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Optical Coherence Tomography Angiography as early vascular biomarker of Multiple Sclerosis

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1. Introduction

The possibility to evaluate the eye as a “window” into the central nervous system (CNS) has attracted great attention in recent years [1].

Different studies have reported the close clinical relationship between the brain and the retina because they are anatomically interconnected, show similar physiological features, an highly isolated and protected vascular system [1,2].

The cerebral and retinal microcirculation presents mechanisms of autoregulation to maintain a relatively constant blood flow despite variation in perfusion pressure [3,4].

The cerebral and retinal neural environments are protected by specialized barriers. The inner blood-retinal barrier is composed by tight junctional complexes between non-fenestrated endothelial cells surrounded by astrocytic end feet, and thus closely resembles the blood-brain barrier [5].

Given the strong anatomical and physiological homologies between these two tissues, it has been suggested that CNS disorders may be associated with distinctive retinal changes.

Recent advances in retinal imaging with the introduction of Optical Coherence Tomography Angiography (OCTA) in clinical practice allowed a non-invasive visualization of retinal microvasculature.

In this perspective, the retina may represent an easy accessible “window” to evaluate the cerebral microvascular damage [1,2,6] (Figure 1).

Monitoring retinal vascularization may add important information to better understand the pathogenesis of neurological diseases and in particular multiple sclerosis (MS) [7].

MS is a chronic inflammatory demyelinating disorder of the CNS associated with progressive neurodegeneration [8].

It is a multifactorial disease in which cerebral hypoperfusion and vascular disfunction are gaining interest as possible factors related to disease risk and progression [9].

Evaluating of retinal vessels by OCTA offers the possibility to detect and monitor the damages of the retinal vascular network, useful to obtain more knowledge about the pathophysiological mechanisms involved in MS [10].

OCTA is a valid and reliable imaging technique that could represent an reliable support in providing potential and useful biomarkers in MS pathogenesis and in disease progression.

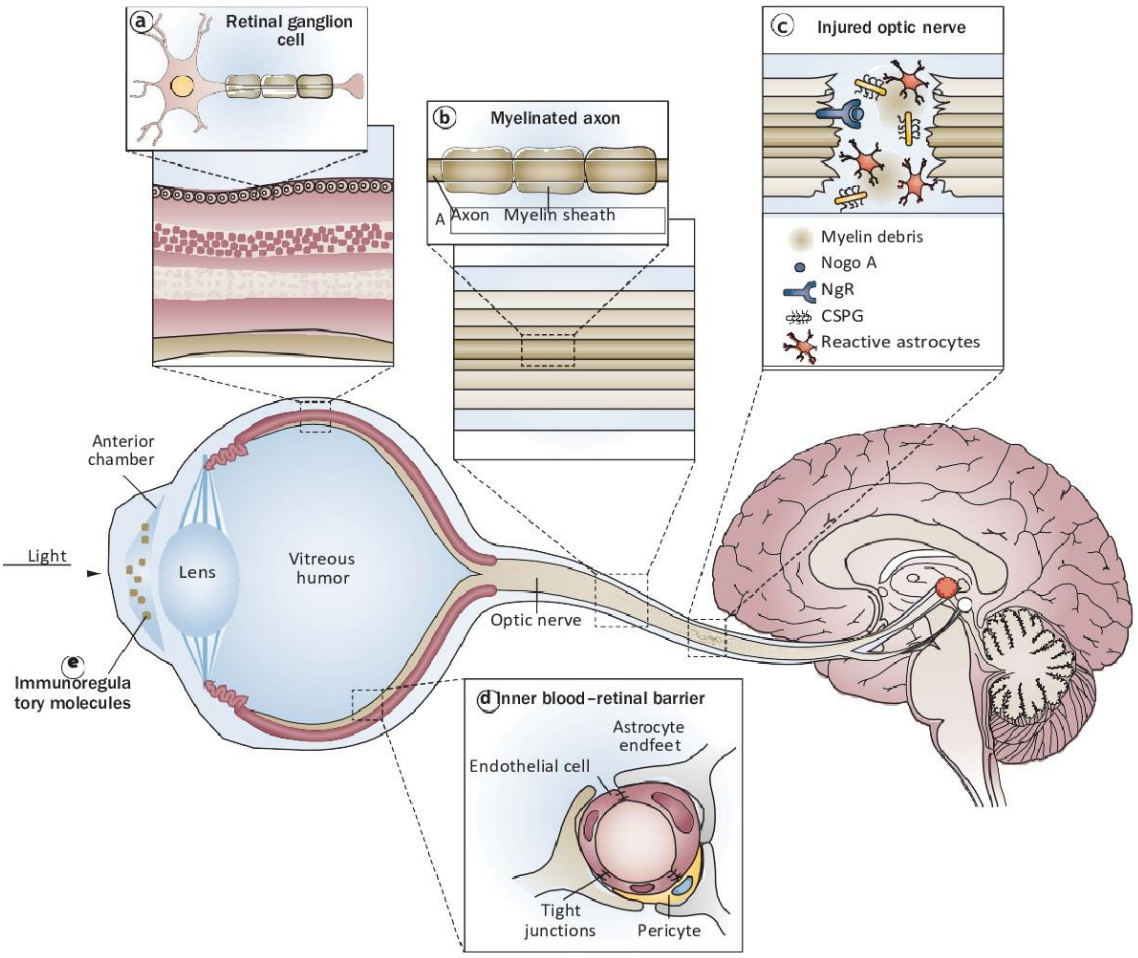

Figure 1. **The eye as a "window" into the central nervous system (CNS).** A. In inner retinal layers are present different neuronal cells including retinal ganglion cells, which share structural morphology with CNS neurons. B. The axons of these cells are myelinated by oligodendrocytes posterior to the globe, and form the optic nerve. C. The optic nerve damages determine, in a manner similar to other CNS neurons, an environment that is both hostile to survival of neurons that were spared in the primary insult and inhibitory to regeneration of severed axons. D. E. Similar to the CNS, the eye presents a relationship with the immune system that involves specialized barriers such as the inner blood–retinal barrier, the retinal counterpart of the CNS blood–brain barrier (D), and the constitutive presence of immunoregulatory molecules (E).

From London et al. 2012

2. State of the art

2.1 Multiple Sclerosis

Multiple sclerosis is an inflammatory, demyelinating and neurodegenerative disease that predominantly affects young adults and it is characterized by heterogeneous manifestations and evolution [11,12].

The main symptoms include motor difficulties, visual defects, cognitive issues, and bladder/bowel dysfunction [13].

MS is a multifactorial disease that is the result of a complex interaction between genetic factors and factors acquired-environmental.

It has been hypothesized that the exposure of individuals with a favorable genetic background (alleles of the MHC DR4, DR15 and DQ6) [14,15] to environmental factors such as smoking and infectious agents (such as Epstein-Barr virus), could contribute to disease risk and progression [11,16].

MS is characterized by an inflammatory component, which is responsible for acute occurrence of clinical relapses and development of focal lesions and by a degenerative component, which is responsible for accrual of progressive physical and cognitive disability [14].

The initial demyelinating event (IDE) of CNS is a first-ever episode of acute/subacute neurological disturbance, lasting ≥ 24 hours, suggestive of demyelination in one or more typical locations, including optic nerve, spinal cord, brainstem, cerebellum, and cerebral hemisphere [17].

At the clinical onset of the disease, the IDE, referred to as clinically isolated syndrome (CIS), might not fulfill the diagnostic criteria for both dissemination in space and time, and in some patients it might even remain as a monophasic illness, without new clinical episodes or paraclinical evidence of disease or an IDE may be the inaugural manifestation of MS [18,19].

According to the later 2017 criteria, the diagnosis of MS can be made in a patient with CIS if the first MRI fulfills dissemination in space (DIS) criteria, and dissemination in time (DIT) criteria, or with positive oligoclonal bands in cerebrospinal fluid [20].

Nevertheless, prospective studies show that the conversion rate of CIS to MS is approximately 60–70% within 20 years [19].

MS clinical course is usually characterized, during the initial stage, by unpredictable clinical and radiological relapses (relapsing-remitting MS -RR-MS), over time the recurrence of relapses tends to decrease and a gradual neurological worsening occurs (secondary progressive MS) [21] (Figure 2).

