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Association between outer retinal alterations and microvascular changes in intermediate stage age-related macular degeneration: an optical coherence tomography angiography study

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ABSTRACT

Aims To investigate associations between changes in retinal vessels and alterations detected by spectral domain optical coherence tomography (SD-OCT) scans in intermediate stage age-related macular degeneration (AMD).

Methods Thirty eyes of 30 patients with intermediate dry AMD were enrolled in the study. Of the cohort study, 15 eyes (changes-AMD group) showed OCT changes preceding the development of drusen-associated atrophy. A control group of healthy subjects was selected for statistical comparisons. All patients underwent an ophthalmologic evaluation, including OCT angiography (OCTA) and SD-OCT scans. Main outcome measures were superficial vessel density, deep vessel density, macular thickness.

Results Foveal macular thickness was 215.2±32.9 µm in changes-AMD patients and was significantly thinner than no changes-AMD patients (248.3±23.3 µm, p=0.002) and healthy subjects (268.1±19.2 µm, p<0.0001). Furthermore, in the parafoveal area, the thicknesses of both the inner retina and the outer retina were reduced in the changes-AMD group, after comparison with the two other groups. Parafoveal superficial vascular plexus flow density was 43.3±2.7% in changes-AMD patients and was decreased compared with the no changes-AMD group (48.7±3.3%, p=0.003) and healthy controls (50.4±6.1%, p=0.001). A direct correlation of the superficial plexus flow density with the inner retina parafoveal macular thickness (R^2=0.761, p=0.028) was found.

Conclusions We demonstrated an association between SD-OCT signs and retinal blood supply in patients with intermediate AMD and we showed that patients with signs predicting development of geographic atrophy have a reduced flow in superficial vascular plexus and damage of the inner and the outer retina.

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of irreversible central vision loss among older individuals in developed countries.[1]

Two subtypes of AMD exist: non-neovascular (dry) AMD and neovascular exudative (wet) AMD.[2] The accumulation of lipo-glyco-proteinaceous deposits (drusen) and the degeneration of the retinal pigment epithelium (RPE) define dry AMD. Moreover, geographic atrophy (GA) is a devastating complication of the advanced form of dry AMD and is characterised by the confined almost complete loss of the RPE layer under the retina, often with an abrupt border between the apparently healthy RPE area and the region without these cells (for that reason, the term 'geographic' is used).[3] Furthermore, GA is accompanied by degeneration of the inner and outer retinal layers.[2 4–6]

Many factors have been implicated in AMD pathogenesis and progression, such as inflammation, oxidative damage, ageing, genetic predisposition and environmental elements.[7 8] In recent years, several studies have reported vascular factors playing an important role in AMD pathogenesis.[9–12] Indeed, there is a growing body of evidence that choroidal and retinal blood flows are diminished in AMD,[9–13] the latter aspect causing hypoxia associated with the progression of AMD.

Spectral domain optical coherence tomography (SD-OCT) scans show bands corresponding to the anatomic layers of the human retina. Recently, Wu *et al*[14] identified the following distinctive features on SD-OCT scans in patients affected by intermediate AMD that portend the development of drusen-associated GA: subsidence of the outer plexiform layer (OPL) and inner nuclear layer (INL); and development of a hyporeflective wedge-shaped band within the limits of the OPL. Indeed, all areas that developed drusen-associated atrophy showed these consistent characteristic features preceding its development.[14] These characteristics were termed 'nascent geographic atrophy' and patients with these signs are considered to have a high risk of drusen-associated atrophy occurrence.

Optical coherence tomography angiography (OCTA) has recently been developed to study retinal and choroidal microvasculature without needing dye injection and has allowed us to study the superficial and the deep retinal vascular plexuses.[15]

The main aim of this study was to investigate superficial and deep retinal vessels, by means of OCTA, in patients affected by intermediate AMD to find an association between features on SD-OCT scans and retinal vessel density.

