asociadas a la enfermedad.

Los parámetros funcionales se correlacionaron con los estructurales mediante la P de Pearson, (nivel de significación $p<0.05$).

3. **Satue M, Rodrigo MJ, Otin S, Bambo MP, Fuertes MI, Ara JR, Martin J, Polo V, Larrosa JM, Pablo L, Garcia-Martin E. Relationship between visual dysfunction and retinal changes in patients with multiple sclerosis. PLoS ONE 2016;11(6):e0157293.**

Se incluyeron 84 pacientes con EM remitente-recurrente (84 ojos) y 84 sujetos sanos (84 ojos), a los que se realizó una exploración neuro-oftalmológica completa, con OCT para análisis estructural de la retina y nervio óptico. Se evaluó el volumen y el espesor macular correspondiente a las 9 áreas del ETDRS, la CFNR de los 4 cuadrantes del nervio óptico y el espesor de la CCG en sus 6 áreas del cubo macular, con el dispositivo Cirrus HD; así como la función visual utilizando la AV de alto y bajo contraste, la SC y la percepción del color.

Se comprobó la normalidad de la distribución mediante el test de Kolmogorov-Smirnov.

Los resultados de cada variable obtenidos en la exploración fueron comparados entre ambos grupos (EP y controles sanos) mediante un test de T de Student, con el objetivo de detectar diferencias en los resultados asociadas a la enfermedad. Igualmente se utilizó el test de la T de Student para encontrar diferencias entre el grupo de pacientes con historia de neuritis óptica frente a al grupo de pacientes sin historia de neuritis óptica (nivel de significación $p<0.05$). Se utilizó la corrección de Bonferroni para comparaciones múltiples, evitando así el incremento de falsos positivos.

Para encontrar diferencias estadísticamente significativas entre el grupo control frente a los grupos de pacientes con o sin historia de neuritis óptica se utilizó el test ANOVA, (nivel de significación $p<0.05$).

Finalmente los parámetros funcionales se correlacionaron con los estructurales mediante la P de Pearson, (nivel de significación $p<0.05$), aplicando igualmente la corrección de Bonferroni.

9. APORTACIONES DEL DOCTORANDO

Los artículos de la presente tesis doctoral aportan a la comunidad científica un análisis de parámetros funcionales de la visión, que son herramientas inocuas para valorar la afectación visual en pacientes con las enfermedades neurodegenerativas de EA, EP y EM.

Nuestro trabajo demuestra la correlación entre la estructura retiniana medida con OCT y la función visual en dichas enfermedades neurodegenerativas.

El diagnóstico y seguimiento de estas patologías, se ha basado tradicionalmente en criterios clínicos, escalas de valoración y alguna prueba de imagen; en muchos casos de disponibilidad limitada en la práctica clínica habitual y de alto coste económico, como es el caso de la resonancia magnética.

El estudio de la estructura retiniana mediante la OCT cumple con varios de los requisitos para postularse como biomarcador en las enfermedades neurodegenerativas; pues se trata de un procedimiento inocuo, no invasivo y sencillo, que permite realizar una medición rápida, objetiva, precisa, reproducible y coste-efectiva del espesor de la retina y de la CCG.

Aunque la OCT, como ya se ha comentado anteriormente, es una prueba rápida y sencilla, necesita de un mínimo de quietud para adquirir las imágenes, que en ciertos pacientes en estadios avanzados de las enfermedades neurodegenerativas se ve limitada por el temblor, rigidez y/o alteraciones posturales.

La correlación encontrada en nuestros trabajos, entre estructura retiniana y función visual, nos permite afirmar que el uso de las pruebas funcionales en pacientes que presentan mala situación motora, pero mantienen una buena función mental (como

ocurre en los estadios tempranos de la EP o en la EM) y que no precisan de colaboración física, es una herramienta útil para aportar más información sobre el estado de la enfermedad de una manera sencilla. Esto sugiere que el análisis con OCT podrían utilizarse como screening ambulatorio en sujetos susceptibles, y en sujetos en etapas preclínicas y/o clínicas, sin necesidad de acudir a centros hospitalarios, con el consecuente ahorro de costes directos e indirectos. Bien es cierto que sería necesario realizar más estudios sobre el tema.

Nuestros resultados sugieren que el análisis de imágenes tomográficas de la retina y el estudio de la función visual aplicadas a la práctica clínica, pueden ser un buen método para el diagnóstico precoz de las enfermedades neurodegenerativas, así como para evaluar su progresión y la eficacia de los tratamientos basándose en la disminución o ralentización del daño neuronal medido con estas herramientas.

Finalmente, estas investigaciones aportan un enfoque multidisciplinar de los pacientes con EA, EP y EM, basado en la colaboración entre Oftalmología y Neurología, donde la evaluación neuroftalmológica jugaría un importante papel, ayudando a optimizar el manejo de estos pacientes.

10. RESULTADOS Y DISCUSIÓN

Carta de aceptación de la publicación "Visual dysfunction and its correlation with retinal changes in patients with Alzheimer´s disease." en la revista EYE.

24th Jan 17

To the author of manuscript EYE-15-1341R, "Visual dysfunction and its correlation with retinal changes in patients with Alzheimer´s disease."

*Thank you for submitting the above manuscript to Eye**. I am pleased to inform you that it has been accepted for publication in Eye***

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Sincerely,

Andrew Lee
Editor
Eye

What was known before:
Recent studies reported retinal thinning in AD patients compared with healthy subjects.AD patients exhibit decreased color vision and contrast sensitivity, and other visual field deficits. Very few published studies have assessed visual dysfunction in AD and its correlation with morphologic parameters

What this study adds:
Patients with AD have visual dysfunction that correlates with structural changes evaluated by SD-OCT. Macular measurements may be reliable indicators of visual impairment in AD patients.

TITLE: Visual dysfunction and its correlation with retinal changes in patients with Alzheimer´s disease.

AUTHORS: Polo V[1,2], Rodrigo MJ[1], Garcia-Martin E[1,2], Otin S[1,2], Larrosa JM[1,2], Fuertes MI[1,2], Bambo MP[1,2], Pablo LE[1,2], Satue M[1,2].

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RUNNING TITLE: Visual dysfunction and OCT in Alzheimer´s disease

KEY WORDS: Alzheimer´s disease, visual function, contrast sensitivity, color vision, retinal nerve fiber layer, optical coherence tomography.

WORD COUNT: 2686 words.

All subjects provided detailed consent to participate in this study, which was conducted in accordance with the guidelines established by the Ethics Committee of the Miguel Servet Hospital and based on the principles of the Declaration of Helsinki.

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The authors disclose no conflict of interest.

The authors have no proprietary interests to disclose.

Abstract

Aim: To evaluate visual dysfunction and its correlation with structural changes in the retina in patients with Alzheimer´s disease (AD).

Methods: Patients with AD (n=24) and controls (n=24) underwent evaluation of visual acuity (VA), color vision (using the Farnsworth and L'Anthony desaturated [D]15 color tests), and contrast sensitivity vision (CSV; using the Pelli-Robson chart and CSV-1000E test) to measure visual dysfunction. Structural measurements of the retinal nerve fiber layer (RNFL) and macular thickness were obtained using spectral domain-optical coherence tomography (SD-OCT).

Results: CSV at three of the four spatial frequencies was significantly worse in AD patients than in controls. Color vision was significantly affected in AD patients based on the Farnsworth color test. Compared with controls, macular thinning was detected in all sectors except the fovea, and the RNFL exhibited significant thinning in the superior quadrant and lower average thickness ($p<0.05$). CSV was the functional parameter most strongly correlated with structural measurements in patients with AD. Color vision was strongly associated with macular volume ($r >0.70$, $p<0.05$). VA at different levels of contrast was associated with macular and RNFL thickness.

Conclusions: Patients with AD had visual dysfunction that correlated with structural changes evaluated by SD-OCT. Macular measurements may be reliable indicators of visual impairment in AD patients.

Introduction

Alzheimer´s disease (AD) is a progressive neurodegenerative disorder and the most frequent cause of dementia worldwide [1]. Although AD is most commonly associated with memory deficits and cognitive impairment, patients with AD also exhibit alterations in sensory perception, such as visual processing [2-4].

Recent studies reported retinal thinning in AD patients compared with healthy subjects, especially in the macular area [5-7]. Defects in the retinal nerve fiber layer (RNFL) may be the earliest sign of AD, even before damage to the hippocampus [8]. These findings may reflect retinal ganglion cell death and axonal loss in the optic nerve beyond the effects of normal aging.

AD patients exhibit decreased color vision and contrast sensitivity, and other visual field deficits that could account for dysfunction in different tasks of basic vision and visual cognition in this disorder [9-11]. Several studies report a correlation between visual dysfunction and axonal loss observed in the optic nerve in other neurodegenerative processes, such as multiple sclerosis and Parkinson disease [12,13]. To our knowledge, however, very few published studies have assessed visual dysfunction in AD and its correlation with morphologic parameters [14].

In the present study, we evaluated visual acuity (VA) using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart, contrast sensitivity vision (CSV) using the CSV-1000E test and Pelli-Robson chart, and color vision using the Farnsworth and L´Anthony tests (by Color Vision Recorder software) in AD patients and healthy controls to examine the association between visual dysfunction and morphologic parameters.

Materials and Methods

Twenty-four patients with mild or moderate AD and 24 age- and sex-matched healthy individuals were recruited for the study. All procedures adhered to the tenets of the Declaration of Helsinki, and the local ethics committee approved the experimental protocol. All participants provided informed consent to participate in the study. For cognitively impaired patients not able

to give signed permission, legal tutors and family members provided signed consent, which was approved by the local ethics committee.

AD diagnosis was based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) established criteria for dementia syndrome (Alzheimer's type) [15] and the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINDS-ADRDA) [16] criteria for probable or possible AD. A trained neurologist blind to the visual and retinal test results evaluated all patients. The stage and severity of AD were determined by a trained neurologist using the MMSE scale, which includes items such as orientation, recall, calculation, registration, attention, repetition, comprehension, naming, reading, writing, and drawing. A score in of 30 on the MMSE scale indicates 100% correct [17]. Disease severity was determined by MMSE score as mild (18–24), moderate (10–18), or severe (<9). Disease duration was also recorded. Patients with severe dementia and/or unable to perform the full exploration protocol due to their functional status were excluded from the study.

Inclusion criteria were confirmed AD diagnosis; best-corrected visual acuity (BCVA) of 0.4 or higher (using a Snellen chart) in each eye to allow for performance of the protocol; and intraocular pressure less than 21 mmHg. Exclusion criteria were the presence of significant refractive errors (>5 diopters of spherical equivalent refraction or 3 diopters of astigmatism); intraocular pressure ≥21 mmHg; media opacifications; concomitant ocular diseases, including history of glaucoma or retinal pathology; and systemic conditions that could affect the visual system. Healthy controls had no history nor evidence of ocular or neurologic disease of any nature; their BCVA was >20/30 based on the Snellen scale. Only one eye per subject was randomly selected and included.

All subjects underwent a complete neuro-ophthalmic evaluation that included pupillary, anterior segment, and funduscopic examination. Visual function was assessed by evaluating BCVA using an ETDRS chart, CSV using the CVS-1000E test and Pelli-Robson chart, and color vision using the Farnsworth D15 and L´Anthony D15 tests. Structural analysis of the retina was performed using spectral domain (SD) optical coherence tomography (OCT) with the Cirrus high definition

(HD) OCT (Carl Zeiss Meditec Inc, Dublin, CA), using two different applications: macular cube 512x128 applications (for macula thickness analysis) and optic disc cube 200x200 (for RNFL thickness analysis).

Visual function evaluation

LogMAR visual acuity (VA) was evaluated at three different contrast levels: 100% (HCVA, using ETDRS chart), 2.50%, and 1.25% (LCVA, using Low-Contrast Sloan Letter Charts - Precision Vision, LaSalle, IL-), The percentage indicating the level of contrast, i.e., 100% representing black letters over white background and 1.25% light grey letters over white background. All measurements were obtained under monocular vision and controlled lighting conditions (photopic: mean luminance of 85 cd/m^2, high mesopic: 5 cd/m^2, and low mesopic: 3 cd/m^2) with best correction.

CSV was evaluated in our patients using the Pelli-Robson chart and the CVS-1000E test. The Pelli-Robson chart comprises horizontal lines of capital letters. The letter sequences are organized into groups of three (triplets) with two triplets per line. Within each triplet, all letters have the same contrast. The contrast decreases from one triplet to the next, even within each line. All patients were evaluated under monocular vision at a distance of 1 meter from the chart and under controlled photopic conditions (85 cd/m^2). The score corresponding to the last triplet of letters seen by the patient was recorded.

The CSV-1000E instrument is used worldwide for standardized CSV and glare testing. All patients were evaluated at a distance of 2.5 m from the chart under monocular vision at 4 different spatial frequencies (3, 6, 12, and 18 cycles per degree [cpd]). The chart comprised four rows of patches. Each row presents 17 circular patches 1.5 inches in diameter. The first patch on the far left in each row presents a very high contrast grating (sample patch). The remaining 16 patches appear in 8 columns across the row. In each column, one patch presents a grating and the other patch is blank. The patches that present a grating decrease in contrast from left to right across the row. The patient indicates whether the grating appears in the top patch or the bottom patch for each column. A CSV curve, in which the visual threshold is represented for each spatial frequency, was analyzed. Each contrast value for each spatial frequency was then transformed into a logarithmic scale according to standardized values.

153 Color vision was assessed using the Color Vision Recorder program (CVR, Optical Diagnostics,
154 Beusichem, The Netherlands). The CVR software is designed for the Windows operating system
155 and analyzes chromatic discrimination by color classification. The CVR includes the classic test
156 of Farnsworth 100-hue, Farnsworth D15, and L´Anthony D15. All patients in the study were
157 evaluated using the Farnsworth D15 and L´Anthony D15 protocols (often used to differentiate
158 between subjects with severe loss of color vision and those with milder color defects or normal
159 color vision) and different output parameters, such as the Age-Corrected Color Confusion index
160 (AC CCI, which represents the ratio between the radius or distance between caps), the Confusion
161 angle (Conf angle, which represents the axis of color deficiency), and the Scatter index (S-index,
162 which represents the parallelism of confusion vectors to the personal confusion angle) were
163 recorded [18,19]. These parameters evaluate the severity of dyschromatopsia. A CCI score
164 higher than 1 indicates altered color vision perception; the higher the score, the worse the
165 condition. The tests were performed under monocular vision.
166
167 *Morphologic evaluation*
168 Structural measurements of the retina were obtained using the Cirrus OCT device. The same
169 experienced operator performed all scans and did not apply manual correction to the OCT
170 output. We used an internal fixation target because it provides the highest reproducibility [20]
171 and rejected poor-quality scans prior to data analysis. We based image quality assessment on the
172 signal strength measurement, which combines the signal-to-noise ratio with the uniformity of the
173 signal within a scan (scale 1–10, where 1 is categorized as poor image quality and 10 as
174 excellent). We included images with a score higher than 7 for evaluation. The Cirrus OCT
175 macular cube 512x128 application provides a macular volume measure and retinal thickness
176 values for nine areas that correspond to the ETDRS. These areas include a central 1-mm circle
177 representing the fovea, an inner ring measuring 3 mm in diameter, and an outer ring measuring 6
178 mm in diameter. The inner and outer rings are divided into four quadrants: superior, nasal,
179 inferior, and temporal. The Cirrus OCT optic disc protocol generates 200 x 200 cubic images
180 with 200 linear scans enabling analysis of the RNFL of a 6-mm^3 area around the optic nerve. For
181 each scan series of RNFL measurements, we assessed the average, superior, inferior, temporal,
182 and nasal thickness.
183

All data analyses were performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was used to assess sample distribution. Due to the normal distribution of the data, differences between evaluations of AD patients and healthy subjects were compared using the Student´s t-test. The linear correlation between structural and functional parameters was determined using Pearson's correlation coefficient. Values of p less than 0.05 were considered to indicate statistical significance. To avoid a high false positive rate, the Bonferroni correction for multiple tests was calculated and the corrected p values were added to the previously calculated data.

Results

Twenty-four patients with AD (48 eyes) and 24 healthy controls (48 eyes) were included in the study. Mean age of the patients with AD was 74.42 ± 8.88 years and the mean age of the healthy controls was 72.94 ±7.40 years. The two groups did not differ significantly in age (p=0.070), sex (p=0.670), or intraocular pressure (p=0.540). Disease duration ranged from 12 months to 8 years with a median of 4.17 years since diagnosis. Mean MMSE score was 15.54 ± 7.1.

Functional parameters

CSV was affected in patients at three of the four spatial frequencies of the CSV-1000E chart (3, 6, and 12 cpd) when analyzed based on the number of correct localized gratings (p=0.020, <0.001, and 0.010 respectively). The Pelli-Robson results also revealed a significant reduction in CSV in AD patients (p=0.020). Color vision results were also significantly affected in AD (Farnsworth´s AC CCI and S-Index, and L´Anthony´s AC CCI). AD patients had a lower BCVA at all three contrast levels of the ETDRS chart compared to controls, but the results did not differ significantly. The results are shown in Table 1 and Figure 1A.

Structural parameters

OCT measurements indicated a significant thickness reduction in AD patients in 8 of the 9 macular sectors analyzed (Table 2, Figure 1B). The RNFL was significantly reduced in the superior quadrant and the average thickness in AD patients (Table 2, Figure 1B).

Correlation between functional and structural parameters

CSV was the functional parameter most frequently associated with structural measurements in AD. The Pelli-Robson CSV results correlated with macular thickness in almost all sectors (Table 3). Thickness in the outer superior (r=0.65, p<0.001), outer nasal (r=0.58, p<0.001), and outer

temporal (r=0.66, p<0.001) regions, and average thickness (r=0.63, p<0.001) were most highly correlated with CSV. The Pelli-Robson results also correlated with the RNFL thickness in different sectors (Table 3). Measurements with the CSV-1000E at different spatial frequencies correlated significantly with most macular parameters and with average and superior RNFL thicknesses (Table 3).

A significant but mild association between color vision (measured with both Farnsworth and L´Anthony tests) and the RNFL and macular parameters was found in isolated sectors (r<0.50, p<0.05). Macular volume was strongly associated with the Farnsworth S-index (r=-0.75, p<0.001).

The VA ETDRS results correlated with macular and RNFL thickness: VA at 100% was significantly associated with all outer macular sectors (Table 4) and macular average thickness correlated with VA at 1.25% (r=0.60, p=0.014) and 2.50% (r=0.66, p<0.001). VA was significantly correlated with average, superior, nasal, and inferior RNFL thickness at all contrast levels (Table 4).

No correlation was observed between disease severity (as measured by the MMSE) and ophthalmological parameters.

Correlations between structural and functional measurements in controls can be observed in Supplemetary Tables 1 and 2.

Discussion

In the present study, we evaluated visual function parameters and assessed the association between visual dysfunction and morphologic changes in the retina of 24 patients with AD. Parameters corresponding to CSV at different spatial frequencies were altered in AD patients compared with healthy subjects. Moreover, contrast sensitivity was the most affected parameter in our study and correlated with most of the structural data. Color vision was measured with two different tests, the Farnsworth and L´Anthony 15 D tests. These tests provide more sensitive information than other tests for differentiating subjects with severe color vision loss from those with milder color defects or normal color vision, and can also be used to evaluate acquired loss of color vision. Both tests are color arrangement tests; however L´Anthony color test is less saturated and therefore more suitable for detecting mild color anomalies. In our study, the AC CCI corresponding to both the Farnsworth and L´Anthony test was significantly altered in AD

patients, corresponding to a worse arrangement of color caps in patients compared to controls. Farnsworth S-Index was also altered in AD subjects suggesting a mild tendency toward protanomaly.

Previous studies demonstrated alterations in sensory perception and visual processing in patients with AD [2,21-23]. Contrast sensitivity (evaluated using the Sloan chart, computerized LogMAR ETDRS chart, frequency-doubling technology, and backward masking tests) disturbances are present even in the early stages of AD [24-26]. Color vision, especially in the blue tone, is altered in these patients [27]. Disease pathology in the pre-cortical visual system is a possible mechanism underlying these visual impairments observed in AD patients. The parvo- and magnocellular ganglion cells are located in the RGC layer and result in two different pathways which identify color and contrast at different frequencies [28]. The parvocellular RGCs are smaller and more numerous than other RGCs and result in the parvocellular pathway, which is specialized to identify patterns and color and is more sensitive to lower temporal frequency and higher spatial frequency. The magnocellular pathway originates in the magnocellular RGCs (larger and less numerous) and is more sensitive to higher temporal frequency and lower spatial frequency. A third set of cells, the koniocellular RGCs (larger than parvocellular RGCs), receives input from shortwave cones that are sensitive to blue-yellow tones [28-30]. Previous studies (histologic, electroretinogram, and imaging studies) of these pathways suggest that the general loss of magnocellular and parvocellular cells is likely to be an important contributory mechanism for visual impairment in AD [31]. Deficits specific to the magnocellular pathway have been identified in individuals with AD in the primary visual cortex and in brain areas devoid of plaques and neurofibrillary tangles [32]. However, dendritic atrophy and loss of retinal ganglion cells have also been observed in the retina of a mouse model of AD, where the accumulation of beta-amyloid in the inner retinal layers was observed. This beta-amyloid deposits may be responsible for the depletion of parvo- and magnocellular cells in the retina. The loss of RGC was recently identified as the ultimate responsible for visual impairment in patients suffering from another neurodegenerative process, multiple sclerosis [33]. Thus, a similar process could be the cause of contrast and color deficiencies in AD individuals.

Previous studies suggested that peripapillary RNFL,[34] macular thickness and the macular inner retinal layers are affected in AD patients [5,7]. Macular RNFL thickness and total macular volume measured by OCT have highly significant sensitivity and specificity for differentiating mild AD patients from healthy subjects [7]. RNFL thickness has also been correlated with AD severity, and is predictive of axonal damage in these patients [35]. Structural measurements, especially those corresponding to the macular area, were affected in our patients, consistent with previous reports [5,7]. Additionally and according to previous published research, our patients did not present significant reduction in foveal thickness.[6, 35, 36] Patients with AD present with decreased visual acuity and contrast sensitivity vision, which depend on large extent on the foveal area. However, visual dysfunction in AD patients seems more likely to be caused by a general loss of the retinal ganglion cells,[36, 37] which ultimately leads to the alteration of the visual pathways responsible for contrast sensitivity and color vision.[31, 36] With the introduction of new OCT software for segmentation and analysis of the different retinal layers, recent studies have focused on the macular ganglion cell layer as responsible for the visual changes in AD and as a possible biomarker for disease progression and neural damage.