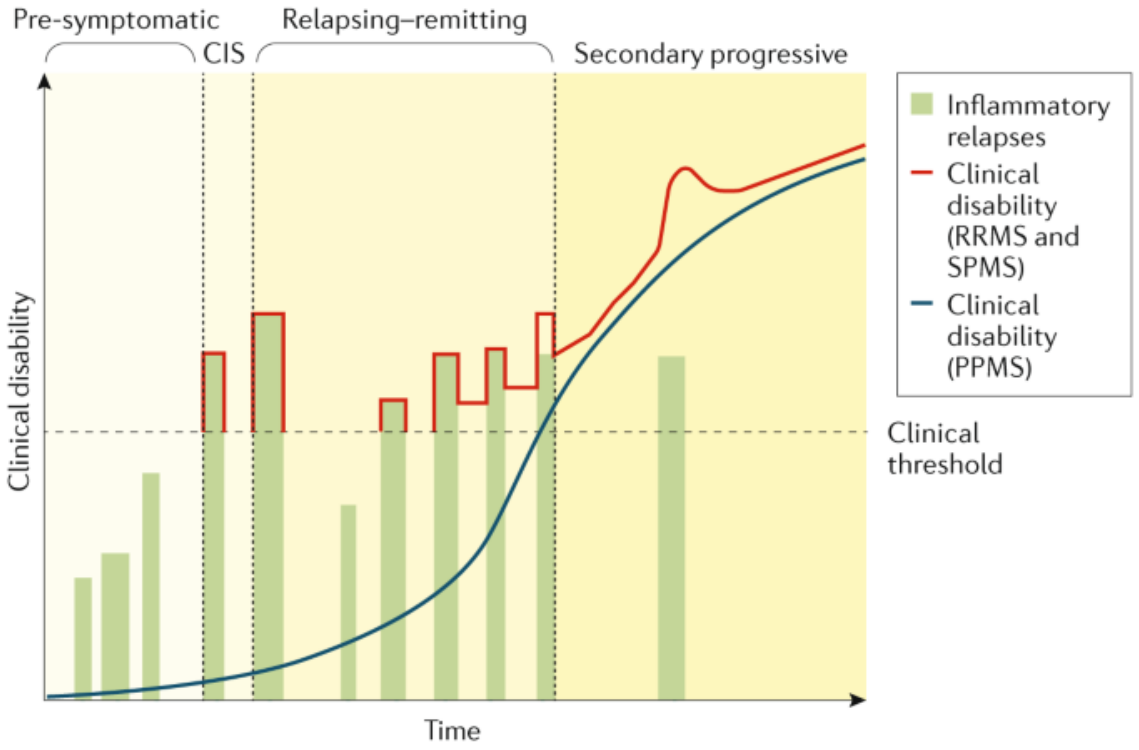

Figure 2. Classification of MS, and disability over time. Clinically isolated syndrome (CIS) refers to a first clinical demyelinating event in central nervous system lasting ≥ 24 hours. Relapsing-Remitting Multiple Sclerosis (RR-MS) exhibits unpredictable clinical and radiological relapses. This may last years or even decades, before some progression to Secondary Progressive MS (SPMS), with a constant degeneration of neurological function and increasing disability. Primary Progressive MS (PPMS) refers to a similar degeneration, but from the onset, in the absence of a relapsing-remitting stage.

2.2 MS pathogenesis

The pathogenesis of MS is characterized by a cascade of events, ranging from focal lymphocytic infiltration and microglia activation to demyelination and axonal degeneration [22-24].

The immune response plays an important part in the pathogenesis of MS. The myelin-specific autoreactive lymphocytes cross the blood-brain barrier and lead to inflammatory demyelinating lesions [25].

The activation of macrophages and microglia mediates the inflammatory cascade contributing to neurodegeneration [11].

The release of inflammatory mediators (nitric oxide, reactive oxygen species, myeloperoxidase, tumor necrosis factor-alfa) from activated microglia and infiltrated macrophages induces neuronal mitochondrial dysfunction. Mitochondrial injury contributes to apoptosis of oligodendrocytes, demyelination and neuro-axonal damage [26-29].

In addition to inflammation, axonal loss can be driven or amplified by a number of other pathological processes including Wallerian degeneration following axonal transection due to focal lesions [30], mutation of mitochondrial DNA [31,32], astrocytes dysfunction [33,34], glutamate excitotoxicity [35], iron accumulation [28,36] and sodium ions accumulation [37].

In MS pathogenesis, microvascular abnormalities and hypoxia

might play a causal role in damage to oligodendrocytes and myelin resulting in the appearance of demyelinating lesions [38,39].

This is supported by perfusion-weighted magnetic resonance imaging (MRI) studies that documented an impairment of cerebral blood flow in the white matter of patients with MS, regardless to the clinical subtype, which does not seem to be secondary to axonal degeneration with reduced metabolic demands [9,40-42].

Also the vascular dysfunction has been described in MS. Indeed, endothelial dysfunction, probably secondary to inflammation and a chronic state of impaired venous drainage from the CNS, seem to play an important role in the development and course of the disease [43].

Another theory is that the inflammatory demyelinating process affects directly the integrity of the cerebral vascular endothelium which results in a reduced blood flow [44] (Figure 3).

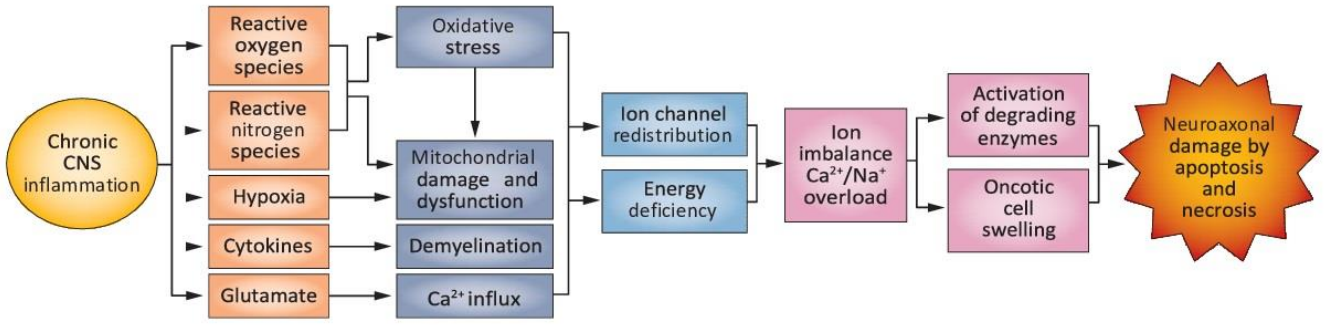

Figure 3. Cascades leading to inflammation-induced neuroaxonal injury. The scheme illustrates the hypothetical sequence of events leading to neuroaxonal degeneration in multiple sclerosis. Chronic central nervous system inflammation determines a deregulation of neuronal and axonal metabolism resulting in neurodegeneration. *From Friese et al. 2014*

3. Optical coherence tomography angiography and multiple sclerosis

The introduction of the OCTA, a novel, non-invasive, fast and highly reproducible technique, has allowed a detailed visualization of the retinal and choroidal microvascular networks and a precise, quantitative and objective analysis of the vessel density in the region of macula and optic disc [45].

Due to the limitations in terms of accessing the brain perfusion, the vascular network of the eye could potentially serve as a mirror reflecting the changes of cerebral microvasculature in MS.

Therefore, OCTA may contribute to greater insights into the pathophysiology of MS and provide helpful biomarkers for detecting retinal and choriocapillaris vessel impairment in early stages and to monitor the vascular changes during the progression of this disease.

Unlike a traditional retinal imaging such as fluorescein angiography (FA) or indocyanine green angiography (ICGA), OCTA is a non-invasive imaging technique. The patients do not have to receive intravenous injections of a dye and this essentially eliminates all the side effects that the conventional retinal angiography techniques have including anaphylaxis, nausea, vomiting, rash, urine and skin discoloration. As a result, OCTA is also much safer and better tolerated compared to FA or ICGA [46].

Moreover, leakage of the dye related to the breakdown of the blood-retinal-barrier did not allow clear visualization of capillary drop out, areas of ischemia, vessel distortion and early neovascularization [47].

OCTA presents a much higher resolution than FA and ICGA and detects small capillaries and microareas of non-perfusion in different retinal vascular plexuses [48].

This technique is time-efficient and it provides the examination of retinal vasculature in 3D in 3 seconds. The advantages of this device led to a quick adoption of this new modality in the clinical routine; it represents a major contribution towards advancing ophthalmic imaging in ocular disease diagnosis, monitoring and treatment [49-51].

OCTA works on the split-spectrum amplitude decorrelation angiography (SSADA) algorithm by collecting multiple cross-sectional scans (B-scans) of the same location and then detecting the differences in motion contrast, amplitude, intensity or phase [52]. Since motionless objects do not produce any change in a signal contrary to moving objects and the retina and choroid are stationary tissues, the differences in values come from the moving of the blood within the vessels. The SSADA algorithm uses the movement of blood flow to visualize the retinal and choriocapillary vascular networks (Figure 4).