METHODS

Study participants

A total of 30 eyes of 30 patients with dry AMD (12 men and 18 women; mean age 71.6±7.6 years) were enrolled in the study. Patients consecutively presented at the University Gabriele D'Annunzio Department of Ophthalmology, between June 2015

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and March 2016. University Gabriele D'Annunzio Ethics Committee approved this study and all patients gave informed consent to the use of their data. The study adhered to the tenets of the Declaration of Helsinki.

All eyes included in our present study were affected by 'intermediate AMD'. The staging was based on the clinical AMD classification proposed by Ferris *et al*[16] in 2013 as follows: persons with medium drusen (63–125 µm), but without pigmentary abnormalities, should be considered to have 'early AMD'; persons with large drusen (>125 µm) or with pigmentary abnormalities associated with at least medium drusen should be considered to have 'intermediate AMD'; and patients with neovascular AMD or GA should be considered to have 'late AMD'. No patients affected by early and late AMD were enrolled. Of the cohort study, 15 eyes (changes-AMD group) showed both OPL and INL subsidence and presence of a hyporeflective wedge-shaped band within the limits of the OPL (changes preceding the development of drusen-associated atrophy)[14] (figure 1). The remaining 15 enrolled eyes did not show these changes (no changes-AMD group).

Staging and assignment to groups were determined on fundus colour images and on SD-OCT scans (to use the measuring tool to evaluate drusen width) by two independent investigators in a blind manner and all the discrepancies were resolved by consensus between these two observers.

Criteria for inclusion were age ≥50 years old, best corrected visual acuity (BCVA) of at least 0.1 LogMAR, and diagnosis of intermediate AMD in at least one eye. Exclusion criteria were diagnosis of early AMD, evidence or history of neovascularisation, presence of GA, previous ocular surgery (included anti-vascular endothelial growth factor therapy), any maculopathy secondary to causes other than AMD (including presence of an epiretinal membrane or vitreomacular traction syndrome), refractive error greater than 3 dioptres and significant media opacities.

All patients had undergone a complete ophthalmologic examination, which included measurement of BCVA, intraocular pressure, fundus examination, colour fundus photography, fluorescein angiography (FA) and OCTA. We only considered scans without significant motion artefacts and with a signal strength index >60.

A control group homogenous for age and sex was also included in the current analysis. All control subjects also underwent a complete ophthalmologic examination, including BCVA, intraocular pressure, fundus examination and OCTA.

