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Vessel density analysis in patients with retinitis pigmentosa by means of optical coherence tomography angiography

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ABSTRACT

Aims To describe the vascular abnormalities in patients affected by retinitis pigmentosa (RP) by means of optical coherence tomography angiography (OCT-A).

Methods Cross-sectional case series; patients with RP presenting at the Medical Retina Service of the Department of Ophthalmology, University Vita-Salute San Raffaele in Milan were recruited. Inclusion criteria were: diagnosis of RP, clear ocular media, adequate pupillary dilation, and stable fixation. Patients underwent best-corrected visual acuity (BCVA), biomicroscopy, short-wavelength fundus autofluorescence (SW-FAF), and 3×3 Swept Source OCT-A. 30 healthy subjects were chosen as controls. The main outcome was identification of abnormalities in density of the superficial capillary plexus (SCP) and deep capillary plexus (DCP), along with abnormalities of the choriocapillaris (CC).

Results 16 patients (32 eyes) were recruited (6 females, 37.4%). Mean age was 53±18 years; mean BCVA was 0.5±0.3 LogMAR. Vessel density analysis disclosed a statistical significant difference in the SCP (29.5±6.8 vs 34.1±4.3; p=0.009) and in the DCP (28.7 ±7.5 vs 35.5±5.7; p=0.001) between the patients and the controls. No difference was found at the level of the CC (51±4.4 vs 51.3±2.2; p=0.716). RP patients showed a bigger foveal avascular zone at the DCP level compared to controls (p<0.001).

Conclusions This study showed that most of the vascular impairment in patients affected by RP localised in the DCP, with relative sparing of the SCP and CC. DCP alterations were more pronounced outside the hyper-autofluorescent ring on SW-FAF. Vascular impairment may preclude good treatment outcomes in RP patients.

INTRODUCTION

Under the same definition of retinitis pigmentosa (RP), a heterogeneous group of inherited dystrophies are characterised by progressive primary degeneration of the photoreceptors of rods and secondary but critical degeneration of cones. The visual impairment typically involves night vision and mid peripheral vision, with gradual central visual acuity deterioration.[1–3] The pathogenesis of RP is complex: loss of rods and cones is accompanied by changes in the retinal pigment epithelium (RPE) and retinal glia; ultimately, the inner retinal neurons, blood vessels, and the optic nerve head are affected by the disease.[1–3]

Arterial narrowing, vessel attenuation, and alterations in vascular flow have been previously described in patients suffering from RP.[4] The availability of optical coherence tomography angiography (OCT-A) allows the detection of vascular abnormalities in the retina and in the choriocapillaris (CC). Two major motion contrast techniques, phase-based and amplitude-based, are used to render depth imaging of retinal and choroidal microvasculature combined with an 'en face' OCT-derived technique.[5–8] This study aims to describe the macular vascular abnormalities in patients affected by RP using OCT-A.

PATIENTS AND METHODS

This was an observational cross-sectional study. A consecutive series of patients affected by RP, referred to the Department of Ophthalmology of San Raffaele Hospital in Milan, were enrolled in the study between July 2015 and March 2016. Written informed consent was obtained from all the subjects. The protocol was approved by the institutional review board of San Raffaele Hospital and the procedures followed the tenets of the Declaration of Helsinki. Inclusion criteria were the diagnosis of RP, along with clear media to allow adequate OCT-A examination. Patients affected by any other ocular disorder were excluded from the study; patients with an advanced form of RP (including extended macular atrophy) were also excluded. Thirty healthy, age-matched patients (30 eyes included in the analysis, one eye for each patient) without any ocular or systemic disease acted as a control group.

Each patient underwent a complete ophthalmic examination, including best-corrected visual acuity (BCVA), biomicroscopy, applanation tonometry, short-wavelength fundus autofluorescence (SW-FAF) (Spectralis, HRA Heidelberg, Heidelberg, Germany), spectral domain OCT (SD-OCT), and OCT-A. In particular, OCT-A was performed using Swept Source DRI OCT Triton (Topcon Corporation, Japan). Images were analysed with the Topcon full spectrum amplitude decorrelation angiography algorithm. This instrument has an A-scan rate of 100 000 scans/s, wavelength-scanning light centred on 1050 nm and in-depth resolution of 2.6 μm (digital). Each OCT-A contains 256 B-scans (each B-scan contains 256 A-scans). To image the motion of scattering particles (erythrocytes), four OCT raster scans are repeated at the same location (assisted by the eye-tracking). Automated segmentation of full-thickness retinal scans into the superficial (SCP) and deep (DCP) inner retinal vascular (capillary) plexus, outer avascular retina, and CC was performed.