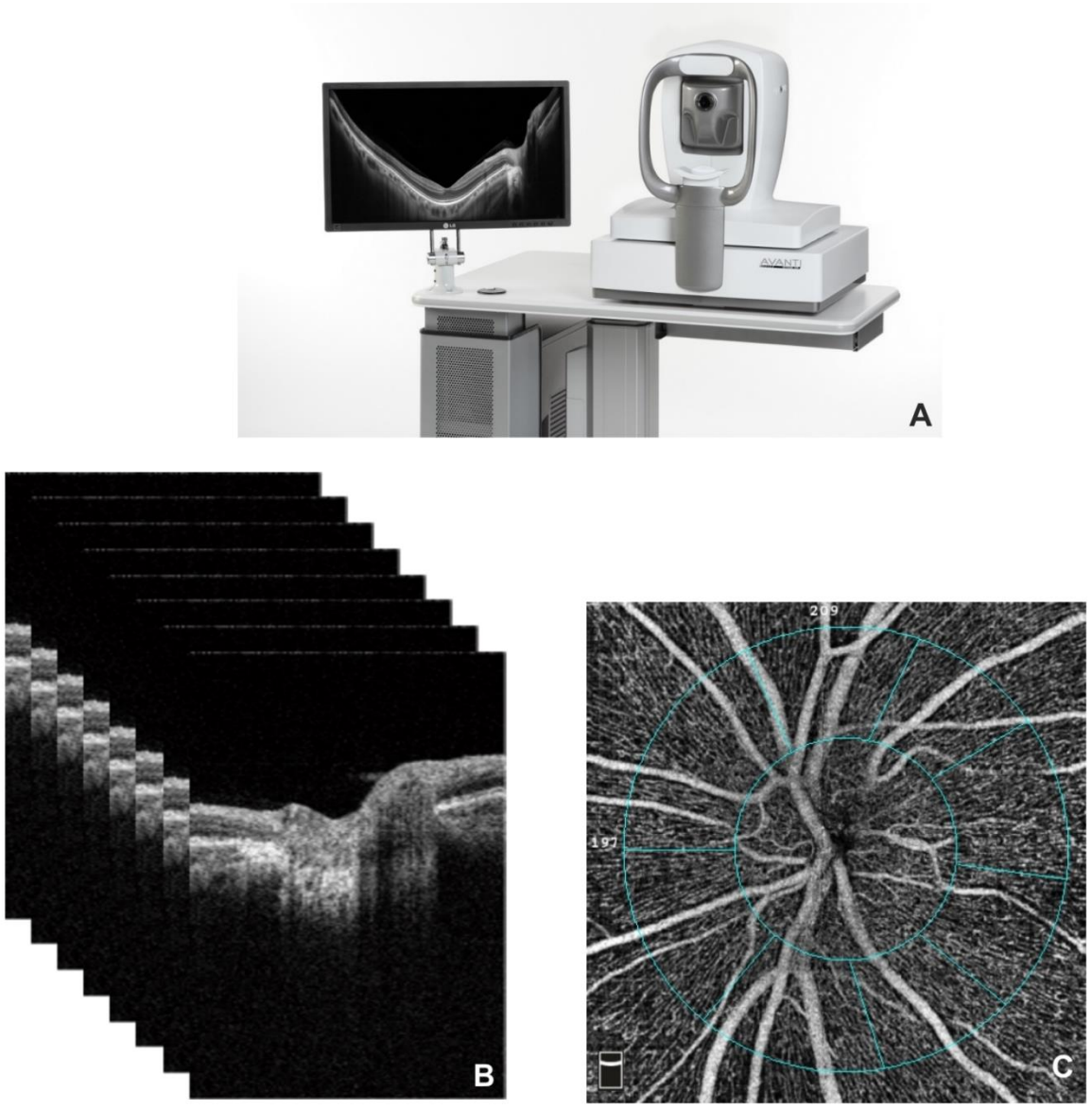

Figure 4. The AngioVue OCTA system Avanti. This device uses the split-spectrum amplitude-decorrelation angiography (SSADA) algorithm (A). The SSADA algorithm compares consecutive B-scans obtained in the same position to detect blood flow in the vessels using "motion contrast" (B).
After processing the scans, the decorrelation of the images is calculated for the purpose of flow signal calculation (C). Decorrelation is a mathematical function that allows to quantify variations without being affected by the average signal strength.

Each scanning is then performed in different axes to create a 3D image [53,54]. Then, on OCTA image it is possible to separately visualize retinal vascular plexuses and choroidal vasculature by en face projection of segmented slab. Each OCTA image is coregistered with OCT B-scan.

Clinically the retinal vasculature is formed by two main and parallel vascular layers: the superficial capillary plexus and deep capillary plexus that are connected by vertical vessels. The superficial capillary plexus is located within the ganglion cell layer, retinal nerve fiber layer and inner plexiform layer. The deep capillary plexus is located below within the inner nuclear layer and outer plexiform layer [55].

The radial peripapillary capillary plexus is constituted by the retinal superficial vascular network that runs parallel with the nerve fiber layer axons in peripapillary region.

Moreover it is possible to analyze also the deeper vascular layers in macular region represented by the choriocapillary plexus.

The OCTA software allows to calculate automatically the vessel density in retinal and choriocapillaris vascular networks [55-57].

The vessel density corresponds to the percentage of the surface occupied by vessels and capillaries based on adaptive thresholding binarization within the desired area.

The ratio is expressed numerically and shown on a scale of false colors. The warm colors are given to high density vascular areas, and then cold colors

show low flow or no flow density. An average vascular density value is obtained for each sector of the map. Vascular density map may be calculated separately for the retinal superficial, deep capillary plexuses, radial peripapillary capillary plexus and choriocapillaris [58].

In macular region the vessel density values were recorded in the whole 3 × 3-mm area or 6 x 6-mm area over the macular scan, in internal annular zone (0.3–1.5 mm from the fovea; defined as 'parafoveal area') and in external annular zone (1.5–3.0 mm from the fovea; defined as 'perifoveal area') [59]. The radial peripapillary capillary plexus is analyzed in the superficial retinal layers of papillary region and extended from the inner limiting membrane (ILM) to the retinal nerve fiber layer posterior boundary. The vessel density measurements of the radial peripapillary capillary plexus is automatically obtained for the whole scanned area, the area inside the optic disc and the peripapillary region. The whole image vessel density is calculated over an area scan of 4.5 × 4.5 mm centered on the optic disc. Inside disc refers to the area inside an ellipse fitted to the optic disc boundary. Peripapillary region is measured in a 0.75 mm-wide elliptical annulus extending outward from the optic disc boundary [60] (Figure 5).

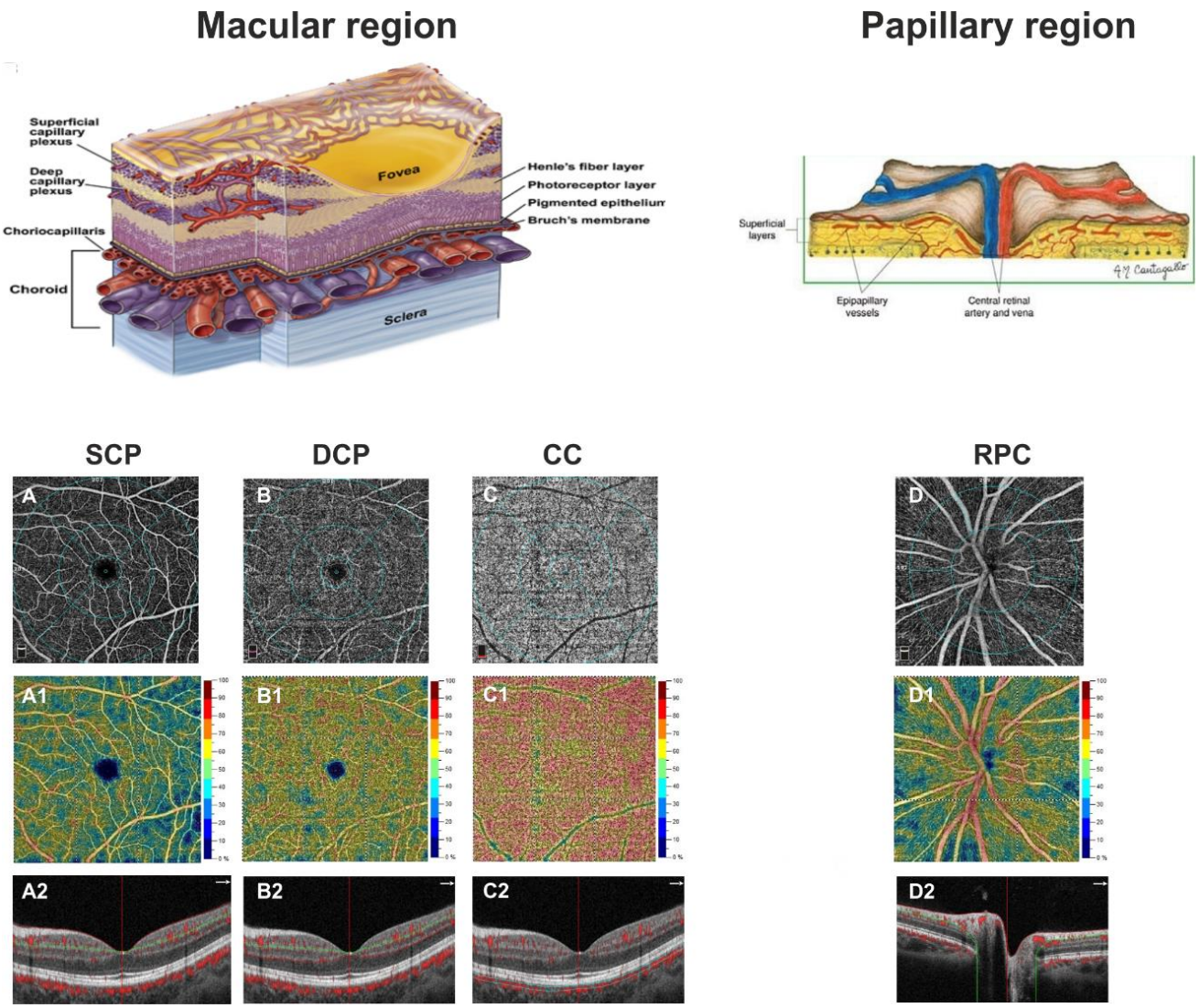

Figure 5. Anatomical localization of retinal and choriocapillary vascular networks at Optical Coherence Tomography Angiography. In the first row are shown en face visualization of superficial capillary plexus (SCP) (A), deep capillary plexus (DCP) (B), choriocapillaris (CC) (C) and radial peripapillary capillary plexus (RPC) (D). The second row reveals the vascular density maps of SCP (A1), DCP (B1), CC (C1) and RPC (D1). The third row shows the OCT B scan related to each OCTA scan with the segmentation of each vascular layer.

4. Objectives

The present project aims to analyze in patients with IDE, defined as the first neurologic symptom referable to demyelination in the CNS, lasting for at least 48 hours, the retinal and choriocapillaris vascular networks by means OCTA and to detect their changes during a longitudinal study. The main objectives are represented by:

1. Optical Coherence Tomography Angiography parameters as early vascular biomarkers in initial demyelinating event

In the first part of the study, the retinal and choriocapillaris vessel density was analyzed in macular and in peripapillary regions in patients with recent onset of multiple sclerosis, at IDE compared with matched RR-MS patients and healthy subjects in order to define which OCTA parameters could be sensitive in identifying the first vascular changes in patients affected by early stages of MS.

2. Retinal and choriocapillary vascular changes in initial demyelinating event: a prospective longitudinal study

The second part of the study focused on the evaluation of retinal and choriocapillaris vascular changes in these IDE patients over two years follow up. OCTA parameters might represent useful vascular biomarkers to monitor the MS progression.