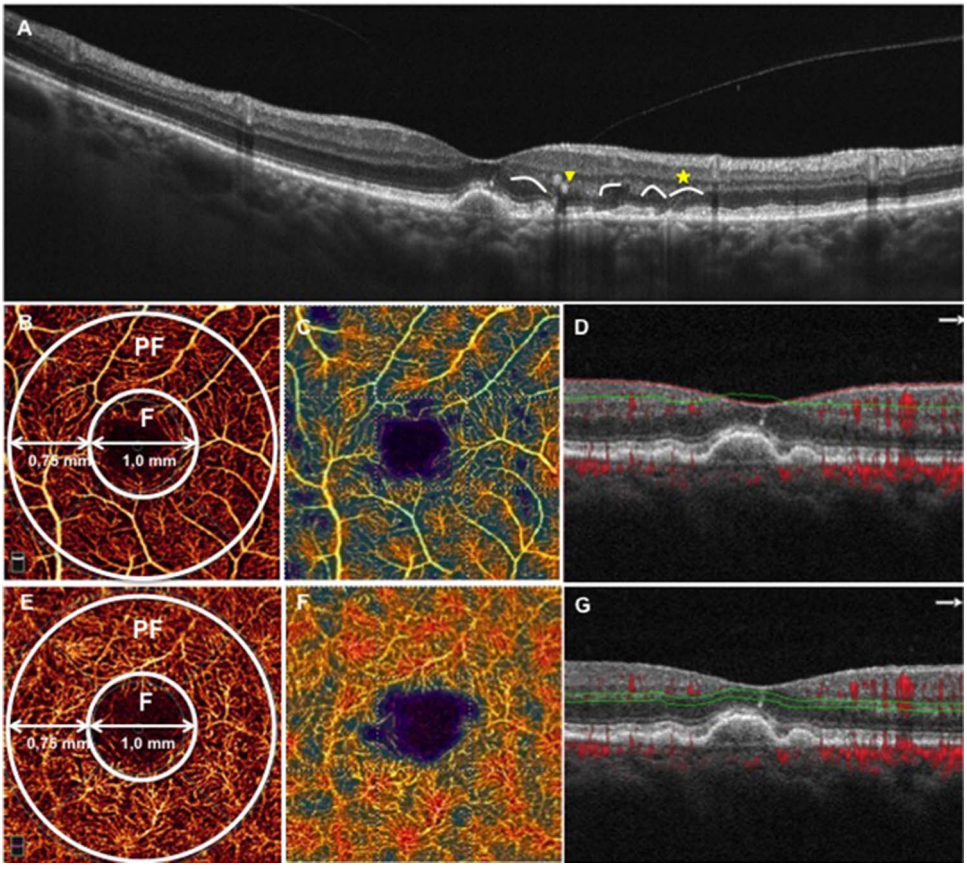

Figure 1 Spectral domain optical coherence tomography (SD-OCT) and optical coherence tomography angiography (OCTA) from an enrolled patient with intermediate age-related macular degeneration. (A) SD-OCT showing outer plexiform layer (OPL) and inner nuclear layer subsidence (yellow arrow) and a hyporeflective wedge-shaped band within the limits of the OPL (yellow star); (B) OCTA macula 3×3 scan showing the superficial vascular plexus. The superficial vascular plexus flow density was defined as the percentage area occupied by vessels in a circular region of interest (ROI) in the centre of the foveal avascular zone and with a diameter of 2.5 mm. The AngioVue software automatically splatted the ROI, as well as the superficial vascular plexus flow density evaluation, into two fields: the foveal area (F), a central circle with a diameter of 1 mm; and the parafoveal area (PF) that constitutes the remaining part inside the ROI. The PF superficial vascular plexus vessel density data were analysed; (C) corresponding colour-coded flow density map of the superficial vascular plexus flow density (the warmer the colour, the greater the flow); (D) OCT B-scan showing the slab set to evaluate the superficial retinal plexus; (E) OCTA macula 3×3 scan showing the deep vascular plexus; (F) corresponding colour-coded flow density map of the deep vascular plexus flow density; (G) OCT B-scan showing the slab set to evaluate the deep retinal plexus.

Outcome measures included superficial vascular plexus (SVP) flow density (SVPFD); deep vascular plexus (DVP) flow density (DVPFD); and macular thickness (MT).

Procedures

SD-OCT angiography with XR Avanti

XR Avanti AngioVue OCTA (Optovue Inc., Fremont, California, USA) is a device using a system based on SSADA algorithm (Version: 2015.100.0.35). The latter algorithm allowed the device to detect the flow as a variation over time in the speckle pattern formed by interference of light scattered from red blood cells and adjacent tissue structure.[17] In addition, the SD-OCT tool was skilled at acquiring the standard structural OCT scans typically used by commercially available devices.

The exam was performed as previously described.[13] In brief, each subject's pupil was dilated with a combination of 0.5% tropicamide and 10% phenylephrine, then study participants underwent SD-OCT imaging following a protocol that included AngioVue OCT 3D volume set of 3×3 mm, consisting of 304×304 pixels in the transverse dimension. Low-quality scans (ie, if the subject blinked or if there were many motion artefacts in the dataset) were excluded and repeated until a good quality was achieved. Three scans for each patient were captured, then the best quality scan (without significant motion artefacts and with a signal strength index >60) was considered for the analysis.

Vascular layer segmentation and flow density analysis

Vascular retinal layers were visualised and segmented as previously described.[13 18–20] To evaluate the superficial vascular retinal plexus we used a layer thickness of 60 µm from the inner limiting membrane (ILM) to include all the vessels of this plexus. To visualise the deep retinal plexus, we used a 30 µm thick layer from the inner plexiform layer (IPL) to visualise the plexus in its entirely (figure 1). Two investigators checked the segmentation quality before testing flow density.