To the best of our knowledge, there are no previously published reports of a correlation between structural changes in the retina of AD patients and alterations observed in contrast sensitivity and color vision. We found that contrast sensitivity results (with both CSV-1000E and Pelli-Robson tests) and color vision (with both L´Anthony and Farnsworth tests) were significantly associated with structural changes, especially macular thickness (not with foveal thickness). Further studies including the analysis of the retinal ganglion cell complex would be needed to better understand the physiopathology of visual impairment in AD patients, and to determine the usefulness of visual function tests in combination with OCT measurements as a biomarker of severity and progression in AD.

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All subjects gave detailed consent to participate in this study, which was conducted in accordance with the guidelines established by the Ethics Committee of the Miguel Servet Hospital and based on the principles of the Declaration of Helsinki.

The authors disclose no conflict of interest.

The authors have no proprietary interests to disclose.

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406 **Titles and legends to figures**

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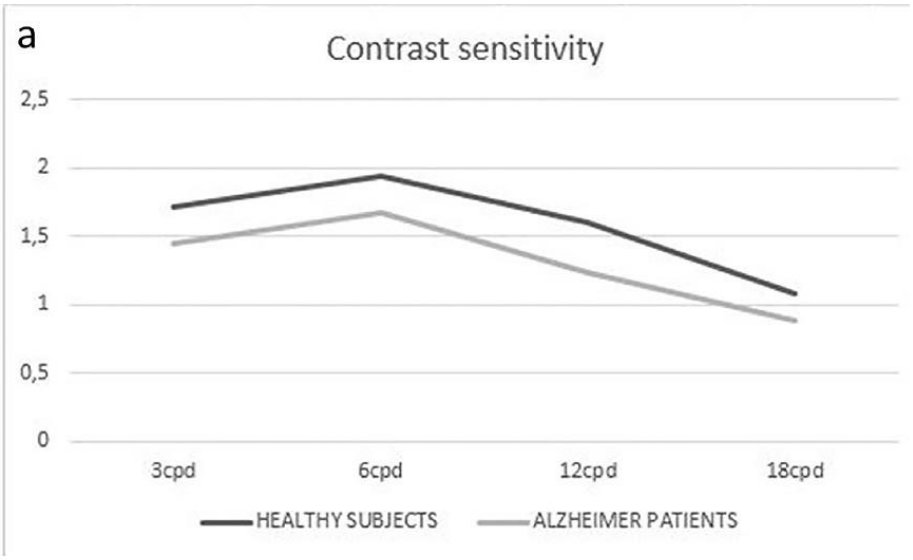
408 **Fig. 1**: (a) Contrast sensitivity curve in patients with Alzheimer´s disease and healthy controls. CPD,
409 cycles per degree. (b) Structural parameters measured by OCT in patients with Alzheimer´s disease and
410 healthy controls. RNFL, retinal nerve fiber layer.



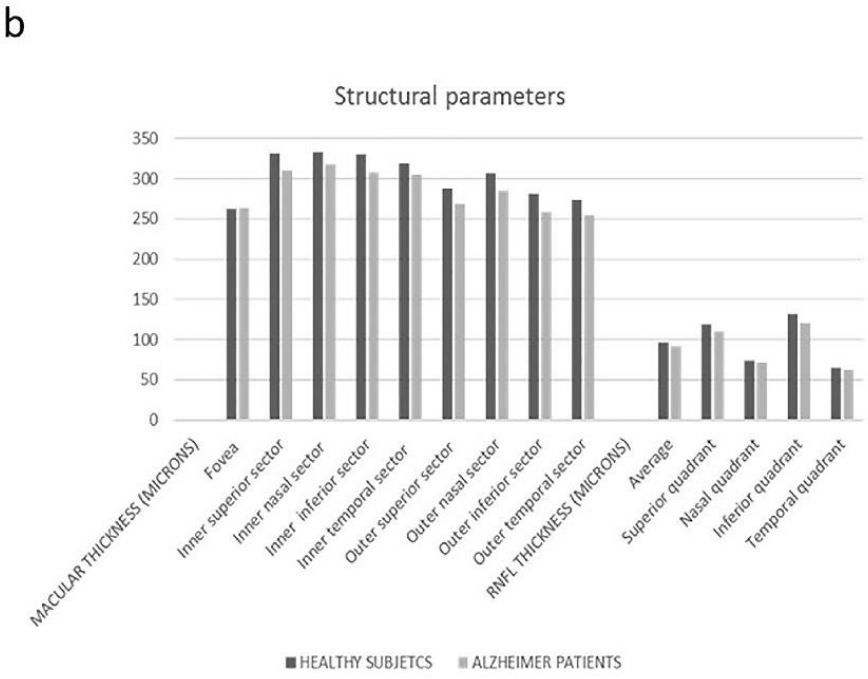


Table 3: Correlation between structural parameters (macular and retinal nerve fiber layer thickness) and contrast sensitivity vision (CSV) evaluated with Pelli-Robson and CSV-1000E tests in patients with Alzheimer´s disease. Data in bold type correspond to statistically significant correlations (p value <0.05). Asterisk indicates a significant difference by Pearson's test after Bonferroni correction for multiple tests ($p \leq 0.0045$ for macular thickness; $p \leq 0.010$ for RNFL thickness).

	CSV-1000E TEST								PELLI ROBSON	
	6 CPD	*P*	*9 CPD*	*P*	*12 CPD*	*P*	*18 CPD*	*P*	R	P
MACULAR THICKNESS										
Fovea	0.043	0.770	0.19	0.900	0.45	0.760	0.14	0.330	-0.02	0.881
Inner superior sector	0.43	**0.030**	0.35	**0.010**	0.44	**0.020**	0.43	**0.030**	0.45	**<0.001***
Inner nasal sector	0.48	**0.010**	0.37	**0.010**	0.44	**0.020**	0.43	**0.040**	0.48	**<0.001***
Inner inferior sector	0.34	**0.020**	0.28	0.050	0.40	**<0.001***	0.38	**0.010**	0.47	**<0.001***
Inner temporal sector	0.42	**0.030**	0.23	0.110	0.36	**0.010**	0.44	**<0.001***	0.43	**<0.001***
Outer superior sector	0.50	**<0.001***	0.36	**0.010**	0.57	**<0.001***	0.50	**<0.001***	0.65	**<0.001***
Outer nasal sector	0.50	**<0.001***	0.50	**<0.001***	0.49	**<0.001***	0.50	**<0.001***	0.58	**<0.001***
Outer inferior sector	0.17	0.240	0.27	0.050	0.38	**<0.001***	0.32	**0.030**	0.38	**<0.001***
Outer temporal sector	0.47	**<0.001***	0.22	0.120	0.50	**<0.001***	0.39	**<0.001***	0.66	**<0.001***
Average thickness	0.43	0.090	0.21	0.420	0.56	**0.030**	0.53	0.050	0.63	**<0.001***
Macular volume	0.48	0.050	0.34	0.150	0.30	0.240	0.27	0.320	0.54	**0.010**
RNFL THICKNESS										
Average	0.41	**0.040**	0.33	**0.020**	0.43	**<0.001***	0.40	**<0.001***	0.46	**<0.001***
Superior quadrant	0.31	**0.030**	0.23	**0.040**	0.46	**<0.001***	0.41	**<0.001***	0.46	**<0.001***
Nasal quadrant	0.37	**0.010***	0.19	0.200	0.20	0.190	0.11	0.440	0.37	**0.010***
Inferior quadrant	0.22	0.140	0.11	0.440	0.23	0.120	0.23	0.110	0.22	0.140
Temporal quadrant	0.23	0.110	0.35	**0.010***	0.26	0.740	0.35	**0.010***	0.12	0.410

Abbreviations: RNFL, retinal nerve fiber layer; CSV, contrast sensitivity vision

Table 4: Correlation between structural parameters (macular and retinal nerve fiber layer thickness) and visual acuity in different contrast levels in patients with Alzheimer´s disease. Data in bold type correspond to statistically significant correlations (p value <0.05). Asterisk indicates a significant difference by Pearson's test after Bonferroni correction for multiple tests ($p \leq 0.0045$ for macular thickness; $p \leq 0.010$ for RNFL thickness).

STRUCTURAL PARAMETERS	AV ETDRS 100%	p	AV ETDRS 2.5%	p	AV ETDRS 1.25%	p
MACULAR THICKNESS						
Fovea	0.21	0.150	0.22	0.140	0.17	0.230
Inner superior sector	0.25	0.090	0.19	0.190	0.30	**0.040**
Inner nasal sector	0.18	0.210	0.12	0.410	0.18	0.210
Inner inferior sector	0.33	**0.020**	0.21	0.150	0.26	0.070
Inner temporal sector	0.16	0.280	0.09	0.540	0.19	0.200
Outer superior sector	0.46	**<0.001***	0.36	**0.010**	0.45	**<0.001***
Outer nasal sector	0.40	**<0.001***	0.25	0.080	0.37	**0.010**
Outer inferior sector	0.42	**<0.001***	0.17	0.240	0.27	0.060
Outer temporal sector	0.40	**<0.001***	0.37	**0.010**	0.40	**<0.001***
Average thickness	0.24	0.360	0.66	**<0.001***	0.60	**0.010**
Macular volume	0.34	0.160	0.41	0.080	0.34	0.160
RNFL THICKNESS						
Average	-0.42	**<0.001***	-0.58	**<0.001***	-0.56	**<0.001***
Superior quadrant	-0.39	**<0.001***	-0.50	**<0.001***	-0.45	**<0.001***
Nasal quadrant	-0.10	0.480	-0.32	**0.020**	-0.33	**0.020**
Inferior quadrant	-0.37	**0.010***	-0.46	**<0.001***	-0.44	**<0.001***
Temporal quadrant	-0.25	0.080	-0.21	0.140	-0.28	0.050

Abbreviations: RNFL, retinal nerve fiber layer; VA, visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study.

Supplementary table 1: Correlation between structural parameters (macular and retinal nerve fiber layer thickness) and contrast sensitivity vision (CSV) evaluated with Pelli-Robson and CSV-1000E tests in healthy controls. Data in bold type correspond to statistically significant correlations (p value <0.05). Asterisk indicates a significant difference by Pearson's test after Bonferroni correction for multiple tests ($p \leq 0.0045$ for macular thickness; $p \leq 0.010$ for RNFL thickness).

	CSV-1000E TEST								PELLI ROBSON	
	6 CPD	*P*	*9 CPD*	*P*	*12 CPD*	*P*	*18 CPD*	*P*	R	P
MACULAR THICKNESS										
Fovea	0.03	0.560	0.22	0.873	0.46	0.434	0.20	0.378	-0.02	0.321
Inner superior sector	0.38	**0.021**	0.35	**0.011**	0.43	**0.016**	0.41	**0.023**	0.46	**<0.001***
Inner nasal sector	0.46	**0.007**	0.36	**0.006**	0.47	**0.034**	0.41	**0.011**	0.49	**<0.001***
Inner inferior sector	0.37	**0.013**	0.31	**0.041**	0.44	**<0.001***	0.39	**0.003***	0.44	**<0.001***
Inner temporal sector	0.44	**0.024**	0.26	0.097	0.38	**0.007**	0.40	**<0.001***	0.40	**<0.001***
Outer superior sector	0.51	**<0.001***	0.38	**0.006**	0.55	**<0.001***	0.54	**<0.001***	0.60	**<0.001***
Outer nasal sector	0.54	**<0.001***	0.44	**0.001***	0.47	**<0.001***	0.52	**<0.001***	0.56	**<0.001***
Outer inferior sector	0.21	0.156	0.30	0.112	0.37	**<0.001***	0.36	**0.023**	0.40	**<0.001***
Outer temporal sector	0.49	**<0.001***	0.26	0.079	0.51	**<0.001***	0.33	**<0.001***	0.62	**<0.001***
Average thickness	0.38	**0.040**	0.20	0.329	0.48	**0.023**	0.50	**0.004***	0.58	**<0.001***
Macular volume	0.45	**0.048**	0.30	0.098	0.35	0.134	0.31	0.221	0.58	**<0.001***
RNFL THICKNESS										
Average	0.43	**0.032**	0.31	**0.024**	0.50	**<0.001***	0.39	**<0.001***	0.44	**<0.001***
Superior quadrant	0.35	**0.021**	0.25	**0.038**	0.45	**<0.001***	0.45	**<0.001***	0.50	**<0.001***
Nasal quadrant	0.39	**0.009***	0.22	0.316	0.31	0.059	0.18	0.843	0.41	**<0.001***
Inferior quadrant	0.27	0.343	0.27	0.239	0.19	0.088	0.22	0.246	0.26	0.079
Temporal quadrant	0.29	0.098	0.31	**0.010***	0.28	0.348	0.40	**<0.001***	0.11	0.125

Abbreviations: RNFL, retinal nerve fiber layer; CSV, contrast sensitivity vision

Supplementary table 2: Correlation between structural parameters (macular and retinal nerve fiber layer thickness) and visual acuity in different contrast levels in healthy controls. Data in bold type correspond to statistically significant correlations (p value <0.05). Asterisk indicates a significant difference by Pearson's test after Bonferroni correction for multiple tests (p≤0.0045 for macular thickness; p≤0.010 for RNFL thickness).

STRUCTURAL PARAMETERS	AV ETDRS 100%	p	AV ETDRS 2.5%	p	AV ETDRS 1.25%	P
MACULAR THICKNESS						
Fovea	0.23	0.215	0.19	0.228	0.20	0.148
Inner superior sector	0.26	0.217	0.22	0.096	0.26	**0.013**
Inner nasal sector	0.20	0.287	0.15	0.550	0.22	0.380
Inner inferior sector	0.29	**0.034**	0.26	0.349	0.31	0.098
Inner temporal sector	0.17	0.203	0.11	0.431	0.25	0.334
Outer superior sector	0.46	**<0.001***	0.40	**<0.001***	0.47	**<0.001***
Outer nasal sector	0.42	**<0.001***	0.26	**0.021**	0.40	**<0.001***
Outer inferior sector	0.39	**<0.001***	0.21	0.137	0.33	**0.045**
Outer temporal sector	0.44	**<0.001***	0.40	**<0.001***	0.42	**<0.001***
Average thickness	0.29	0.457	0.59	**<0.001***	0.54	**<0.001***
Macular volume	0.37	0.401	0.44	0.111	0.29	0.160
RNFL THICKNESS						
Average	-0.39	**<0.001***	-0.60	**<0.001***	-0.57	**<0.001***
Superior quadrant	-0.40	**<0.001***	-0.47	**<0.001***	-0.48	**<0.001***
Nasal quadrant	-0.15	0.099	-0.36	**<0.001***	-0.40	**<0.001***
Inferior quadrant	-0.41	**0.010***	-0.51	**<0.001***	-0.46	**<0.001***
Temporal quadrant	-0.24	0.076	-0.25	0.090	-0.25	0.110

Abbreviations: RNFL, retinal nerve fiber layer; VA, visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study.

BMJ Open

Visual dysfunction and its correlation with retinal changes in patients with Parkinson's disease: an observational cross-sectional study

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Study design and setting: an observational cross-sectional study, carried out at Miguel Servet University Hospital, Zaragoza, Spain

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ABSTRACT

Objectives: To evaluate visual dysfunction and its correlation with structural changes in the retina in patients with Parkinson's disease (PD).

Methods: Patients with PD (n=37) and controls (n=37) were included in an observational cross-sectional study, and underwent visual acuity (VA), colour vision (using the Farnsworth and Lanthony desaturated D15 colour tests) and contrast sensitivity vision (CSV; using the Pelli-Robson chart and CSV 1000E test) evaluation to measure visual dysfunction. Structural measurements of the retinal nerve fibre layer (RNFL), and macular and ganglion cell layer (GCL) thicknesses, were obtained using spectral domain optical coherence tomography (SD-OCT). Comparison of obtained data, and correlation analysis between functional and structural results were performed.

Results: VA (in all different contrast levels) and all CSV spatial frequencies were significantly worse in patients with PD than in controls. Colour vision was significantly affected based on the Lanthony colour test. Significant GCL loss was observed in the minimum GCL+inner plexiform layer. A clear tendency towards a reduction in several macular sectors (central, outer inferior, outer temporal and superior (inner and outer)) and in the temporal quadrant of the RNFL thickness was observed, although the difference was not significant. CSV was the functional parameter most strongly correlated with structural measurements in PD. Colour vision was associated with most GCL measurements. Macular thickness was strongly correlated with macular volume and functional parameters ($r>0.70$, $p<0.05$).

Conclusions: Patients with PD had visual dysfunction that correlated with structural changes evaluated by SD-OCT. GCL measurements may be reliable indicators of visual impairment in patients with PD.

Strengths and limitations of this study

- This study includes a complete assessment of visual function parameters and the evaluation of different retinal structures using spectral domain optical coherence tomography in patients with Parkinson's disease.
- There are only two other published articles evaluating the association between visual dysfunction and morphological parameters. Results provided by these previous studies differ from our results, possibly due to different measurement methods and sample size.
- Colour vision in our study was assessed by Lanthony and Farnsworth D15 colour tests, which may provide more specific information about colour deficiencies. These tests are not commonly used to evaluate colour deficiencies in patients with PD.
- An important limitation to our study is the inclusion of one randomly selected eye per patient. The incorporation of both eyes of each patient in Parkinson's disease studies is usually recommended due to asymmetrical involvement of the retina in this process.

INTRODUCTION

Foveal vision alterations are associated with Parkinson's disease (PD), and seem to be caused by dysfunction of the intraretinal dopaminergic circuitry and final retinal output to the brain.[1] Recent studies demonstrated retinal thinning in patients with PD compared with healthy participants.[2–5]

Several studies report a correlation between functional disability and axonal loss observed in the optic nerve in multiple sclerosis, another neurodegenerative process.[6 7] Patient with PD are also reported to have decreased contrast sensitivity and colour vision, and altered visual evoked potentials.[1 8–13] To our knowledge, however, very few studies have assessed visual dysfunction in PD and its correlation with morphological parameters.[14 15]

In the present study, we evaluated visual acuity (VA), using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart,

contrast sensitivity vision (CSV), using the CSV-1000E test and Pelli-Robson chart, and colour vision, using the Farnsworth and Lanthony tests in patients with PD and healthy controls, to examine the association between visual dysfunction and morphological parameters.

MATERIALS AND METHODS

Thirty-seven eyes of 37 patients with definite PD and 37 eyes of 37 age-matched and sex-matched healthy individuals were recruited for an observational cross-sectional study. The study was performed at Miguel Servet University Hospital in Zaragoza, Spain, and all evaluations were performed in one single visit. All procedures adhered to the tenets of the Declaration of Helsinki, and all participants provided informed consent to participate in the study.

The diagnosis of PD was based on standard clinical and neuroimaging criteria.[16] Information about disease severity was assessed using the Hoehn-Yahr scale[17] and the Unified Parkinson Disease Rating Scale part III score (UPDRS III).[18] Disease duration and treatment were recorded. Exclusion criteria were the presence of significant refractive errors (>5 dioptres of spherical equivalent refraction or 3 dioptres of astigmatism); intraocular pressure≥21 mm Hg; media opacification; concomitant ocular diseases, including history of glaucoma or retinal pathology; and systemic conditions that could affect the visual system. The healthy controls had no history and no evidence of ocular or neurological disease of any nature; their best-corrected VA (BCVA) was >20/30 based on the Snellen scale.

All participants underwent a complete neuro-ophthalmic evaluation, which included pupillary, anterior segment and funduscopic examination. Visual function was assessed by evaluating BCVA, using an ETDRS chart, CSV, using the CVS-1000E test and Pelli-Robson chart, and colour vision, using the Farnsworth desaturated D15 and Lanthony desaturated D15 tests. Structural analysis of the retina was performed using spectral domain (SD) optical coherence tomography (OCT) with a Cirrus high definition OCT (Carl Zeiss Meditec Inc, Dublin, California, USA), which included three different protocols: macular protocol (for macular thickness analysis), retinal nerve fibre layer (RNFL) protocol and ganglion cell protocol (for individual analysis of this layer).

LogMAR VA was evaluated at three different contrast levels: 100% (HCVA, using ETDRS chart), 2.50% and 1.25% (LCVA, using Low-Contrast Sloan Letter Charts, Precision Vision, LaSalle, Illinois, USA), with the percentage indicating the level of contrast, that is, 100% representing black letters over white background and 1.25% representing light grey letters over white background. All measurements were obtained under monocular vision and controlled lighting conditions with best correction.

Contrast sensitivity provides more complete information about visual function than do VA tests. CSV was evaluated in our patients, using the Pelli-Robson chart and the CVS-1000E test. The Pelli-Robson chart comprises horizontal lines of capital letters organised into groups of three (triplets), with two triplets per line. Within each triplet, all letters have the same contrast. The contrast decreases from one triplet to the next, even within each line. All patients were evaluated under both monocular and binocular vision at a distance of 1 m from the chart, and under controlled fotopic conditions (85 cd/m^2). The score corresponding to the last triplet of letters seen by the patient was recorded.

The CSV-1000E instrument is used worldwide for standardised CSV and glare testing. All patients were evaluated at a distance of 2.5 m from the chart, under monocular vision, at four different spatial frequencies (3, 6, 12 and 18 cycles per degree (cpd)). The chart comprises four rows with 17 circular patches each. The patches present a grating that decreases in contrast, moving from left to right across the row. The patient indicates whether the grating appears in the top patch or the bottom patch for each column. Each contrast value for each spatial frequency was transformed into a logarithmic scale according to standardised values.

Colour vision was assessed using the Color Vision Recorder (CVR) program. CVR software analyses chromatic discrimination by classification of colours. The program includes the classic test of Farnsworth 100-hue (FM-100), Farnsworth–Munsell D15 and Lanthony D15. All patients in the study were evaluated using the Farnsworth–Munsell D15 and Lanthony D15 protocols, and different output parameters such as the confusion index (C-index), the colour confusion index (CCI), the confusion angle (Conf Ang) and the scatter index (S-index) were recorded.[19 20] The tests were performed under monocular vision.