5. Optical Coherence Tomography Angiography parameters as early vascular biomarkers in initial demyelinating event

5.1 Introduction

Multiple sclerosis (MS) is characterised by inflammation, demyelination and axonal loss throughout the central nervous system. Markers for disease pathology are highly needed. The introduction of the optical coherence tomography (OCT), fast and non-invasive imaging technique, allowed to investigate and monitor the structural retinal damages, in particular the ganglion cell and retinal nerve fiber layers in neurological diseases. Previous studies, analysing the different disease stages often accompanied by optic neuritis, demonstrated that the retinal changes reflect not only the neurodegenerative processes but also the inflammatory disease activity. Since the strong association described between the retinal impairment and brain atrophy on MRI, the OCT parameters play a significant role as biomarkers for MS diagnosis and follow up [61-63]. Several reports also described cerebral hypo-perfusion and vascular pathology as pathological changes underpinning MS aetiology and evolution [9,42,64]. Besides the application of advanced MRI techniques such as arterial spin-labelling, optical coherence tomography angiography (OCTA) offers the unique opportunity to assess

integrity of brain vasculature by looking at vascular networks within the retina [65,66]. A previous study described a reduction of retinal vessel density (VD) on OCTA in MS compared with controls. Reduced VD was associated with higher disability, as measured with Expanded Disability Status Scale (EDSS) [67]. However, the inclusion of very early cases of MS would have allowed a better understanding about implication of retinal vasculature changes in the disease pathogenesis. Feucht et al. recently studied retinal vasculature network in patients with clinically isolated syndrome (CIS), and found vessel rarefaction of superficial and deep retinal plexus only in eyes suffering from previous optic neuritis, while a higher VD in choriocapillaris layer was associated with recent relapses and MRI activity [68].

The aim of this study is to investigate VD in macular and peripapillary regions in patients with an initial demyelinating event (IDE), namely patients experiencing the first neurologic symptom referable to demyelination in the central nervous system regardless they meet MS or CIS diagnosis at MRI scan according with 2017 McDonald criteria [20]. We also aimed to compare vascular changes in the retina between controls, IDE and relapsing-remitting MS (RR-MS) patients through OCTA.

5.2 Methods

In this cross-sectional study, we enrolled IDE patients and healthy controls (HCs) at the MS Centre of the University of Naples "Federico II", from January 2018 to December 2018. IDE was defined as the first neurologic symptom referable to demyelination in the central nervous system, lasting for at least 48 hours, regardless patients met RR-MS or CIS diagnosis according with 2017 McDonald criteria [20]. We excluded patients with any history of optic neuritis, in order to avoid a bias related to optic nerve direct damage. Family history, motor disability assessed through EDSS, disease duration and previous relapses were recorded for all patients. HC presented with normal neurological and ophthalmic examinations. IDE patients and HCs were compared with age-matched RR-MS patients with low disease disability.

Exclusion criteria were i) a relapse and/or corticosteroid use in the previous month ii) the presence of systemic vascular diseases (high blood pressure, diabetes and heart diseases), iii) clinically relevant lens opacities, iv) low-quality images obtained with Spectral Domain (SD)-OCT and OCTA, v) myopia greater than 6 diopters, vi) history of intraocular surgery, vitreoretinal and retinal vascular diseases, uveitis, congenital eye disorders.

All subjects underwent evaluation of best-corrected visual acuity according to the Early Treatment of Diabetic Retinopathy Study [69], slit-

lamp biomicroscopy, fundus examination For each subject, we also assessed the mean deviation and pattern standard deviation as measures of visual field for subject with visual fixation above 20%. Finally, we performed both SD-OCT and OCTA. Ophthalmological evaluation was blinded to subjects' clinical status. The study was approved by the Institutional Review Board of the University of Naples "Federico II" and all investigations adhered to the tenets of the Declaration of Helsinki (protocol number: 142/19). Written informed consents were obtained from the subjects enrolled in the study.

5.2.1 Spectral Domain Optical Coherence Tomography

The retinal nerve fiber layer and ganglion cell complex thickness were obtained with SD-OCT (software RTVue XR version 2018.1.1.60, Optovue Inc., Fremont, CA, USA). The acquisition protocol for optic nerve head map was used to calculate the circumpapillary retinal nerve fiber layer thickness and it was based on measurements around a circle 3.45 mm in diameter centered on the optic disc. The ganglion cell complex thickness was obtained centering the scan 1-mm temporal to the fovea and covering a 7 x 7 mm area over the macular region. The ganglion cell complex thickness included the measurements from the internal limiting membrane to the outer boundary of the inner plexiform layer [70]. Each OCT scan was analyzed in alignment following APOSTEL

recommendations and applying the OSCAR-IB protocol for quality control [71,72]. These guidelines were adapted for our device.

5.2.2 Spectral Domain Optical Coherence Tomography Angiography

OCTA images were performed using the RTVue XR Avanti, Optovue, Inc. (software RTVue XR version 2018.1.1.60 Freemont, California, USA) following a standardized protocol based on the split-spectrum amplitude de-correlation algorithm, as previously described [73]. Macular capillary plexus was visualized performing a 6 × 6 mm scan over the macular region and the percentage area occupied by the large vessels and microvasculature in the analyzed region defined the vessel density (VD) [58]. The software identified the VD in whole area of the macular scan considering the two retinal vascular networks(namely the superficial and deep capillary plexuses) and choriocapillaris. The Angio-Vue disc mode automatically segmented the radial peripapillary capillary plexus VD analyzing the whole papillary region with a scanning area of 4.5 x 4.5 mm. VD for the radial peripapillary capillary plexus was analyzed in the superficial retinal layers and extended from the inner layer membrane to the retinal nerve fiber layer posterior boundary [60]. From the analysis were excluded the images with a signal strength index less

than 80 and residual motion artifacts. A summary of measures evaluated through SD-OCT and OCTA is reported in Figure 6.

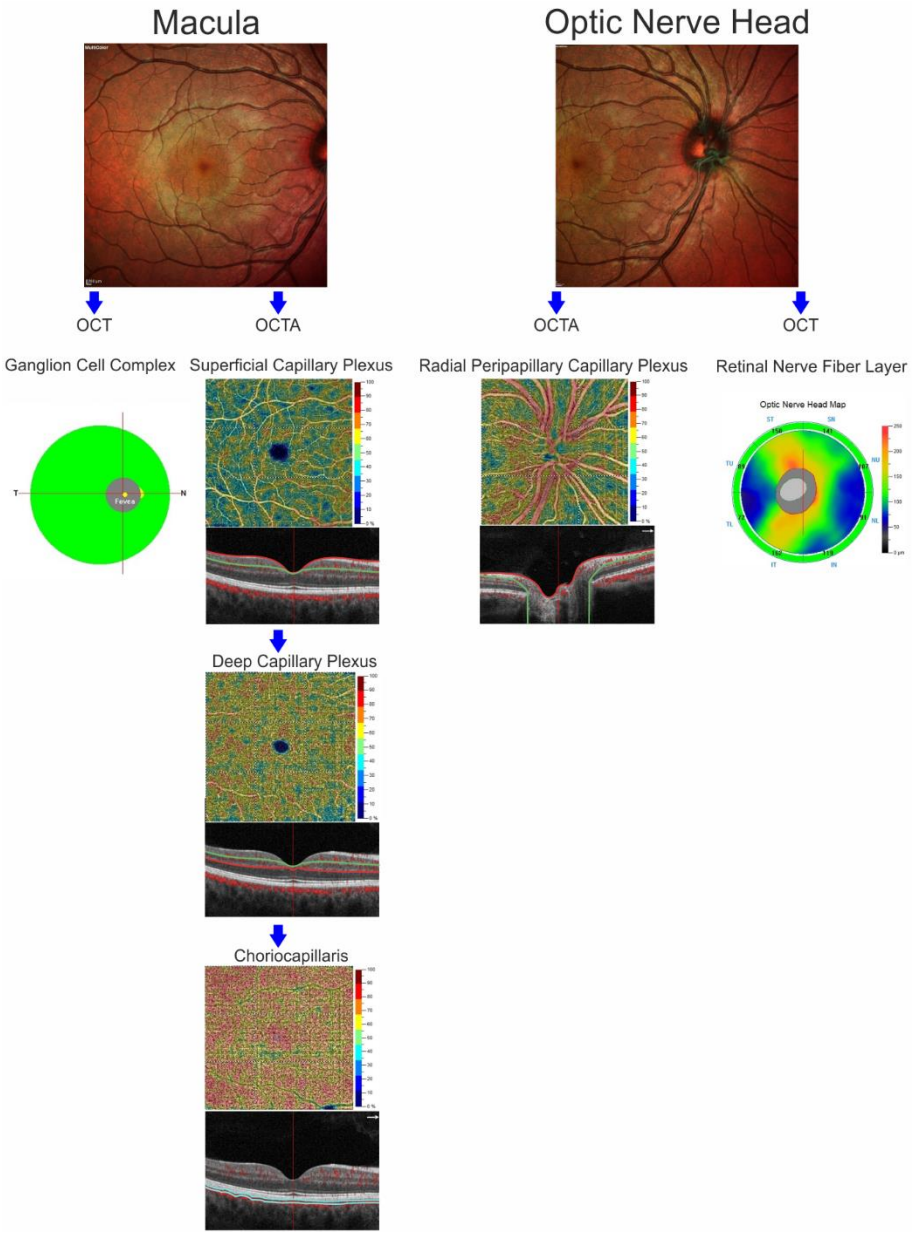

Figure 6. Anatomic illustration of macular and optic nerve head regions showing the retinal structures analyzed by optical coherence tomography (ganglion cell complex and retinal nerve fiber layer) and the retinal and choriocapillaris vascular networks evaluated by OCT-Angiography.

