



# Widefield optical coherence tomography angiography in diabetic retinopathy

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Received: 8 July 2019 / Accepted: 19 August 2019 / Published online: 29 August 2019  
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## Abstract

**Aims** To evaluate superficial capillary plexus (SCP), deep capillary plexus (DCP) and choriocapillaris (CC) perfusion in macular and near/mid periphery regions in diabetic patients using widefield swept-source optical coherence tomography angiography (WSS-OCTA).

**Methods** Ninety-four diabetic patients (94 eyes) classified as diabetics without diabetic retinopathy (no DR) (25 eyes), mild DR (23 eyes), moderate/severe DR (26 eyes), proliferative DR (20 eyes) and a control group of 25 healthy subjects (25 eyes) were imaged with the WSS-OCTA system (PLEX Elite 9000, Carl Zeiss Meditec Inc., Dublin, CA, USA). Quantitative analysis was performed in the macular and peripheral regions. The main outcome measures were perfusion density (PD) and vessel length density of SCP, DCP and CC.

**Results** Peripheral retina (all sectors) showed lower SCP and DCP PD compared to the macular region ( $p < 0.001$ ). In diabetics without DR and DR in different stages, SCP and DCP PD significantly decreased at advancing stages of DR ( $p < 0.001$ ). At DCP level, central PD was significantly directly related to peripheral PD (superior,  $R = 0.682$  and  $0.479$ ; temporal,  $R = 0.918$  and  $0.554$ ; inferior,  $R = 0.711$ ). A good sensitivity and an excellent specificity were found in terms of prediction of disease worsening, especially for central and temporal sectors in all plexuses and for all sectors both central and peripheral of DCP.

**Conclusions** The widefield OCTA is useful for the study of central and peripheral retina in diabetic patients with or without diabetic retinopathy, assessing good correlation between central and peripheral retina.

**Keywords** Widefield optical coherence tomography angiography · Diabetic retinopathy · Retinal periphery · Retinal vessel density · Perfusion density

Managed by Giuseppe Querques.

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## Introduction

Optical coherence tomography angiography (OCTA) is a recent retinal imaging method useful for the evaluation of the retinal and choroidal circulation without dye injection [1, 2].

Due to the limited field of view, conventional OCTA allows depth-resolved qualitative and quantitative assessment of retinal and choroidal vascular network in the macular area, being particularly useful in retinal pathologies of the central retina [1, 2].

Several researches report the use of OCTA in diabetic retinopathy (DR) with detection of vascular alterations in different retinal layers as opposed to fluorescein angiography, unable to provide topographic three-dimensional images of the retina [3, 4].

Conventional OCTA allows visualization of all DR anomalies such as microaneurysms, non-perfusion areas, vascular loops, venous beading, intraretinal microvascular abnormalities and neovascularization.

It has been proven to be useful mainly to detect early vascular changes in diabetic patients without retinopathy, to evaluate DR severity and to predict responsiveness of diabetic macular edema (DME) to treatment [5, 6].

Retinal vascular anomalies such as areas of capillary non-perfusion, enlargement of the foveal avascular zone and impairment of choriocapillaris (CC) can be detected even in diabetic patients without evident signs of retinopathy [7, 8].

Progressive decrease in perfusion density in retinal capillary network both in the superficial and deep plexuses and in the CC has been reported in patients with DR compared with controls, with a significant decrease in capillary perfusion density values as retinopathy progresses [9–11].

Nevertheless, the small scan size with a limited field of view, without retinal periphery, may represent a limit to the use of OCTA for disease screening and monitoring.

Widefield swept-source OCTA (WSS-OCTA) imaging has been recently introduced employing a longer wavelength and a higher speed thus allowing better visualization of the CC and the study of a wider retinal field of view [12–14].

Recent studies showed high sensitivity and specificity of WSS-OCTA for detection of peripheral diabetic retinopathy abnormalities such as non-perfusion areas and retinal neovascularization [13, 15].

The possibility of OCTA evaluation of retinal abnormalities in a wider retinal area might provide more insight into DR correlating central and peripheral vascular modifications in different stages of the disease.

The aim of our study was to evaluate flow features of superficial capillary plexus (SCP), deep capillary plexus (DCP) and CC in macular and near/mid periphery regions in diabetic patients using WSS-OCTA.