Structural measurements of the retina were obtained using the Cirrus OCT device. The same experienced operator performed all scans and did not apply manual correction to the OCT output. We used an internal fixation target because it provides the highest reproducibility and rejected poor quality scans prior to data analysis. The Cirrus OCT macular cube 512×128 protocol provides a macular volume measure and retinal thickness values for nine areas. These areas include a central 1 mm circle representing the fovea, and inner and outer rings measuring 3 and 6 mm in diameter, respectively. The inner and outer rings are divided into four quadrants each. The Cirrus OCT optic disc protocol generates images with 200 linear scans enabling analysis of the RNFL of a 6 mm^3 area around the optic nerve. For each scan series of RNFL measurements, we assessed the average, superior, inferior, temporal and nasal thickness. Cirrus segmentation analysis for retinal layers also provides measurements of the ganglion cell layer (GCL) thickness, evaluating six areas of the macular cube (superior, superonasal, inferonasal, inferior, inferotemporal and superotemporal sectors), and measurements of the average and minimum GCL plus the inner

Table 1 Epidemiological and disease characteristics of patients with PD and healthy participants, and statistical significance (p)

Parameter	Controls	Parkinson disease	p Value
Number of eyes (n)	37	37	–
Age, years, range	68 (60–76)	69 (58–74)	0.361
Men:women (% of men)	24:13 (64.9)	23:14 (62.2)	0.441
Intraocular pressure	15.58 (2.71)	15.12 (2.98)	0.720
Disease duration, years, mean (SD)	–	13.2 (5.77)	–
Hoehn-Yahr, mean (SD)	–	2.7 (0.64)	–
UPDRS III, mean (SD)	–	25.06 (8.24)	–

UPDRS III, Unified Parkinson Disease Rating Scale part III.

plexiform layer (GCL+IPL) value of a set of 360 spokes, where each average represents the mean number of the pixels along the spoke that lies within the measurement annulus. The minimum is selected because the thinnest portion of the GCL+IPL in the perifoveal region is considered to indicate damage to the ganglion cells.

All data analyses were performed using SPSS software V.20.0 (SPSS Inc, Chicago, Illinois, USA). Owing to the parametric distribution of the data, differences between evaluations of patients with PD and healthy participants were compared using Student's t test. To avoid a high false-positive rate, the Bonferroni correction for multiple comparisons was calculated. The level of significance for each variable was established based on Bonferroni calculations.

The linear correlation between structural and functional parameters was determined using Pearson's correlation coefficient. Values of $p<0.05$ were considered to indicate a significant correlation. Each eye was considered separately, and one eye from each patient was randomly selected for analysis.

RESULTS

Thirty-seven patients with PD and 37 healthy controls were included in the study. The mean age of the patients with PD was 69 years (range 58–74 years) and the mean age of the healthy controls was 68 years (range 60–76 years). Age (p=0.361), sex (p=0.441) and intraocular pressure (p=0.720) did not differ significantly between healthy controls and patients with PD. Mean time from diagnosis of PD was 13.2 years. The median Hoehn-Yahr stage was 2.7, and the stage of PD based on the UPDRS III was 25.06 (range 7–39; table 1).

Treatment was divided into three different categories: 'drugs that enhance dopamine levels' (carbidopa, levodopa and rasagiline), 'dopaminergic drugs' (pramipexole, ropirinol and rotigotine) and 'other'(amitriptiline, propranolol and clonazepam). 'Drugs that enhance dopamine levels' was the most prescribed category (89% of patients), and combination therapy with levodopa and carbidopa was the most frequent treatment (44%). Sixty-four per cent of treatments were categorised as 'dopaminergic', most of which were used in combination with drugs included in the previous category. A small percentage of patients (9%) were prescribed drugs with no dopaminergic effects.

Functional parameters

Patients with PD had a lower BCVA at all three contrast levels of the ETDRS chart compared with the controls (0.18±0.26 in patients vs −0.065±0.9 in controls at 100%, p=0.001; 0.59±0.21 vs 0.44±0.13 at 2.50%, p=0.010; and 0.61±0.23 vs 0.58±0.16 at 1.25%, p=0.009). CSV was affected in patients at all four spatial frequencies of the CSV 1000E chart (3, 6, 12 and 18 cpd) when analysed based on the number of correct localised gratings (p=0.001, p<0.001, p<0.001 and p=0.004, respectively). The Pelli-Robson results also revealed a significant reduction in CSV in patients with PD (1.71 in patients vs 1.89 in controls, p=0.02). Colour vision (Conf Angle in Lanthony test) was also affected in PD. The results are shown in table 2.

Structural parameters

Based on Bonferroni corrections, OCT measurements indicated a significant difference in the minimum GCL +IPL value (80.18±6.19 vs 82.45±3.60 µm; p=0.005). However, we observed a clear tendency towards a reduction in superior macular sectors in the outer inferior, outer temporal and central macular thickness in patients with PD compared with controls: the p value for these variables was <0.05 but did not meet Bonferroni significance (results are shown in table 2). The segmentation analysis revealed a tendency towards reduced GCL in patients with PD in the superior (81.64±7.08 µm in patients vs 84.55±4.32 µm in controls; p=0.032) and superonasal sectors (81.04±7.23 vs 85.28±4.78 µm; p=0.029); and the RNFL was reduced in the temporal quadrant in patients with PD (table 3). These parameters, however, did not meet the level of significance established by Bonferroni correction.

Correlation between functional and structural parameters

CSV was the functional parameter most frequently associated with structural measurements in PD. The Pelli-Robson CSV results correlated with GCL thickness in all sectors, although the association was not strong (r<0.5). The superonasal (r=0.40, p=0.010), inferonasal (r=0.40, p=0.010), inferior (r=0.43, p=0.005),

Table 2 Mean and SD of visual functional parameters in healthy controls and participants with Parkinson's disease

	Healthy controls		Parkinson's disease patients		
	Mean	SD	Mean	SD	Significance (p)
VA ETDRS 100	−0.06	0.096	0.18	0.26	0.001*
VA ETDRS 2.5	0.44	0.13	0.59	0.22	0.010*
VA ETDRS 1.25	0.58	0.16	0.62	0.23	0.009*
Pelli-Robson	1.89	0.11	1.71	0.17	0.002*
CSV 1000 3 cpd	1.72	0.16	1.49	0.35	0.001*
CSV 1000 6 cpd	1.94	0.13	1.62	0.34	<0.001*
CSV 1000 12 cpd	1.62	0.17	1.26	0.41	<0.001*
CSV 1000 18 cpd	1.11	0.22	0.73	0.34	0.004*
Farnsworth AC CCI	1.11	0.22	0.73	0.34	0.851
Farnsworth C-index	1.10	0.20	1.24	0.42	0.093
Farnsworth CCI	1.07	0.12	1.14	0.24	0.110
Farnsworth Conf Angle	63.90	11.15	65.84	7.49	0.392
Farnsworth S-index	1.56	0.22	1.64	0.39	0.278
Farnsworth time	78.67	28.96	82.91	33.10	0.616
Lanthony AC CCI	1.05	0.19	1.02	0.18	0.489
Lanthony C-index	1.43	0.39	1.64	0.53	0.058
Lanthony CCI	1.30	0.23	1.44	0.37	0.066
Lanthony Conf Angle	62.31	14.74	71.91	9.25	0.002*
Lanthony S-index	1.69	0.43	1.95	0.48	0.020
Lanthony time	77.14	25.99	84.09	39.31	0.431

The asterisk indicates those values with statistical significance after Bonferroni correction for multiple tests (p<0.0125 for VA ETDRS 100, 2.50 and 1.25; p<0.0125 for Pelli-Robson and CSV 1000E measurements; p<0.0083 for Farnsworth and Lanthony tests).
AC CCI, age-corrected colour confusion index; CCI, colour confusion index; C-index, confusion index; Conf Angle, confusion angle; cpd, cycles per degree; ETDRS, Early Treatment Diabetic Retinopathy Study; PD, Parkinson's disease; S-index, scatter index; VA, visual acuity.

superotemporal sector (r=0.43, p=0.006) and average GCL+IPL (r=0.45, p=0.004) values had the highest correlations. The Pelli-Robson results also correlated with the thickness in different sectors of the RNFL (average, superior and inferior sectors). Measurements with the CSV 1000E at different spatial frequencies correlated significantly with most GCL measurements. The superonasal (r=0.40, p=0.013) and superotemporal (r=0.44, p=0.006) thickness, average GCL+IPL thickness (r=0.40, p=0.012) and the minimum GCL+IPL (r=0.40, p=0.011) at a spatial frequency of 6 cpd; and the superotemporal (r=0.41, p=0.01) thickness and the minimum GCL+IPL thickness (r=0.43, p=0.006) at a spatial frequency of 18 cpd, had the strongest correlations between CSV 1000E and GCL thickness. Spatial frequencies of 6 and 18 cpd were strongly correlated with average macular thickness (r=0.79, p=0.012; r=0.77, p=0.016, respectively) and macular volume (r=0.78, p=0.013; r=0.78, p=0.014, respectively, figure 1).

Colour vision assessed by the Lanthony test was also associated with the structural parameters: the C-index and CCI results were significantly correlated with all outer macular parameters and most of the GCL measurements (see table 4). A significant association between colour vision and the RNFL parameters was only found in isolated sectors (see table 4).

The strongest correlation was between the average macular thickness and macular volume, and the Lanthony CCI, C-index and S-index results. No significant correlations were found between the Farnsworth's test parameters and structural measurements.

The VA ETDRS results correlated strongly with average macular thickness and macular volume (see table 5, figures 2 and 3). There were significant but mild associations between the GCL parameters and VA at 100% (superonasal, inferonasal and average GCL+IPL thickness, r=−0.38, p=0.016; r=−0.35, p=0.016; and r=0.35, p=0.029, respectively) and 2.50% (superonasal sector, r=−0.36, p=0.023).

There was a significant correlation between Hoehn-Yahr score and VA contrast level 2.50% (r=0.48, p=0.040), and CS measured with CSV 1000 at a space frequency of 12 cpd (r=−0.59, p=0.038). No correlations were detected between structural and disease severity parameters.

DISCUSSION

In the present study, we evaluated the visual function parameters, and assessed the association between visual dysfunction and morphological changes in the retina of 37 patients with PD. Parameters corresponding to VA at different contrast levels, and all CSV test results, were altered in patients with PD in comparison with healthy participants, prior to and after statistical correction for multiple tests. Moreover, contrast sensitivity was the most affected parameter in our study and correlated with most of the structural data. Colour vision was measured

Table 3 Mean and SD of structural parameters (RNFL, GCL and macular thicknesses) obtained with the Cirrus HD optical coherence tomography device in healthy controls and participants with Parkinson's disease

	Controls		Parkinson's disease		
Structural parameters	**Mean**	**SD**	**Mean**	**SD**	**p Value**
Macular measurements					
Central macular thickness	254.75	17.903	248.96	17.765	0.028
Inner superior macular thickness	327.34	13.094	325.73	19.329	0.019
Inner nasal macular thickness	328.52	13.263	325.45	17.098	0.091
Inner inferior macular thickness	326.14	13.179	324.82	17.921	0.106
Inner temporal macular thickness	315.90	13.615	312.82	15.760	0.945
Outer superior macular thickness	284.76	9.418	279.44	17.981	0.008
Outer nasal macular thickness	302.41	12.167	299.18	17.064	0.074
Outer inferior macular thickness	277.79	10.755	273.76	16.798	0.045
Outer temporal macular thickness	271.52	10.992	266.23	18.987	0.013
GCL thickness					
Superior	84.55	4.323	81.61	7.087	0.032
Superonasal	85.28	4.780	81.04	7.234	0.029
Inferonasal	84.66	5.314	81.82	7.521	0.135
Inferior	84.34	5.052	81.91	6.252	0.389
Inferotemporal	85.79	4.003	83.73	4.860	0.233
Temporal	83.76	3.324	82.27	5.312	0.069
Average IPL+GCL	84.83	4.071	82.73	6.230	0.095
Minimum IPL+GCL	82.45	3.601	80.18	6.194	0.005*
RNFL thickness					
Average	96.17	6.714	94.88	11.505	0.105
Superior	117.90	10.965	118.68	16.861	0.115
Nasal	73.59	12.724	72.40	15.182	0.345
Inferior	128.14	14.060	123.20	22.907	0.075
Temporal	64.97	8.218	61.48	10.553	0.027

The asterisk indicates those values with statistical significance after Bonferroni correction for multiple tests ($p<0.0055$ for macular measurements; $p<0.0062$ for ganglion cell measurements and $p<0.01$ for RNFL measurements).
GCL, ganglion cell layer; HD, high definition; IPL, inner plexiform layer; RNFL, retinal nerve fibre layer.

using two different tests, the Farnsworth and Lanthony 15 D tests. These tests provide information for differentiating participants with severe loss of colour vision from those with milder colour defects or normal colour vision, and also can be used to evaluate acquired loss of colour vision. In our study, only the Lanthony Conf Ang was significantly altered in patients with PD. The Lanthony test is less saturated than the Farnsworth colour test, thus it is designed to detect more subtle colour deficiencies. Our patients performed worse than controls in both tests (higher C-index and S-index, reaching ranges similar to protanomalies) although these differences did not reach statistical significance as established by Bonferroni correction. Lanthony S-index p value was <0.05, indicating that our patients had a (subtle) tendency to protanomaly (S-index of 1.95).

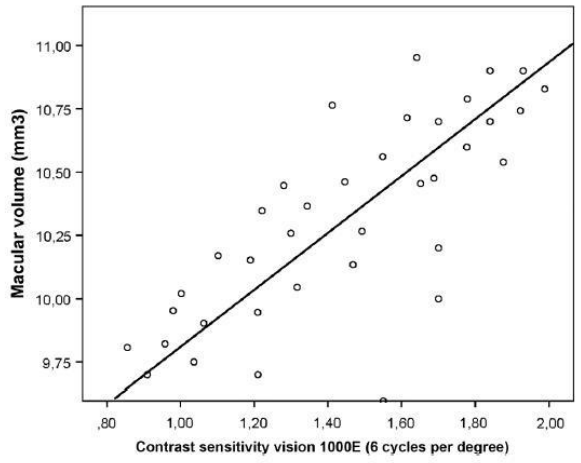

Figure 1 Correlation between the average macular thickness and contrast sensitivity vision as measured with the CSV 1000E test, at a spatial frequency of 6 cycles per degree, in patients with Parkinson's disease. CSV, contrast sensitivity vision.

One important limitation of this study is that only one eye was tested per person. Some recent studies suggest asymmetrical involvement of the retina in PD and recommend the incorporation of both eyes of each patient in the study.[21] Thus, the diagnostic yield in this study may have been lowered by including a potentially lesser affected eye. In a similar way, including a randomly selected eye could be inappropriate for other neurological conditions, for example, a tumour compressing one optic nerve. However, incorporating both eyes of a patient may sometimes be controversial since minimum symmetric structural and functional alterations could

Table 4 Correlation between macular and GCL structural measurements and colour vision evaluated with Lanthony colour test in patients with Parkinson's disease

	Lanthony colour test					
	C-index	p Value	CCI	p Value	S-index	p Value
Macular thickness						
Central	−0.019	0.905	−0.059	0.716	−0.017	0.915
Inner superior	−0.146	0.369	−0.119	0.463	−0.167	0.302
Inner nasal	−0.055	0.735	−0.044	0.788	−0.040	0.807
Inner inferior	−0.073	0.654	−0.064	0.697	−0.074	0.649
Inner temporal	−0.049	0.764	−0.031	0.850	−0.126	0.439
Outer superior	**−0.377**	0.017	**−0.380**	0.015	−0.271	0.090
Outer nasal	**−0.341**	0.031	**−0.323**	0.042	−0.310	0.051
Outer inferior	**−0.360**	0.022	**−0.353**	0.025	**−0.375**	0.017
Outer temporal	**−0.360**	0.023	**−0.361**	0.022	**−0.350**	0.027
Macular average	**−0.691**	0.019	**−0.657**	0.028	**−0.709**	0.015
Macular volume	**−0.686**	0.020	**−0.647**	0.032	**−0.709**	0.015
GCL thickness						
Superior	**−0.380**	0.015	**−0.369**	0.019	−0.287	0.072
Superonasal	**−0.383**	0.015	**−0.337**	0.033	**−0.350**	0.027
Inferonasal	**−0.338**	0.033	**−0.313**	0.049	−0.268	0.094
Inferior	**−0.341**	0.031	−0.311	0.051	−0.282	0.078
Inferotemporal	−0.252	0.116	−0.263	0.101	−0.203	0.208
Temporal	**−0.403**	0.010	**−0.437**	0.005	**−0.314**	0.048
Average IPL+GCL	**−0.381**	0.015	**−0.358**	0.023	**−0.319**	0.045
Minimum IPL+GCL	**−0.338**	0.033	**−0.326**	0.040	−0.290	0.069

Data in bold type correspond to statistically significant correlations (p<0.05).
CCI, colour confusion index; C-index, confusion index; GCL, ganglion cell layer; IPL, inner plexiform layer; S-index, scatter index.

have been masked and generated a per cent of dependence between measurements.

Previous studies have indicated that patients with PD lose foveal contrast sensitivity regarding patterns to which normal observers are most sensitive (ie, requiring the least contrast for detection).[8 9] Ganglion cells in the retina show adaptation to visual contrast and pool visual inputs over their receptive fields through an array of parallel bipolar cells with smaller receptive fields.[22] The parvocellular and magnocellular ganglion cells are located in the retinal ganglion cells (RGC) layer and take two different pathways for the identification of colour and

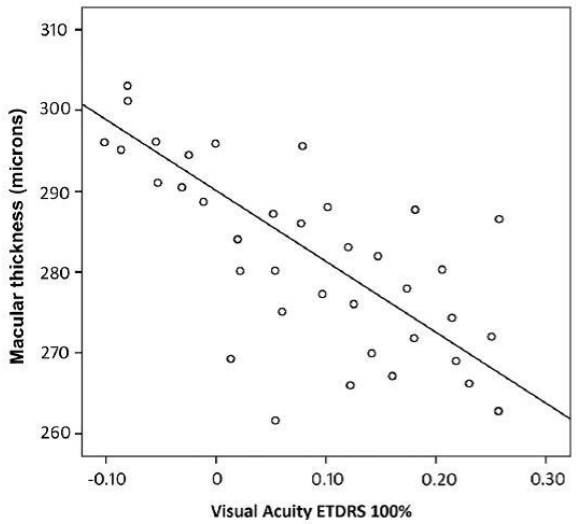

Figure 2 Correlation between the average macular thickness and visual acuity as measured with ETDRS optotype at a contrast level of 100% in patients with Parkinson's disease. ETDRS, Early Treatment Diabetic Retinopathy Study.

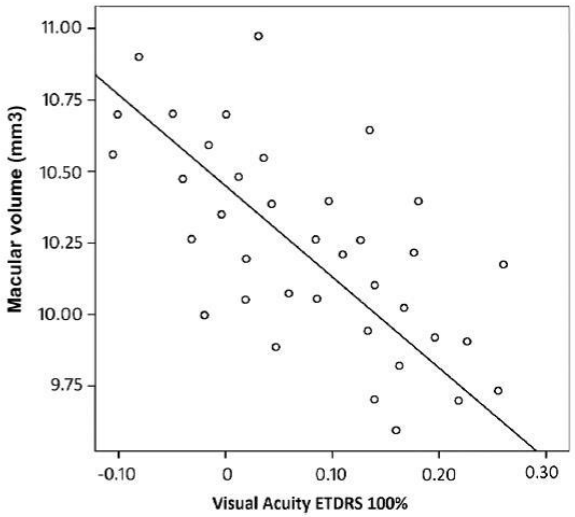

Figure 3 Correlation between macular volume and visual acuity as measured with ETDRS optotype at a contrast level of 100% in patients with Parkinson's disease. ETDRS, Early Treatment Diabetic Retinopathy Study.

Table 5 Correlation between visual acuity measured with ETDRS chart at different levels of contrast (in %) and macular structural measurements (thickness and volume) in patients with Parkinson's disease

	Macular thickness	p Value	Macular volume	p Value
VA ETDRS 100	−0.765	0.006	−0.761	0.007
VA ETDRS 1.25	−0.718	**0.013**	−0.715	**0.013**
VA ETDRS 2.50	−0.738	**0.010**	−0.729	**0.011**

Correlation data in bold type are statistically significant (p<0.05).
ETDRS, Early Treatment Diabetic Retinopathy Study; VA, visual acuity.

contrast at different frequencies.[23] RGC loss (as observed using SD-OCT) was recently identified as the cause of visual impairment in patients suffering from another neurodegenerative process, multiple sclerosis.[24] Thus, a similar process could be the cause of the contrast and colour deficiencies in patients with PD. In addition, in the mammalian retina, colour vision and contrast sensitivity are modulated through D1 and D2 receptors. These dopaminergic receptors are differentially located in the retinal layers and a complete lack of activation leads to signal dispersion, and alterations in colour vision and contrast sensitivity.[2]

Alteration of the retinal layers in PD was first demonstrated in 2004.[25] Since then, various studies have shown different results.[3–5 25–28] Previous studies performed by our team confirmed that both macular thickness and the RNFL were affected in patients with PD, especially in the inferior and temporal quadrants.[4 5 29] Moreover, Garcia-Martin *et al*[30] demonstrated that the inner retinal layers were most affected in these patients, and that the GCL thickness was inversely correlated with disease duration and PD severity, and was predictive of axonal damage in these patients. The present study included a smaller number of patients, which may have affected the significance of our results compared with previous studies. We could only detect a significant reduction in the minimum GCL+IPL thickness in patients with PD compared with healthy participants, after correction for multiple comparisons. However, we detected a clear tendency towards a reduction in the macular, RNFL and GCL thicknesses. A significant reduction in the temporal sectors of the peripapillary RNFL thickness has been repeatedly observed by different groups,[31 32] and this reduction was also observed in the present study. Two recent studies, however, detected no differences in the peripapillary RNFL thickness of patients with PD compared with healthy controls, using SD-OCT,[27 28] and one study only found significant differences in the nasal quadrant.[33] More studies are required to clarify these contradictory observations.

In a previous study, we demonstrated that the retinal thickness corresponding to the papillomacular bundle (as measured with the Axonal Analytics software for Spectralis OCT) correlated (r>0.70) with some functional parameters (such as the mean defect and the pattern SD of the automated perimetry) in patients with PD.[34] The GCL was not investigated at that time, however, and visual function parameters were reduced to perimetry and colour vision was measured with the Ishihara colour test. The current study evaluated not only the RNFL but also the GCL thickness, and more visual function parameters were analysed. The GCL correlated most with the visual function parameters: GCL thickness was directly associated with VA and CSV measured at all different spatial frequencies, and inversely correlated with the colour vision indexes. Thus, GCL thinning is linked to colour deficiencies, contrast sensitivity loss and lower vision at different contrast levels in patients with PD.