5.2.3 Statistical analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (Version 20.0 for Windows; SPSS Inc, Chicago, Ill, USA). One-way analysis of variance followed by Bonferroni post hoc analysis was used to evaluate differences in visual field parameters, age and best-corrected visual acuity among HCs, IDE and RR-MS patients. Chi-squared test was used to determine sex differences among groups. Linear mixed models, including subject, age and sex as covariates, was used to evaluate VD differences in each retinal vascular network (superficial capillary plexus, deep capillary plexus, radial peripapillary capillary plexus) and in choriocapillaris, using group as factor of interests. The same model was used to analyze differences in structural OCT parameters (ganglion cell complex average and retinal nerve fiber layer average) among the groups. Correlations between SD-OCT and OCTA parameters were assessed using linear mixed model for both IDE and RR-MS. Moreover we analyzed the correlations between best-corrected visual acuity, mean deviation and pattern standard deviation, neurological (EDSS, annualized relapse rate, and disease duration) and OCT-A parameters. Since we evaluated VD in four different regions as dependent variables for the linear mixed models, to correct analysis for multiple regressions, we set the p-value for significance at p=0.05/4 (0.012).

5.3 Results

5.3.1 Demographic and clinical features

Thirty patients (20 with IDE and 10 with RR-MS) for a total of 60 eyes and 15 HCs for a total of 30 eyes, were enrolled. There were no significant differences for age, sex, best-corrected visual acuity, and visual field parameters in the three groups. After MRI evaluation, 16 out of 20 IDE (80%) patients met criteria for CIS whereas 4 IDE patients met MRI criteria for RR-MS. Demographic, clinical and OCT features are summarized in Table 1.

Table 1. Demographic and clinical characteristics of IDE, RRMS patients and healthy controls.

	Control	**IDE**	**RR-MS**
Eyes (N.)	30	40	20
Age, mean ± SD (years)	28.2 ± 8.6	28.9 ± 9.5	29.7 ± 6.3
Sex (female/male)	10/5	10/10	7/3
EDSS, mean ± SD (Range)	-	1.85 ± 0.95 (0-2.5)	2.3 ± 0.57 (1.5-3.5)
Annualised Relapse Rate, mean ± SD	-	-	0.99 ± 1.05
Disease duration, mean ± SD (years)	-	1.7 ± 2.3	4 ± 1.3
Onset modality			
Brainstem, N. (%)	-	6 (30%)	4 (40%)
Pyramidal, N. (%)	-	5 (25%)	2 (20%)
Cerebellar, N. (%)	-	1 (5%)	1 (10%)
Sensory, N. (%)	-	7 (35%)	3 (30%)
Bowel/Bladder, N. (%)	-	0 (0%)	0 (0%)
Cerebral, N. (%)	-	1 (5%)	0 (0%)
OCT-A parameters (%)			
Superficial Capillary Plexus, mean ± SD	53.63 ± 2.53	50.43 ± 4.47	48.75 ± 4.41
Deep Capillary Plexus, mean ± SD	55.97 ± 4.86	55.15 ± 6.53	53.25 ± 7.06
Choriocapillaris, mean ± SD	74.10 ± 2.66	74.08 ± 2.34	74.15 ± 2.54
Radial Peripapillary Plexus, mean ± SD	53.23 ± 3.35	49.62 ± 2.90	45.9 ± 3.93
OCT parameters (µm)			
Ganglion cell complex average, mean ± SD	100.2 ± 6.79	98.61 ± 9.89	89.54 ± 9.85
Retinal nerve fiber layer average, mean ± SD	103.1 ± 8.19	101.83 ± 10.88	95.15 ± 13.18
Visual Field parameters (dB)			
Mean Deviation, mean ± SD	-0.51 ± 1.18	-0.59 ± 1.61	-1.19 ± 2.09
Pattern Standard Deviation, mean ± SD	2.1 ± 0.46	2.42 ± 1.12	2.25 ± 0.83
Best-corrected visual acuity, mean ± SD (logMAR)	0.03 ± 0.04	0.02 ± 0.04	0.01 ± 0.03

IDE: Initial Demyelinating Event, RRMS: Relapsing–Remitting Multiple Sclerosis; EDSS: Expanded Disability Status Scale; OCT-A: Optical Coherence Tomography Angiography; OCT: Optical Coherence Tomography; dB: decibel; logMAR: logarithm of the minimum angle of resolution, SD: Standard Deviation. Data expressed as mean ± standard deviation.

5.3.2 Spectral Domain Optical Coherence Tomography

At SD-OCT exam, RR-MS patients showed lower ganglion cell complex values compared with IDE patients (89.54 ± 9.85 vs 98.61 ± 9.89; p=0.017) and HCs (89.54 ± 9.85 vs 100.2 ± 6.79; p=0.006). Ganglion cell complex thickness was not different between IDE group and HCs. Retinal nerve fiber layer did not differ between HCs, IDE and RR-MS patients.

5.3.3 Spectral Domain Optical Coherence Tomography Angiography

The VD in radial peripapillary capillary plexus was significantly lower in IDE group compared with HCs (coeff. β = -3.578; p= 0.002). VD for both superficial capillary plexus and radial peripapillary capillary plexus was lower for RR-MS patients compared with HCs (coeff. β = -4.955; p= 0.002, and coeff. β = -7.446; p<0.001, respectively; see Table 2). RR-MS patients showed a lower VD in radial peripapillary capillary plexus compared with IDE patients (coeff. β = -3.868; p= 0.003; see Table 2). The VD in choriocapillaris and deep capillary plexus did not differ between the three groups (see Figure 7). There were no significant correlations between OCT-A measures and visual field parameters (mean deviation and pattern standard deviation) while VD in the deep capillary plexus showed a significant correlation with best-corrected visual acuity (coeff. β = -0.002; p=0.007; Table 3). No correlation was found between

OCT-measures and neurological parameters (EDSS, annualized relapse-rate and disease duration).

Table 2. Differences in OCTA parameters among IDE, RRMS and control groups.

OCTA parameters	**IDE vs Control**		
	β	**(95% CI)**	**P-value**
Superficial Capillary Plexus	-3.180	(-5.696 to -0.664)	0.015
Deep Capillary Plexus	-0.534	(-4.021 to 2.952)	0.758
Choriocapillaris	-0.111	(-1.518 to 1.296)	0.874
Radial Peripapillary Capillary Plexus	-3.578	(-5.724 to -1.431)	0.002
	RRMS vs Control		
	β	**(95% CI)**	**P-value**
Superficial Capillary Plexus	-4.955	(-7.933 to -1.977)	0.002
Deep Capillary Plexus	-2.996	(-7.122 to 1.131)	0.15
Choriocapillaris	0.129	(-1.536 to 1.794)	0.877
Radial Peripapillary Capillary Plexus	-7.446	(-9.906 to -4.986)	<0.001
	RRMS vs IDE		
	β	**(95% CI)**	**P-value**
Superficial Capillary Plexus	-1.775	(-4.361 to 1.080)	0.216
Deep Capillary Plexus	-2.461	(-6.418 to 1.496)	0.216
Choriocapillaris	0.240	(-1.357 to 1.837)	0.763
Radial Peripapillary Capillary Plexus	-3.868	(-6.289 to -1.448)	0.003

IDE: Initial Demyelinating Event, RR-MS: Relapsing–Remitting Multiple Sclerosis; OCT-A: Optical Coherence Tomography Angiography; CI: Confidence Interval.

Table 3. Correlations between vessel density, visual field and visual acuity for MS patients.

Regions	**Mean Deviation**		
	β	**(95% CI)**	**P-value**
Superficial Capillary Plexus	0.079	(-0.023 to 0.182)	0.127
Deep Capillary Plexus	0.035	(-0.016 to 0.087)	0.173
Choriocapillaris	-0.046	(-0.189 to 0.097)	0.517
Radial Peripapillary Capillary Plexus	-0.103	(-0.200 to -0.005)	0.038
	Pattern Standard Deviation		
	β	**(95% CI)**	**P-value**
Superficial Capillary Plexus	-0.018	(-0.098 to 0.062)	0.654
Deep Capillary Plexus	-0.015	(-0.058 to 0.028)	0.480
Choriocapillaris	-0.001	(-0.116 to 0.113)	0.978
Radial Peripapillary Capillary Plexus	0.017	(-0.057 to 0.092)	0.637
	Best-corrected visual acuity		
	β	**(95% CI)**	**P-value**
Superficial Capillary Plexus	0.003	(-0.0001 to 0.006)	0.061
Deep Capillary Plexus	-0.002	(-0.005 to 0.0008)	0.007
Choriocapillaris	-0.0008	(-0.005 to 0.004)	0.733
Radial Peripapillary Capillary Plexus	-0.0002	(-0.003 to 0.002)	0.855

CI=Confidence Interval.