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All 3×3 OCT-A images were exported from the system as a Joint Photographic Experts Group file into the National Institutes of Health ImageJ 1.50 (National Institutes of Health, Bethesda, Maryland, USA) software. Capillary vessel density was calculated through a new macro. The image was converted from 8-bit into red green blue (RGB) colour type and then was split into the three channels (red, green, and blue); the red channel was chosen as the reference. The adjust threshold tool set to default was applied; the dark-background option was selected. This tool automatically set lower and upper threshold values (110–255 in our case, respectively), and segments greyscale images into features of interest and background. Processed images were converted to RGB. The foveal avascular zone (FAZ) area was manually outlined through the free-hand selection tool, and its dimension was expressed as squared millimetres, using a previously published method.[9–10] The FAZ area was coloured to pure blue. White pixels were considered as vessel, black pixels as background, and blue pixels were automatically excluded from the analysis. Vessel density was expressed as the ratio between vessel pixels and the total area. SCP, DCP and CC of the patients and the healthy controls were analysed using this method (see online supplementary figure S1). Sets of obtained values were compared to controls for each different layer of segmentation (SCP, DCP and CC by means of Student's t-test) with GraphPad Prism software V.5.0 (GraphPad software, Inc, San Diego, California, USA). Statistical analysis included descriptive statistics for demographics and main clinical records, comparative analysis (Student's t-test analysis for independent samples), as well as qualitative descriptions of the imaging findings. Tukey correction has been used for post-hoc analysis to find means that are significantly different from each other. The chosen level of statistical significance was $p<0.05$.

RESULTS

Overall 16 patients (32 eyes) were recruited for the study. Demographic characteristics of patients and controls are listed in table 1.

OCT-A evaluation was performed both at the central macular area and at the level of the hyper-autofluorescent ring identified on SW-FAF. Qualitative analysis of OCT-A at the central macular area revealed an abnormal SCP and DCP with temporal parafoveal reduction of vessel density (figures 1 and 2). The examination of the DCP disclosed the most profound reduction in vessel density within the whole macular area, with the temporal area more affected. One patient disclosed bilateral macular oedema on SD-OCT and showed focal interruptions in the vascular network at the level of the DCP on the OCT-A, referring to the serous intraretinal cyst displacing the neural tissue and the vascular plexus (figure 3).

Table 1 Demographic characteristics of patients with retinitis pigmentosa and controls

	N (%)	Eyes	Age (y)	BCVA (logMAR)	CMO (%)
Patients	16	32	53±18	0.5±0.3 (20/63)	1 (6.25)
Males	10 (62.6)				
Females	6 (37.4)				
Controls	30	30	53±17	0.0±0.0 (20/20)	
Males	14 (46.7)				
Females	8 (53.3)				

BCVA, best-corrected visual acuity; CMO, cystoid macular oedema; logMAR, logarithm of the minimum angle of resolution (approximation of Snellen Equivalent in brackets); N, number; y, years.

Quantitative analysis of OCT-A centred on the macular area revealed a statistical significant difference in the mean density of the SCP ($p=0.009$) and the DCP ($p=0.001$), while no statistically significant difference was found at the level of the CC ($p=0.716$), comparing the patients and the control group (table 2). No correlation was found between vessel density and age of either patients or controls ($p>0.05$).

The FAZ area was measured at both the SCP and DCP level in patients and controls; the mean FAZ was significantly larger in RP patients considering the DCP ($p<0.001$), while it was not different at the level of the SCP ($p=0.350$) (table 2; figures 1 and 2).

DISCUSSION

Attenuation of the retinal blood vessels, along with perivascular pigment deposits and retinal atrophy, are funduscopic hallmarks of RP. Histopathologic studies have shown that vessel narrowing and sclerosis are associated with progressive thickening of blood vessel walls and occlusion of their lumina in more advanced forms of the disease. In detail, when cells of the RPE detach from Bruch's membrane and migrate around inner retinal blood vessels, they stimulate the deposition of the dense layer of extracellular matrix (ECM) resembling ectopic Bruch's membrane, just below the thin endothelial wall of the venules and capillaries.[4] This perivascular ECM progressively thickens and completely occludes the vessel lumina, seriously compromising retinal blood flow.[11]

Because of the close interdependence of the retinal vasculature, the RPE and photoreceptor cells, it is still not clear whether the capillary bed is primarily affected by the disease or its thinning is secondary to photoreceptor and neighbouring RPE cell degeneration, a phenomenon that has been previously demonstrated in healthy animal eyes.[12] Moreover, it is unknown whether the retinal capillary plexuses can be re-established after longstanding relocation of the RPE, as vascular bed restoration would be essential for transplanted photoreceptors and RPE cell survival and function.

