• Clinical Research •

Neurodegeneration and choroidal vascular features on OCT in the progression to advanced age-related macular degeneration

Inês Costa[1], Ana Carvalho[2], Helton Andrade[2], Bruno Pereira[1,3,4], Pedro Camacho[1,2,4]

[1]H&TRC-Health & Technology Research Center, ESTeSL Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, Lisbon 1990-096, Portugal
[2]Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa 1990-096, Portugal
[3]Instituto de Retina de Lisboa, IRL, Lisbon 1050-085, Portugal
[4]iNOVA4Health, NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, Lisbon 1150-82, Portugal
Correspondence to: Pedro Camacho. ESTeSL–Escola Superior de Tecnologia da Saúde de Lisboa, Av. D. João II, Lote 4.69.01, Lisboa 1990-096, Portugal. pedro.camacho@estesl.ipl.pt
Received: 2023-08-17 Accepted: 2024-10-28

Abstract

● **AIM:** To quantify and compare longitudinal thickness changes of the ganglion cell complex (GCC) and the choroid in patients with different patterns of age-related macular degeneration (AMD) progression.

● **METHODS:** Retrospective cohort analysis of anonymized data from participants aged 50y or more and diagnosed with early/intermediate AMD in at least one eye (with no evidence of advanced AMD). A total of 64 participants were included from the Instituto de Retina de Lisboa (IRL) study (IPL/2022/MetAIlAMD_ESTeSL) and divided into 4 groups according to the Rotterdam classification for AMD. Spectral domain optical coherence tomography (SD-OCT) was used to assess and quantify GCC and choroid thickness at two time points (first visit *vs* last visit) with a minimum interval of 3y.

● **RESULTS:** In the GCC inner ring, a thinner thickness (P=0.001) was observed in the atrophic AMD group (51.3±21.4 μm) compared to the early AMD (84.3±11.5 μm), intermediate AMD (77.6±16.1 μm) and neovascular AMD (88.9±16.3 μm) groups. Choroidal thickness quantification showed a generalized reduction in the central circle (P=0.002) and inner ring (P=0.001). Slight reductions in retinal thickness were more accentuated in the inner ring in the atrophic AMD (-13%; P<0.01).

● **CONCLUSION:** The variation of the analyzed structures could be an indicator of risk of progression with neurodegenerative (GCC) or vascular (choroid) pattern in the intermediate and atrophic AMD. The quantification of both structures can provide important information about the risk of disease progression in the early and intermediate stages but also for the evolution pattern into late stages (atrophic or neovascular).

● **KEYWORDS:** age-related macular degeneration; ganglion cell complex; choroid, geographic atrophy; choroidal neovascularization; spectral domain optical coherence tomography

DOI:10.18240/ijo.2025.01.12

Citation: Costa I, Carvalho A, Andrade H, Pereira B, Camacho P. Neurodegeneration and choroidal vascular features on OCT in the progression to advanced age-related macular degeneration. *Int J Ophthalmol* 2025;18(1):103-110

INTRODUCTION

Age-related macular degeneration (AMD), a condition that affects the macular area of the retina, is considered to be the leading cause of blindness in people over the age of 50[1]. Given the increasing aging of the world's population, the number of people with AMD is expected to rise from 196 million in 2020 to 288 million in 2040[2]. In Europe, an estimated 67 million people have some degree of AMD and the economic impact of the disease in England, Italy, France, and Germany could reach €101.1 million[1]. Therefore, the increasing disease frequency as well as the economic burden make it necessary to improve the understanding of AMD pathology and the potential biomarkers that could minimize this public health problem[3].

AMD can be classified into early/intermediate stages, characterized by the presence of drusen and retinal pigment epithelium (RPE) changes; or late stages, characterized by the presence of geographic atrophy (atrophic AMD; aAMD) or development of choroidal neovascularization (neovascular AMD; nAMD)[4]. Despite the drusen being the main biomarker of AMD and their quantification, size, and distribution being

associated with disease severity and progression, it is crucial to identify new biomarkers that can help establish the risk of disease progression and the appropriate therapeutic response[5]. Several studies have explored different approaches to better understand the progression and severity of AMD such as the study of choroidal circulation[6], segmentation of the different retinal layers[7] and more recently using artificial intelligence[8]. With increasing interest in the study of AMD progression, neurodegenerative and circulatory factors have been described. The age-related accumulation of lipofuscin in the RPE appears to influence choroidal blood flow[9] with repercussions on the integrity of the RPE, photoreceptors and outer retinal layers[10-12]. These features seem to promote transneuronal degeneration, with a decrease in ganglion cell complex (GCC) thickness in the advanced stages compared to early/intermediate AMD[13-14]. However, the idea that neurodegeneration is secondary to vascular alterations is still unclear[4] and the description of a decrease or increase in choroidal thickness is not consensual[15-16].

Given the possibility that the vascular and neurodegenerative pathways may influence the progression and severity of AMD differently[17], it is crucial to study these two structures in participants with different patterns of AMD progression in an era where therapeutic options for nAMD are diverse and options for aAMD are emerging. Thus, this work, through an automatic and semi-automatic segmentation approach, aims to quantify the thickness of the GCC and choroid to highlight potential neurodegenerative and vascular pathways in the different patterns of AMD progression.

PARTICIPANTS AND METHODS

Ethical Approval This retrospective case-control analysis was performed through the database from the study *Differential DNA METhylation across ALL stages of Age-related Macular Degeneration: New therapeutic approaches* (IPL/2022/MetAllAMD_ESTeSL), conducted at the Instituto de Retina de Lisboa (IRL), approved by the Ethics Committee of the Escola Superior de Tecnologia da Saúde de Lisboa (CE-ESTeSL-No.84-2022) and the Ethics Committee of the IRL. All procedures of this study and data collection followed the principles of the Declaration of Helsinki. All study participants signed the informed consent protocol.