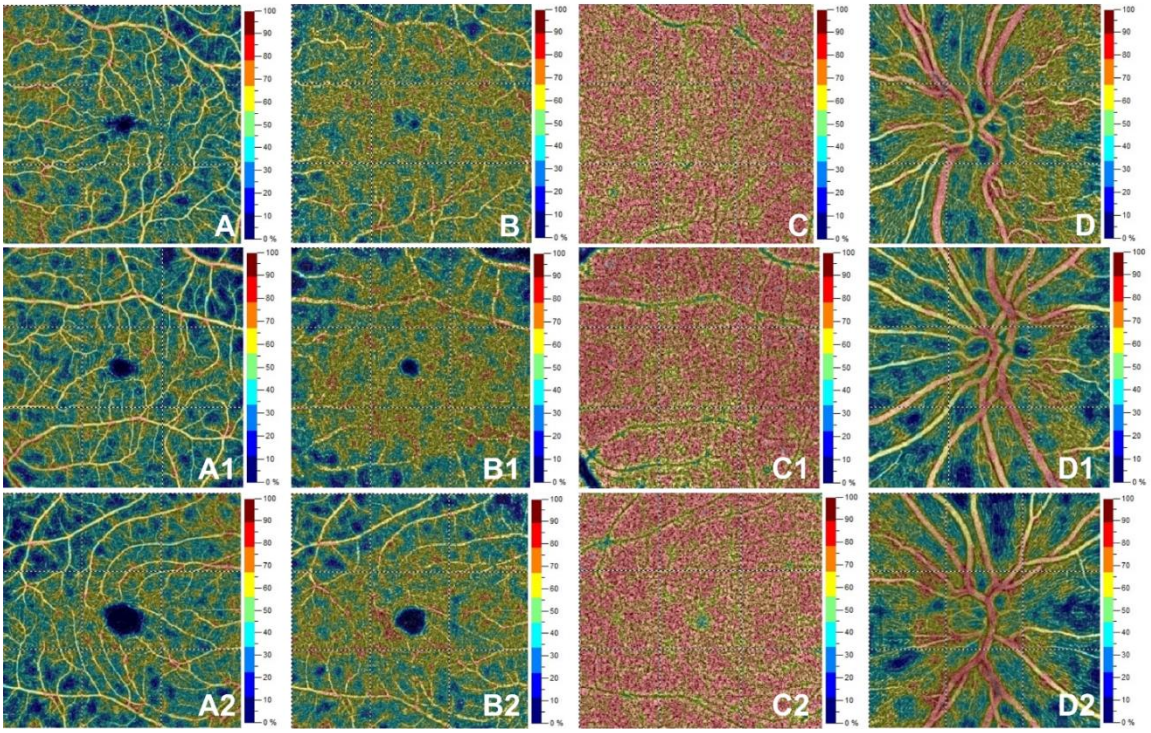

Figure 7. Optical coherence tomography angiography (OCT-A) images from a healthy subject' s left eye (male, 28 years) in the first row show normal vessel density in superficial capillary plexus (A), deep capillary plexus (B), choriocapillaris (C) and radial peripapillary capillary plexus (D). The second row depicts OCTA features in the left eye for a patient (female, 28 years) with initial demyelinating event. OCTA reveals normal vessel density in superficial capillary plexus (A1), deep capillary plexus (B1), choriocapillaris (C1) with a decrease for vessel density in the radial peripapillary capillary plexus (D1). The bottom row shows a patient's right eye (male, 29 years) affected by relapsing-remitting multiple sclerosis. Here, vessel density is reduced in the superficial capillary plexus (A2) and radial peripapillary capillary plexus (D2) without vessel density changes in the deep capillary plexus (B2) and choriocapillaris (C2)

5.3.4 OCT-A correlates to SD-OCT

In patients, ganglion cell complex thickness was associated with VD in superficial capillary plexus and radial peripapillary capillary plexus (coeff. $\beta = 1.474$; $p<0.001$ and coeff. $\beta = 1.101$; $p<0.001$). Similarly, retinal nerve fiber layer thickness was associated with VD in radial peripapillary capillary plexus (coeff. $\beta = 0.817$; $p=0.009$; Table 4).

5.4 Discussion

Notwithstanding the many progresses achieved over the last years in uncovering different mechanisms contributing to tissue damage and clinical disability in MS, a full understanding of the disease pathogenesis is still hampered by the impossibility to study the premorbid stages of the disease. To overcome this obstacle, a valuable opportunity is provided by the exploration of pathology abnormalities occurring in very early disease phases. Specifically, we hereby investigated the role of vascular abnormalities in MS pathogenesis. We evaluated their role as early marker of disease, analyzing retinal and choriocapillaris VD in patients with recent onset of their first demyelinating episode. OCTA is a reliable marker of disease and disability accrual in definite MS [67,73,74] especially in later stages. However, its role in earlier disease stages is less clear. To our knowledge, only two studies exploring VD variations enrolled early-stage MS patients, but in both cases these were considered

in a pooled analysis including also RR-MS patients, making impossible to draw specific conclusions to early stage MS [68,74]. In the present study, the comparison of patients with IDE, RR-MS and HCs in terms of retinal VD suggests an early involvement of the radial peripapillary capillary plexus, regardless of the presence of retinal atrophy or ongoing inflammation. Feucht and colleagues recently reported a reduced VD of the superficial capillary plexus and deep capillary plexus in eyes of MS and CIS patients affected by optic neuritis, with no changes in the healthy eye [68]. In addition, Murphy and colleagues described a reduction of superficial capillary plexus in eyes affected by optic neuritis and, to a lesser extent, in the healthy eye [74]. In our sample, we identified a rarefaction of radial peripapillary capillary plexus both in IDE and RRMS with no history of optic neuritis. Both previous studies [68,74] described an association between inner retinal layer volumes and density of both the superficial and deep vascular plexuses, suggesting a relationship between retinal atrophy and the consequent reduction in vascularization, induced by the reduced metabolic request of the atrophic layers. In our study, similar associations were identified between OCT-A and structural-OCT parameters in the entire patients' group but, as no retinal atrophy was present in IDE patients, VD reduction in radial peripapillary capillary plexus should not be ascribed to macroscopic structural abnormalities of the retina nor to the presence of optic nerve atrophy. Radial peripapillary

capillary plexus rarefaction could be indeed the proxy of a more diffuse vascular involvement in MS pathogenesis or, alternatively, it might be related to subtle microstructural changes of the optic nerve fibres, which might explain the selective VD reduction in radial peripapillary capillary plexus rather than in all the explored vascular districts. Fibres within the retinal nerve fibre layer might suffer indirectly from vascular damage of the optic nerve, in the frame of a more diffuse white matter microstructural damage, that has been described as an early finding in CIS patients [75,76]. As per the insight gained from RR-MS patients, later on in the disease course, radial peripapillary capillary plexus rarefaction increased, with superficial capillary plexus showing VD reduction too, mirroring the development of atrophy in retinal nerve fibre layer and ganglion cell complex. Finally, similarly to what reported by Feucht et al. for CIS/MS patients [68], abnormalities in choriocapillaris VD were not detected in our IDE/RRMS patients. Unfortunately, no formal analysis of the association between choriocapillaris VD and previous relapse rate could be performed, as the majority of our sample was constituted by IDE subjects for whom, by definition, no past disease activity is present in terms of more than one relapse. Furthermore, the lack of correlation between OCTA and clinical parameters, which might seem counterintuitive, considering previous reports in MS [67,73,74], might be similarly accounted for by the mild clinical status of IDE patients.

Eventually, due to the cross-sectional nature of the study, we can not completely rule out that changes in VD without structural-OCT abnormalities might depend on the lower inter-subject variability of OCTA measures compared with structural OCT measures. When analyzed over the follow-up, SD-OCT shows high level of sensibility for detecting retinal structural changes [77,78]. Longitudinal studies are highly needed to evaluate the sensitivity for OCTA in detecting progressive VD loss over the disease course regardless of the inter-subject variability and to assess the contribute of this technique to the already validated standard-OCT.

In conclusion, our data suggest that retinal vascular abnormalities are possibly driven by primary vessel involvement, or secondary to structural damage ongoing in the retina and optic nerve during the disease course. The role played by each mechanism seems to differ according to the disease stage, with VD being the proxy of primary vessel involvement or subclinical white matter macrostructural abnormalities in an early stage, and retinal atrophy in a later stage. Regardless of the causative mechanism, our results confirm the relevant role of retinal VD as a non-invasive, early biomarker of disease, independently from the presence of inflammation, although we recognize that the applications of radial peripapillary capillary plexus VD measurements as diagnostic marker in clinical settings will require further studies to explore the specificity of such vessel density rarefaction.

6. Retinal and choriocapillary vascular changes in initial demyelinating event: a prospective longitudinal study

6.1 Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating disorder of the central nervous system with progressive neuroaxonal degeneration [79,80].

Previous studies demonstrated the cerebral hypoperfusion as possible contribute to the appearance and progression of this chronic disease [9,81].

However, it is still a source of debate if the cerebral perfusion loss can precede the brain tissue impairment or it is a consequence to a reduced metabolic demand [82].

Increasing evidences showed the common morphological and physiological characteristics of the retinal and cerebral vascularization, assuming that retinal vessels could be a potential marker of the cerebrovascular state [2,83,84].

Therefore studying the retinal perfusion may provide an insight into the role of vascular dysfunction in the pathogenesis of MS

OCTA, a non-invasive imaging technique, is a useful tool to detect and to quantify the retinal and choriocapillary blood flow in macular and papillary regions in neurodegenerative diseases [10,48,85,86].

Few reports, using OCTA, evaluated the retinal and choriocapillaris vascular networks in early stages of MS but no study focused on their changes in longitudinal analysis [68,87,88].

The purpose of this prospective longitudinal study was to investigate the changes of retinal and choriocapillary vessel density (VD) in macular and papillary regions in patients with an initial demyelinating event (IDE) by means OCTA in order to better define the vascular involvement in MS pathogenesis and to individuate a vascular biomarker in progression of this disease.

6.2 Methods

This is a 2-years prospective longitudinal study. We enrolled IDE patients at the MS Centre of the University of Naples "Federico II", from January 2018 to December 2018. IDE was considered as the first neurologic symptom referable to demyelination in the central nervous system, lasting for at least 48 hours, regardless patients met RR-MS or CIS diagnosis following 2017 McDonald criteria [20].

Each patient underwent a complete neurological and ophthalmological examination at baseline and after 1 and 2 years from IDE diagnosis, including the assessment of physical disability through the expanded disability status scale (EDSS).

All patients underwent evaluation of best-corrected visual acuity, slit-lamp biomicroscopy, fundus examination and SD-OCT and OCTA. Ophthalmological evaluation was blinded to subjects' clinical status.

We excluded patients with history of optic neuritis in order to avoid a bias related to optic nerve direct damage. We also excluded patients with a relapse and/or corticosteroid use in the previous month.

Patients did not present systemic vascular diseases (high blood pressure, diabetes and heart diseases), clinically relevant lens opacities, low-quality images obtained with Spectral Domain (SD)-OCT and OCTA, myopia greater than 6 diopters, history of intraocular surgery, vitreo-retinal and retinal vascular diseases, uveitis, congenital eye disorders.