Functional dysregulation of retinal and choroidal haemodynamics seems to occur in advanced RP patients. In particular, experimental and clinical data, using laser Doppler flowmetry,[13–16] MRI,[17 18] and/or ocular pulse amplitude,[19] have demonstrated a reduction in the choroidal and retinal blood flow velocity and vascular diameter correlating with retinal vessel attenuation and tortuosity,[20] in agreement with the histopathologic changes. The drop in blood flow has been detected not only in retinal and choroidal vessels but also in retroocular circulation.[21]

Our comparative analysis between early-stage RP patients and healthy subjects revealed retinal vasculature (both superficial and deep) signal reduction on OCT-A, with apparently a more profound involvement of the DCP. The FAZ area turned out to be more seriously enlarged also at the level of the DCP. However, since OCT-A relies on change between consecutive b-scans, it will detect flow only above a minimum threshold, the slowest detectable flow, which is determined by the time between the two sequential OCT b-scans. Vessels with a blood flow lower than the threshold would therefore not be visualised using OCT-A. In view of this limitation, we cannot determine whether retinal vessels, especially in the DCP, have completely disappeared or have only narrowed.

We can speculate that vascular signal changes on OCT-A could be the early demonstration of what has been histologically and functionally proven in more advanced forms of RP (as

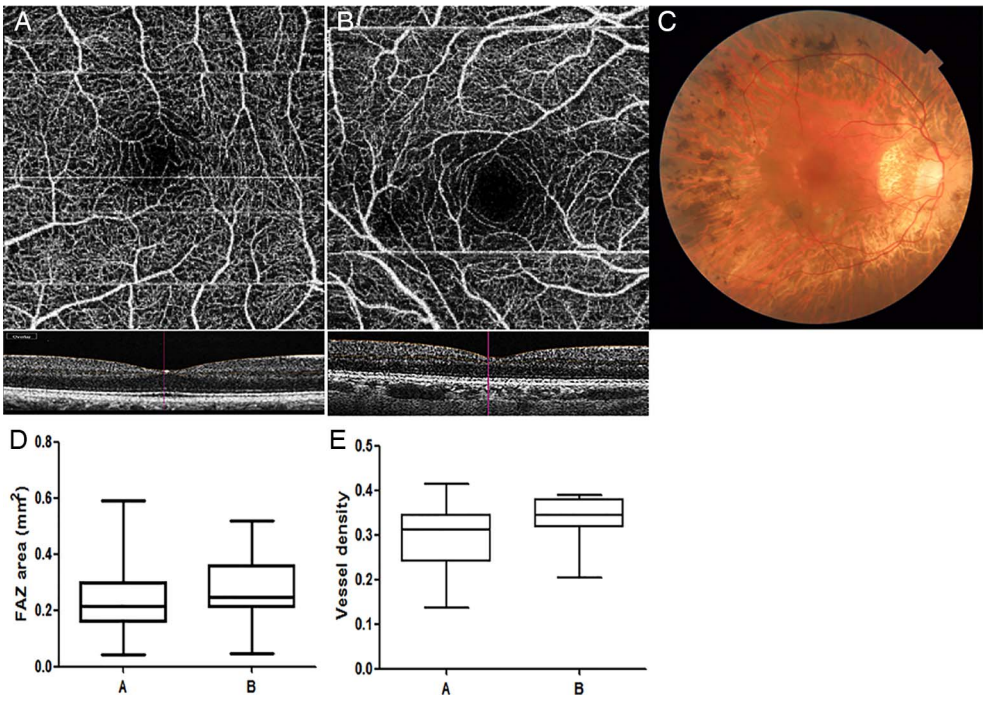

Figure 1 Vascular network differences between patients and controls at the superficial capillary plexus level. (A) 3×3 optical coherence tomography angiography (OCT-A) of the superficial capillary plexus of a healthy 18-year-old man. (B) 3×3 OCT-A of the superficial capillary plexus of a 20-year-old patient affected by retinitis pigmentosa (RP): defects in the perimacular vascular network are recognisable in the RP patient. (C) Image of the fundus of the RP patient showing foveal sparing and peripheral atrophy with pigment mottling. (D) Box plot showing a non-significant difference in the foveal avascular zone (FAZ) area (p=0.350) between patients (A) and controls (B). (E) Box plot showing a significant difference in the superficial plexus vascular density between patients (A) and controls (B) (p=0.009).

mentioned above). If so, the reduced signal on OCT-A corresponds to slower retinal blood flow into partially occluded capillaries. However, the literature lacks histological studies in the early phases of RP, and it is difficult to state when exactly vascular involvement starts in the natural history of the disease.