The degree of correlation is usually classified as low (<0.30), moderate (0.30–0.70) or strong (>0.70). Our results revealed a low and moderate degree of correlation between most parameters, consistent with findings in other neurodegenerative diseases.[35] Macular thickness and macular volume, however, were strongly associated with functional parameters (VA, CS, and Lanthony CCI, C-index and S-index). This strong association, to the best of our knowledge, has not been previously demonstrated in PD.

There are very few studies of the correlation between functional and structural parameters in patients with PD. Adam *et al*[14] demonstrated a significant reduction in the inner retinal layer complex (RNFL+GCL+IPL) in patients with PD, but no association with contrast sensitivity (measured with the Pelli-Robson chart). A very recent study by Kaur *et al*[15] demonstrated a correlation between functional parameters and GCL thinning, consistent with our results. Kaur *et al*, however, found no significant alterations in VA and colour vision in patients with PD, and the severity of the disease was not correlated with structural parameters, in contrast to other studies that demonstrate an association between macular and GCL thickness and disease duration and severity.[29 30] Although the severity of the disease in our sample (based on the Hoehn-Yahr scale) was similar to that in Kaur's study, the duration of the disease in our study was longer than that in Kaur's study (13 vs 5 years), which may account for some of the differences in the results between the two. These discrepancies (and similarities) support the need for more studies on this topic. Our results, together with previously published studies,[15 30] suggest that the GCL could be a reliable indicator of structural alterations in the retina of patients with PD, demonstrating a significant correlation with functional tests in these patients. The results of the present study

have important implications for clinical diagnosis and functional deficits in patients with PD, and highlight the importance of visual function tests in the evaluation of these patients.

In conclusion, visual dysfunction was significantly correlated with morphological parameters in patients with PD. Patients with PD present with a reduction in GCL thickness, which is closely associated with visual dysfunction.

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RESEARCH ARTICLE

Relationship between Visual Dysfunction and Retinal Changes in Patients with Multiple Sclerosis

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Abstract

Aim

To evaluate structural changes in the retina and their correlation with visual dysfunction in patients with multiple sclerosis.

Methods

Patients with multiple sclerosis (n = 84) and healthy controls (n = 84) underwent structural evaluation of the retinal nerve fiber layer, and macular and ganglion cell layer thicknesses using Spectral domain optical coherence tomography (SD-OCT). All subjects underwent high and low contrast visual acuity, color vision (using the Farnsworth and L´Anthony desaturated D15 color tests), and contrast sensitivity vision using the Pelli Robson chart and CSV 1000E test.

Results

Macular, retinal nerve fiber layer, and ganglion cell layer thinning was observed in multiple sclerosis patients compared to healthy controls ($p<0.05$). High- and low-contrast visual acuity and contrast sensitivity vision at four different spatial frequencies were significantly reduced in comparison with healthy subjects ($p<0.05$). Macular, retinal nerve fiber layer and ganglion cell layer measurements correlated with high and low contrast visual acuity, and contrast sensitivity vision. Contrast sensitivity vision was the functional parameter that most strongly correlated with the structural measurements in multiple sclerosis and was associated with ganglion cell layer measurements. The L´Anthony color vision score (age-corrected color confusion index) was associated with macular measurements.

Conclusions

Patients with multiple sclerosis had visual dysfunction that correlated with structural changes evaluated by SD-OCT. Macular and ganglion cell layer measurements may be good indicators of visual impairment in multiple sclerosis patients.

Introduction

Optic nerve atrophy and thinning of the peripapillary retinal nerve fiber layer (RNFL) are two typical findings of patients with multiple sclerosis (MS), with or without a history of optic neuritis (ON). Axonal loss is considered to be the main cause of disability in MS.[1–3] Neuronal loss is, however, increasingly recognized as a biomarker that correlates with disability in MS patients.[4–7]

MS is often associated with involvement of the visual pathway that can lead to clinically evident manifestations (such as ON and diplopia) and, more frequently, to subclinical alterations. Several studies have reported a correlation between axonal loss observed in the optic nerve and visual dysfunction in MS.[1,8]. More recently, segmentation analysis of the various retinal layers made possible by new software for digital imaging techniques in ophthalmology have provided a more specific measurement of the retinal ganglion cell layer and the inner plexiform layer complex (GCIPL) and suggest a correlation not only between axonal but also neuronal loss and visual dysfunction in MS patients.[9,10]

In the present study, we assessed macular, RNFL, and GCIPL thicknesses measured by Spectral domain-optical coherence tomography (SD-OCT) using segmentation analysis and evaluated the correlation between structural measurements and visual dysfunction in MS patients.

Material and Methods

Patients with definite MS and age and sex matched healthy individuals were included in the study. All procedures adhered to the tenets of the Declaration of Helsinki, and the experimental protocol was approved by the Ethics Committee of the Miguel Servet Hospital. All participants provided written informed consent to participate in the study. One eye per subject was randomly selected and included in the study.

The diagnosis of MS was based on standard clinical and neuroimaging criteria and related medical records were carefully evaluated. Information about Expanded Disability Status Scale (EDSS) scores, disease duration, treatments, acute MS attacks, and prior episodes of ON were recorded. The diagnosis of ON was based on clinical findings, which included the presence of decreased visual acuity, relative afferent pupil defect, color vision loss, visual field defect, and a compatible fundus examination. Patients with active ON in the 6 months preceding enrollment in the study or during follow-up were excluded from the study. Participants had no concomitant ocular diseases and no history of glaucoma, retinal pathology, or systemic conditions that could affect the visual system. Eyes with significant refractive errors (>5 D of spherical equivalent refraction or >3 D of astigmatism) were not included in the study.

All subjects underwent a complete neuro-ophthalmic evaluation that included pupillary, anterior segment, and funduscopic examination. We assessed high contrast (HCVA) and low contrast visual acuity (LCVA) using ETDRS and Low-Contrast Sloan Letter Charts, contrast sensitivity vision (CSV) using CVS 1000E and Pelli Robson charts, and color vision (CV) using Farnsworth-Munsell D15 and Lanthony D15 tests. Structural analysis of the retina was performed with SD-OCT using the Cirrus High Definition (HD) OCT (Carl Zeiss Meditec Inc, Dublin, CA), which included 3 applications: macular application (for macular thickness analysis), and RNFL and ganglion cell applications (for individual analysis of these layers). All functional and structural tests were performed during a single visit per patient and measurements were obtained under monocular vision using best correction.

LogMAR visual acuity (VA) was evaluated at three different contrast levels: 100% (HCVA, using ETDRS chart), 2.50%, and 1.25% (LCVA, using Low-Contrast Sloan Letter Charts -Precision Vision, LaSalle, IL-), The percentage indicating the level of contrast, i.e., 100% representing black letters over white background and 1.25% light grey letters over white background. All

measurements were obtained under controlled lighting conditions (photopic: mean luminance of 85 cd/m^2, high mesopic: 5 cd/m^2, and low mesopic: 3 cd/m^2).

CSV was evaluated using the Pelli-Robson chart and the CVS 1000E test. The Pelli-Robson chart comprises horizontal lines of capital letters organized into groups of three (triplets) with two triplets per line. Within each triplet, all letters have the same contrast. The contrast decreases from one triplet to the next, even within each line. All patients were evaluated at a distance of 1 meter from the chart, under controlled photopic conditions (85 cd/m^2). The score corresponding to the last triplet of letters seen by the patient was recorded.

The CSV 1000E instrument is used worldwide for standardized CSV and glare testing. All patients were evaluated at a distance of 2.5 meters from the chart, at four spatial frequencies (3, 6, 12, and 18 cycles per degree [cpd]). The chart comprises 4 rows with 17 circular patches each. The patches present a grating that decreases in contrast moving from left to right across the row. The patient indicates whether the grating appears in the top patch or the bottom patch for each column. Each contrast value for each spatial frequency was transformed into a logarithmic scale according to standardized values.

Color vision was assessed using the Color Vision Recorder program (CVR, Optical Diagnostics, Beusichem, The Netherlands). CVR software is designed for the Windows operating system and analyzes chromatic discrimination by classification of colors. CVR includes several classic color tests. All patients in the study were evaluated using the Farnsworth D15 and L'Anthony D15 protocols (often used to differentiate between subjects with severe loss of color vision and those with milder color defects or normal color vision) and different output parameters, such as the Age-Corrected Color Confusion index (AC CCI, which represents the ratio between the patient's major radius–largest difference between caps–and the major radius of a perfect arrangement for the subject's age group), the Confusion angle (Conf angle, which represents the axis of color deficiency), and the Scatter index (S-index, which represents the parallelism of confusion vectors to the personal confusion angle) were recorded.[11,12] All these parameters evaluate the severity of dyschromatopsia. For example, an AC CCI score higher than 1, indicates altered color vision perception; the higher the score in the AC CCI and the S-index, the worse the condition.

Structural measurements of the retina were obtained using the Cirrus OCT device. The same experienced operator performed all scans and did not apply manual correction to the OCT output. We used an internal fixation target because it provides the highest reproducibility and rejected poor quality scans prior to data analysis.[13] We based image quality assessment on the signal strength measurement that combines the signal-to-noise ratio with the uniformity of the signal within a scan (scale 1–10, where 1 is categorized as poor image quality and 10 as excellent). We included images with a score $\geq$7 for evaluation. The Cirrus OCT macular cube 512 x 128 protocol provides a macular volume measure and retinal thickness values for 9 areas that correspond to the ETDRS. These areas include a central 1 mm circle representing the fovea, and inner and outer rings measuring 3 mm and 6 mm in diameter, respectively. The inner and outer rings are divided into four quadrants each: superior, nasal, inferior, and temporal. The Cirrus OCT optic disc protocol generates 200 x 200 cube images with 200 linear scans enabling analysis of the RNFL of a 6-mm^3 area around the optic nerve. For each scan series of RNFL measurements, we assessed the average, superior, inferior, temporal, and nasal thickness. The Cirrus segmentation analysis for retinal layers also provides measurements of the GCIPL thickness, evaluating six areas of the macular cube (superior, superonasal, inferonasal, inferior, inferotemporal, and superotemporal sectors). The segmentation analysis also includes measurements of the average and minimum GCIPL. These values are obtained from a set of 360 spokes, where each average represents the mean number of the pixels along that spoke that lies within the measurement annulus. The average and minimum values were selected because they are more sensitive than other retinal measurements for detecting retinal thickness changes.[14]

All data analyses were performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was used to assess sample distribution. Differences between evaluations of MS patients and healthy subjects were compared using the Student's t test. Patients were divided in two groups (history of ON vs no history of ON) and a second analysis to calculate the differences between these two groups was performed using the Student's t test. The linear correlation between structural and functional parameters was determined using the Pearson correlation coefficient. P values less than 0.05 were considered to indicate statistical significance. To avoid a high false positive rate, the Bonferroni correction for multiple comparisons was calculated and the corrected p values were added to the previously calculated data.

Each eye was considered separately, and one eye from each patient was randomly selected for analysis.

Results

Eighty-four patients with MS and 84 healthy controls were included in the study. The mean age of the patients with MS was 45.69 (SD = 9.60) and the mean age of the healthy controls was 47.86 (SD = 9.62). Age, sex, and intraocular pressure did not differ significantly between healthy controls and patients with MS (p = 0.140; 0.090 and 0.770 respectively).

All patients had been diagnosed with MS relapse-remitting subtype and were under treatment with interferon 1b (14.3%), glatiramer acetate (4.7%), fingolimod (39.3%), or interferon 1a (10.7%). Only 28.6% of the patients were not under any current treatment. Mean EDSS score was 1.64 (SD: 2.07) and 58% of the patients (n = 49) had a previous ON episode.

Functional parameters

MS patients showed significant reduction in best-corrected visual acuity at the three contrast levels compared to the controls (0.14±0.67 in patients vs -0.09 ± 0.09 in controls at 100%, p = 0.010; 0.55±0.17 vs 0.42±0.12 at 2.5%, p<0.001; 0.71±0.16 vs 0.55±0.14 at 1.25%, p<0.001). CSV was affected in patients in all four spatial frequencies of the CSV 1000E chart (3, 6, 12, and 18 cpd) when analyzed based on the number of correct localized gratings (p<0.001). The Pelli Robson results revealed a significant reduction in CSV in MS patients (p<0.001). L'Anthony AC-CCI and Conf angle results were also significantly worse in MS patients. The results are shown in Table 1.

ANOVA was used to calculate differences between controls and patients with and without a previous episode of ON (ON vs no-ON) (S1 Table). The EDSS score was significantly worse in patients without a previous episode of ON (2.43±2.50 in patients without an ON episode vs 1.14 ±1.47 in patients with an ON episode, p<0.001). The post hoc analysis revealed that functional parameters were only different between controls and patients (with and/or without a history of ON); no differences were observed between the two groups of patients (S1 Table).

Structural parameters

OCT measurements indicated significant differences in almost all macular sectors (except in the central and outer temporal thickness; Table 1). The segmentation analysis revealed reduced GCIPL thickness in MS patients in the inferotemporal (73.9±13.1 μm in patients vs 84.2 ±6.7 μm in controls; p = 0.04) and superotemporal sectors (73.4±10.7 μm vs 83.9±6.2 μm; p = 0.01). The minimum GCIPL value was significantly reduced (70.2±13.8 μm vs 82.4 ±6.5 μm; p<0.001). The RNFL was significantly reduced in the average thickness and temporal quadrant in MS patients (Table 1).

The ANOVA analysis revealed a significant difference in all GCIPL measurements (S2 Table). Post hoc analysis of macular measurements revealed significant differences between

Table 1. Visual function and structural parameters in healthy controls and subjects with multiple sclerosis.

	CONTROL	MS	P
FUNCTIONAL EXAMINATION			
VISUAL ACUITY			
ETDRS 100	-0.09 (0.09)	0.14 (0.67)	**0.010***
ETDRS 2.5	0.42 (0.12)	0.55 (0.17)	**<0.001***
ETDRS 1.25	0.55 (0.14)	0.71 (0.16)	**<0.001***
CONTRAST SENSITIVITY			
Pelli Robson	1.89 (0.11)	1.74 (0.23)	**<0.001***
CSV 1000 3 cpd	1.73 (0.19)	1.44 (0.23)	**<0.001***
CSV 1000 6 cpd	1.98 (0.19)	1.50 (0.31)	**<0.001***
CSV 1000 12 cpd	1.62 (0.22)	1.00 (0.27)	**<0.001***
CSV 1000 18 cpd	1.16 (0.21)	0.56 (0.11)	**<0.001***
CHROMATIC VISION			
CVR Farnsw AC CCI	1.11 (0.43)	1.13 (0.24)	0.660
CVR Farnsw ConfAngle	56.08 (7.93)	59.10 (8.63)	0.520
CVR Farnsw S- Index	1.66 (0.43)	1.74 (0.48)	0.310
CVR L'Anthony AC CCI	1.13 (0.36)	1.26 (0.30)	**0.030**
CVR L'Anthony ConfAngle	55.49 (4.02)	40.27 (6.49)	**0.040**
CVR L'Anthony S-Index	1.79 (0.50)	1.81 (0.41)	0.856
STRUCTURAL EXAMINATION			
MACULAR THICKNESS			
Fovea	257.07 (16.33)	249.01 (16.34)	**0.012**
Inner superior sector	325.99 (13.37)	309.86 (22.47)	**<0.001***
Inner nasal sector	326.35 (13.56)	309.61 (24.02)	**<0.001***
Inner inferior sector	321.96 (14.00)	304.44 (23.52)	**<0.001***
Inner temporal sector	311.14 (12.24)	297.67 (21.70)	**<0.001***
Outer superior sector	283.21 (11.66)	273.44 (19.06)	**<0.001***
Outer nasal sector	299.80 (14.97)	282.06 (23.60)	**<0.001***
Outer inferior sector	272.04 (13.02)	262.11 (27.91)	**0.030**
Outer temporal sector	264.79 (11.86)	254.67 (14.27)	0.540
Average	291.55 (15.70)	273.70 (18.47)	**<0.001***
Volume	10.12 (0.54)	9.88 (0.66)	**<0.001***
GCIPL THICKNESS			
Superior sector	85.58 (6.67)	75.91 (8.46)	**0.040**
Superonasal sector	85.85 (7.46)	75.87 (9.25)	**0.030**
Inferonasal sector	84.61 (7.55)	75.26 (9.58)	0.140
Inferior sector	83.59 (7.70)	74.30 (9.56)	0.130
Inferotemporal sector	84.17 (6.73)	73.91 (13.11)	**0.040**
Superotemporal sector	83.87 (6.23)	73.35 (10.70)	**0.010**
Average GCIPL	84.68 (6.74)	74.87 (8.54)	0.080
Min GCIPL	82.42 (6.50)	70.17 (13.83)	**<0.001***
RNFL THICKNESS			
Average	94.35 (9.62)	84.34 (13.14)	**0.010***
Superior sector	117.10 (17.20)	107.08 (17.71)	0.550
Nasal sector	69.93 (11.79)	67.50 (14.07)	0.230
Inferior sector	124.1 (14.81)	107.63 (18.70)	**0.010***

(Continued)

Table 1. (*Continued*)

	CONTROL	MS	P
Temporal sector	64.14 (8.94)	55.5 (18.52)	**<0.001***

Mean and standard deviation (SD) of visual function and structural parameters in healthy controls and subjects with multiple sclerosis. The Student T test was performed to compare controls and patients with MS. Results in bold letters indicate statistical significance (p<0.050). Asterisk indicates a significant difference by Student's t test after Bonferroni correction for multiple tests (p≤0.017 for ETDRS; p≤0.010 for CSV 1000E measurements; p≤0.0083 for Farnsworth and L'Anthony tests; p≤0.0056 for macular thickness values; p≤0.00625 for GCIPL thickness and p≤0.010 for RNFL thickness). Abbreviations: ETDRS, Early Treatment Diabetic Retinopathy Study; cpd, cycles per degree; AC CCI, age-corrected color confusion index; Conf Angle, confusion angle; S-index, scatter index; GCIPL, ganglion cell +inner plexiform layer; RNFL, retinal nerve fiber layer; HD, high definition; MS, multiple sclerosis.

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healthy controls and patients (with and without a previous ON episode) in several sectors; however, differences between the two subgroups of patients were only observed in the foveal and average macular thickness, and macular volume.

Post hoc analysis of GCIPL thickness revealed statistical differences between controls and patients (with and without a previous ON episode) in the superior and superonasal sectors, and in the minimum GCIPL thickness.

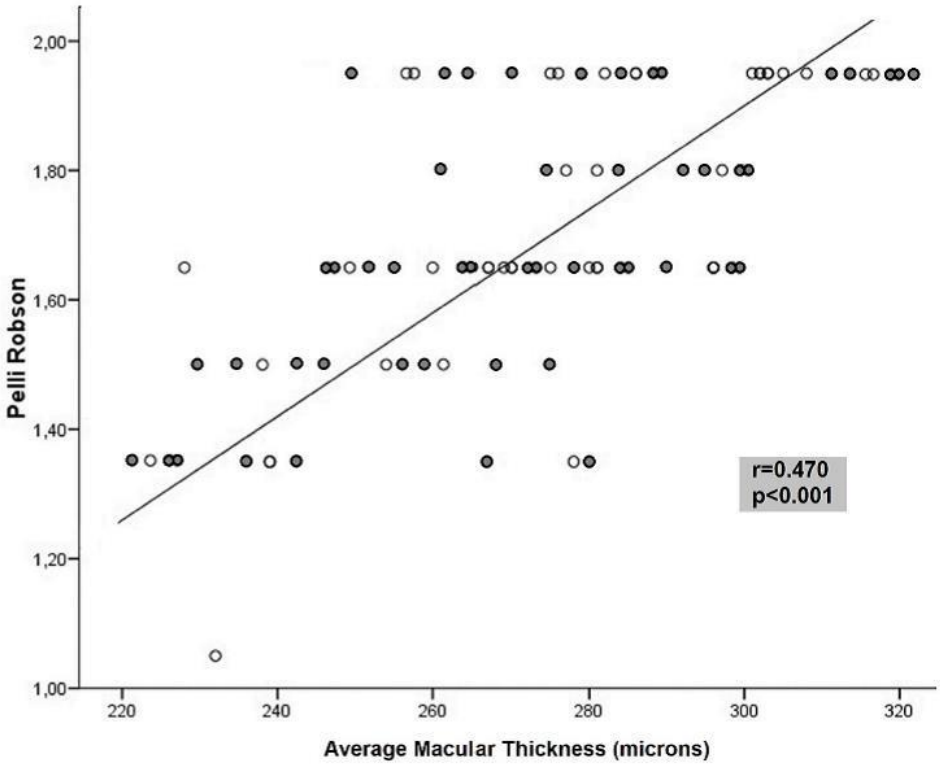

Fig 1. Correlation between the average macular thickness and contrast sensitivity vision measured with the Pelli Robson test in patients with multiple clerosis. Dark symbols represent data from patients with a previous episode of optic neuritis, whereas light symbols represent patients without a previous episode of optic neuritis.