Objective quantification of flow density was evaluated for each eye using the SSADA software. Quantitative analysis was performed on the OCTA en face image using the AngioVue software. The flow density was defined as the percentage area occupied by vessels in a circular region of interest (ROI) in the centre of the foveal avascular zone and with a diameter of 2.5 mm. The AngioVue software automatically splatted the ROI into two fields: the foveal area, a central circle with a diameter of 1 mm; and the parafoveal area that constitutes the remaining part inside the ROI (figure 1).

The AngioVue software automatically outputs the flow density percentage inside the parafoveal area, as previously described.[21 22]

The software output the mean MT in the foveal (FMT) and the parafoveal (PFMT) areas. Furthermore, the software automatically segmented the innermost retinal layers, from the ILM to the outer portion of the IPL, and the outermost retinal layers, from the inner portion of the INL to the outer portion of the hyper-reflective line corresponding to the RPE. The latter segmentations allowed the software to separately test the parafoveal thickness of the inner retina and the outer retina.

Statistical analysis

Statistical calculations were performed using Statistical Package for Social Sciences (V.20.0, SPSS Inc., Chicago, Illinois, USA). To detect departures from normality distribution, Shapiro–Wilk's test was performed for all variables. All quantitative variables were presented as media and SD in the results and in the tables. The difference among the three groups was generated by conducting analysis of variance (ANOVA) analysis followed by Bonferroni's post hoc test. Pearson's correlation was performed to evaluate the linear correlation among variables in patients with AMD.

The chosen level of statistical significance was $p<0.05$.

RESULTS

No difference in age (71.6±7.6, 72.8±6.5 and 69.7±8.2 years in changes-AMD, no changes-AMD and control groups, respectively) and BCVA (0.03±0.05 LogMAR in changes-AMD group, 0.03±0.04 LogMAR in no changes-AMD group and 0.01 ±0.04 LogMAR in healthy subjects) was found among the three groups. All the subjects enrolled were Caucasian and not affected by diabetes. Furthermore, there were no significant differences in systemic hypertension or the use of systemic antihypertensive medications among the groups.

Macular thickness analysis

Foveal MT was 215.2±32.9 µm in changes-AMD patients and was significantly thinner than no changes-AMD patients (248.3 ±23.3 µm, $p=0.002$) and healthy subjects (268.1±19.2 µm, $p<0.0001$).

Full parafoveal MT was 266.5±44.5 µm in the changes-AMD group, 302.9±18.8 µm in the no changes-AMD group and 316.1±13.8 µm in healthy controls ($p=0.002$ and $p<0.0001$, respectively, after comparison with the changes-AMD group). Furthermore, in the parafoveal area, the thickness of the inner retina was significantly thinner in the changes-AMD group (98.7±18.2 µm) compared with the no changes-AMD group (112.7±10.0 µm, $p=0.023$) and the healthy group (117.3 ±12.1 µm, $p=0.007$). Finally, considering the parafoveal area, the outer retina thickness was 167.7±27.6 µm in changes-AMD patients and was significantly thinner than no changes-AMD patients (190.1±14.1 µm, $p=0.004$) and healthy subjects (198.8 ±4.8 µm, $p<0.0001$) (table 1).

Flow density analysis

Parafoveal SVPFD was 43.3±2.7% in changes-AMD patients and was decreased after comparison with the no changes-AMD group (48.7±3.3% and $p=0.003$). Moreover, parafoveal SVPFD was 50.4±6.1% in healthy controls ($p=0.001$ in the comparison with the changes-AMD group). Furthermore, no difference was found in parafoveal SVPFD between the no changes-AMD group and healthy controls ($p=0.715$).

Parafoveal DVPFD was 51.9±1.9% in changes-AMD patients, 53.6±4.9% in no changes-AMD patients and 51.6±6.0% in healthy controls ($p=1.0$ in all the comparisons among the groups) (table 1).

Correlation analysis

The Pearson test showed a significant direct correlation among all the parafoveal thicknesses (full, inner and outer) in changes-AMD patients (table 2). Moreover, in the latter group, we found a direct correlation of the superficial plexus flow density with the full parafoveal MT ($R^2=0.734$, $p=0.038$) and with the innermost layers' parafoveal MT ($R^2=0.761$, $p=0.028$). Table 2 shows all the correlations among MTs and vascular plexus densities.