Selection and Staging Participants First, our team reviewed all consecutive AMD patients followed at IRL between January 2009 and May 2023. Second, searched patients with confirmed diagnosis of early/intermediate AMD and late AMD with at least 36mo of follow-up. After screening, participants were divided into cases (AMD patients who progressed to late stages during follow-up) and controls (AMD patients with no progression during follow-up). Finally, with a convenience non-probable sampling, a total of sixty-four participants were included and further classified into 4 groups according to the Rotterdam classification of AMD[18]: stage 0a (no signs of amd); stage 0b (hard drusen only with <63 μm); stage 1a (soft distinct drusen with ≥63 μm); stage 1b (pigmentary abnormalities only, without soft drusen ≥63 μm); stage 2a (soft indistinct drusen with ≥125 μm or subretinal drusenoid deposits only); stage 2b (soft distinct drusen with ≥63 μm plus pigmentary abnormalities); stage 3 (soft indistinct drusen with ≥125 μm or subretinal drusenoid deposits with pigmentary abnormalities); stage 4 (atrophic AMD or neovascular AMD). The early AMD group includes participants who maintained stage 1 and the intermediate AMD group includes participants of stages 2 and 3. Participants who progressed to stage 4 during the study period constitute the nAMD group and the aAMD group. The different groups were analyzed in order to report possible changes in the retina, retinal nerve fiber layer (RNFL), GCC and choroid.

Only participants with complete ophthalmological data, including best corrected visual acuity (BCVA) converted to logMAR, digital color fundus photographs (CFP), and spectral domain optical coherence tomography (SD-OCT) imaging (Spectralis; Heidelberg Engineering, Heidelberg, Germany) were included. Age, intraocular pressure (IOP), spherical equivalent (SE), clinical follow-up period was also obtained for each participant.

Timeline Assessment and Group Definition As mentioned, for the analysis and comparison of GCC and choroidal thickness, two time points were defined with a minimum follow-up period of 36mo: the initial visit (baseline or t0) and the conversion/maintenance visit (final visit or t1).

The early and intermediate AMD groups (control group) consisted of participants aged 50y or older with a confirmed medical diagnosis of AMD and no AMD progression between the follow-up period (t0 to t1). For control group, t1 was defined as the last visit performed with no signs of conversion to late AMD.

The late AMD groups (case group) consisted of participants aged 50y or older, with a confirmed clinical diagnosis of AMD and with clear progression to late AMD (nAMD or aAMD) between the follow-up period (t0 to t1). For case group, t1 was defined as the first visit with clinical classification progression. Importantly, participants included in the aAMD group did not show any signs of choroidal neovascularization and participants in the nAMD group did not show any signs of atrophy at the level of the outer retinal layers and/or RPE.

Exclusion criteria were presence of high ametropia (>6.00 D), opacification of ocular structures, subfoveal haemorrhage, ocular inflammation, history of retinal detachment, subjects undergoing intravitreal injections with anti-vascular endothelial growth factor (VEGF), having undergone ocular surgery in

Table 1 Overall characterization of the sample according to study group mean±SD

Parameters	eAMD (*n*=20)	iAMD (*n*=25)	nAMD (*n*=8)	aAMD (*n*=11)	Total (*n*=64)	*P*
Age (y)	78.3±7.9	83.8±7.2	84.6±5.5	83.8±5.8	82.2±7.4	0.037[a]
Sex, M/F, *n* (%)	8 (40.0)/12 (60.0)	11 (44.0)/14 (56.0)	3 (37.5)/5 (62.5)	2 (18.2)/9 (81.8)	24 (37.5)/40 (62.5)	0.540[b]
Study eye, R/L, *n* (%)	11 (55.0)/9 (45.0)	15 (60.0)/10 (40.0)	3 (37.5)/5 (62.5)	3 (27.3)/8 (72.7)	32 (50.0)/32 (50.0)	0.271[b]
Follow-up (y)	6.1±2.8	6.9±2.8	7.6±3.9	6.36±2.6	6.7±2.9	0.663[c]
Initial BCVA, logMAR	0.05±0.048	0.09±0.109	0.09±0.163	0.16±0.186	0.09±0.163	0.014[c]
Final BCVA, logMAR	0.05±0.048	0.16±0.124	0.16±0.186	0.22±0.029	0.09±0.163	0.002[c]
IOP (mm Hg)	14.7±3.1	13.0±3.7	14.0±2.1	13.5±3.9	13.8±3.4	0.727[a]
SE	-1.25±2.3	0.35±1.5	-0.45±1.5	-0.92±0.8	-0.36±1.8	0.256[c]

The composition of the groups presented is according to the final progression (end of study classification) and not according to the baseline. AMD: Age-related macular degeneration; eAMD: Early AMD; iAMD: Intermediate AMD; nAMD: Neovascular AMD; aAMD: Atrophic AMD; SD: Standard deviation; M: Male; F: Female; R: Right eye; L: Left eye; BCVA: Best corrected visual acuity converted to logMAR; IOP: Intraocular pressure; SE: Spherical equivalent in diopters. [a]ANOVA test; [b]Fisher Freeman-Halton exact test; [c]Kruskal-Wallis test.

the year prior to the first OCT, glaucoma, IOP>20 mm Hg. Participants with a history of stroke, transient ischaemia, dementia and/or other neurological disorders were also excluded.