Grunwald *et al*[14] suggested that reduced retinal blood flow could be a response to decreased metabolic load after ganglion cell death and further loss of oxygen-consuming photoreceptors, leading to capillary vasoconstriction as part of a systemic vascular dysregulation syndrome. Recently, this theory has been confirmed by the finding of a significant increase of endothelin-1, a powerful endogenous vasoconstrictor factor, in the body and locally in the eye of patients affected by RP.[22–23] Functional correlations have been performed by means of a

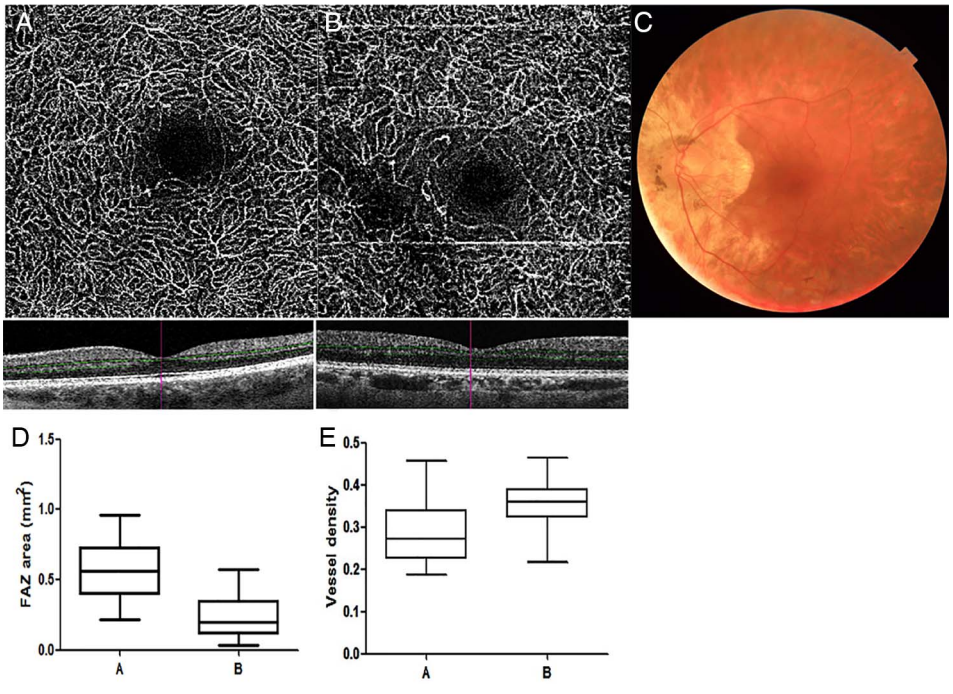

Figure 2 Vascular network differences between patients and controls at the deep capillary level. (A) 3×3 optical coherence tomography angiography (OCT-A) of the deep capillary plexus of a healthy 40-year-old man. (B) 3×3 OCT-A of the deep capillary plexus of a 42-year-old patient affected by retinitis pigmentosa (RP): reduction in the perimacular vascular network density is recognisable. (C) Image of the fundus of the RP patient showing foveal sparing, and peripapillary and peripheral atrophy with pigment mottling. (D) Box plot showing a significant difference in the foveal avascular zone (FAZ) area (p<0.001) between patients (A) and controls (B). (E) Box plot showing a significant difference in the deep plexus vascular density between patients (A) and controls (B) (p=0.001).