DISCUSSION

In this prospective study we investigated the association between retinal vessel changes and SD-OCT in patients affected by intermediate AMD. Overall we found patients showing signs

Table 1 Macular thickness and retinal vascular plexus flow density in intermediate AMD patients and controls

				p Value		
	Changes-AMD group (n=15)	No changes-AMD group (n=15)	Controls (n=15)	Changes-AMD group vs no changes-AMD group	Changes-AMD group vs healthy controls	No changes-AMD group vs healthy controls
Foveal macular thickness (μm)	215.2±32,9	248.3±23.3	268.1±19.2	**0.002**	**<0.0001**	0.091
Full parafoveal macular thickness (μm)	266.5±44.5	302.9±18.8	316.1±13.8	**0.002**	**<0.0001**	0.427
Innermost layers' parafoveal macular thickness (μm)	98.7±18.2	112.7±10.0	117.3±12.1	**0.023**	**0.007**	0.949
Outermost layers' parafoveal macular thickness (μm)	167.7±27.6	190.1±14.1	198.8±4.8	**0.004**	**<0.0001**	0.428
Superficial vascular plexus flow density (%)	43.3±2.7	48.7±3.3	50.4±6.1	**0.003**	**0.001**	0.715
Deep vascular plexus flow density (%)	51.9±1.9	53.6±4.9	51.6±6.0	1.0	1.0	1.0

Bold text represents **p<0.05.**
Values were compared by one-way analysis of variance (ANOVA), followed by Bonferroni post hoc test.
AMD, age-related macular degeneration.

Table 2 Correlation between macular thickness and flow density in patients with intermediate AMD

	Full parafoveal macular thickness	Innermost layers' parafoveal macular thickness	Outermost layers' parafoveal macular thickness	Superficial vascular plexus flow density	Deep vascular plexus flow density
Changes-AMD group (n=15)					
Full parafoveal macular thickness	–	**0.955** **<0.0001**	**0.981** **<0.0001**	**0.734** **0.038**	0.394 0.334
Innermost layers' parafoveal macular thickness	**0.955** **<0.0001**	–	**0.879** **0.004**	**0.761** **0.028**	0.352 0.392
Outermost layers' parafoveal macular thickness	**0.981** **<0.0001**	**0.879** **0.004**	–	0.679 0.064	0.402 0.324
Superficial vascular plexus flow density	**0.734** **0.038**	**0.761** **0.028**	0.679 0.064	–	0.284 0.495
Deep vascular plexus flow density	0.394 0.334	0.352 0.392	0.402 0.324	0.284 0.495	–
No changes-AMD group (n=15)					
Full parafoveal macular thickness	–	**0.659** **<0.0001**	**0.851** **<0.0001**	0.053 0.802	−0.086 0.683
Innermost layers' parafoveal macular thickness	**0.659** **<0.0001**	–	0.165 0.431	0.009 0.967	−0.030 0.887
Outermost layers' parafoveal macular thickness	**0.851** **<0.0001**	0.165 0.431	–	−0.119 0.324	0.090 0.669
Superficial vascular plexus flow density	0.053 0.802	0.009 0.967	−0.119 0.324	–	**0.559** **0.004**
Deep vascular plexus flow density	−0.086 0.683	−0.030 0.887	0.090 0.669	**0.559** **0.004**	–

Bold text represents **p<0.05.**
Correlation analysis was obtained by Pearson correlation.
AMD, age-related macular degeneration.

predicting GA development had a reduction in inner and outer retinal layers, as well as an alteration in superficial vascular retinal plexus.