The study was approved by the Institutional Review Board of the University of Naples "Federico II" and all investigations adhered to the tenets of the Declaration of Helsinki (protocol number: 142/19). Written informed consents were obtained from the subjects enrolled in the study.

6.2.1 Spectral Domain Optical Coherence Tomography

The retinal nerve fiber layer and ganglion cell complex thickness were obtained with SD-OCT (software RTVue XR version 2018.1.1.60, Optovue Inc., Fremont, CA, USA). The circumpapillary retinal nerve fiber layer thickness was analyzed by the optic nerve head map protocol using a 3.45 mm radius ring centered on the optic disc. The ganglion cell

complex thickness was analyzed from the internal limiting membrane to the outer boundary of the inner plexiform layer centered the scan 1-mm temporal to the fovea and covering a 7 x 7 mm area over the macular region [70].

Each OCT scan was evaluated according to APOSTEL recommendations and the OSCAR-IB protocol for quality control [71,72]. These guidelines were adapted for our device.

6.2.2 Optical Coherence Tomography Angiography

OCTA images were performed by the Optovue Angiovue System (software ReVue XR version 2018.1.1.60, Optovue Inc., Fremont, CA, USA) that is based on a split-spectrum amplitude de-correlation algorithm (SSADA) and which uses blood flow as intrinsic contrast [52].

The macular capillary network was evaluated in a 6x6 mm scan centered on the fovea and the AngioAnalytics™ software automatically calculated the VD that represents the percentage area occupied by the vessels in the analyzed region [58].

The OCTA software analyzed the whole area of the macular region in each vascular network of the retina (namely the superficial and deep capillary plexuses) and the choriocapillaris.

The Angio-Vue disc mode automatically calculated the VD of radial peripapillary capillary plexus analyzing the whole papillary region with a

scanning area of 4.5 x 4.5 mm centered on the optic disc (whole image). VD for the radial peripapillary capillary plexus was analyzed in the superficial retinal layers and extended from the inner layer membrane to the retinal nerve fiber layer posterior boundary [60].

Images with a signal strength index less than 80 and residual motion artifacts were excluded from the analysis.

6.2.3 Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (Version 25 for Windows; SPSS Inc, Chicago, Ill, USA). We explored VD changes over time in each retinal vascular network (superficial capillary plexus, deep capillary plexus, radial peripapillary capillary plexus) and in choriocapillaris, as well as structural OCT parameters (ganglion cell complex average and retinal nerve fiber layer average) changes through general linear models (GLM), including age, sex, EDSS at baseline and relapse over the follow-up as covariates and time points as factor of interest to evaluate. Subject ID was included in all models as random factor to account for within-subject inter-eye correlation. A p value of < 0.05 was considered statistically significant.

6.3 Results

6.3.1 Demographic and clinical features

During the enrollment period, 40 eyes of 20 IDE patients were assessed. Ten eyes were excluded because five patients were lost to follow up. A total of fifteen IDE patients for a total of 30 eyes (7 females, 8 males; mean age 28.4 ± 9.6 years) were enrolled.

BCVA did not differ from baseline and each time point (p=0.10). Demographic, clinical features at baseline are summarized in Table 4. Only one patient experienced a relapse 10 months after the baseline OCT examination.

Table 4. Demographic and clinical characteristics of IDE patients.

	IDE patients
Eyes (n.)	30
Age, mean ± SD (years)	28.4 ± 9.6
Sex (female/male)	7/8
EDSS, mean ± SD	1.81 ± 0.56
Onset modality	
Brainstem, N. (%)	4 (27%)
Pyramidal, N. (%)	4 (27%)
Cerebellar, N. (%)	1 (7%)
Sensory, N. (%)	5 (32%)
Bowel/Bladder, N. (%)	0 (0%)
Cerebral, N. (%)	1 (7%)
BCVA (logMAR)	0.02 ± 0.04

IDE: Initial Demyelinating Event; EDSS: Expanded Disability Status Scale; BCVA: Best Corrected Visual Acuity; logMAR: logarithm of the minimum angle of resolution.

6.3.2 Spectral –Domain Optical Coherence Tomography and Optical Coherence Tomography Angiography

At SD-OCT exam, ganglion cell complex and retinal nerve fiber layer thicknesses did not change over time (Figure 8, Table 5).

Retinal VD of the macular and papillary regions did not change after one year. We did report a decrease for VD in superficial, deep capillary plexuses and radial peripapillary capillary plexus after 2 years compared with baseline (coeff. β = -2.787; p= 0.013, coeff. β = -4.049; p=0.013 and coeff. β = -2.693; p<0.001, respectively). Specifically, VD of the superficial, deep capillary plexuses and radial peripapillary capillary plexus reduced between 1 and 2 years of follow-up (coeff. B = -2.651; p= 0.018, coeff. β = -3.953; p=0.015 and coeff. β = -1.810; p= 0.005, respectively) (Figure 9, Table 5). Conversely, VD of the choriocapillaris did not show statistically significant differences from baseline and each time point.

Table 5. Clinical characteristics of IDE patients at baseline and each time point.

	Baseline	1 year	2 years	Baseline vs 1 year		Baseline vs 2 years		1 year vs 2 years	
				β	p-value	β	p-value	β	p-value
OCT-A parameters (%)									
SCP Whole	50.23 ± 4.56	50.09 ± 3.82	47.53 ± 4.71	-0.137	0.894	-2.787	0.013*	-2.651	0.018*
DCP Whole	55.12 ± 6.07	55.02 ± 6.23	50.99 ± 6.97	-0.097	0.948	-4.049	0.013*	-3.953	0.015*
CC Whole	74.01 ± 2.20	73.37 ± 3.40	72.85 ± 4.39	-0.647	0.380	-1.199	0.135	-0.553	0.491
RPC Whole	49.62 ± 2.85	48.74 ± 2.24	46.90 ± 2.45	-0.883	0.131	-2.693	<0.001*	-1.810	0.005*
OCT parameters (µm)									
GCC average	98.23 ± 7.09	96.66 ± 10.17	94.68 ± 11.71	-1.570	0.470	-4.083	0.084	-2.516	0.287
RNFL average	101 ± 10.44	99.53 ± 10.58	98.22 ± 8.52	-1.470	0.499	-3.276	0.166	-1.809	0.444

Data expressed as mean ± standard deviation.
IDE: Initial Demyelinating Event; OCT-A: Optical Coherence Tomography Angiography; SCP: Superficial Capillary Plexus; DCP: Deep Capillary Plexus; CC: Choriocapillaris; RPC: Radial Peripapillary Capillary; OCT: Optical Coherence Tomography; GCC: Ganglion Cell Complex; RNFL: Retinal Nerve Fiber Layer.
*p<0.05

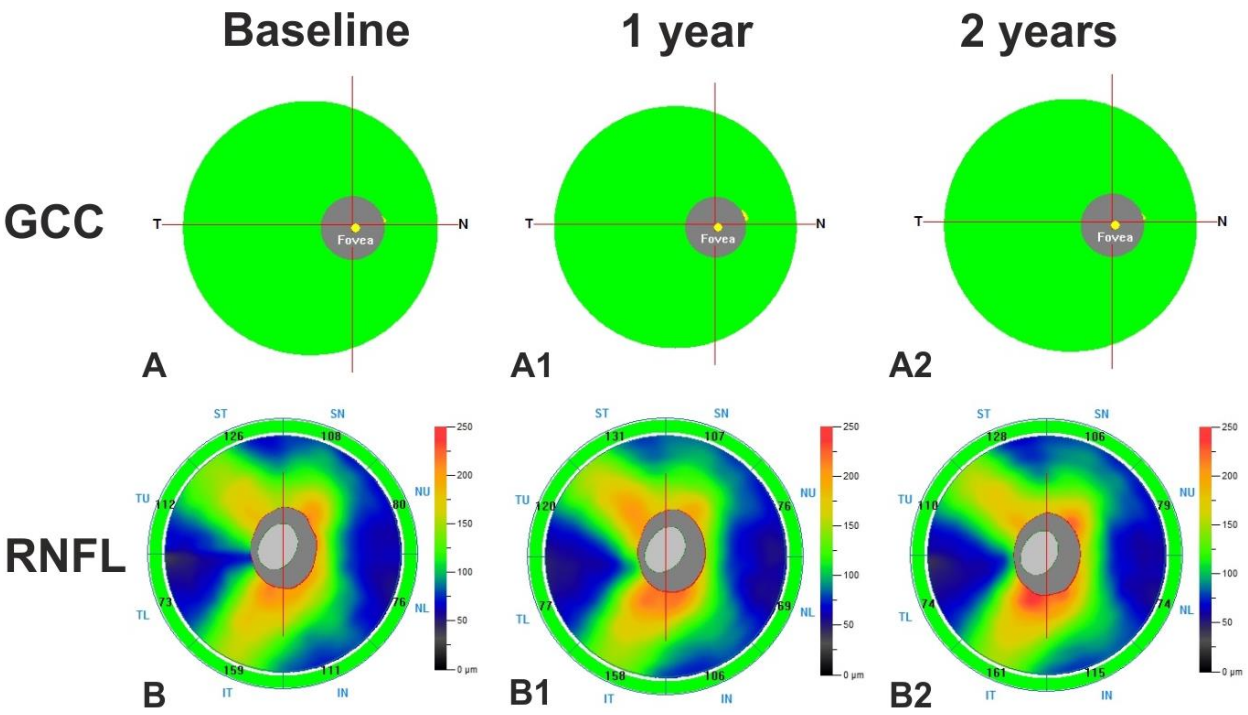

Figure 8. The measurements of the structural spectral-domain optical coherence tomography parameters in right eye of a IDE patient (27 years-old male) reveals no significant changes in ganglion cell complex and in retinal nerve fiber layer at 1 year (A1, B1) and 2 years (A2, B2) of follow up respect to baseline (A, B).