Quantitative Assessment by SD-OCT The SD-OCT acquisition protocol includes a high-resolution macular volume scan (20°×20°, 49 B-scans, 7 frames per scan), 49 raster horizontal B-scans with 1024 A-scans per B-scans and a depth resolution of 3.9 μm. Additionally, a similar volume scan (high-density SD-OCT raster volume scan) with enhanced depth imaging (EDI) mode[19] was performed for the study of the choroid.

Data collection consisted of the extraction of the central retinal thickness, the RNFL, the ganglion cell layer (GCL), and the inner plexiform layer (IPL), in the two time periods analyzed. The quantification of the thickness of these structures was obtained from the automatic segmentation performed on the 49 B-scans by the SD-OCT Spectralis software (Heidelberg Engineering®), previously confirmed and corrected, whenever necessary, by an operator. For each of the above layers, the values of the 9 sectors of the ETDRS grid were obtained. The GCC thickness values were obtained from the sum of the GCL and IPL thickness values in the 9 sectors of the ETDRS grid.

Choroidal segmentation was performed semi-automatically, using the 1:1 μm viewing mode, according to the technical protocol of Zhao *et al*[20], which consisted of the following steps: after quantification of the global retinal thickness [inner limiting membrane (ILM)–Bruch membrane (BM)] obtained through automatic segmentation performed on the 49 B-scans, the BM reference line was manually transposed to the posterior limit of the choroid on the 49 B-scans. With this transposition, the map of the combined retinal and choroidal thickness was obtained. Finally, the choroidal thickness was calculated by subtracting the retinal thickness (ILM-BM) from the thickness of the choroidal-retinal combination (ILM-choroidoscleral interface) in the 9 sectors of the ETDRS grid.

Statistical Analysis The collected data was analyzed using the Statistical Package for the Social Sciences (IMB SPSS 27). ANOVA and Kruskal-Wallis tests were used, after the verification of normality by the Shapiro-Wilk test, to verify the existence of differences between groups. To compare retinal thickness changes (total retina, RNFL, GCC and choroid) between t0 and t1 with 2-sided paired *t*-test or Wilcoxon signed rank test were used. The 95% confidence interval (CI) and a 5% significance level were considered.

RESULTS

Table 1 summarized the participants characteristics according to baseline clinical records. A total of 64 participants (37.5% male and 62.5% female) with a mean age of 82.2±7.4y, with no differences in follow-up years and with different AMD progression pattern were included. All participants had early/intermediate AMD (eAMD/iAMD) at baseline, however, to highlight possible progression of AMD pattern the participants were divided according to the last classification (t1): eAMD group consisted of 20 participants (31.3%); iAMD group of 25 participants (39.1%); nAMD group of 8 participants (12.5%); and aAMD group of 11 participants (17.2%).

The eAMD group had a lower mean age (78.3±7.9y) than the other groups (P=0.037). Statistically significant differences were observed in the final and initial BCVA (P<0.05), with the eAMD group presenting higher values (initial BCVA: 0.05±0.048 and final BCVA: 0.05±0.048) and the aAMD group having the lowest BCVA values (initial BCVA: 0.16±0.186 and final BCVA: 0.22±0.029). No statistically significant differences were found between the groups for the remaining variables.

It is important to note that, although all participants were classified as early/intermediate AMD at t0, some subtle structural differences between participants with different patterns of progression were presented in Table 2. The group that progressed to nAMD at the final visit had thicker

Table 2 Retinal layers thicknesses in the central, inner, and outer rings of the ETDRS grid by studied groups at baseline (t0) mean±SD

Retinal layers thickness	eAMD (*n*=20)	iAMD (*n*=25)	nAMD (*n*=8)	aAMD (*n*=11)	*P*
Retina, central circle	276.3±22.4	270.3±19.9	305.0±26.2	250.0±43.2	0.001[a,b,c]
Retina, inner ring	336.1±16.9	327.0±14.7	350.3±17.4	308.8±26.1	0.002[c,d,f,e]
Retina, outer ring	294.5±12.8	286.0±13.6	300.4±17.1	283.0±21.8	0.067[f]
RNFL, central circle	12.8±2.8	13.4±2.7	14.0±2.3	11.3±2.3	0.110[f]
RNFL inner ring	22.7±3.6	22.6±3.8	23.3±3.6	21.3±2.9	0.325[f]
RNFL, outer ring	37.7±5.5	37.4±6.0	38.1±7.1	38.0±6.9	0.996[f]
GGC, central circle	35.4±7.7	35.6±6.3	42.5±6.9	35.6±13.6	0.077[f]
GGC inner ring	87.2±9.6	83.8±11.5	90.9±8.6	78.5±13.4	0.221[f]
GGC, outer ring	60.9±5.8	58.6±6.9	64.7±6.4	58.9±8.1	0.147[a]
Choroid, central circle	208.6±70.1	194.5±58.3	187.5±43.3	182.6±59.8	0.674[a]
Choroid, inner ring	210±64.5	193.2±56.7	186.5±39.6	170.3±52.2	0.312[a]
Choroid, outer ring	202.1±70.0	186.2±56.1	180.5±38.7	154.6±45.0	0.197[a]

The composition of the groups presented is according to the final progression (end of study classification) and not according to the baseline. AMD: Age-related macular degeneration; eAMD: Early AMD; iAMD: Intermediate AMD; nAMD: Neovascular AMD; aAMD: Atrophic AMD; SD: Standard deviation. [a]ANOVA test; [b]iAMD with nAMD; [c]nAMD with aAMD; [d]iAMD with aAMD; [e]eAMD with aAMD; [f]Kruskal-Wallis test.