Several studies reported that the presence of OPL and INL subsidence[23] and the development of a hyporeflective wedge-shaped band in OPL[24] were SD-OCT features of GA areas. Nevertheless, recently, Wu *et al*[14] demonstrated that the latter SD-OCT hallmarks were also found in patients with intermediate AMD. The latter longitudinal study included 221 eyes affected by intermediate AMD and the enrolled patients were followed up every 3 months for up to 30 months. The authors demonstrated these two SD-OCT signs portend the development of drusen-associated atrophy and termed these characteristics as 'nascent geographic atrophy'. In fact, the presence of

OPL and INL subsidence and a hyporeflective wedge-shaped band in OPL were unique to areas that developed drusen-associated atrophy, unlike features such as hyperreflective foci and drusen characteristics,[25] parameters already reported as risk factors for the development of atrophy.

Several study showed that both the inner and the outer retina were thinner in patients with AMD, the latter aspect suggesting AMD is also featured by a neuroretinal layer damage.[2 4–6] In the current study we showed that MT is reduced as soon as patients have the intermediate stage, but only considering patients with signs predicting the development of atrophy associated with drusen. Moreover, interestingly, our data demonstrated that the inner and the outer retina were significantly thinner in these patients. The latter aspect, added to the fact that the inner and outer retina thicknesses were significantly correlated in these patients, suggested the damage started in all retinal layers and confirmed that AMD is not confined to the outer segments.

It is known that retinal vasculature and choroidal vasculature are damaged in AMD eyes and that this impairment might contribute to AMD progression.[9 10] These features would not be surprising because of systemic hypertension,[26] dietary fat intake[27 28] and a history of coronary, carotid and peripheral vascular disease[29] are risk factors for AMD and for vascular disease. Indeed, our group recently showed that the superficial retinal plexus is damaged in patients not affected by late stage dry AMD.[13] Moreover, it demonstrated retinal and choroidal vessel damage following the same trend in AMD, as the vascular injury starts as soon as the intermediate stage is reached.

In the current study, we showed that patients with signs predicting GA on SD-OCT scans also showed a reduction in SVPFD. Moreover, we found a significant correlation between inner retina layer thickness and SVPFD. The authors think that the association between inner retina thinning and reduction of blood flow in the SVP is of interest and could be explained by several mechanisms: the reduction in thickness of the neuroretina might be followed by a reduction of metabolic demand and a resulting reduction in blood flow; and the inner retina may perish from progressive hypoperfusion secondary to vascular damage. Indeed, several authors demonstrated reduced blood flow in the choroid and retina causes chronic ischaemia in Bruch's membrane, RPE and neuroretina.[30–32] Vascular deficits due to reduced choroidal and retinal blood flow have been identified in early and late AMD using FA and Doppler imaging.[32–34] Further studies should evaluate the relationship between the reduction in SVPFD and in neuroretinal thickness to clarify the causal relationship.

Moreover, we did not find differences in the deep retinal capillary plexus. This difference could be explained by the dissimilarity between the two plexuses. Indeed, the two vascular plexuses have different patterns: the SVP is composed of capillaries developing an interconnected vascular network between arterioles and venules; the DVP is compounded by polygonal units, in which the capillaries converge radially towards an epicentre called a capillary vortex.[15 18] The different structure might elucidate the preservation of the deep capillary plexus in intermediate AMD.

Our study has several limitations. The series presented here is relatively small. However, one should consider the strict inclusion criteria for patients and the control group, as well as the similarity of groups with respect to meaningful characteristics such as age. Another limitation is that the OPL and INL subsidence could in theory influence the deep capillary plexus segmentation. Finally, a further major limitation is that we did not evaluate choroidal vessels. However, the OCTA choriocapillaris evaluation should be interpreted with caution because drusen could lead to an attenuation artefact confusing the evaluation.

In conclusion, we provide the first study evaluating the association between SD-OCT signs and retinal blood supply in patients with intermediate AMD and we show that patients with signs predicting the development of GA had a reduced flow in SVP and damage of the inner and the outer retina. This study raises many questions and prompts further investigation, including evaluation of new therapeutic approaches targeting reduction of retinal vessel damage progression and the possible slowdown toward GA.

Contributors LT, EB, PC and LM conceived the study. LT and EB conducted the study. EB collected the data. EB performed the statistical analysis. LT, EB, LDA, RM wrote the article.

Competing interests None declared.

Patient consent Obtained.

Ethics approval University Gabriele D'Annunzio Ethics Committee.

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