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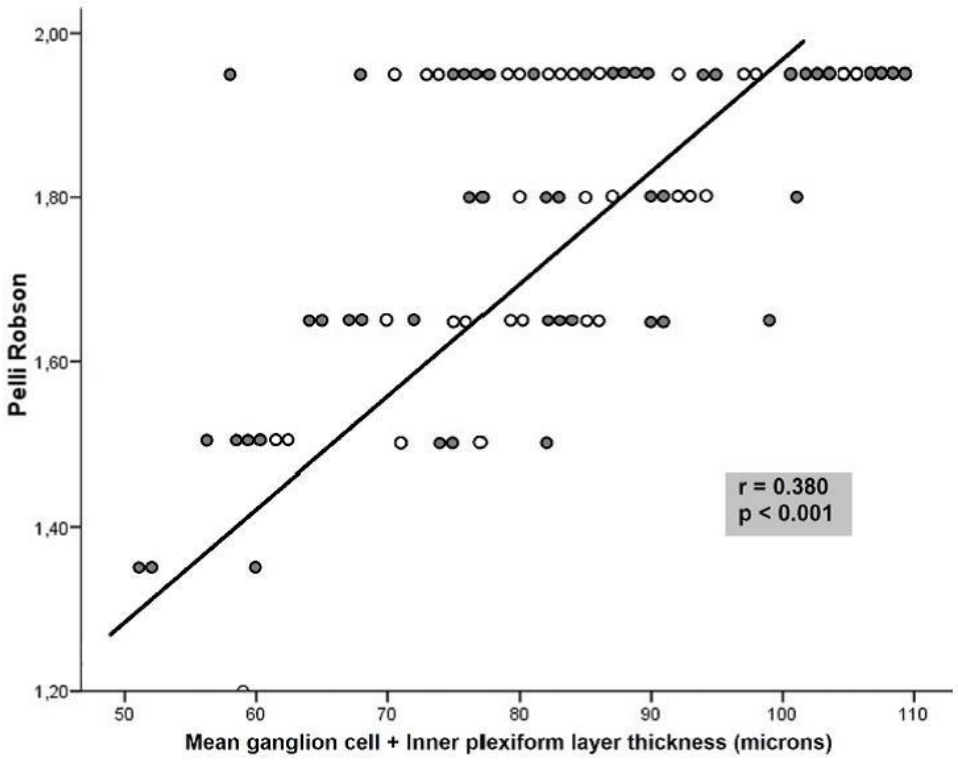

Fig 2. Correlation between the average ganglion cell + inner plexiform layer thickness and contrast sensitivity vision measured with the Pelli Robson test in patients with multiple sclerosis. Dark symbols represent data from patients with a previous episode of optic neuritis, whereas light symbols represent patients without a previous episode of optic neuritis.

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Correlation between functional and structural parameters

CSV was the functional parameter most frequently associated with structural measurements in MS. The Pelli Robson CSV results correlated with GCIPL thickness in all sectors and macular thickness in 8 of 9 sectors, although the association was not strong ($r < 0.5$) (Figs 1 and 2). The outer temporal ($r = 0.41$, $p<0.001$) and average macular thickness ($r = 0.47$, $p<0.001$) values had the highest correlations. The Pelli Robson results also correlated with the thickness in different sectors of the RNFL (average, superior, inferior, and temporal sectors, $r<0.50$, $p<0.05$). Measurements with the CSV 1000E at different spatial frequencies correlated significantly with most GCIPL measurements ($r<0.50$). The superotemporal ($r = 0.50$, $p< 0.001$) thickness, average GCIPL thickness ($r = 0.48$, $p< 0.001$), and minimum GCIPL ($r = 0.50$, $p< 0.001$) thickness had the strongest correlations at a spatial frequency of 18 cpd (Table 2).

Color vision was also associated with structural parameters: Farnsworth's AC-CCI was significantly associated with the GCIPL thickness (superior, superonasal, inferonasal, and the average GCIPL thickness), although the association was not strong ($r<0.30$; Table 3). L'Anthony's AC-CCI was statistically correlated with different macular sectors (Table 3). Macular average thickness showed the highest correlations with the color vision indexes (Farnsworth's AC-CCI $r = -0.53$, $p<0.001$ [data not shown in tables]; L'Anthony's AC-CCI $r = -0.47$, $p<0.001$). The color vision parameters were not significantly correlated with the RNFL thickness.

Table 2. Correlation between structural parameters and contrast sensitivity vision in patients with multiple sclerosis.

	CSV 1000E test								Pelli Robson	
STRUCTURAL MEASUREMENTS	*3 cpd*	*P*	*6 cpd*	*p*	*12 cpd*	*p*	*18 cpd*	*p*	*r*	*P*
MACULAR THICKNESS										
Fovea	0.15	0.080	0.15	0.080	0.12	0.180	0.18	**0.040**	0.12	0.150
Inner superior sector	0.32	**<0.001***	0.41	**<0.001***	0.41	**<0.001***	0.42	**<0.001***	0.36	**<0.001***
Inner nasal sector	0.34	**<0.001***	0.45	**<0.001***	0.41	**<0.001***	0.44	**<0.001***	0.35	**<0.001***
Inner inferior sector	0.32	**<0.001***	0.42	**<0.001***	0.42	**<0.001***	0.41	**<0.001***	0.35	**<0.001***
Inner temporal sector	0.30	**<0.001***	0.37	**<0.001***	0.38	**<0.001***	0.41	**<0.001***	0.31	**<0.001***
Outer superior sector	0.30	**<0.001***	0.33	**<0.001***	0.36	**<0.001***	0.37	**<0.001***	0.39	**<0.001***
Outer nasal sector	0.35	**<0.001***	0.43	**<0.001***	0.38	**<0.001***	0.44	**<0.001***	0.34	**<0.001***
Outer inferior sector	0.17	0.050	0.28	**0.001***	0.27	**0.002***	0.31	**<0.001***	0.27	**<0.001***
Outer temporal sector	0.33	**<0.001***	0.30	**<0.001***	0.40	**<0.001***	0.41	**<0.001***	0.41	**<0.001***
Average	0.29	**0.020**	0.38	**0.003***	0.39	**0.002***	0.44	**0.001***	0.47	**<0.001***
Volume	0.17	0.180	0.34	**0.006**	0.27	**0.030**	0.30	**0.018**	0.15	0.230
GCIPL THICKNESS										
Superior sector	0.22	**0.020**	0.37	**<0.001***	0.37	**<0.001***	0.44	**<0.001***	0.40	**<0.001***
Superonasal sector	0.21	**0.030**	0.38	**<0.001***	0.36	**<0.001***	0.41	**<0.001***	0.38	**<0.001***
Inferonasal sector	0.18	**0.060**	0.37	**<0.001***	0.36	**<0.001***	0.41	**<0.001***	0.37	**<0.001***
Inferior sector	0.19	**0.050**	0.41	**<0.001***	0.35	**<0.001***	0.45	**<0.001***	0.38	**<0.001***
Inferotemporal sector	0.23	**0.020**	0.36	**<0.001***	0.34	**0.001***	0.42	**<0.001***	0.25	**<0.001***
Superotemporal sector	0.31	**0.002***	0.45	**<0.001***	0.44	**<0.001***	0.50	**<0.001***	0.36	**<0.001***
Average GCIPL	0.25	**0.010**	0.43	**<0.001***	0.40	**<0.001***	0.48	**<0.001***	0.38	**<0.001***
Min GCIPL	0.27	**0.007**	0.43	**<0.001***	0.42	**<0.001***	0.50	**<0.001***	0.36	**<0.001***
RNFL THICKNESS										
Average	0.18	**0.030**	0.19	**0.020**	0.14	0.090	0.15	0.070	0.33	**<0.001***
Superior sector	0.15	0.070	0.13	0.110	0.13	0.120	0.16	0.060	0.30	**<0.001***
Nasal sector	0.02	0.790	0.08	0.350	0.16	0.060	0.15	0.070	0.08	0.310
Inferior sector	0.15	0.060	0.16	**0.040**	0.11	0.200	0.09	0.260	0.32	**<0.001***
Temporal sector	0.24	**0.004***	0.34	**<0.001***	0.34	**<0.001***	0.35	**<0.001***	0.24	**<0.001***

Correlation between structural parameters (macular, ganglion cell layer and retinal nerve fiber layer thickness) and contrast sensitivity vision (CSV) evaluated with the CSV 1000E and Pelli Robson tests in patients with multiple sclerosis. Data in bold type correspond to statistically significant correlations (p-value <0.05). Asterisk indicates a significant difference after Bonferroni correction for multiple tests ($p \leq 0.0056$ for macular thickness values; $p \leq 0.00625$ for GCIPL thickness and $p \leq 0.010$ for RNFL thickness). Abbreviations: GCIPL, ganglion cell+inner plexiform layer; RNFL, retinal nerve fiber layer; cpd, cycles per degree.

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VA using the ETDRS chart correlated with macular, GCIPL, and RNFL thickness (Table 4, Fig 3). There were significant but mild associations between 8 of the 9 macular parameters and LCVA at 2.50% and 1.25%, where the macular average thickness had the highest correlations ($r = -0.41$, $p = 0.01$ and $r = -0.36$, $p = 0.04$, respectively).

Discussion

In the present study, we evaluated different structural and functional parameters and assessed the association between visual dysfunction and morphologic changes in the retina of 84 patients with MS and 84 healthy controls. We demonstrated that structural parameters, such as macular, RNFL, and GCIPL thicknesses, are reduced in MS patients, but they also exhibited visual impairment (VA and CSV reduction). Moreover, contrast sensitivity was the most affected parameter in our study and correlated with most of the structural data. We have used color vision tests that provide information for differentiating subjects with severe loss of color vision from those with milder color defects or normal color vision, and also can be used to

Table 3. Correlation between structural measurements and color vision in patients with multiple sclerosis.

FARNSWORTH COLOR TEST		
GCIPL THICKNESS	AC-CCI	p
Superior sector	-0.21	**0.030**
Superonasal sector	-0.23	**0.020**
Inferonasal sector	-0.23	**0.010**
Inferior sector	-0.16	0.100
Inferotemporal sector	-0.16	0.100
Superotemporal sector	-0.21	**0.030**
Average GCIPL	-0.23	**0.020**
Min GCIPL	-0.13	0.190
L´ANTHONY COLOR TEST		
MACULAR THICKNESS	AC-CCI	p
Fovea	-0.01	0.900
Inner superior sector	-0.21	**0.020**
Inner nasal sector	-0.25	**<0.001***
Inner inferior sector	-0.22	**0.010**
Inner temporal sector	-0.17	0.060
Outer superior sector	-0.18	0.050
Outer nasal sector	-0.10	0.290
Outer inferior sector	-0.05	0.580
Outer temporal sector	-0.06	0.530
Average thickness	-0.47	**<0.001***

Correlation between structural measurements (ganglion cell layer and macular thickness) and color vision evaluated with the Farnsworth and L´Anthony color tests in patients with multiple sclerosis. Correlation data in bold type are statistically significant (p-value <0.05). Asterisk indicates a significant difference after Bonferroni correction for multiple tests ($p \leq 0.00625$ for GCIPL thickness and $p \leq 0.0056$ for macular thickness values). Abbreviations: GCIPL, ganglion cell+inner plexiform layer; AC-CCI: age-corrected color confusion index.

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evaluate acquired loss of color vision. Both the Farnsworth and L´Anthony tests are color arrangement tests (based on the arrangement of different color caps); however, the L´Anthony color test is less saturated, and is thus more suitable for detecting mild color anomalies that may not be detected using the Farnsworth color test alone. In our study, only the L´Anthony test results (AC CCI and Conf angle) were significantly worse in the MS group compared to healthy controls, showing a mild tendency toward protanomaly.

Visual dysfunction may occur in up to 80% of MS patients during the course of the disease. [15] Diminished contrast sensitivity and color vision deficiencies in MS have been widely reported.[16,17] Measures of low-contrast vision as tested by line gratings, letter charts, and by Pelli-Robson charts in MS patients were sensitive to visual impairment, even in patients with VA of 20/20 or better as measured with a Snellen chart. This alteration in low-contrast vision is also associated with visual impairment of everyday tasks, such as reading, driving, and facial recognition.[18] The introduction in the last few decades of OCT in the study of optic nerve neuropathies has provided new information on correlations between visual deficiencies and retinal alterations. Recent studies using OCT showed that low contrast letter acuity scores reflect the axonal and neuronal losses in the anterior visual pathways.[9,19,20] This axonal loss is also associated with disease progression, worsening disability, and lower quality of life in MS patients [21,22].

Table 4. Correlation between structural parameters and visual acuity in patients with MS.

STRUCTURAL PARAMETERS	Visual Acuity ETDRS LogMar					
	100%	*p*	*2.50%*	*p*	*1.25%*	*p*
MACULAR THICKNESS						
Central	-0.07	0.400	-0.07	0.430	-0.05	0.530
Inner superior	-0.09	0.310	-0.30	**<0.001***	-0.30	**0.001***
Inner nasal	-0.08	0.360	-0.28	**0.001***	-0.26	**0.002***
Inner inferior	-0.09	0.270	-0.32	**<0.001***	-0.30	**<0.001***
Inner temporal	-0.11	0.220	-0.25	**0.003***	-0.26	**0.002***
Outer superior	-0.06	0.460	-0.31	**<0.001***	-0.32	**<0.001***
Outer nasal	-0.04	0.670	-0.32	**<0.001***	-0.29	**0.001***
Outer inferior	-0.07	0.420	-0.26	**0.002***	-0.25	**0.003***
Outer temporal	-0.08	0.370	-0.33	**<0.001***	-0.31	**<0.001***
Average	-0.01	0.960	-0.41	**0.001***	-0.36	**0.004***
Volume	-0.04	0.720	-0.15	0.230	-0.08	0.520
GCIPL THICKNESS						
Superior	-0.42	**<0.001***	-0.27	**0.005***	-0.27	**0.006***
Superonasal	-0.39	**<0.001***	-0.29	**0.003***	-0.28	**0.005***
Inferonasal	-0.39	**<0.001***	-0.31	**0.001***	-0.28	**0.004***
Inferior	-0.36	**<0.001***	-0.26	**0.008**	-0.14	0.150
Inferotemporal	-0.26	**0.008**	-0.14	0.140	-0.10	0.299
Superotemporal	-0.32	**0.001***	-0.23	**0.020**	-0.12	**0.040**
Average GCIPL	-0.38	**<0.001***	-0.27	**0.005***	-0.23	**0.020**
Minimum GCIPL	-0.31	**0.001***	-0.21	**0.030**	-0.15	0.130
RNFL THICKNESS						
Average	-0.21	**0.008***	-0.27	**0.001***	-0.35	**<0.001***
Superior	-0.21	**0.010***	-0.28	**<0.001***	-0.33	**<0.001***
Nasal	-0.05	0.490	-0.04	0.630	-0.07	0.400
Inferior	-0.26	**0.001***	-0.24	**0.004***	-0.29	**<0.001***
Temporal	-0.16	**0.040**	-0.19	**0.020**	-0.29	**<0.001***

Correlation between structural parameters (macular, ganglion cell layer and retinal nerve fiber layer thickness) and visual acuity in different contrast levels in patients with multiple sclerosis. Data in bold type correspond to statistically significant correlations (p value <0.05). Asterisk indicates a significant difference after Bonferroni correction for multiple tests ($p \leq 0.0056$ for macular thickness values; $p \leq 0.00625$ for GCIPL thickness and $p \leq 0.010$ for RNFL thickness). Abbreviations: ETDRS, Early Treatment Diabetic Retinopathy Study; GCIPL, ganglion cell+inner plexiform layer; RNFL, retinal nerve fiber layer.

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New SD-OCT segmentation software allows for the measurement of the various retinal layers. Previous studies on MS found a reduction in RNFL not only in eyes with a previous episode of ON, but also in patients who have never had an acute clinical episode of ON.[23,24] Current studies using segmentation analysis of the retinal layers have demonstrated a thinning of the GCIPL, suggesting ganglion cell loss.[9,25,26] This GCIPL thinning was significantly associated with reduced visual function and vision-specific quality of life in MS patients. Additionally GCIPL thinning occurs 3 to 6 months following acute ON.[10]

Histopathologic evaluation of postmortem MS eyes revealed the loss of inner nuclear layer neurons and significant GCIPL atrophy [27], even in cases where the number of axons remained intact.[28] Thus, GCIPL thickness has rapidly emerged as a useful structural marker

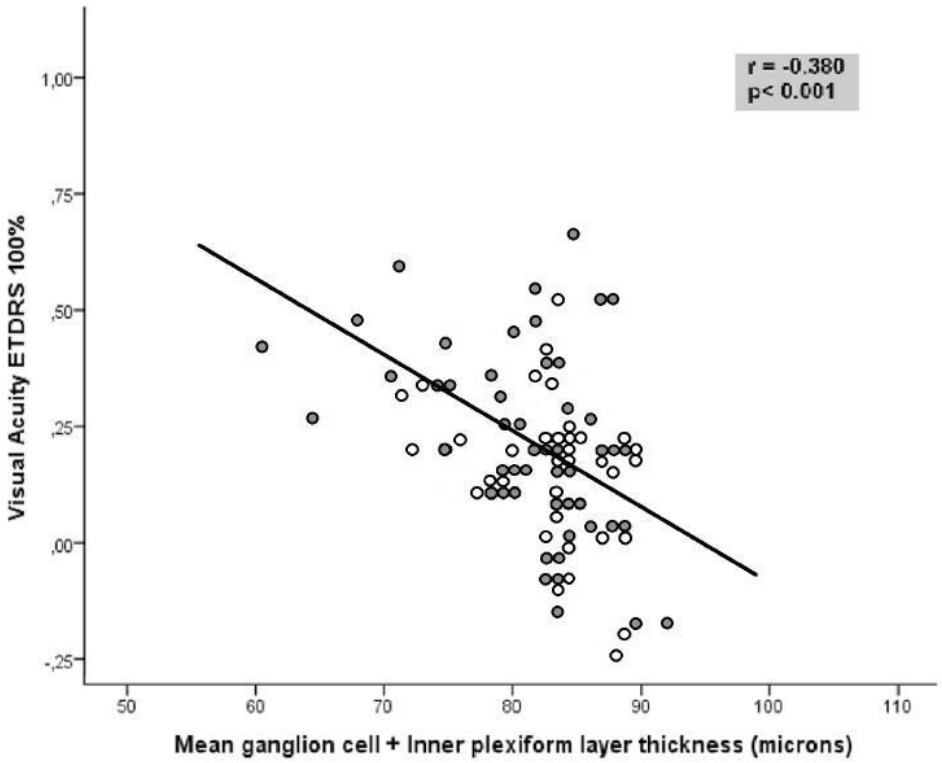

Fig 3. Correlation between visual acuity as measured with ETDRS optotipe at a contrast level of 100% and the average ganglion cell + inner plexiform layer thickness in patients with multiple sclerosis. Dark symbols represent data from patients with a previous episode of optic neuritis, whereas light symbols represent patients without a previous episode of optic neuritis.

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in MS, even better than RNFL thickness. A positive correlation between the average GCIPL and the peripapillary RNFL thickness was recently demonstrated.[14]. Additionally, GCIPL thickness is suggested to have better sensitivity than temporal peripapillary RNFL thickness for detecting retinal thickness changes in patients with MS.[14,29]. In the present study, macular thickness was reduced in most sectors, but the peripapillary RNFL thickness was only reduced in the inferior and temporal quadrants. Segmentation analysis of the GCIPL thickness revealed a clear tendency towards a reduction in the superior and temporal (superotemporal and inferotemporal) sectors ($p<0.05$) although only the minimum GCIPL thickness was significantly reduced ($p<0.001$). These findings suggest a topographic match between defects in the GCIPL and decreased peripapillary RNFL thickness, supporting previous research, where superior macular areas (including superotemporal and superonasal sectors) anatomically correspond with the RNFL bundle to the temporal quadrant in the optic disc.[30] The thickness of these layers (especially the GCIPL) was also significantly worse in patients with a previous history of ON, corroborating previously reported results.[10, 31] Our results did not demonstrate a higher sensitivity of the GCIPL thickness to detect axonal damage. A clear tendency toward GCIPL loss in patients with MS, however, was observed (higher than in the RNFL), and comparison between groups revealed greater effects on GCIPL thickness in subjects with previous ON. Thus, further studies with a larger sample size are needed to confirm that the GCIPL reduction is a better marker of axonal damage in these patients.

Correlations between visual dysfunction and structural measurements in MS have been also reported,[9, 25, 26, 32, 33] although few of these studies include CSV and color vision analysis. A reduction in the RNFL is associated with lower LCVA, alterations in color vision, and lower quality of life in MS patients.[33, 34] The reduction of the macular and GCIPL thickness are significantly correlated with VA (high and low contrast).[9,25,26] In accordance with these previous studies, our results showed an association between macular and GCIPL thinning and worse LCVA and CSV (measured by the CSV 1000E and Pelli Robson tests), highlighting the importance of CSV tests and analysis of the GCIPL and macular thickness in the clinical evaluation of MS patients. Moreover, in our study, macular and GCIPL thicknesses were inversely correlated with L'Anthony and Farnsworth's color indexes, respectively. These color tests evaluate the severity of dyschromatopsia and are not frequently included in studies assessing visual dysfunction and MS. In a recent study, the Farnsworth D-100 color test (based on the same principle as the Farnsworth and L'Anthony D15 color tests) was demonstrated to be more sensitive than pattern visual evoked potentials in detecting subclinical visual pathway alterations in MS patients making this color test a valuable tool for evaluating these patients.[35]

In conclusion, MS patients had reduced macular, RNFL, and GCIPL thicknesses, with the changes in the GCIPL being most closely associated with visual dysfunction. These results may be important for future investigations of neuronal and axonal loss in MS and other neurodegenerative diseases. Further studies are needed to evaluate the association between axonal injury and ganglion cell loss and to investigate the role of the GCIPL as a possible biomarker of the efficacy of neuroprotective agents.

Supporting Information

S1 Table. Mean and standard deviation (SD) of visual function parameters in healthy controls and subjects with multiple sclerosis. ANOVA test was used to compare controls and patients with history of ON and without ON. Results in bold letters indicate statistical significance ($p<0.050$). The brackets indicate the groups that had statistically differences in post hoc comparisons. Abbreviations: ETDRS, Early Treatment Diabetic Retinopathy Study; cpd, cycles per degree; AC CCI, age-corrected color confusion index; Conf Angle, confusion angle; S-index, scatter index; MS, multiple sclerosis; ON, optic neuritis; C, controls.
(DOCX)

S2 Table. Mean and standard deviation (SD) of structural parameters in healthy controls and subjects with multiple sclerosis. ANOVA test was used to compare controls and patients with history of ON and without ON (no-ON). Results in bold letters indicate statistical significance ($p<0.050$). The brackets indicate the groups that had statistical differences in post hoc comparisons. Abbreviations: GCIPL, Ganglion cell + inner plexiform layer; RNFL, retinal nerve fiber layer; MS, multiple sclerosis; ON, optic neuritis; C, controls.
(DOCX)

Author Contributions

Conceived and designed the experiments: MS MJR SO MPB MIF JRA JM VP JML LP EGM. Performed the experiments: MS MJR JRA JM EGM. Analyzed the data: MS MJR EGM. Contributed reagents/materials/analysis tools: MS MJR SO MPB MIF EGM. Wrote the paper: MS MJR EGM.