## Materials and methods

### Study participants

In this prospective observational study, 94 diabetic patients (94 eyes), classified according to the Clinical Diabetic Retinopathy Scale proposed by the Diabetic Retinopathy Project Group as diabetics without diabetic retinopathy (no DR, 25 eyes), mild DR (23 eyes); moderate/severe DR (26 eyes); proliferative DR (PDR, 20 eyes) and a control group of 25 healthy age-frequency matched subjects (25 eyes), were referring to the University G. d'Annunzio, Chieti-Pescara, Italy.

This prospective study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. Written informed consent was obtained from all subjects.

Criteria for inclusion were: (1) age > 8 years old; (2) best-corrected visual acuity (BCVA) greater than 0.5 log-MAR in the study eye at baseline examination (to ensure proper execution of examination); (3) confirmed diagnosis of diabetes mellitus with or without DR/DME.

The exclusion criteria were: (1) any ocular surgery (included intravitreal injections of anti vascular endothelial growth factor or dexamethasone implant) in the study eye in the last 9 months; (2) laser treatment in the study eye; (3) history of glaucoma; (4) medium lens opacity in the study eye; (5) ocular axial length included between 23 and 24 mm.

All patients recruited between June 2018 and December 2018 underwent a complete ophthalmic evaluation, including assessment of BCVA, tonometry, slit-lamp biomicroscopy and indirect fundus ophthalmoscopy. In addition, all patients were tested using PLEX Elite 9000 device (Carl Zeiss Meditec Inc., Dublin, CA, USA).

Diabetic retinopathy stage was classified according to the Clinical Diabetic Retinopathy Scale proposed by the Diabetic Retinopathy Project Group using retinal digital photographs [15]. The stages are as follows: (I) no DR; (II) mild non-proliferative diabetic retinopathy (NPDR); (III) moderate/severe (NPDR); (IV) PDR.

Outcome measures included: (1) SCP perfusion density (SCP PD); DCP PD and CC PD (2) SCP vessel length density (VLD); DCP VLD; CC VLD, in macular and near/mid periphery.

### Imaging protocol

Patients underwent OCTA imaging using the PLEX Elite 9000 device (Carl Zeiss Meditec Inc., Dublin, CA, USA), which uses a swept laser source with a central wavelength of 1050 nm (1000–1100 nm full bandwidth) and operates at 100,000 A-scans per second. This instrument employs a

full-width at half-maximum (FWHM) axial resolution of approximately 5  $\mu\text{m}$  in tissue, and a lateral resolution at the retinal surface estimated at approximately 14  $\mu\text{m}$  [10]. For each eye, two 15  $\times$  9-mm OCTA volume scans (one of the superior retina and one of the inferior retina) with patient fixating centrally were acquired. FastTrac motion correction software was used, while the images were acquired. These scans were then automatically montaged by the software to create a single widefield image with an approximately 50 degree field of view (or covering an approximately 15  $\times$  15 mm region of the retina) and automatic segmentation was performed by the software of the device with default settings. Poor-quality images (signal strength index (SSI) < 8) with either significant motion artifact or extensive incorrect segmentation were excluded and repeated. For all the participants, both eyes were imaged separately three times each, and the best-quality image of the right eye from each subject was selected to be analyzed in the study.

All selected images were carefully visualized by two retinal specialist independently (LT and LDA) to ascertain the correctness of the position of the upper and lower boundaries of segmentation such as the inner limiting membrane (ILM) and retinal pigment epithelium (RPE), respectively, and in case of erroneous boundaries recognition, manual correction was performed using the segmentation and propagation editing tool from the device. Then automatic segmentation by PLEX Elite 9000 device was used to define vascular beds obtaining three depth-resolved retinal slabs: the SCP extends from the ILM to the inner plexiform layer (IPL), the DCP extends from the IPL to the outer plexiform layer (OPL) and the CC consisting of a 20- $\mu\text{m}$ -thick uniform layer extending 29  $\mu\text{m}$  below the RPE to 49  $\mu\text{m}$  below the RPE.