Table 3 Retinal layers thicknesses in the central, inner, and outer rings of the ETDRS grid by studied groups at end of the study (t1) mean±SD

Retinal layers thickness	eAMD (*n*=20)	iAMD (*n*=25)	nAMD (*n*=8)	aAMD (*n*=11)	*P*
Retina, central circle	274.7±26.1	267.5±26.1	289.6±44.7	213.1±49.9	<0.001[a,c,d,e]
Retina, inner ring	330.9±19.3	317.3±22.4	341.0±31.9	268.8±30.3	<0.001[a,c,d,e]
Retina, outer ring	290.8±14.4	280.4±15.4	299.9±32.5	266.2±24.4	0.002[a,c,e]
RNFL, central circle	12.4±3.1	11.5±2.9	14.4±3.8	10.0±4.5	0.641[a]
RNFL inner ring	21.7±2.9	21.9±4.5	25.5±7.4	17.4±4.9	0.021[c,d,f,e]
RNFL, outer ring	36.2±5.5	36.1±7.7	37.5±8.3	33.5±8.1	0.640[a]
GCC, central circle	34.6±7.9	33.8±7.5	43.5±14.1	30.2±13.3	0.078[f]
GCC inner ring	84.3±11.5	77.6±16.1	88.9±16.3	51.3±21.4	0.001[c,d,f,e]
GCC, outer ring	60.4±5.9	55.4±7.6	64.6±13.2	49.7±9.5	0.001[a,c,e]
Choroid, central circle	217.2±73.4	167.9±72.9	196.4±83.4	120.6±33.9	0.002[c,d,e,f,g]
Choroid, inner ring	216.3±70.7	164.1±63.6	191.5±106.0	117.9±33.4	0.001[c,d,e,f,g]
Choroid, outer ring	199.1±66.9	155.2±51.5	170.0±64.4	122.2±33.6	0.004[a,e]

The composition of the groups presented is according to the final progression (end of study classification) and not according to the baseline. AMD: Age-related macular degeneration; eAMD: Early AMD; iAMD: Intermediate AMD; nAMD: Neovascular AMD; aAMD: Atrophic AMD; RNFL: Retinal nerve fiber layer; GCC: Ganglion cell complex; SD: Standard deviation. [a]ANOVA test; [b]iAMD with nAMD; [c]nAMD with aAMD; [d]iAMD with aAMD; [e]eAMD with aAMD; [f]Kruskal-Wallis test; [g]eAMD with iAMD.

retinal thickness (central circle) than participants with iAMD (305±26.2 *vs* 270.3±19.9 μm) with no progression over the study period (*P*=0.001). Additionally, participants who progressed to aAMD at the final visit were found to have a thinner central retinal thickness at baseline (250±43.2 *vs* 305±26.2 μm) compared to the nAMD group (*P*=0.001).

Some inner ring differences were found at baseline between the groups with different progression patterns. The aAMD group showed thinner inner ring retinal thickness (308.8±26.1 μm) compared to all groups (*P*=0.002): eAMD (336.1±16.9 μm), iAMD (327±14.7 μm) and nAMD (350.3±17.4 μm).

Despite no statistical differences, two aspects stand out clinically: participants who progressed to aAMD at the final visit showed at baseline a thinner GCC compared to nAMD participants and a decrease in choroidal thickness seems more evident in the aAMD group.

Table 3 showed the main differences found between the studied groups at the last visit (t1). The aAMD group showed a thinner retinal thickness in both the central circle (*P*<0.001) and the inner ring (*P*<0.001) compared to the eAMD, nAMD, and iAMD groups. This decrease was less evident in the outer ring (*P*=0.002) but presented in the aAMD compared to the eAMD and nAMD group.

Regarding the RNFL, a thinner thickness was observed in the inner ring (*P*=0.021) in the aAMD group (17.4±4.9 μm) compared to the eAMD (21.7±2.9 μm), iAMD (21.9±4.5 μm), and nAMD (25.5±7.4 μm) groups.