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Review Article

Optical Coherence Tomography as a Biomarker for Diagnosis, Progression, and Prognosis of Neurodegenerative Diseases

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Neurodegenerative diseases present a current challenge for accurate diagnosis and for providing precise prognostic information. Developing imaging biomarkers for multiple sclerosis (MS), Parkinson disease (PD), and Alzheimer's disease (AD) will improve the clinical management of these patients and may be useful for monitoring treatment effectiveness. Recent research using optical coherence tomography (OCT) has demonstrated that parameters provided by this technology may be used as potential biomarkers for MS, PD, and AD. Retinal thinning has been observed in these patients and new segmentation software for the analysis of the different retinal layers may provide accurate information on disease progression and prognosis. In this review we analyze the application of retinal evaluation using OCT technology to provide better understanding of the possible role of the retinal layers thickness as biomarker for the detection of these neurodegenerative pathologies. Current OCT analysis of the retinal nerve fiber layer and, specially, the ganglion cell layer thickness may be considered as a good biomarker for disease diagnosis, severity, and progression.

1. Introduction

Neurodegenerative disorders present a current challenge for accurate diagnosis and for providing precise prognostic information. Some diseases, such as multiple sclerosis (MS), present with an unpredictable course, whereas others, such as Parkinson disease (PD) and Alzheimer's disease (AD), may take several years to obtain a definitive diagnosis. Due to increased aging population in developed countries, neurodegenerative diseases such as PD and AD have become more prevalent and thus new technologies and more accurate tests are needed to improve and accelerate the diagnostic procedure in early stages of these diseases.

Developing imaging biomarkers for MS, PD, and AD in order to provide early diagnosis and predict the clinical course and future disability will improve the clinical management of these patients and may be useful for monitoring treatment effectiveness as well.

Optical coherence tomography (OCT) provides cross-sectional imaging of internal structures in biological tissues [1, 2]. Recent research using OCT technology has demonstrated that parameters provided by OCT are accurate to detect various inner retinal or optic nerve pathologies. In the last decade this technique has also been applied in several areas in neurology, demonstrating its potential role as a fundamental tool in the study of neurodegenerative diseases, such as MS, PD, or AD [3–9]. At the present time, however, no clear guidelines are available on whether one, several, or all of the retinal parameters measured by OCT can be used in the diagnosis of these pathologies, and therefore the use of OCT technology in the clinical management of neurological patients is limited to follow disease progression in several common pathologies.

In the present article, we review the application of retinal evaluation using OCT technology to provide better understanding of the possible role of the retinal layers thickness as

biomarker for the detection of neurodegenerative pathologies such as MS, PD, and AD.

2. Multiple Sclerosis

Multiple sclerosis (MS) is a neurodegenerative disease characterized by demyelination and axonal degeneration in the central nervous system, leading to progressive neurologic deficits [10, 11]. Axonal damage already occurs in the early stages of the disease, not being related to inflammatory or autoimmune episodes against myelin [12, 13].

The retina of patients with MS displays inflammatory and neurodegenerative findings, such as perivascular inflammatory infiltrates and atrophy of the inner retinal layers [14]. In 1999, Parisi et al. reported for the first time a significant reduction in the retinal nerve fiber layer (RNFL) of patients with MS and previous optic neuritis (MSON) compared to healthy subjects and its correlation with pattern electroretinogram changes in these eyes [15]. Since then, an increased interest in the application of OCT technology led to a large number of studies on the retinal changes in MS patients with and without previous ON episodes (non-ON). So far, studies using spectral-domain OCT have revealed that the retina in non-ON eyes shows thinner peripapillary RNFL (pRNFL) than healthy controls [16–19].

2.1. RNFL Thickness as a Biomarker of Disease Severity and Progression in MS. The introduction in the last few decades of OCT in the study of MS has provided new information on correlations between visual deficiencies and retinal alterations in these patients and also between pRNFL thinning and disability [9]. Recent studies using OCT showed that low contrast letter acuity scores in MS patients reflect the axonal and neuronal losses in the anterior visual pathway (observed as RNFL and retinal neuronal layer thinning quantified using OCT technology) [20, 21]. Saidha et al. [20] demonstrated the presence of retinal ganglion layer thinning in patients with relapsing-remitting and progressive MS and its correlation with high and low contrast visual acuity scores. Similarly, Burkholder et al. showed a significant correlation between altered visual function scores and reduced macular volume in these patients [21]. More importantly, retinal measures in MS patients evaluated using OCT technology seem to correlate directly with brain-substructure volumes and grey and white matter volumes and inversely with FLAIR-lesion volume, as objectified by MRI, thus reflecting a possible correlation with general central nervous system pathology in MS [22].

This axonal loss in MS, as observed by OCT, is associated with physical and cognitive disability as measured by the Expanded Disability Status Scale (EDSS) [23–25] and has demonstrated its utility as a biomarker of disease progression [5, 22, 26, 27]. Recently, pRNFL atrophy was associated with worsening disability and lower quality of life [28]. Garcia-Martin et al. analyzed the structural change in the retina of MS patients for a time lapse of 3 years and demonstrated that a reduction in the pRNFL thickness in these patients was associated with lower quality of life (measured using the MSQOL-54 questionnaire) and greater disability. The MSQOL-54 questionnaire is a multidimensional health-related quality of life measure that combines both generic and MS-specific items into a single instrument. This 54-item questionnaire generates 12 subscales (physical function, role limitations: physical, role limitations: emotional, pain, emotional well-being, energy, health perceptions, social function, cognitive function, health distress, overall quality of life, and sexual function) along with two summary scores (the physical health composite summary and the mental health composite) and two additional single-item measures (satisfaction with sexual function and change in health). In Garcia-Martin's study the physical health composite of the MSQOL-54 questionnaire (composed of different questions about the patient's perception of their physical condition to fulfill every day's tasks) was especially correlated with pRNFL thickness. Additionally, the baseline mean and superior pRNFL thicknesses appear to predict decreases in the quality of life in patients with MS [28].

RNFL thickness decreases with normal aging [29]. However, compared to healthy subjects, MS patients present with a higher reduction and more affected sectors of the pRNFL thickness (Figures 1 and 2) and this reduction seems to be even greater in untreated patients [30]. More recently, the pRNFL thickness was pointed out as a good predictor of the likelihood of disability worsening in MS patients over time [9]. Patients who had a pRNFL $\leq$ 92-93 μm showed a 58% increase in the risk of disability worsening, and patients in the lower pRNFL thickness tertile displayed increased risk of disability worsening compared to those in the higher tertile. Patients with pRNFL $\leq$ 87/88 μm doubled the risk of disability worsening at any time after the first year and until the third year of follow-up. This disability worsening prediction by pRNFL seems to be dependent on the follow-up time, since this risk almost increased fourfold after the third year and until the fifth year of follow-up. This increased risk of disability worsening was present in patients with and without a previous ON episode, although it was higher in patients with MSON [9].

Combined RNFL parameters were also demonstrated to improve the ability of this technology to distinguish between eyes from MS patients and eyes from healthy subjects, by calculating a linear discriminant function [6]. Mathematical analysis showed that a linear discriminant function where different RNFL parameters (thickness in different sectors) were combined yielded the highest sensitivity at a high specificity compared to any single sector of the OCT parameters [6].

2.2. Retinal Segmentation Analysis: Ganglion Cell Layer Thickness as a Biomarker for MS. Histopathologic evaluation of postmortem MS eyes revealed the loss of inner nuclear layer neurons and significant ganglion cell and inner plexiform layer (GCIPL) atrophy [14], even in cases where the number of axons remained intact [31]. New spectral-domain (SD) OCT segmentation software allows for the measurement of the various retinal layers separately, taking in vivo measurements one step closer to histologic observations. Current studies using this segmentation analysis software demonstrated a reduction of the inner retinal layers, including the GCIPL, suggesting ganglion cell loss [20, 32–34]. Moreover,

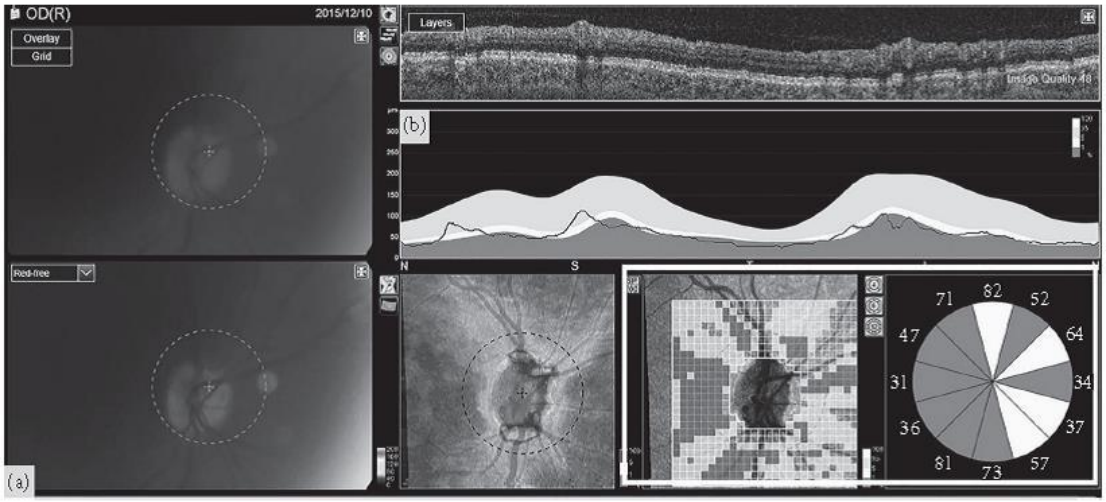

FIGURE 1: Optic nerve head (a) and retinal nerve fiber layer analysis (b) as obtained with swept-source optical coherence tomography in a 43-year-old patient with multiple sclerosis who suffered a previous episode of optic neuritis 5 years ago. The pixel map and the clock sector analysis (marked with the white square) of the optic disc shows important retinal nerve fiber layer loss in most sectors of the peripapillary area.

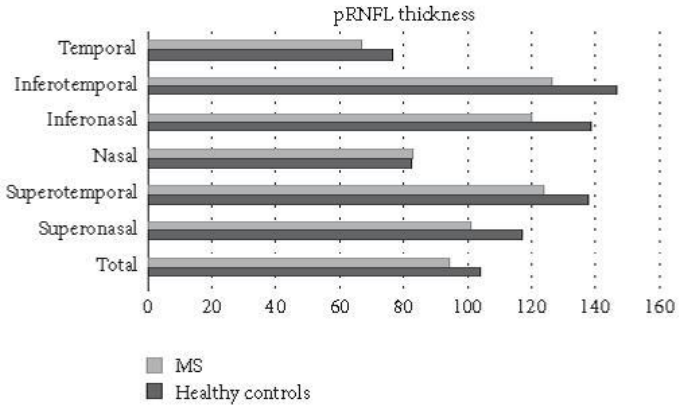

FIGURE 2: Mean peripapillary retinal nerve fiber layer (pRNFL) thickness of 100 multiple sclerosis (MS) patients compared with 97 healthy controls, as measured with optical coherence tomography. The peripapillary area is divided into 6 different sectors (superonasal, superotemporal, nasal, inferonasal, inferotemporal, and temporal) and average thickness. All measurements except nasal thickness were found to be significantly reduced in MS patients compared to controls (Garcia-Martin et al., data not published).

this GCIPL reduction is also correlated with reduced visual function, functional disability as measured by the EDSS, and vision-specific quality of life in MS patients [32, 34]. Does this all mean that the GCIPL could be a more precise biomarker than the RNFL? In a recent study comparing both GCIPL and pRNFL thickness, average GCIPL was altered more frequently than average pRNFL, and GCIPL thickness was demonstrated to have better sensitivity than temporal pRNFL thickness for detecting retinal thickness changes in patients with MS [35, 36]. Additionally, logistic regression analysis demonstrated that GCIPL thickness is a potential predictor of axonal damage in patients with MS, whereas the thickness of all other retinal layers (including the RNFL) was not predictive of axonal atrophy [34]. Thus, GCIPL thickness has rapidly emerged as a useful structural biomarker in MS, even better than RNFL thickness, probably because the neuronal cell bodies suffer an earlier affectation than the retinal axons in MS. Some authors, ophthalmologists and neurologists, have suggested that OCT measurements may be more accurate than MRI parameters to determine progression in MS patients [9, 34].

3. Parkinson Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the developed world (after Alzheimer's disease) and is characterized by motor symptoms, such as resting tremor, bradykinesia, and rigidity. However, a large variety of nonmotor symptoms are also present in this disease: mood [37, 38], cognitive dysfunction

[39], autonomic failure [40], and sleep disorders [41] are highly common in PD patients.

Vision is one of the nonmotor systems altered in PD, reporting decreased visual acuity, contrast sensitivity, or colour vision reduction [42–48].

Postmortem neurochemical analysis of eyes of deceased patients diagnosed with PD has shown decreased retinal dopamine concentration [49, 50]. Dopamine in the human retina is released by a set of amacrine cells located in the proximal inner nuclear layer of the retina. These dopaminergic cells communicate with other types of amacrine cells modulating the interconnections between bipolar and retinal ganglion cells and also send long processes to other retinal layers, thus playing a pivotal role in channelling visual information "vertically" through the retina [51]. Dopamine in the mammalian retina modulates colour vision and contrast sensitivity through dopaminergic receptors (D1 and D2), which are differentially located in the retinal layers. A complete lack of this dopaminergic receptor activation leads to signal dispersion and alterations in colour vision and contrast sensitivity.

3.1. RNFL Thickness as a Possible Biomarker for PD Diagnosis. Retinal changes in PD were first reported in 2004 by Inzelberg et al. [52], who demonstrated RNFL thickness reduction in the peripapillary area in a small group of 10 PD patients. In the following years, research on this topic increased dramatically and Inzelberg's results were confirmed by other studies using time-domain OCT [53–55]. Mean and temporal pRNFL thickness seem to be most affected based on these studies. Later, studies using SD-OCT also demonstrated significant reduction of the inferior pRNFL thickness (along with mean and temporal reduction) [56]. Retinal thickness in the macular area and total macular volume are also significantly reduced in PD [53, 56–58]; however not all studies demonstrating macular thinning in these patients could find similar differences in the pRNFL measurements [59–61].

3.2. Macular Thickness as a Biomarker for Disease Progression and Severity. Macular measurements appear to be an important feature in PD. Based on Spectralis OCT measurements, a linear discriminant function was designed by Garcia-Martin et al. to combine parameters improving the diagnostic ability of OCT: a calculated retinal linear discriminant function including different macular thickness measurements yielded the highest sensitivity at a high specificity compared to any single parameter determined using OCT or any other linear discriminant function calculated from pRNFL measurements, suggesting that macular measurements in PD could be a stronger marker for PD diagnosis [8]. A remodelling of the foveal pit caused by PD has also been suggested [62].

There is an association between macular thinning and disease progression and severity in PD. Altintaş et al. demonstrated a relation between PD severity and alterations in foveal thickness using time-domain OCT [54]. An association between disease severity as measured by the Hoehn Yahr scale and macular thickness was also found using SD-OCT [57, 63]. Disease severity based on the commonly used Unified Parkinson's Disease Rating Scale III (UPDRS III) and quality of life (based on the Schwab England scale) is also correlated with macular measurements, especially temporal and inferior sectors [57, 63]. Contrary to macular measurements, correlations between disease severity/duration and pRNFL thickness have proved to be scarce or nonexistent [55, 57].

3.3. Retinal Segmentation Analysis: Ganglion Cell Layer Thickness as a Biomarker for PD. As research on PD moved forward, SD-OCT segmentation analysis was also applied to the evaluation of the retina of PD patients (Figures 3 and 4). In a recent study by Blennow et al., the inner retinal layer (IRL, defined as the internal limiting membrane + nerve fiber layer + ganglion cell layer + inner plexiform layer down to the inner nuclear layer interface) was found to be reduced in the perifoveal area of PD patients compared to healthy subjects [64]. Furthermore, when single retinal layers were measured, reductions in the macular RNFL, the ganglion cell layer (GCL), the inner plexiform layer (IPL), the inner nuclear layer, and the outer plexiform layer were demonstrated [63]. However, only the GCL predicts axonal damage in PD patients [63]. Segmentation analysis also revealed that the inner retinal layers of the macular area (RNFL, GCL, and IPL) are more affected with disease duration and that GCL thickness is inversely correlated with disease duration and disease severity [63]. Therefore and based on these recent segmentation studies the inner retinal layers of the macular area should be pointed at as the strongest biomarkers for PD diagnosis and progression.

4. Alzheimer's Disease

Alzheimer's disease (AD) is the most frequent cause of dementia worldwide [64]. Although it is most commonly associated with memory deficits and cognitive impairment, patients with AD also exhibit alterations in visual processing [65–67]. Colour vision and contrast sensitivity alterations are frequently present and have been suggested as predictors for cognitive dysfunction [66].

4.1. RNFL Measurements in Alzheimer's Disease. It has been postulated that defects in the pRNFL may be the earliest sign of AD, even before damage to the hippocampus occurs [68]. A reduction in the pRNFL thickness was observed in AD patients [69–71], especially in mean and inferior sectors [72, 73]. However, one study did not find significant differences in the pRNFL thickness between AD patients and healthy controls [74].

4.2. Macular Measurements as a Biomarker of Disease Severity in AD. Macular thickness and macular volume are importantly affected in patients with AD [72–75] and a correlation between macular volume and cognitive impairment was suggested [71]. Although foveal thickness is not considered a useful parameter to detect atrophy in AD [76], the inner and outer ETDRS sectors of the macula seem to be highly affected in these patients [73]. However, it is the combination of the pRNFL parameters (in a calculated linear discriminant function) that seems to show the highest diagnostic

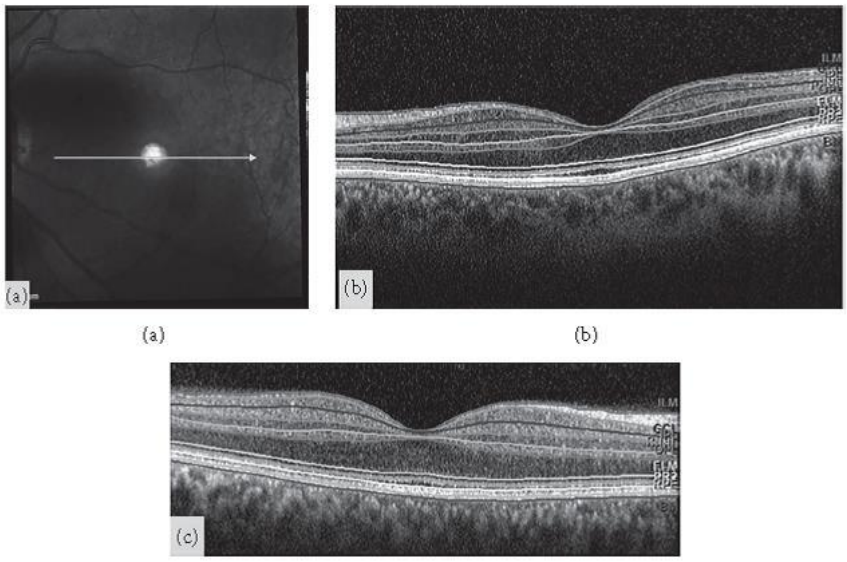

FIGURE 3: Example of segmentation analysis of the different retinal layers, in a cross-sectional linear scan of the macular area (a), obtained with Spectralis optical coherence tomography, in a healthy control (b) and a patient diagnosed with Parkinson disease (c). The marked lines are automatically provided by the segmentation software and represent the different layers of the retina. Corresponding acronyms are also provided by the segmentation software: ILM: inner limiting membrane; GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; ELM: external limiting membrane; PR: photoreceptors; MB: Bruch's membrane.

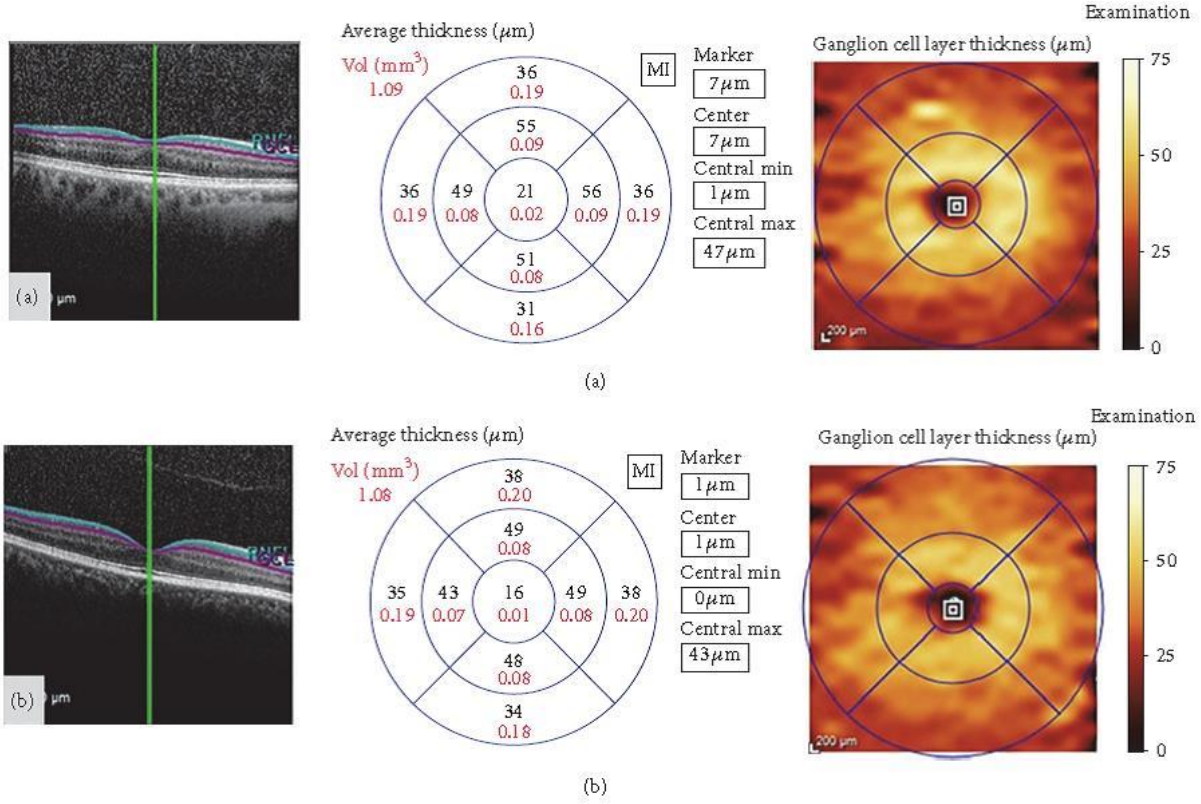

FIGURE 4: Example of segmentation analysis of the macular ganglion cell layer, obtained with Spectralis optical coherence tomography, in a healthy control (a) and a patient diagnosed with Parkinson disease (b). The segmentation report shows the ganglion cell layer thickness (in microns) and total volume (in mm^3) of the ETDRS macular area. In this patient (b), the central and inner macular areas present thinning of the ganglion cell layer, compared with the healthy control (a).

accuracy in AD, compared to combined macular thickness measurements or single thickness sectors [73]. More studies on discriminant linear function including macular volume are needed to corroborate whether macular measurements are a good biomarker for AD diagnosis.