To analyze and quantify in detail the main outcome measures (SCP PD; DCP PD; CC PD; SCP VLD; DCP VLD; CC VLD), a previously described protocol was adopted [14, 15]. For each eye, en face OCTA images segmented at the SCP, DCP and CC levels were imported into ImageJ software version 1.50 (National Institutes of Health, Bethesda, MD; available at <http://rsb.info.nih.gov/ij/index.html>).

Each SCP and DCP en face image was duplicated and binarized with two different binarization methods (“Huang’s fuzzy” method and “median local” thresholding) to calculate perfusion density, as previously described [16, 17].

To evaluate CC perfusion density, en face images were binarized using the Phansalkar method and then processed with the “Analyze Particles” command as previously shown [16, 18].

The DCP and CC directly beneath major superficial retinal vessels were excluded from analysis to eliminate potentially confounding shadow or projection artifacts as previously described [18].

Successively, the SCP, DCP and CC images obtained after binarization were skeletonized and these images were employed to measure VLD [16–18].

The quantitative analysis was performed in the macular region, which was defined as a circular annulus around the fovea with diameter of 5.5 mm and in the peripheral region which was assessed in three circles tangential (superior, temporal and inferior) to the macula and with diameters of 4.5 mm (Fig. 1).

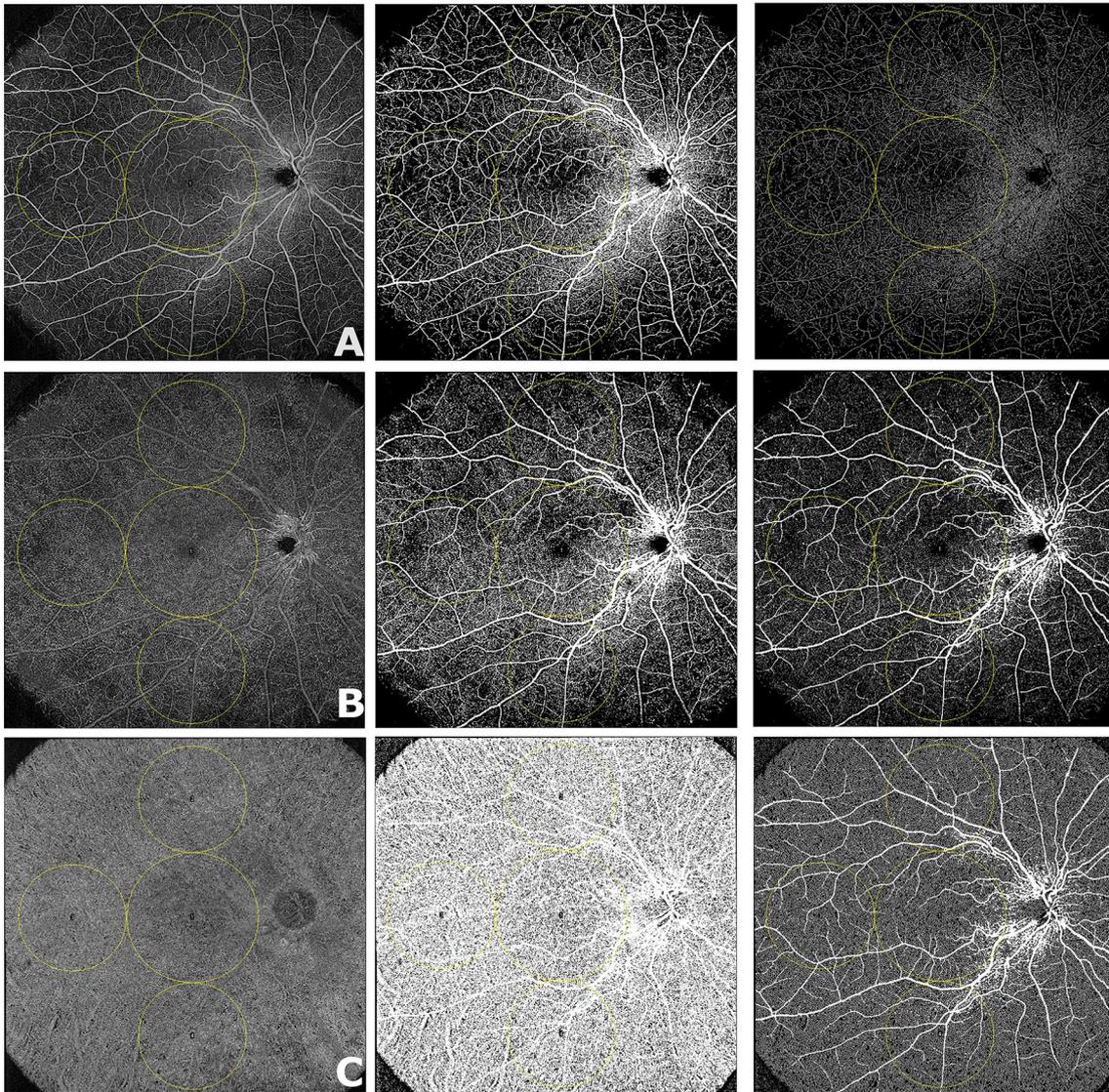
## Statistical analysis

Qualitative variables were presented as frequency and percentage. Continuous variables were tested for normal distribution with Shapiro–Wilks test and reported as mean and standard deviation (SD). Results were reported separately for each groups. One-way analysis of variance (ANOVA) test was performed to compare quantitative variables among groups. Chi-squared test was performed to compare for qualitative variables. Lin’s concordance correlation coefficient (CCC) with the 95% confidence intervals was calculated to assess the interobserver reproducibility of measurements. Each patient was attributed to the mean value between observer 1 and observer 2 for WSS-OCTA. Two-way analysis of variance (ANOVA) was used to determine the effects of type of sector (central, superior, temporal, inferior) and group (control, no DR, mild NPDR, moderate or severe NPDR and PDR), as well as the interaction of these two variables, on perfusion density and vessel length density. In all models, to assess differences respect to control, no DR and mild NPDR