In the GCC inner ring, a thinner thickness (*P*=0.001) was also

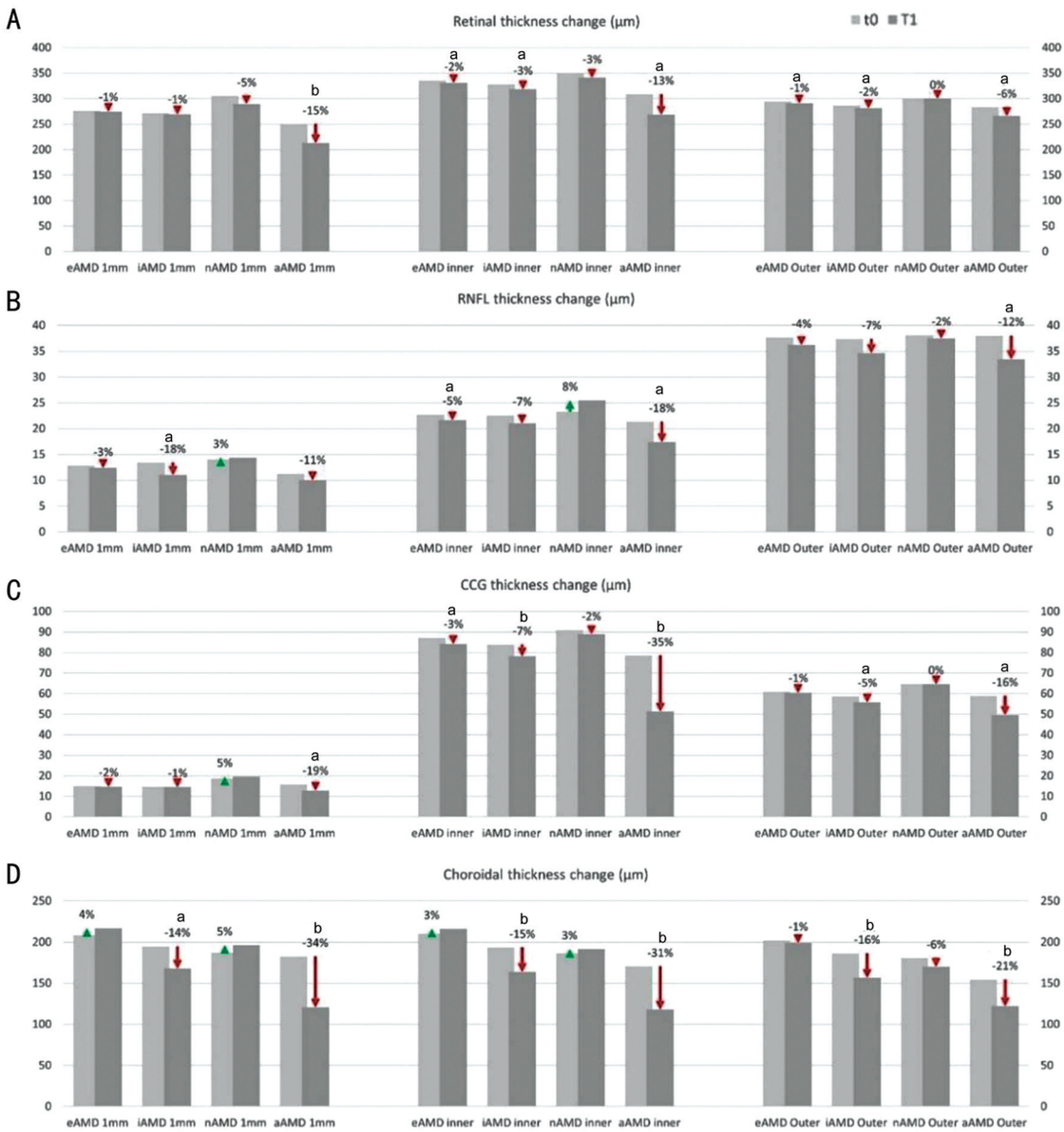

Figure 1 Mean thickness comparison of the retinal layers and choroid at the beginning (t0) and at the end of the study (t1) The arrows indicate the percentage change of the structures at the beginning (t0) and at the end of the study (t1). 1 mm: ETDRS central circle; inner: ETDRS inner circle; outer: ETDRS outer circle; [a]*P*<0.01; [b]*P*<0.001. AMD: Age-related macular degeneration; eAMD: Early AMD; iAMD: Intermediate AMD; nAMD: Neovascular AMD; aAMD: Atrophic AMD.

observed in the aAMD group (51.3±21.4 μm) compared to the eAMD (84.3±11.5 μm), iAMD (77.6±16.1 μm) and nAMD (88.9±16.3 μm) groups. In the outer ring the decrease in GCC was evident (*P*=0.001) between aAMD (49.7±9.5 μm), eAMD (60.4±5.9 μm) and nAMD (64.6±13.2 μm) groups.

Choroidal quantification showed a generalized reduction in thickness in the central circle (*P*=0.002) and inner ring (*P*=0.001) in aAMD group. In the central circle the aAMD showed a thinner thickness (120.6±33.9 μm) compared to the eAMD (217.2±73.4 μm), iAMD (167.9±72.9 μm), and nAMD (196.4±83.4 μm). A statistically significant (*P*=0.001) decrease in thickness was also found at this location in iAMD (167.9±72.9 μm) compared to eAMD (217.2±73.4 μm).

Figure 1A showed a decrease in central retinal ring thickness (-15%; *P*<0.001) for aAMD. Slight decreases in retinal thickness were more accentuated in the inner ring in the aAMD (-13%; *P*<0.01). Similar but less marked thinning was found in the outer ring of the aAMD group (-6%; *P*<0.01).

In Figure 1B the iAMD group showed a thinner RNFL in the central circle (-18%) between t0 and t1 (*P*<0.01). In the inner ring, the aAMD group showed the greatest reduction in RNFL thickness (-18%) between t0 and t1 (*P*<0.01). In the RNFL outer ring a significant decrease in RNFL thickness (-12%) was again found in the aAMD group (*P*<0.01).

In the assessment of GCC change, in Figure 1C, it was observed that only the aAMD group showed a significant decrease in the central circle thickness (-19%; *P*<0.01). In the inner ring all groups showed a significant decrease in GCC

thickness except nAMD. In this location the major changes (P<0.001) were found in iAMD (-7%) and aAMD (-35%) over the follow-up period (t0-t1). In the outer ring the thickness changes found were smaller (P<0.01) and again in the iAMD (-5%) and aAMD (-16%) groups.

Concerning the assessment of choroidal thickness between the two time points studied, Figure 1D showed that at central circle the major differences were found in the iAMD (-14%; P<0.01) and aAMD (-34%; P<0.001) groups. At the inner ring differences were again observed in the iAMD (-15%; P<0.001) and aAMD (-31%; P<0.001) groups. Similar results were found at the outer ring in the iAMD (-16%; P<0.001) and aAMD (-21%; P<0.001) groups.