4.3. Retinal Segmentation Analysis: Ganglion Cell Layer Thickness as a Biomarker for Diagnosis and Disease Severity in AD. Taking a step further into research on retinal biomarkers for AD, segmentation analysis of the retinal layers was recently introduced in AD studies. A previous study on postmortem AD patients did not find any evidence for ganglion cell loss compared to controls [77]. However, the sample size in Curcio study was extremely small. Other histopathological studies have suggested that disease pathology in the precortical visual system (i.e., the retina and optic nerve) is a possible mechanism underlying visual impairments observed in AD patients and may be related to ganglion cell alterations. Different sets of ganglion cells (parvocellular, magnocellular, and koniocellular ganglion cells) located in the retinal GCL result in three different pathways which identify colour and spatial contrast at different frequencies [78–80]. Previous studies (histologic, electroretinogram, and imaging studies) of these pathways suggest that the general loss of magnocellular and parvocellular cells of the retina is likely to be an important contributory mechanism for visual impairment in AD [81]. Dendritic atrophy and loss of retinal ganglion cells have also been observed in the retina of a mouse model of AD, where the accumulation of beta-amyloid in the inner retinal layers was observed [82]. These beta-amyloid deposits may be responsible for the depletion of parvo- and magnocellular cells in the retina and may be linked to visual function impairment. Moreover, results of this study suggest that dendritic atrophy of the retinal ganglion cells precedes ganglion cell loss. Since dendrites of the ganglion cells are confined to the IPL, this layer could also play a major role as a biomarker for neuronal damage in AD [82].

According to previous studies, recent animal research showed inner retinal dysfunction in a mouse model for AD [83]. Retinal segmentation analysis with OCT in this animal model demonstrated RNFL thinning, but no associated changes were observed in the ganglion cell complex [83]. Contrary to the animal model, patients with AD present a reduction of the RNFL, GCL, and IPL observable with the OCT segmentation software [84, 85], and these inner layers are also more affected in those patients with longer course of the disease [85]. Importantly, when compared to RNFL thickness, GCIPL presents higher sensitivity to discriminate AD patients from controls [84]. Moreover, the GCL and IPL are predictors of axonal damage in these patients and GCL is associated with disease duration and severity [85]. Based on these findings, it is possible that the combination of measurements of the retinal inner layers might be the ultimate biomarker for diagnosis and progression in AD.

5. Future Directions

The unique accessibility of the retina and optic nerve to in vivo measurements and the structure-function correlations provided by the afferent visual system in multiple sclerosis, Parkinson's disease, and Alzheimer's disease make the analysis of the retinal structures a useful model system to test new therapies. However, there are currently very few studies focusing on the evaluation of treatment effectiveness through OCT analysis. Further research remains to be done in a number of areas, including practical aspects of implementing clinical outcome measures in multicentre studies, further validation of other biomarkers (fluid-based biomarkers and other imaging techniques) development, and the evaluation of new different therapies effectiveness. Longitudinal studies are also key in the development of biomarkers for disease progression. Most studies evaluated in this review include only cross-sectional data, which is an important limitation for the analysis of imaging biomarkers, especially for disease progression and treatment effectiveness. We believe more longitudinal studies should be carried out, especially in PD and AD patients, since progressive changes in these two diseases have not yet been investigated.

6. Conclusions

In the past decade, OCT technology has proved its utility in the diagnosis and progression of neurodegenerative diseases. Numerous clinical studies have demonstrated that the RNFL and macular thickness are useful markers for disease progression and prognosis in MS, PD, and AD. New OCT segmentation software has also allowed better understanding of the physiopathology of axonal degeneration in these neurological diseases through the objective observation of the different retinal layers. Recent research using the latest imaging technology in ophthalmology has demonstrated that an early damage of the anterior visual pathway occurs in MS, PD, and AD and that the ganglion cell layer is the ultimate biomarker for disease diagnosis, severity, and progression. Thus, OCT technology should be used as a common and very useful clinical complement in the diagnosis and control of neurodegenerative disorders.

Consent

All subjects provided detailed consent to participate in this study, which was conducted in accordance with the guidelines established by the Ethics Committee of the Miguel Servet Hospital and based on the principles of the Declaration of Helsinki.

Competing Interests

The authors declare no competing interests.

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11. LIMITACIONES DEL ESTUDIO

La correcta realización de las pruebas en algún caso pudo verse obstaculizada por el mal estado general del paciente o su falta de cooperación, lo que podría haber causado la exclusión de los resultados de la prueba en los sujetos con mayor severidad.

Los exámenes mediante OCT precisan de una AV mínima, que permita la fijación visual del paciente.

En algunos casos no fue posible realizar alguna de las pruebas del protocolo exploratorio a pacientes con muy mala función visual o mal estado general. Para evitar el sesgo que se produciría al excluir a estos pacientes más graves del estudio, se tomaron como valores perdidos los parámetros de las pruebas que no se pudieron realizar, sin excluir a estos sujetos del análisis estadístico final.

El pequeño tamaño muestral en el caso de los estudios de EA y EP es una importante limitación, pudiendo afectar a la significación estadística.

Todos los estudios se realizaron con el OCT Cirrus HD, sin embargo hay estudios publicados que sugieren una mayor sensibilidad con Spectralis OCT. (AE[15], EP[59], EM[37, 80]).

12. CONCLUSIONES

I. Los sujetos con las enfermedades neurodegenerativas de Alzheimer, Parkinson y esclerosis múltiple presentan cambios estructurales en la retina y en el nervio óptico, detectables mediante tomografía de coherencia óptica tipo Cirrus. Se registra disminución del espesor de sus capas con respecto a los sujetos sanos de su misma edad.

II. Los espesores retinianos se encuentran más disminuidos cuanto mayor es el tiempo de evolución de la enfermedad.

III. Los pacientes con las enfermedades neurodegenerativas de Alzheimer, Parkinson y esclerosis múltiple presentan alteraciones en su capacidad visual, detectables en los test de agudeza visual, sensibilidad al contraste y percepción del color. La sensibilidad al contraste es el parámetro más frecuentemente afectado, incluso en etapas precoces, en las tres enfermedades.

IV. Las exploraciones en los pacientes afectos de las enfermedades neurodegenerativas de Alzheimer, Parkinson y esclerosis múltiple, presentan correlación función-estructura. La sensibilidad al contraste es el parámetro funcional que más se correlaciona con las medidas estructurales en las tres enfermedades.

V. En la enfermedad de Alzheimer las mediciones de los espesores maculares se correlacionan con la agudeza visual, con la sensibilidad al contraste y con la percepción del color medida mediante test de Farnsworth, por lo que el espesor macular puede considerarse un indicador fiable de la alteración de la función visual en dichos pacientes.

VI. En la enfermedad de Parkinson, las mediciones de los espesores maculares se correlacionan con los parámetros funcionales y la medición del espesor de la capa de células ganglionares se correlaciona principalmente con la percepción del color medida

con el test L'Anthony. El espesor macular y de la capa de células ganglionares pueden considerarse indicadores de la función visual en dichos pacientes.

VII. En la esclerosis múltiple, las mediciones de los espesores maculares se correlacionan con los parámetros funcionales y principalmente con el test de percepción del color L'Anthony. La medición del espesor de la capa de células ganglionares se correlaciona con la sensibilidad al contraste. Las mediciones de los espesores maculares y de la capa de células ganglionares pueden considerarse buenos indicadores de la función visual en dichos pacientes.

VIII. Pacientes con grados más severos de enfermedad presentan peores mediciones en función y calidad visual. En el caso de la esclerosis múltiple no se encontraron diferencias entre los grupos con y sin antecedente de neuritis óptica, sugiriendo que la disfunción visual es secundaria a la neurodegeneración.

IX. La evaluación neurooftalmológica funcional y estructural de los pacientes afectos de las enfermedades neurodegenerativas de Alzheimer, Parkinson y esclerosis múltiple puede servir como biomarcador en estas patologías.

CONCLUSIONS

I. Subjects with neurodegenerative diseases of Alzheimer, Parkinson and multiple sclerosis present structural changes in the retina and optic nerve and these changes are detectable with Cirrus OCT. Decreased thickness is detected in patients compared to healthy controls.

II. The longer disease duration, the fewer retinal thickness is found.

III. Patients with neurodegeneratives diseases such as Alzheimer, Parkinson and multiple sclerosis show alterations in visual capacity detectable with visual acuity, contrast sensitivity and color perception tests. Contrast sensitivity is the parameter more frequently affected, even at early stages, in all the three pathologies.

IV. Functional-structural correlation is detected in examinations of patients with neurodegeneratives diseases of Alzheimer, Parkinson and multiple sclerosis. Contrast sensitivity is the functional parameter that is most strongly correlated with structural measurements in all the three pathologies.

V. Macular thickness measurements, in patients with Alzheimer's disease, correlate with visual acuity, contrast sensitivity and color perception, using Farnsworth's test, so macular thickness can be considered a reliable indicator of visual impairment in these patients.

VI. Macular thickness measurements, in patients with Parkinson's disease, correlate with functional parameters, and ganglion cell layer thickness mainly correlate with color perception, using L'Anthony's test. Macular and ganglion cell layer thickness can be considered indicators of visual function in these patients.

VII. Macular thickness measurements, in patients with multiple sclerosis, correlate with functional parameters and mainly with L'Anthony test for color perception. Ganglion cell layer thickness measurements correlate with contrast sensitivity. Macular and ganglion cell layer thickness measurements can be considered good indicators of visual function in these patients.

VIII. Patients with more severe degrees of disease present worse measurements in function and visual quality. In case of multiple sclerosis no differences were found

between groups with and without history of optic neuritis, suggesting that visual dysfunction is secondary to neurodegeneration.

IX. Functional and structural neurophthalmologic examination of patients with neurodegeneratives diseases such as Alzheimer, Parkinson and multiple sclerosis can be used as biomarker in these pathologies.

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14. APÉNDICES

Apéndice I. Factor de impacto de las revistas y áreas temáticas.

1. Autores: Polo V, **Rodrigo MJ**, Garcia-Martin E, Otin S, Larrosa JM, Fuertes MI, Bambo MP, Pablo LE, Satue M**.**

   Título: Visual dysfunction and its correlation with retinal changes in patients with Alzheimer´s disease.

   Revista: EYE. (en prensa).

   ISSN: 0950-222X

   Índice de Impacto (JCR 2015): 2,213

   Posición entre las revistas de oftalmología en el JCR 2015: 19/56. Cuartil 2.

   Área temática de la revista: Oftalmología.

2. Autores: Polo V, Satue M, **Rodrigo MJ**, Otin S, Alarcia R, Bambo MP, Fuertes MI, Larrosa JM, Pablo LE, Garcia-Martin E.

   Título: Visual dysfunction and its correlation with retinal changes in patients with Parkinson disease.

   Revista: British Medical Journal Open (BMJ Open).

   ISSN: 2044-6055

   PMID: 27154474

   Índice de Impacto (JCR 2015): 2,562

   Posición entre las revistas de Medicina General e Interna (Medicine, General & Internal) en el JCR 2014: 40/154. Cuartil 1.

   Área temática de la revista: Medicina.

3. Autores: Satue M, **Rodrigo MJ**, Otin S, Bambo MP, Fuertes MI, Ara JR, Martin J, Polo V, Larrosa JM, Pablo LE, Garcia-Martin E**.**

   Título: Relationship between visual dysfunction and retinal changes in patients with multiple sclerosis.

   Revista: Plos One.

   ISSN: 1932-6203

   PMID: 27351450

   Índice de Impacto (JCR 2015): 3,54

   Posición entre las revistas de Ciencias Multidisciplinar (Multidisciplinary Sciences) en el JCR 2015: 9/57. Cuartil 1.

   Área temática de la revista: Ciencias multidisciplinares.

4. Autores: Satue M, Obis J, **Rodrigo MJ**, Otin S, Fuertes MI, Vilades E, Gracia H, Ara JR, Alarcia R, Polo V, Larrosa JM, Pablo LE, Garcia-Martin E.

   Título: Optical Coherence Tomography as a Biomarker for Diagnosis, Progression, and Prognosis of Neurodegenerative Diseases.

   Revista: Journal of Ophthalmology.

   ISSN: 2090-004X (Print), ISSN: 2090-0058 (Online)

   PMID: 27840739

   Índice de Impacto (JCR 2015): 1.463.

   Posición entre las revistas de oftalmología: Cuartil 2.

   Área temática de la revista: Oftalmología.

Apéndice II. Justificación de la contribución del doctorando en cada publicación.

1. **Polo V, Rodrigo MJ, Garcia-Martin E, Otin S, Larrosa JM, Fuertes MI, Bambo MP, Pablo LE, Satue M. Visual dysfunction and its correlation with retinal changes in patients with Alzheimer´s disease. Eye (Lond) 2017 (en prensa).**

El doctorando realizó todo el trabajo de campo con los pacientes y las pruebas oftalmológicas, coordinó el resto de pruebas, realizó la recogida de datos, y participó en la redacción del artículo, en la realización de los diversos análisis estadísticos, en la revisión y en la corrección del artículo científico.

2. **Polo V, Satue M, Rodrigo MJ, Otin S, Alarcia R, Bambo MP, Fuertes MI, Larrosa JM, Pablo LE, Garcia-Martin E. Visual dysfunction and its correlation with retinal changes in patients with Parkinson's disease: an observational cross-sectional study. BMJ Open 2016;6:e009658.**

El doctorando realizó todo el trabajo de campo con los pacientes y las pruebas oftalmológicas, coordinó el resto de pruebas, realizó la recogida de datos, la redacción del artículo y participó en la realización de los diversos análisis estadísticos, en la redacción, la revisión y la corrección del artículo científico.

3. **Satue M, Rodrigo MJ, Otin S, Bambo MP, Fuertes MI, Ara JR, Martin J, Polo V, Larrosa JM, Pablo L, Garcia-Martin E. Relationship between visual dysfunction and retinal changes in patients with multiple sclerosis. PLoS ONE 2016;11(6):e0157293.**

El doctorando realizó todo el trabajo de campo con los pacientes y las pruebas oftalmológicas, coordinó el resto de pruebas, realizó la recogida de datos, la redacción del artículo y participó en la realización de los diversos análisis estadísticos, en la redacción, la revisión y la corrección del artículo científico.

4. **Satue M, Obis J, Rodrigo MJ, Otin S, Fuertes MI, Vilades E, Gracia H, Ara JR, Alarcia R, Polo V, Larrosa JM, Pablo LE, Garcia-Martin E. Optical coherence tomography as a biomarker for diagnosis, progression, and prognosis of neurodegenerative diseases. J Ophthalmol. 2016;2016:8503859. Review.**

El doctorando realizó la revisión de los artículos científicos recientes en torno al tema revisado, y participó en la posterior redacción, así como en la revisión y en la corrección del artículo definitivo.

Apéndice III. Renuncia de los coautores no doctores a usar el artículo en su propia tesis doctoral.

Yo, Javier Obis Alfaro, que figuro como coautor del siguiente artículo:

Satue M, Obis J, Rodrigo MJ, Otin S, Fuertes MI, Vilades E, Gracia H, Ara JR, Alarcia R, Polo V, Larrosa JM, Pablo LE, Garcia-Martin E. Optical coherence tomography as a biomarker for diagnosis, progression, and prognosis of neurodegenerative diseases. J Ophthalmol. 2016;2016:8503859. Review.

Renuncio a presentar dicho trabajo como parte de mi tesis doctoral.

En Zaragoza, a 15 de Enero de 2017

Fdo: Javier Obis Alfaro

Yo, Elisa Viladés Palomar, que figuro como coautor del siguiente artículo:

Satue M, Obis J, Rodrigo MJ, Otin S, Fuertes MI, Vilades E, Gracia H, Ara JR, Alarcia R, Polo V, Larrosa JM, Pablo LE, Garcia-Martin E. Optical coherence tomography as a biomarker for diagnosis, progression, and prognosis of neurodegenerative diseases. J Ophthalmol. 2016;2016:8503859. Review.

Renuncio a presentar dicho trabajo como parte de mi tesis doctoral.

En Zaragoza, a 15 de Enero de 2017

Fdo: Elisa Viladés Palomar

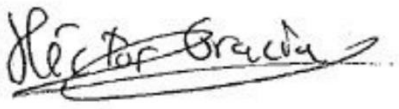
Yo, Héctor Gracia Cabrera, que figuro como coautor del siguiente artículo:

Satue M, Obis J, Rodrigo MJ, Otin S, Fuertes MI, Vilades E, Gracia H, Ara JR, Alarcia R, Polo V, Larrosa JM, Pablo LE, Garcia-Martin E. Optical coherence tomography as a biomarker for diagnosis, progression, and prognosis of neurodegenerative diseases. J Ophthalmol. 2016;2016:8503859. Review.

Renuncio a presentar dicho trabajo como parte de mi tesis doctoral.

En Zaragoza, a 15 de Enero de 2017

Fdo: Héctor Gracia Cabrera



Apéndice IV: Consentimientos informados: EA, EP y EM.

Evaluación de la función visual y de la capa de fibras nerviosas de la retina.
Versión 2, fecha de 12 / Febrero /2012.

HOJA DE INFORMACIÓN AL PACIENTE

Se le va a realizar una evaluación de su función visual. Con el objetivo de mejorar continuamente la calidad asistencial a los pacientes, los Servicios de Oftalmología, Neurología y Neurofisiología del Hospital Miguel Servet de Zaragoza están desarrollando un estudio de investigación en que se pretende cuantificar la afectación visual en pacientes del área sanitaria.

Para ello se le llevarán a cabo las exploraciones enumeradas a continuación: agudeza visual (que consiste en cuantificar cuántas letras puede usted discriminar a 6 metros de distancia), sensibilidad al contraste (que consiste en cuantificar cuántas letras puede usted discriminar a 1 metro de distancia variando la luminosidad de las letras), visión de colores (que consiste en cuantificar si es capaz de distinguir los colores entre si), Tomografía de coherencia óptica (que consiste en una fotografía del ojo que nos permite observar sus estructuras), Potenciales evocados visuales y Electro-retinograma (que son dos pruebas en las que usted deberá mirar un punto fijo en una pantalla mientras le registramos su actividad cerebral con un casco colocado sobre su cabeza). Todas estas exploraciones son no invasivas ni dolorosas y se llevarán a cabo en las consultas externas del Hospital Miguel Servet, en una revisión a cargo del Servicio de Oftalmología y otra del Servicio de Neurofisiología. Estas pruebas nos permiten evaluar el estado de su sistema visual y detectar la presencia de patologías del mismo en caso de que existan, posibilitando así su posterior tratamiento si se considerara adecuado.

Su participación en este estudio no implica la realización de exploraciones complementarias que no le serían realizadas en caso de no participar en el mismo, sino la aceptación de que, de modo absolutamente confidencial, sean recogidos y utilizados los resultados de sus exploraciones con el objetivo de dicho proyecto de investigación. Su participación es voluntaria y puede abandonar el estudio en el momento en que lo decida, sin que esto tenga repercusión alguna en su atención sanitaria futura.

El equipo investigador encargado de dicho estudio serán las doctoras Elena García Martín y María Pilar Bambó Rubio, oftalmólogas del Hospital Miguel Servet de Zaragoza; con las que podrá contactar a lo largo del estudio en cualquier momento que así lo desee, acudiendo al servicio de oftalmología de dicho hospital.

Evaluación de la función visual y de la capa de fibras nerviosas de la retina. Versión 2, fecha de 12 / Febrero /2012.

CONSENTIMIENTO INFORMADO

Título del proyecto: "Evaluación de la función visual y de la capa de fibras nerviosas de la retina".

Yo, …… (nombre y apellidos) he leído la hoja de información que se me ha entregado, he podido hacer preguntas sobre el estudio, habiendo recibido suficiente información sobre el estudio.

Comprendo que mi participación es voluntaria y que puedo retirarme del estudio en el momento en que lo desee, sin tener que dar explicaciones y sin que esto repercuta en mis cuidados médicos.

De este modo, presto libremente mi conformidad para participar en el estudio y para que mis datos clínicos sean revisados para los fines del mismo, consciente de que este consentimiento es revocable.

Firma del paciente

DNI:

Fecha:

Firma del representante legal:

DNI:

Fecha:

Como investigador del estudio he explicado la naturaleza y el propósito del mismo al paciente mencionado.

Firma del investigador

Nº Colegiado:

Fecha:

Evaluación de la función visual y de la capa de fibras nerviosas de la retina en pacientes con Enfermedad de Parkinson.

Versión 2, fecha de 17/Marzo/2012

HOJA DE INFORMACIÓN AL PACIENTE

Se le va a realizar una evaluación de su función visual. Con el objetivo de mejorar continuamente la calidad asistencial a los pacientes, los Servicios de Oftalmología, Neurología y Neurofisiología del Hospital Miguel Servet de Zaragoza están desarrollando un estudio de investigación en el que se pretende evaluar si existe afectación visual debida a la enfermedad de Parkinson.