group, a post hoc analysis was performed with Dunnett’s test. The Pearson correlation coefficient (*R*) was performed to evaluate the correlation among central perfusion density and central vessel length density and corresponding peripheral parameter. The area under the receiver operating characteristic (ROC) curve (AUC) was estimated to determine the ability of each image size and parameter to predict moderate, severe NPDR or PDR. The true positive rate was plotted versus the false positive rate. The area under the curves (AUC) was calculated as a measure of classification model performance. Statistical analysis was performed using IBM® SPSS Statistics v 20.0 software (SPSS Inc, Chicago, Illinois, USA).

## Results

### Study population characteristics

A total of 119 eyes (no DR, 25 eyes; mild NPDR, 23 eyes; moderate/severe NPDR, 26 eyes; PDR, 20 eyes; control group, 25 eyes) met the required image quality criteria and were considered in the analysis. DME was present in 0 eyes of no DR group, in 5 eyes of mild NPDR group, in 10 eyes of moderate/severe NPDR group, in 10 eyes of PDR group.



**Fig. 1** Widefield OCTA assessment of superficial capillary plexus (**a**), deep capillary plexus (**b**) and choriocapillaris (**c**). **a–c** (left images) SCP, DCP and CC were investigated in two different regions: macular region (central circle with a diameter of 5.5 mm); and mid-periphery region (three circles with diameters of 4.5 mm tangential to the central circle). The SCP, DCP and CC binarized (**a–c**, mid-

dle) and skeletonized (**a–c**, right) images were obtained with ImageJ to investigate perfusion and vessel length density, respectively. The superficial capillary plexus' big retinal vessels were identified in the DCP and CC images and finally excluded from the analysis, thus avoiding shadowing and projection artifacts from SCP

The demographic and clinical characteristics of study population are reported in Table 1. For all eyes, two expert operators (LT and LDA) performed all the examinations in a in a masked fashion.

All eyes imaged and examined were included in the analysis.

Concordance correlation coefficient between the two readers was 0.99 (95% CI 0.98–0.99).

## Widefield OCTA analysis of the retinal vessels

### Perfusion density and vessel length density analysis of SCP

At SCP level, perfusion density showed statistically significant differences between different groups and between retinal sectors (Table 2). The interaction between groups and retinal sectors was not statistically significant.