Interestingly, the nAMD group presented no statistically significant changes in choroidal thickness but with a slight increase of 5% and 3% in the central and inner ring, respectively.

DISCUSSION

SD-OCT segmentation of the retinal and choroidal layers, due to its high reliability and reproducibility, is critical for monitoring choroidal and retinal pathologies[21-22] and is the basis for the most recent AMD nomenclatures[23-24]. Previous studies have shown that differences in the thickness of the choroid and inner retinal layers such as the RNFL, IPL, and GCC can be detected at specific retinal locations in early/intermediate AMD[25-26]. Also in late AMD there seems to be a reduction in the choroidal and GCC thickness[16] although its involvement in disease progression is unclear. In this sense, our group hypothesized that changes in the thickness of the retinal neural layers or choroid, related to neurodegeneration and vascular pathways, may be associated with different patterns of AMD progression.

There is consensus on the importance of age and disease duration in the prevalence and severity of AMD[4]. In this study, despite statistical differences in age between the eAMD group and the other groups, no differences in age were found between the iAMD, nAMD and aAMD groups. Another significant issue observed, was that follow-up in the different groups was similar, so the main differences between the groups studied were retinal metrics and differences in AMD progression. With the normal human ageing process, several changes occur in the retina[27], such as a decrease in retinal thickness, due to internal neural modifications, with a decrease in the GCC and IPL[28]. The data found show that, even with no statistical differences in age and follow-up, the thickness of the central retina circle of the participants who evolved to late AMD was altered compared with early/intermediate AMD group.

Recently, Farinha *et al*[29] described some changes in retinal thickness in eAMD patients suggesting a continuous process of atrophy or neurodegeneration most pronounced in the outer layers but extending to the inner layers in ETDRS inner and outer ring. The changes that our group found in the central retinal ring were also clear in the inner ring supporting two findings: 1) at t0 those participants who progressed to late AMD already had differences in retinal thickness compared to those with no progression; 2) the retinal thickness pattern in patients who later progressed to late AMD was clinically distinct with thinner thickness in the aAMD group and thicker thickness in nAMD. Although retinal thickness at t0 has been shown to provide a pattern of predisposition to late AMD progression, it was at t1 that statistically significant differences were found in the inner retinal and choroidal layers supporting the potential of these structures as quantitative AMD biomarkers[29].

Chauhan *et al*[30] reported that central RNFL quantification is not susceptible to age variation. Although this is a positive aspect for the disease change assessment, due to the age factor being minimized in RNFL quantification, the contribution of this structure as a biomarker for late AMD progression did not seem to be relevant at t0. In contrast, despite no statistical or clinical differences at t0 that would support its importance as a biomarker of progression, a thinner RNFL thickness at t1 was found in the aAMD group. Reduced RNFL thickness had already been described in early/intermediate AMD[31] but not in late AMD progression.

The assessment of the GCC, probably due to its localization and cellular concentration in the macular region[32], has been raising interest in retinal pathologies[33]. The first studies using SD-OCT to study the inner layers in AMD described a decrease in GCC thickness in non-exudative AMD[13]. Later a new implication of the GCC was described and, after ROC analysis, the potential to distinguish changes related to early/intermediate AMD was investigated[14]. Following the recent report of the potential interest of neuronal retinal layer thicknesses as quantitative biomarkers of disease progression in early AMD[29], this paper suggests the utility of these layers, GCC in specific, as a potential biomarker of progression to late AMD.

In this work, in addition to the thinner thickness of the GCC in the aAMD group compared to the early/intermediate AMD groups, it was found that these thickness differences also occur when compared to the nAMD group strengthening the distinct mechanisms involved. The GCC thinner thickness between the two time points studied (t0 *vs* t1) was more evident in the inner ring of aAMD. In the Zucchiatti *et al*[34] study, the mean values obtained for GCC thickness in participants with aAMD was 50.4±17.9 μm, being close to the values obtained at t1. Clinically interesting, despite no statistical differences, were the GCC values found in the nAMD group, that were consistently higher than the early/intermediate group even at t0 before progression to late AMD had occurred.

The study of the choroid, given its decrease over age[27], the inconsistency of decrease[35-36], or increase[9] of its thickness in the different stages of AMD[35-36], was the most challenging parameter. Considering that choroidal thickness decreases with age[27], and that its decrease is accentuated in AMD progression[29], it is important to highlight that at t0 participants who progressed to late AMD had already a decrease in choroidal thickness compared to participants who did not progress. In this structure we want to highlight two key points: 1) the differences found were not only in the central circle, and it is a central issue related to the relevance of the metrics used in most studies-subfoveal choroidal thickness; 2) the fact that choroidal thickness has an inverse behaviour in the nAMD group and increases from t0 to t1.

Several studies have described a loss of choriocapillary density in participants with aAMD[10], which, by decreasing choroidal blood flow, leads to impairment of the outer retinal layers such as RPE or photoreceptors[9]. Our choroidal data were close to the study by Sigler and Randolph[37]. who described a mean choroidal thickness in participants with iAMD of 161±39 μm compared to the decrease of 115± 40 μm described in aAMD.

The main limitations of this work were the study of two visits only (t0 and t1) which removes the insight into the retinal layers changes/conversion profile at different time points. The sample size was another limitation, which resulted from the study's criteria of inclusion/exclusion for the case group (AMD patients who progressed to late stages during follow-up) and controls (AMD patients with no progression during follow-up).

In conclusion, the role of the choroid in the impairment of the outer layers[9] and consequent repercussion on the inner neural layers of the retina by transneuronal degeneration seems clear[10-11] from the reported differences in choroidal and GCC thickness.