Para ello se le llevarán a cabo las exploraciones enumeradas a continuación: agudeza visual (que consiste en cuantificar cuántas letras puede usted discriminar a 6 metros de distancia), sensibilidad al contraste (que consiste en cuantificar cuántas letras puede usted discriminar a 1 metro de distancia variando la luminosidad de las letras), visión de colores (que consiste en cuantificar si es capaz de distinguir los colores entre si), Tomografía de coherencia óptica (que consiste en una fotografía del ojo que nos permite observar sus estructuras), Potenciales evocados visuales y Electro-retinograma (que son dos pruebas en las que usted deberá mirar un punto fijo en una pantalla mientras le registramos su actividad cerebral con un casco colocado sobre su cabeza). Todas estas exploraciones son no invasivas ni dolorosas y se llevarán a cabo en las consultas externas del Hospital Miguel Servet, en una revisión a cargo del Servicio de Oftalmología y otra del Servicio de Neurofisiología. Estas pruebas nos permiten evaluar el estado de su sistema visual y detectar la presencia de patologías del mismo en caso de que existan, posibilitando así su posterior tratamiento si se considerara adecuado. Los resultados de las pruebas realizadas serán analizados estadísticamente y comparados con

la población sana para poder evaluar si existe una alteración en la función visual debida a la enfermedad de Parkinson.

Su participación en este estudio no implica la realización de exploraciones complementarias que no le serían realizadas en caso de no participar en el mismo, sino la aceptación de que, de modo absolutamente confidencial y siguiendo la LO 15/99 de protección de datos de carácter personal, sean recogidos y utilizados los resultados de sus exploraciones con el objetivo de dicho proyecto de investigación. Su participación es voluntaria y puede abandonar el estudio en el momento en que lo decida, sin que esto tenga repercusión alguna en su atención sanitaria futura.

En el caso de encontrar alguna alteración oftalmológica inesperada que requiera de tratamiento se derivará al paciente al departamento correspondiente para su evaluación y tratamiento adecuado.

El equipo investigador encargado de dicho estudio serán las doctoras Elena García Martín y María Satué Palacián, oftalmólogas del Hospital Miguel Servet de Zaragoza; con las que podrá contactar a lo largo del estudio en cualquier momento que así lo desee, acudiendo al servicio de oftalmología de dicho hospital. Así mismo, el equipo investigador estará en contacto con la Asociación de Parkinson de Aragón, para la divulgación de resultados obtenidos en el estudio mediante charlas orientativas y artículos que promueve dicha Asociación.

Ningún componente del equipo investigador va a obtener beneficio económico ni de otra clase a través de la realización de este estudio.

Evaluación de la función visual y de la capa de fibras nerviosas de la retina en pacientes con Enfermedad de Parkinson.

Versión 2, fecha de 17/Marzo/2012

CONSENTIMIENTO INFORMADO

Título del proyecto: "Evaluación de la función visual y de la capa de fibras nerviosas de la retina en pacientes con Enfermedad de Parkinson".

Yo, …… (nombre y apellidos) he leído la hoja de información que se me ha entregado, he podido hacer preguntas sobre el estudio, habiendo recibido suficiente información sobre el estudio.

Comprendo que mi participación es voluntaria y que puedo retirarme del estudio en el momento en que lo desee, sin tener que dar explicaciones y sin que esto repercuta en mis cuidados médicos.

De este modo, presto libremente mi conformidad para participar en el estudio y para que mis datos clínicos sean revisados para los fines del mismo, consciente de que este consentimiento es revocable.

Firma del paciente

DNI:

Fecha:

Firma del representante legal:

DNI:

Fecha:

Como investigador del estudio he explicado la naturaleza y el propósito del mismo al paciente mencionado.

Firma del investigador

Nº Colegiado:

Fecha:

Evaluación de los tratamientos para la Esclerosis Múltiple empleando el análisis periódico de la capa de fibras nerviosas de la retina como marcador de degeneración axonal.

Versión 2, fecha de 12/Febrero/2012.

HOJA DE INFORMACIÓN AL PACIENTE

Se le va a realizar una evaluación de su función visual para valorar si se encuentra afectada como consecuencia de la degeneración neuronal que aparece de modo subagudo a lo largo de la evolución de la enfermedad de Esclerosis Múltiple. Con el objetivo de mejorar continuamente la calidad asistencial a los pacientes, los Servicios de Oftalmología, Neurología y Neurofisiología del Hospital Miguel Servet de Zaragoza están desarrollando un estudio de investigación en que se pretende cuantificar la afectación visual de pacientes que como usted padecen Esclerosis Múltiple y determinar si dicha evaluación puede ser útil como marcador de progresión de la enfermedad y de respuesta a los tratamientos administrados.

Para ello se le llevarán a cabo las exploraciones enumeradas a continuación: agudeza visual (que consiste en cuantificar cuántas letras puede usted discriminar a 6 metros de distancia), sensibilidad al contraste (que consiste en cuantificar cuántas letras puede usted discriminar a 1 metro de distancia variando la luminosidad de las letras), visión de colores (que consiste en cuantificar si es capaz de distinguir los colores entre si), Tomografía de coherencia óptica (que consiste en una fotografía del ojo que nos permite observar sus estructuras), Potenciales evocados visuales y Electro-retinograma (que son dos pruebas en las que usted deberá mirar un punto fijo en una pantalla mientras le registramos su actividad cerebral con un casco colocado sobre su cabeza). Todas estas exploraciones son no invasivas ni dolorosas y se llevarán a cabo en las consultas externas del Hospital Miguel Servet, en una revisión a cargo del Servicio de

Oftalmología y otra del Servicio de Neurofisiología. Estas pruebas nos permiten evaluar el estado de su sistema visual y detectar la presencia de patologías del mismo en caso de que existan, posibilitando así su posterior tratamiento si se considerara adecuado.

Su participación en este estudio no implica la realización de exploraciones complementarias que no le serían realizadas en caso de no participar en el mismo, sino la aceptación de que, de modo absolutamente confidencial, sean recogidos y utilizados los resultados de sus exploraciones con el objetivo de dicho proyecto de investigación. Su participación es voluntaria y puede abandonar el estudio en el momento en que lo decida, sin que esto tenga repercusión alguna en su atención sanitaria futura.

El equipo investigador encargado de dicho estudio serán las doctoras Elena García Martín y Raquel Herrero Latorre, oftalmólogas del Hospital Miguel Servet de Zaragoza; con las que podrá contactar a lo largo del estudio en cualquier momento que así lo desee, acudiendo al servicio de oftalmología de dicho hospital.

Evaluación de los tratamientos para la Esclerosis Múltiple empleando el análisis periódico de la capa de fibras nerviosas de la retina como marcador de degeneración axonal.

Versión 2, fecha de 12/Febrero/2012.

CONSENTIMIENTO INFORMADO

Título del proyecto: “Evaluación de los tratamientos para la Esclerosis Múltiple empleando el análisis periódico de la capa de fibras nerviosas de la retina como marcador de degeneración axonal.”

Yo, …… (nombre y apellidos) he leído la hoja de información que se me ha entregado, he podido hacer preguntas sobre el estudio, habiendo recibido suficiente información sobre el estudio.

Comprendo que mi participación es voluntaria y que puedo retirarme del estudio en el momento en que lo desee, sin tener que dar explicaciones y sin que esto repercuta en mis cuidados médicos.

De este modo, presto libremente mi conformidad para participar en el estudio y para que mis datos clínicos sean revisados para los fines del mismo, consciente de que este consentimiento es revocable.

Firma del paciente

DNI:

Fecha:

Como investigador del estudio he explicado la naturaleza y el propósito del mismo al paciente mencionado.

Firma del investigador

Nº Colegiado:

Fecha:

Apéndice V: Documentación del Comité Ético de Investigación Clínica de Aragón (CEICA).

GOBIERNO DE ARAGON
Departamento de Sanidad, Bienestar Social y Familia

Informe Dictamen Favorable
Proyecto Investigación Biomédica
C.P. - C.I. PI12/000
15 de febrero de 2012

/CEIC Aragón (CEICA)

Dña. María González Hinjos, Secretaria del CEIC Aragón (CEICA)

CERTIFICA

1º. Que el CEIC Aragón (CEICA) en su reunión del día 15/02/2012, Acta Nº 03/2012 ha evaluado la propuesta del investigador referida al estudio:

Título: Evaluación de la función visual y de la capa de fibras nerviosas de la retina en los pacientes con Enfermedad de Alzheimer.

Versión Protocolo: enero 2012
Versión hoja de información al paciente y consentimiento informado V2, de 12/02/2012

1º. Considera que

- El proyecto se plantea siguiendo los requisitos de la Ley 14/2007, de 3 de julio, de Investigación Biomédica y su realización es pertinente.
- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
- Son adecuados tanto el procedimiento para obtener el consentimiento informado como la compensación prevista para los sujetos por daños que pudieran derivarse de su participación en el estudio.
- El alcance de las compensaciones económicas previstas no interfiere con el respeto a los postulados éticos.
- La capacidad de los Investigadores y los medios disponibles son apropiados para llevar a cabo el estudio.

2º. Por lo que este CEIC emite un **DICTAMEN FAVORABLE.**

3º. Este CEIC acepta que dicho estudio sea realizado en los siguientes Centros por los Investigadores:

Elena García Martín

Lo que firmo en Zaragoza, a 15 de febrero de 2012

Fdo:

GOBIERNO DE ARAGON · DEPARTAMENTO DE SANIDAD, BIENESTAR SOCIAL Y FAMILIA · COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA

Dña. María González Hinjos
Secretaria del CEIC Aragón (CEICA)

Departamento de Salud y Consumo del Gobierno de Aragón
Avda Gómez Laguna 25 Zaragoza 50009 Zaragoza España
Tel. 976 71 48 57 Fax. 976 71 55 54 Correo electrónico mgonzalezh.ceic@aragob.es

GOBIERNO DE ARAGÓN
Departamento de Salud y Consumo

COMITÉ ÉTICO DE INVESTIGACIÓN
CLÍNICA DE ARAGÓN (CEICA)
Avda. Gómez Laguna, 25 planta 11
50009 Zaragoza

COMPOSICIÓN DEL COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA DE ARAGÓN

Dra. María González Hinjos, Secretaria del Comité Ético de Investigación Clínica de Aragón,

CERTIFICA

1º En la reunión celebrada el día 15 de febrero de 2012, correspondiente al Acta nº **CP03/2012**, se cumplieron los requisitos establecidos en la legislación vigente -Real Decreto 223/2004 y Decreto 26/2003 del Gobierno de Aragón, modificado por el Decreto 292/2005– para que la decisión del citado CEIC sea válida.

3º El CEIC de Aragón, tanto en su composición, como en sus PNT, cumple con las normas de BPC.

4º La composición del CEIC de Aragón en la citada fecha, era la siguiente:

- **Presidente:** Cesar Loris Pablo; Médico. Servicio de Pediatría. Hospital Universitario Miguel Servet. Representante de Comisión de Investigación.
- **Vicepresidente:** Carlos Aibar Remón; Médico. Servicio de Medicina Preventiva y Salud Pública. Hospital Clínico Universitario Lozano Blesa. Profesional Sanitario experto en epidemiología clínica.
- **Secretaria:** María González Hinjos; Farmacéutica.
- Pilar Comet Cortés; Enfermera. Unidad Mixta de Investigación. Hospital Clínico Universitario Lozano Blesa.
- Marina Heredia Ríos; Representante de las Organizaciones de Consumidores y Usuarios.
- Gabriel Hernández Delgado; Médico. Servicio de Radiología. Hospital Universitario Miguel Servet. Representante de Comisión de Investigación.
- Angela Idoipe Tomás; Farmacéutica. Servicio de Farmacia. Hospital Universitario Miguel Servet. Farmacéutica de Hospital.
- María Jesús Lallana Álvarez. Farmacéutica de Atención Primaria de Zaragoza Sector III.
- Jesús Magdalena Bello; Médico. Centro de Salud de Azuara. Médico con labor asistencial y representante del Comité de Ética Asistencial del Área de Atención Primaria II y V.
- Mariano Mateo Arrizabalaga; Médico. Servicio de Farmacología Clínica. Hospital Clínico Universitario Lozano Blesa.
- Elisa Moreu Carbonell; Jurista. Profesora de la Facultad de Derecho, Universidad de Zaragoza.
- Javier Perfecto Ejarque; Médico. Centro de Salud Arrabal. Médico con labor asistencial.
- Alexandra Prados Torres; Médico. Instituto Aragonés de Ciencias de la Salud. Representante de Comisión de Investigación.
- José Puzo Foncillas; Médico. Servicio de Bioquímica. Hospital General San Jorge. Representante de Comisión de Investigación.
- Mónica Torrijos Tejada; Médico. Instituto Aragonés de Ciencias de la Salud.

Para que conste donde proceda, y a petición del promotor,

Zaragoza, a 15 de febrero de 2012

GOBIERNO DE ARAGÓN · DEPARTAMENTO DE SANIDAD, BIENESTAR SOCIAL Y FAMILIA · INVESTIGACIÓN CLÍNICA

Firmado: María González Hinjos

GOBIERNO DE ARAGON
Departamento de Sanidad, Bienestar Social y Familia

Informe Dictamen Favorable
Proyecto Investigación Biomédica
C.P. - C.I. PI12/0028
21 de marzo de 2012

/CEIC Aragón (CEICA)

Dña. María González Hinjos, Secretaria del CEIC Aragón (CEICA)

CERTIFICA

1º. Que el CEIC Aragón (CEICA) en su reunión del día 21/03/2012, Acta Nº CP06/2012 ha evaluado la propuesta del investigador referida al estudio:

Título: Evaluación de la función visual y de la capa de fibras nerviosas de la retina en los pacientes con Enfermedad de Parkinson.

Versión Protocolo: v 2 de 17/03/2012
Versión hoja de información al paciente y consentimiento informado **v 2 de 17/03/2012**

1º. Considera que

- El proyecto se plantea siguiendo los requisitos de la Ley 14/2007, de 3 de julio, de Investigación Biomédica y su realización es pertinente.
- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
- Son adecuados tanto el procedimiento para obtener el consentimiento informado como la compensación prevista para los sujetos por daños que pudieran derivarse de su participación en el estudio.
- El alcance de las compensaciones económicas previstas no interfiere con el respeto a los postulados éticos.
- La capacidad de los Investigadores y los medios disponibles son apropiados para llevar a cabo el estudio.

2º. Por lo que este CEIC emite un **DICTAMEN FAVORABLE**.

3º. Este CEIC acepta que dicho estudio sea realizado en los siguientes Centros por los Investigadores:

Elena García Martín , Hospital Universitario Miguel Servet

Lo que firmo en Zaragoza, a 21 de marzo de 2012

Fdo:

COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA

Dña. María González Hinjos
Secretaria del CEIC Aragón (CEICA)

GOBIERNO DE ARAGON
Departamento de Salud y Consumo

COMITÉ ÉTICO DE INVESTIGACIÓN
CLÍNICA DE ARAGÓN (CEICA)
Avda. Gómez Laguna, 25 planta 11
50009 Zaragoza

COMPOSICIÓN DEL COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA DE ARAGÓN

Dra. María González Hinjos, Secretaria del Comité Ético de Investigación Clínica de Aragón,

CERTIFICA

1º En la reunión celebrada el día 21 de marzo de 2012, correspondiente al Acta nº **CP06/2012**, se cumplieron los requisitos establecidos en la legislación vigente -Real Decreto 223/2004 y Decreto 26/2003 del Gobierno de Aragón, modificado por el Decreto 292/2005– para que la decisión del citado CEIC sea válida.

3º El CEIC de Aragón, tanto en su composición, como en sus PNT, cumple con las normas de BPC.

4º La composición del CEIC de Aragón en la citada fecha, era la siguiente:

- **Presidente:** Cesar Loris Pablo; Médico. Servicio de Pediatría. Hospital Universitario Miguel Servet. Representante de Comisión de Investigación.
- **Vicepresidente:** Carlos Aibar Remón; Médico. Servicio de Medicina Preventiva y Salud Pública. Hospital Clínico Universitario Lozano Blesa. Profesional Sanitario experto en epidemiología clínica.
- **Secretaria:** María González Hinjos; Farmacéutica.
- Pilar Comet Cortés; Enfermera. Unidad Mixta de Investigación. Hospital Clínico Universitario Lozano Blesa.
- Marina Heredia Ríos; Representante de las Organizaciones de Consumidores y Usuarios.
- Gabriel Hernández Delgado; Médico. Servicio de Radiología. Hospital Universitario Miguel Servet. Representante de Comisión de Investigación.
- Angela Idoipe Tomás; Farmacéutica. Servicio de Farmacia. Hospital Universitario Miguel Servet. Farmacéutica de Hospital.
- María Jesús Lallana Álvarez. Farmacéutica de Atención Primaria de Zaragoza Sector III.
- Jesús Magdalena Bello; Médico. Centro de Salud de Azuara. Médico con labor asistencial y representante del Comité de Ética Asistencial del Área de Atención Primaria II y V.
- Mariano Mateo Arrizabalaga; Médico. Servicio de Farmacología Clínica. Hospital Clínico Universitario Lozano Blesa.
- Elisa Moreu Carbonell; Jurista. Profesora de la Facultad de Derecho, Universidad de Zaragoza.
- Javier Perfecto Ejarque; Médico. Centro de Salud Arrabal. Médico con labor asistencial.
- Alexandra Prados Torres; Médico. Instituto Aragonés de Ciencias de la Salud. Representante de Comisión de Investigación.
- José Puzo Foncillas; Médico. Servicio de Bioquímica. Hospital General San Jorge. Representante de Comisión de Investigación.
- Mónica Torrijos Tejada; Médico. Instituto Aragonés de Ciencias de la Salud.

Para que conste donde proceda, y a petición del promotor,

Zaragoza, a 21 de marzo de 2012

Firmado: María González Hinjos

GOBIERNO DE ARAGON
Departamento de Sanidad, Bienestar Social y Familia

Informe Dictamen Favorable
Proyecto Investigación Biomédica
C.P. - C.I. PI12/0015
15 de febrero de 2012

/CEIC Aragón (CEICA)

Dña. María González Hinjos, Secretaria del CEIC Aragón (CEICA)

CERTIFICA

1º. Que el CEIC Aragón (CEICA) en su reunión del día 15/02/2012, Acta Nº 03/2012 ha evaluado la propuesta del investigador referida al estudio:

Título: Evaluación de los tratamientos para la Esclerosis Múltiple y de su impacto sobre la calidad de vida del paciente mediante el análisis periódico de la capa de fibras nerviosas de la retina.

Versión Protocolo: 2011
Versión hoja de información al paciente y consentimiento informado V2, de 12/02/2012

1º. Considera que

- El proyecto se plantea siguiendo los requisitos de la Ley 14/2007, de 3 de julio, de Investigación Biomédica y su realización es pertinente.
- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
- Son adecuados tanto el procedimiento para obtener el consentimiento informado como la compensación prevista para los sujetos por daños que pudieran derivarse de su participación en el estudio.
- El alcance de las compensaciones económicas previstas no interfiere con el respeto a los postulados éticos.
- La capacidad de los Investigadores y los medios disponibles son apropiados para llevar a cabo el estudio.

2º. Por lo que este CEIC emite un **DICTAMEN FAVORABLE.**

3º. Este CEIC acepta que dicho estudio sea realizado en los siguientes Centros por los Investigadores:

Luis Emilio Pablo Júlvez

Lo que firmo en Zaragoza, a 15 de febrero de 2012

Fdo:

GOBIERNO DE ARAGON · DEPARTAMENTO DE SANIDAD, BIENESTAR SOCIAL Y FAMILIA · COMISIÓN DE INVESTIGACIÓN CLÍNICA

Dña. María González Hinjos
Secretaria del CEIC Aragón (CEICA)

Departamento de Salud y Consumo del Gobierno de Aragón
Avda Gómez Laguna 25 Zaragoza 50009 Zaragoza España
Tel. 976 71 48 57 Fax. 976 71 55 54 Correo electrónico mgonzalezh.ceic@aragob.es

GOBIERNO DE ARAGON
Departamento de Salud y Consumo

COMITÉ ÉTICO DE INVESTIGACIÓN
CLÍNICA DE ARAGÓN (CEICA)
Avda. Gómez Laguna, 25 planta 11
50009 Zaragoza

COMPOSICIÓN DEL COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA DE ARAGÓN

Dra. María González Hinjos, Secretaria del Comité Ético de Investigación Clínica de Aragón,

CERTIFICA

1º En la reunión celebrada el día 15 de febrero de 2012, correspondiente al Acta nº **CP03/2012**, se cumplieron los requisitos establecidos en la legislación vigente -Real Decreto 223/2004 y Decreto 26/2003 del Gobierno de Aragón, modificado por el Decreto 292/2005– para que la decisión del citado CEIC sea válida.

3º El CEIC de Aragón, tanto en su composición, como en sus PNT, cumple con las normas de BPC.

4º La composición del CEIC de Aragón en la citada fecha, era la siguiente:

- **Presidente:** Cesar Loris Pablo; Médico. Servicio de Pediatría. Hospital Universitario Miguel Servet. Representante de Comisión de Investigación.
- **Vicepresidente:** Carlos Aibar Remón; Médico. Servicio de Medicina Preventiva y Salud Pública. Hospital Clínico Universitario Lozano Blesa. Profesional Sanitario experto en epidemiología clínica.
- **Secretaria:** María González Hinjos; Farmacéutica.
- Pilar Comet Cortés; Enfermera. Unidad Mixta de Investigación. Hospital Clínico Universitario Lozano Blesa.
- Marina Heredia Ríos; Representante de las Organizaciones de Consumidores y Usuarios.
- Gabriel Hernández Delgado; Médico. Servicio de Radiología. Hospital Universitario Miguel Servet. Representante de Comisión de Investigación.
- Angela Idoipe Tomás; Farmacéutica. Servicio de Farmacia. Hospital Universitario Miguel Servet. Farmacéutica de Hospital.
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- Mariano Mateo Arrizabalaga; Médico. Servicio de Farmacología Clínica. Hospital Clínico Universitario Lozano Blesa.
- Elisa Moreu Carbonell; Jurista. Profesora de la Facultad de Derecho, Universidad de Zaragoza.
- Javier Perfecto Ejarque; Médico. Centro de Salud Arrabal. Médico con labor asistencial.
- Alexandra Prados Torres; Médico. Instituto Aragonés de Ciencias de la Salud. Representante de Comisión de Investigación.
- José Puzo Foncillas; Médico. Servicio de Bioquímica. Hospital General San Jorge. Representante de Comisión de Investigación.
- Mónica Torrijos Tejada; Médico. Instituto Aragonés de Ciencias de la Salud.

Para que conste donde proceda, y a petición del promotor,

Zaragoza, a 15 de febrero de 2012

GOBIERNO DE ARAGÓN DEPARTAMENTO DE SANIDAD, BIENESTAR SOCIAL Y FAMILIA COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA

Firmado: María González Hinjos

DNV