**Table 1** Demographic data of patients and controls

| Variable                              | Control group ( <i>n</i> = 25) | Diabetic patients groups ( <i>n</i> = 94) |                            |  |                      | <i>p</i> value <sup>a</sup> |
|---------------------------------------|--------------------------------|---|----------------------------|--|----------------------|-----------------------------|
|                                       |                                | No DR ( <i>n</i> = 25)                    | Mild NPDR ( <i>n</i> = 23) | Moderate or severe NPDR ( <i>n</i> = 26) | PDR ( <i>n</i> = 20) |                             |
| Age (years) (mean ± SD)               | 55.8 ± 10.7                    | 57.8 ± 10.1                               | 56.8 ± 10.8                | 54.7 ± 13.9                              | 54.3 ± 11.8          | 0.846                       |
| Gender [ <i>n</i> (%)]                |                                |   |                            |  |                      | 0.674 <sup>b</sup>          |
| Male                                  | 8 (40.0)                       | 9 (39.1)                                  | 12 (57.1)                  | 13 (54.2)                                | 10 (50.0)            |                             |
| Female                                | 12 (60.0)                      | 14 (60.8)                                 | 9 (42.9)                   | 11 (45.8)                                | 10 (50.0)            |                             |
| BCVA (LogMAR) (mean ± SD)             | 0.00 ± 0.01                    | 0.00 ± 0.02                               | 0.04 ± 0.05                | 0.13 ± 0.10                              | 0.41 ± 0.14          | < <b>0.001</b>              |
| Diabetes duration (years) (mean ± SD) | –                              | 5.4 ± 5.1                                 | 15.1 ± 10.8                | 15.2 ± 8.3                               | 18.2 ± 10.8          | < <b>0.001</b>              |
| HbA1c (%) (mean ± SD)                 | –                              | 6.8 ± 0.1                                 | 7.0 ± 1.4                  | 7.2 ± 0.9                                | 7.8 ± 0.8            | <b>0.004</b>                |

Statistically significant values are shown in bold

DR diabetic retinopathy; NPDR non proliferative diabetic retinopathy; PDR proliferative diabetic retinopathy; SCP superficial capillary plexus; DCP deep capillary plexus; CC choriocapillaries