The concept that the development of both neovascular and atrophic AMD is associated with distinct prognostic features[38] is certainly an area for further exploration. With the understanding that there is still little knowledge on how the inner retinal layers change over time or by stage of AMD progression[29] and which prognostic value of retinal layers should be enhanced[38] this initial work made an important input. The relevance was the characterization of the different patterns of retinal layer changes in participants with different patterns of progression (at t0 *vs* t1) and to highlight the possible relevance of the GCC and choroid which have also recently been addressed in an artificial intelligence project[39].

ACKNOWLEDGEMENTS

Foundations: Supported by FCT/MCTES UIDB/05608/2020 (https://doi.org/10.54499/UIDB/05608/2020) and UIDP/05608/2020 (https://doi.org/10.54499/UIDP/05608/2020), IDI&CA grant IPL/2022/MetAllAMD_ESTeSL by H&TRC-Health & Technology Research Center, ESTeSL-Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, and by Retina Institute of Lisbon (IRL).

Conflicts of Interest: Costa I, None; **Carvalho A,** None; **Andrade H,** None; **Pereira B,** None; **Camacho P,** None.

REFERENCES

1 Li JQ, Welchowski T, Schmid M, Mauschitz MM, Holz FG, Finger RP. Prevalence and incidence of age-related macular degeneration in Europe: a systematic review and meta-analysis. *Br J Ophthalmol* 2020;104(8):1077-1084.

2 Keenan TDL, Cukras CA, Chew EY. Age-related macular degeneration: epidemiology and clinical aspects. *Adv Exp Med Biol* 2021;1256:1-31.

3 Lains I, Han X, Gil J, *et al*. Plasma metabolites associated with OCT features of age-related macular degeneration. *Ophthalmol Sci* 2024;4(1):100357.

4 Fleckenstein M, Keenan TDL, Guymer RH, Chakravarthy U, Schmitz-Valckenberg S, Klaver CC, Wong WT, Chew EY. Age-related macular degeneration. *Nat Rev Dis Primers* 2021;7(1):31.

5 Heesterbeek TJ, Lorés-Motta L, Hoyng CB, Lechanteur YTE, den Hollander AI. Risk factors for progression of age-related macular degeneration. *Ophthalmic Physiol Opt* 2020;40(2):140-170.

6 Cabral D, Fradinho AC, Zhang Y, Zhou H, Ramtohul P, Ramakrishnan MS, Pereira T, Wang RK, Freund KB. Quantitative assessment of choriocapillaris flow deficits and type 1 macular neovascularization growth in age-related macular degeneration. *Sci Rep* 2023;13(1):8572.

7 Camacho P, Dutra-Medeiros M, Salgueiro L, Sadio S, Rosa PC. Manual segmentation of 12 layers of the retina and choroid through SD-OCT in intermediate AMD: repeatability and reproducibility. *J Ophthalmic Vis Res* 2021;16(3):384-392.

8 Damian I, Nicoară SD. SD-OCT Biomarkers and the current status of artificial intelligence in predicting progression from intermediate to advanced AMD. *Life (Basel)* 2022;12(3):454.

9 Koh LHL, Agrawal R, Khandelwal N, Sai Charan L, Chhablani J. Choroidal vascular changes in age-related macular degeneration. *Acta Ophthalmol* 2017;95(7):e597-e601.

10 Hadziahmetovic M, Malek G. Age-related macular degeneration revisited: from pathology and cellular stress to potential therapies. *Front Cell Dev Biol* 2020;8:612812.

11 Fragiotta S, Scuderi L, Iodice CM, Rullo D, di Pippo M, Maugliani E, Abdolrahimzadeh S. Choroidal vasculature changes in age-related macular degeneration: from a molecular to a clinical perspective. *Int J Mol Sci* 2022;23(19):12010.

12 Camacho P, Dutra-Medeiros M, Cabral D, Silva R. Outer retina and choroidal thickness in intermediate age-related macular degeneration: reticular pseudodrusen findings. *Ophthalmic Res* 2018;59(4):212-220.

13 Yenice E, Şengün A, Soyugelen Demirok G, Turaçlı E. Ganglion cell complex thickness in nonexudative age-related macular degeneration. *Eye(Lond)* 2015;29(8):1076-1080.

14 Camacho P, Dutra-Medeiros M, Páris L. Ganglion cell complex in

early and intermediate age-related macular degeneration: evidence by SD-OCT manual segmentation. *Ophthalmologica* 2017;238(1-2):31-43.

15 Invernizzi A, Torre A, Parrulli S, *et al*. Retinal findings in patients with COVID-19: results from the SERPICO-19 study. *EClinicalMedicine* 2020;27:100550.

16 Abouelregal AR, Mahmoud AF, Eliwa TF, Naguib KM. Correlation between subfoveal choroidal thickness and disease activity in wet age-related macular degeneration using spectral domain optical coherence tomography. *QJM* 2021;114(Supplement_1):hcab109.003.

17 Tricco AC, Thomas SM, Lillie E, *et al*. Anti-vascular endothelial growth factor therapy for age-related macular degeneration: a systematic review and network meta-analysis. *Syst Rev* 2021;10(1):315.

18 van Leeuwen R, Klaver CC, Vingerling JR, Hofman A, de Jong PT. The risk and natural course of age-related maculopathy: follow-up at 6 1/2 years in the Rotterdam study. *Arch Ophthalmol* 2003;121(4):519-526.

19 Spaide RF. Enhanced depth imaging optical coherence tomography of retinal pigment epithelial detachment in age-related macular degeneration. *Am J Ophthalmol* 2009;147(4):644-652.