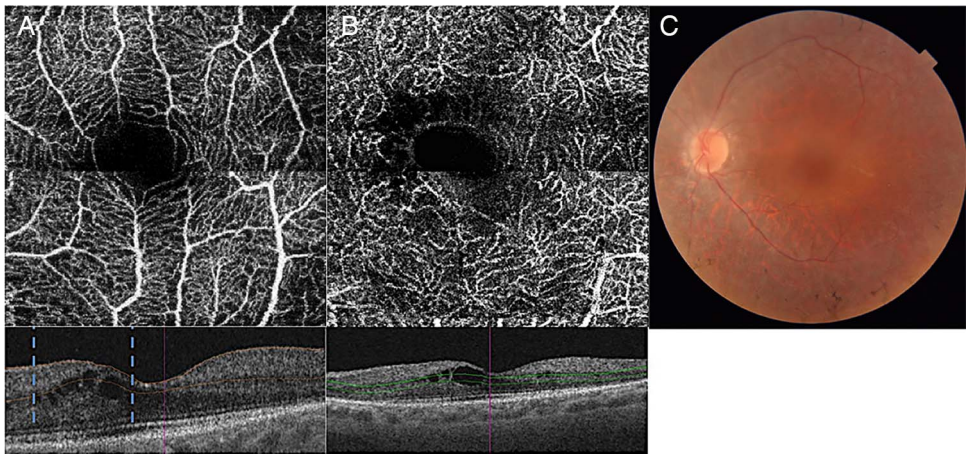

Figure 3 Optical coherence tomography angiography (OCT-A) and corresponding B-scan OCT of a patient affected by retinitis pigmentosa complicated by macular oedema. (A) 3×3 OCT-A segmentation at the superficial capillary level, showing focal dislocation of the vascular network in correspondence of the serous intraretinal cysts (left eye). B-scan shows the level of segmentation; dotted blue lines indicates intraretinal oedema. (B) 3×3 OCT-A segmentation at the deep capillary level, showing more pronounced changes of the vascular network where macular oedema is more evident (left eye). (C) Colour fundus image of the same eye.

computer-based Interactive Vessel Analysis program, disclosing a direct relationship between vessel calibre and visual acuity and visual field.[24]

Our data reveal that vascular impairment of the retinal capillary plexus is qualitatively more pronounced outside the hyper-autofluorescent ring as visualised on SW-FAF. This hyper-autofluorescence represents the demarcation line between a relatively normal and severely affected macula, it being the area within the hyper-autofluorescent ring associated with a still identifiable ellipsoid zone.[25 26] These results reflect the anatomic and functional findings described in the literature, with the outer retinal degeneration typical of RP.

One patient in our series presented with cystoid macular oedema, an RP-related relatively common complication. The presence of macular oedema seems to significantly alter macular cytoarchitecture and then global vessel distribution, as assessed by OCT-A. Moreover, the DCP seems to be more severely distorted in comparison to the superficial plexus and the CC, suggesting that the fluid accumulates preferentially between the inner plexiform layer (IPL) and the outer plexiform layer (OPL).

New encouraging treatments have been proposed for RP, including stem cell transplantation, neurotrophic growth factors, and retinal prosthesis.[27–29] Morphological vascular evaluation in patients affected by RP may become an important step for therapies aimed at promoting the survival of mutant photoreceptors, their replacement with normal photoreceptors, or the electric stimulation of their function. Any degenerative change in the inner retinal neurons and blood vessels is a potential limitation for treatment outcome.

Limitations of this study include the small number of patients and their poor genetic characterisation; different gene mutations may lead to diverse pathologic phenotypes, including vascular pattern. We arbitrarily decided to exclude a more advanced form of RP, characterised by extensive atrophy at the posterior pole, in order to obtain a consistent comparison between patients and controls at all vascular layers (including the CC). Moreover, signal strength index could be significantly reduced in RP patients compared to healthy subjects and captured images could suffer from a number of motion artefacts related to fixation instability. This technical limitation may have negatively influenced the interpretation of the images included in the study. Finally, functional correlation between the perfusion rate estimated on OCT-A and retinal sensitivity (measured either with objective methods such as multifocal electroretinography (mfERG) or subjective ones such as microperimetry) could better clarify whether any functional impairment corresponds to vascular signal changes.

Table 2 Quantitative analysis of the macular vascular density and the FAZ between retinitis pigmentosa patients and controls

	Patients	Controls	p Value
SCP			
Vessel density (%)	29.5±6.8	34.1±4.3	0.009*
FAZ (mm^2)	0.277±0.133	0.243±0.127	0.350
DCP			
Vessel density (%)	28.7±7.5	35.5±5.7	0.001*
FAZ (mm^2)	0.541±0.211	0.243±0.157	<0.001*
CC			
Vessel density (%)	51±4.4	51.3±2.2	0.716

*Statistically significant value.
CC, choriocapillary; DCP, deep capillary plexus; FAZ, foveal avascular zone; SCP, superficial capillary plexus.

Competing interests FB: consultant for AllerganInc (Irvine, California, USA), Novartis (Basel, Switzerland), Farmila-Thea (Clermont-Ferrand, France), Bayer Shering-Pharma (Berlin, Germany), Alcon (Fort Worth, Texas, USA), Bausch And Lomb (Rochester, New York, USA), Genentech (San Francisco, California, USA), AlimeraSciences (Alpharetta, Georgia, USA), Sanofi-Aventis (Paris, France), Thrombogenics (Heverlee, Belgium), Hoffmann-La-Roche (Basel, Switzerland), NovagaliPharma (Évry, France).

Ethics approval Institutional Review Board of San Raffaele Hospital.

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