<sup>a</sup>*p* value derived from analysis of variance (ANOVA) between groups

<sup>b</sup>Chi-squared test

**Table 2** Differences of perfusion density among controls and diabetics with different stages of diabetic retinopathy

| Variable   | Control group | Diabetic patients groups |                           |                                |                                | Overall     | <i>p</i> value |                |             |
|------------|---------------|--------------------------|---------------------------|--------------------------------|--------------------------------|-------------|----------------|----------------|-------------|
|            |               | No DR                    | Mild NPDR                 | Moderate or severe NPDR        | PDR                            |             | Sector         | Group          | Interaction |
| <i>SCP</i> |               |                          |                           |                                |                                |             | < <b>0.001</b> | <b>0.006</b>   | 0.230       |
| Central    | 44.24 ± 4.00  | 44.30 ± 4.25             | 43.28 ± 2.29              | 36.34 ± 5.91 <sup>++oo*</sup>  | 36.18 ± 3.81 <sup>++oo*</sup>  | 39.3 ± 5.7  |                |                |             |
| Superior   | 37.18 ± 7.38  | 32.23 ± 6.07             | 27.75 ± 4.33              | 30.08 ± 7.67                   | 29.95 ± 7.27                   | 31.0 ± 7.0  |                |                |             |
| Temporal   | 26.10 ± 4.18  | 24.09 ± 3.35             | 23.93 ± 3.17              | 23.24 ± 4.15                   | 18.92 ± 4.61 <sup>++</sup>     | 22.7 ± 4.5  |                |                |             |
| Inferior   | 32.74 ± 9.49  | 27.78 ± 5.39             | 24.48 ± 7.53              | 27.73 ± 5.85                   | 27.70 ± 6.03                   | 27.9 ± 6.6  |                |                |             |
| <i>DCP</i> |               |                          |                           |                                |                                |             | < <b>0.001</b> | < <b>0.001</b> | 0.864       |
| Central    | 44.54 ± 3.11  | 44.89 ± 5.39             | 37.92 ± 2.57              | 33.80 ± 4.27 <sup>++++oo</sup> | 32.53 ± 5.09 <sup>++++oo</sup> | 37.6 ± 6.8  |                |                |             |
| Superior   | 32.95 ± 5.55  | 34.11 ± 11.13            | 21.82 ± 7.05              | 23.98 ± 9.21                   | 22.86 ± 8.51                   | 26.5 ± 9.8  |                |                |             |
| Temporal   | 38.79 ± 7.27  | 39.07 ± 6.83             | 29.09 ± 4.58              | 23.82 ± 6.98 <sup>++++oo</sup> | 23.31 ± 5.63 <sup>++++oo</sup> | 29.4 ± 9.2  |                |                |             |
| Inferior   | 34.66 ± 18.52 | 31.30 ± 8.51             | 19.48 ± 8.86 <sup>+</sup> | 18.76 ± 5.83                   | 18.44 ± 8.32                   | 23.3 ± 11.3 |                |                |             |
| <i>CC</i>  |               |                          |                           |                                |                                |             | < <b>0.001</b> | <b>0.004</b>   | 0.316       |
| Central    | 83.54 ± 1.47  | 83.42 ± 1.02             | 81.51 ± 2.36              | 80.87 ± 2.77                   | 76.85 ± 3.60 <sup>++++oo</sup> | 80.7 ± 3.6  |                |                |             |
| Superior   | 82.92 ± 0.67  | 81.70 ± 1.17             | 78.84 ± 1.61              | 78.99 ± 4.82                   | 79.06 ± 1.87                   | 80.0 ± 3.0  |                |                |             |
| Temporal   | 84.72 ± 1.46  | 84.38 ± 1.12             | 84.46 ± 1.99              | 83.49 ± 1.97                   | 82.28 ± 1.99                   | 83.6 ± 1.9  |                |                |             |
| Inferior   | 82.21 ± 2.80  | 80.22 ± 3.97             | 79.75 ± 4.09              | 79.57 ± 5.32                   | 76.81 ± 5.28                   | 79.3 ± 4.7  |                |                |             |

Statistically significant values are shown in bold

DR diabetic retinopathy; NPDR non proliferative diabetic retinopathy; PDR proliferative diabetic retinopathy; SCP superficial capillary plexus; DCP deep capillary plexus; CC choriocapillaries

<sup>+</sup>*p* < 0.05; <sup>++</sup>*p* < 0.01; <sup>+++</sup>*p* < 0.001 Dunnett's post hoc test comparison vs control group

<sup>o</sup>*p* < 0.05; <sup>oo</sup>*p* < 0.01; <sup>ooo</sup>*p* < 0.001 vs No DR

\**p* < 0.05 versus mild NPDR

SCP showed a lower PD in all peripheral sectors compared to the macular region (*p* < 0.001), with the lowest perfusion density value detected in the temporal sector if compared with the other retinal sectors (post hoc test *p* < 0.05, Table 2).

In detail, a significant lower central PD was found in moderate/severe NPDR group and PDR group compared to control group, diabetics without retinopathy group and mild NPDR group (post hoc analysis), and only temporal PD of

**Table 3** Differences of vessel length density among controls and diabetics with different stages of diabetic retinopathy