20 Zhao M, Alonso-Caneiro D, Lee R, Cheong AMY, Yu WY, Wong HY, Lam AKC. Comparison of choroidal thickness measurements using semiautomated and manual segmentation methods. *Optom Vis Sci* 2020;97(2):121-127.

21 Yamashita T, Yamashita T, Shirasawa M, Arimura N, Terasaki H, Sakamoto T. Repeatability and reproducibility of subfoveal choroidal thickness in normal eyes of Japanese using different SD-OCT devices. *Invest Ophthalmol Vis Sci* 2012;53(3):1102-1107.

22 Parravano M, Oddone F, Boccassini B, Menchini F, Chiaravalloti A, Schiavone M, Varano M. Reproducibility of macular thickness measurements using Cirrus SD-OCT in neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2010;51(9):4788-4791.

23 Sadda SR, Guymer R, Holz FG, *et al*. Consensus definition for atrophy associated with age-related macular degeneration on OCT: classification of atrophy report 3. *Ophthalmology* 2018;125(4):537-548.

24 Spaide RF, Jaffe GJ, Sarraf D, *et al*. Consensus nomenclature for reporting neovascular age-related macular degeneration data: consensus on neovascular age-related macular degeneration nomenclature study group. *Ophthalmology* 2020;127(5):616-636.

25 Brandl C, Brücklmayer C, Günther F, Zimmermann ME, Küchenhoff H, Helbig H, Weber BHF, Heid IM, Stark KJ. Retinal layer thicknesses in early age-related macular degeneration: results from the German AugUR study. *Invest Ophthalmol Vis Sci* 2019;60(5):1581-1594.

26 Savastano MC, Minnella AM, Tamburrino A, Giovinco G, Ventre S, Falsini B. Differential vulnerability of retinal layers to early age-related macular degeneration: evidence by SD-OCT segmentation analysis. *Invest Ophthalmol Vis Sci* 2014;55(1):560-566.

27 Wood A, Binns A, Margrain T, Drexler W, Považay B, Esmaeelpour M, Sheen N. Retinal and choroidal thickness in early age-related macular degeneration. *Am J Ophthalmol* 2011;152(6):1030-1038.e2.

28 Nieves-Moreno M, Martínez-de-la-Casa JM, Morales-Fernández L, Sánchez-Jean R, Sáenz-Francés F, García-Feijoó J. Impacts of age and sex on retinal layer thicknesses measured by spectral domain optical coherence tomography with Spectralis. *PLoS One* 2018;13(3):e0194169.

29 Farinha C, Silva AL, Coimbra R, Nunes S, Cachulo ML, Marques JP, Pires I, Cunha-Vaz J, Silva R. Retinal layer thicknesses and neurodegeneration in early age-related macular degeneration: insights from the Coimbra eye study. *Graefes Arch Clin Exp Ophthalmol* 2021;259(9):2545-2557.

30 Chauhan BC, Vianna JR, Sharpe GP, Demirel S, Girkin CA, Mardin CY, Scheuerle AF, Burgoyne CF. Differential effects of aging in the macular retinal layers, neuroretinal rim, and peripapillary retinal nerve fiber layer. *Ophthalmology* 2020;127(2):177-185.

31 Lee EK, Yu HG. Ganglion cell-inner plexiform layer and peripapillary retinal nerve fiber layer thicknesses in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2015;56(6):3976-3983.

32 Nguyen-Ba-Charvet KT, Rebsam A. Neurogenesis and specification of retinal ganglion cells. *Int J Mol Sci* 2020;21(2):451.

33 Kim JM, Lee MW, Lim HB, Won YK, Shin YI, Lee WH, Kim JY. Longitudinal changes in the ganglion cell-inner plexiform layer thickness of age-related macular degeneration. *Acta Ophthalmol* 2021;99(7):e1056-e1062.

34 Zucchiatti I, Parodi MB, Pierro L, Cicinelli MV, Gagliardi M, Castellino N, Bandello F. Macular ganglion cell complex and retinal nerve fiber layer comparison in different stages of age-related macular degeneration. *Am J Ophthalmol* 2015;160(3):602-607.e1.

35 Manjunath V, Goren J, Fujimoto JG, Duker JS. Analysis of choroidal thickness in age-related macular degeneration using spectral-domain optical coherence tomography. *Am J Ophthalmol* 2011;152(4):663-668.

36 Govetto A, Sarraf D, Figueroa MS, Pierro L, Ippolito M, Risser G, Bandello F, Hubschman JP. Choroidal thickness in non-neovascular versus neovascular age-related macular degeneration: a fellow eye comparative study. *Br J Ophthalmol* 2017;101(6):764-769.

37 Sigler EJ, Randolph JC. Comparison of macular choroidal thickness among patients older than age 65 with early atrophic age-related macular degeneration and normals. *Invest Ophthalmol Vis Sci* 2013;54(9):6307-6313.

38 Thiele S, Nadal J, Pfau M, Saßmannshausen M, von der Emde L, Fleckenstein M, Holz FG, Schmid M, Schmitz-Valckenberg S. Prognostic value of retinal layers in comparison with other risk factors for conversion of intermediate age-related macular degeneration. *Ophthalmol Retina* 2020;4(1):31-40.

39 Tvenning AO, Hanssen SR, Austeng D, Morken TS. Deep learning identify retinal nerve fibre and choroid layers as markers of age-related macular degeneration in the classification of macular spectral-domain optical coherence tomography volumes. *Acta Ophthalmol* 2022;100(8):937-945.