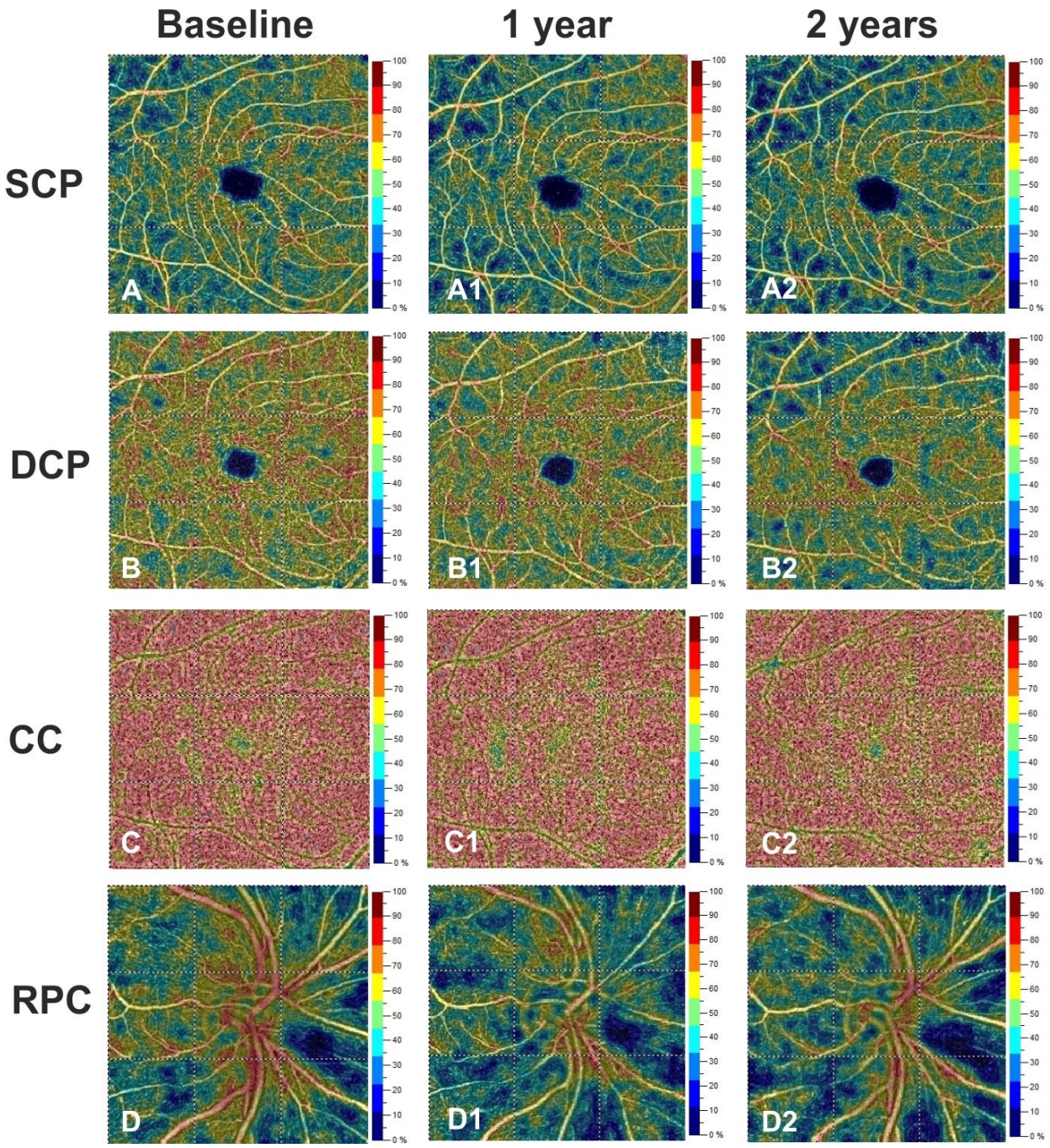

Figure 9. Right eye of a IDE patient (27 years-old male) shows at 1 year of follow up no significant reduction in vessel density of superficial capillary plexus (SCP) (A1), deep capillary plexus (DCP) (B1) and radial peripapillary capillary plexus (RPC) (D1) respect to baseline (A, B, C, D). No difference is shown in vessel density of choriocapillaris (CC) during follow up.

Several focal significant reduction in vessel density in SCP (A2), DCP (B2), RPC (D2) are found at 2 years respect to the first year and baseline except for CC that does not reveal any change.

6.4 Discussion

This longitudinal study in eyes of patients with IDE showed that, while no changes could be detected on structural OCT measures over the first two years after disease onset, VD reduced in almost all explored retinal areas between 1 and 2 years after disease onset. To our acknowledge, this is the first longitudinal study to investigate the retinal and choriocapillaris VD by means OCTA in IDE patients. The absence of structural OCT changes was not surprising since it confirms a previous study revealing the absence of abnormalities in retinal neuronal component in IDE subjects respect to controls [87], thus demonstrating no retinal structural involvement in early stages of MS.

Conversely, several reports, analyzing all MS phenotypes (clinically isolated syndrome, relapsing and progressive MS), reported a significant annualized rate of change for ganglion cell layer and retinal nerve fiber layer over time suggesting a possible retrograde trans-synaptic degeneration as mechanism of retinal neuronal damage [89-93]. We may speculate that this process starts later in the disease course, paralleling the accumulation of demyelination throughout the central nervous system, particularly over visual pathways.

The introduction of OCTA, a non-invasive diagnostic tool that provides a detailed analysis of the retinal and choriocapillary perfusion damages in MS, allows evaluating the retinal vascular involvement in IDE

subjects, possibly exploring another pathogenetic aspect of MS, namely perfusion changes.

In this study the absence of any changes in structural SD-OCT respect to significant abnormalities of OCTA parameters after 2 years of follow up might confirm the crucial role of retinal vascular impairment, preceding the neuronal damage in early stages of MS.

It is possible to speculate that the retinal perfusion damage due to an inflammatory, demyelinating event, could affect the axoplasmic flow and determine a subsequent retinal neurodegeneration over time [94].

Moreover this longitudinal study revealed that in the first year of follow up there were no changes in OCTA parameters respect to baseline while at 2 years the retinal VD was significantly decreased respect to the first year in both macular and papillary regions. This was true even in the absence of new relapses or episodes of optic neuritis during follow up. This suggests that the single demyelinating phenomenon, occuring in IDE patients, could have caused a subclinical and progressive inflammatory process that persisted over time.

This would demonstrate an initial but not yet significant retinal vascular impairment in the first year, while at the second year follow up the persistent inflammation may have led to further vascular perfusion damages.

Conversely, a previous study have analyzed in MS subjects the retinal vascular changes by OCTA after 1 year follow up showing a significant increase in superficial capillary plexus in parafoveal region and this variation over time correlated with lower disability on EDSS. This improved vascularization could be due to therapeutic efficacy since these patients were all steadily on DMTs [73].

These different results could be due to the fact that the subjects enrolled were MS patients with severe disease disability and they were evaluated only after one year. Also, some subjects presented previous episodes of optic neuritis.

Therefore, in this study the analysis of the retinal microvasculature by means OCTA demonstrated the crucial involvement of the vascular dysfunction in pathogenesis and progression of MS.

Indeed, extensive evidences showed the involvement of the breakdown of the blood-brain barrier, the inflammatory infiltrates and the presence of immune soluble mediators in inflammatory processes determining acute demyelinating lesions [22,79,80].

Studies on animal models of MS have provided interesting insights into the role of inflammation demonstrating a subtle and continuous leucocyte infiltration in chronic MS lesions even in the absence of a breakdown in the blood-brain barrier [22,95].

Moreover, the cerebral hypoperfusion represents a factor of great interest that turns to contribute to disease risk and progression [9,81].

Our results did not show any significant changes in choriocapillaris in IDE patients over time excluding a possible involvement of this vascular network in MS pathogenesis.

In conclusion, the results of this study demonstrated a retinal vascular rarefaction in early stages of MS, that could possibly reflect a neurovascular degenerative progression even in absence of relapse activity during the follow up. OCTA could be considered as an early vascular biomarker of disease progression before the appearance of subsequent neuroaxonal loss.

Further longitudinal studies on larger cohorts and longer follow up periods, involving the analysis of choroidal vasculature and brain magnetic resonance imaging, would be necessary to validate these results.

6.4 Conclusions and future perspectives

This study investigated in IDE patients the retinal and choriocapillaris microvasculature, using OCTA, monitoring its changes over two years follow up

In the first part of the study, a significant loss of vessel density in radial peripapillary capillary plexus was found in IDE patients respect to RRMS patients and healthy subjects.

The impairment of this vascular network might be considered as an early event in MS and might be relevant as a vascular biomarker of this disease.

These results would confirm the crucial role of OCTA to shed light on the vascular involvement in the pathogenesis of MS.

Afterwards, IDE patients underwent OCTA examination in a 2-years prospective longitudinal study.

The vessel density of the retinal vascular plexuses in macular and papillary regions showed a significant decrease at the second year respect to the first year and baseline in absence of retinal axonal loss, as shown at structural SD-OCT.

The retinal vascular rarefaction in IDE during the follow up could reflect an neurovascular degenerative progression even in absence of relapse activity.

OCTA parameters could represent an helpful biomarkers of the retinal perfusion damage before the appearance of neuroaxonal loss confirming the vascular dysfunction in pathophysiological mechanisms and in progression of MS.

In the future, further analysis with larger cohorts and longer follow up with brain magnetic resonance imaging are needed to evaluate the changes in retinal blood flow and the relationship between the retinal microvascular impairment and the neuronal damage. Moreover, the evaluation of blood flow alterations by means OCTA could be useful to monitor not only the evolution of disease but also the effectiveness of therapy in MS patients.

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