| Variable   | Control group | Diabetic patients groups |            |                               |                               | p value    |                  |                  |             |
|------------|---------------|--------------------------|------------|-------------------------------|-------------------------------|------------|------------------|------------------|-------------|
|            |               | No DR                    | Mild NPDR  | Moderate or severe NPDR       | PDR                           | Overall    | Sector           | Group            | Interaction |
| <i>SCP</i> |               |                          |            |                               |                               |            | <b>&lt;0.001</b> | <b>0.003</b>     | 0.169       |
| Central    | 18.94±1.97    | 19.51±2.24               | 19.21±1.10 | 15.97±2.92 <sup>+°°</sup>     | 15.92±1.81 <sup>+°°</sup>     | 17.6±2.71  |                  |                  |             |
| Superior   | 14.56±3.04    | 13.33±2.65               | 11.44±1.95 | 12.21±3.17                    | 11.88±2.77                    | 12.58±2.87 |                  |                  |             |
| Temporal   | 11.91±1.41    | 11.08±1.69               | 11.20±1.74 | 9.97±1.53                     | 8.76±2.11 <sup>+°</sup>       | 10.34±2.06 |                  |                  |             |
| Inferior   | 13.45±3.79    | 12.01±2.37               | 10.37±3.54 | 11.25±2.57                    | 11.31±2.53                    | 11.62±2.87 |                  |                  |             |
| <i>DCP</i> |               |                          |            |                               |                               |            | <b>&lt;0.001</b> | <b>&lt;0.001</b> | 0.562       |
| Central    | 20.28±1.32    | 21.17±2.00               | 18.30±1.00 | 16.07±1.99 <sup>++++°°°</sup> | 15.67±2.03 <sup>++++°°°</sup> | 17.95±2.96 |                  |                  |             |
| Superior   | 15.19±2.63    | 16.21±4.15               | 11.18±3.12 | 12.24±3.56                    | 11.45±3.91                    | 13.10±4.04 |                  |                  |             |
| Temporal   | 17.29±2.96    | 18.62±2.72               | 14.36±2.09 | 11.63±3.03 <sup>++++°°°</sup> | 11.68±2.44 <sup>++++°°°</sup> | 14.3±4.02  |                  |                  |             |
| Inferior   | 15.34±7.73    | 15.28±3.80               | 10.20±4.17 | 9.47±2.84 <sup>+°</sup>       | 9.63±3.76 <sup>+°</sup>       | 11.67±4.99 |                  |                  |             |
| <i>CC</i>  |               |                          |            |                               |                               |            | <b>0.010</b>     | 0.128            | 0.749       |
| Central    | 31.66±0.27    | 31.18±0.16               | 31.18±0.46 | 31.30±0.50                    | 30.74±0.81                    | 31.29±0.66 |                  |                  |             |
| Superior   | 30.69±0.48    | 30.62±0.67               | 30.62±0.24 | 30.16±2.13                    | 30.94±0.52                    | 30.71±1.16 |                  |                  |             |
| Temporal   | 31.08±0.28    | 30.74±0.17               | 30.74±0.45 | 31.07±0.36                    | 31.11±0.43                    | 31.10±0.40 |                  |                  |             |
| Inferior   | 31.22±0.45    | 31.13±0.83               | 31.13±0.86 | 30.67±1.40                    | 30.77±1.15                    | 30.95±1.05 |                  |                  |             |

Statistically significant values are shown in bold

DR diabetic retinopathy; NPDR non proliferative diabetic retinopathy; PDR proliferative diabetic retinopathy; SCP superficial capillary plexus; DCP deep capillary plexus; CC choriocapillaries

<sup>+</sup> $p < 0.05$ ; <sup>++</sup> $p < 0.01$ ; <sup>+++</sup> $p < 0.001$  Dunnett's post hoc test comparison vs control group

<sup>°</sup> $p < 0.05$ ; <sup>°°</sup> $p < 0.01$ ; <sup>°°°</sup> $p < 0.001$  vs No DR

\*  $p < 0.05$  versus mild NPDR

PDR group was significantly lower compared to the control group ( $p < 0.01$ , Table 2).

Vessel length density showed statistically significant differences between groups (Table 3;  $p = 0.003$ ) and between sectors within the same group (Table 3;  $p < 0.001$ ). The interaction between group and sectors was not statistically significant.

SCP showed a lower VLD in all peripheral sectors compared to the macular region (post hoc analysis  $p < 0.05$ ), with the lowest perfusion density value detected in the temporal region if compared with the other retinal sectors ( $p < 0.05$ ; Table 3).

In detail, a significant lower central VLD was found in moderate/severe retinopathy group and PDR group compared to control group and diabetics without retinopathy group, and only temporal VLD of PDR group was significantly lower compared to the control group and no DR group ( $p < 0.05$ , Table 3).

#### Perfusion density and vessel length density analysis of DCP

At DCP level, perfusion density showed statistically significant differences between different groups and between retinal sectors (Table 2). The interaction between groups and retinal sectors was not statistically significant.

At DCP level, a lower perfusion density was detected in all peripheral sectors compared to the macular region both

in healthy controls and diabetics with/without retinopathy ( $p < 0.001$ , Table 2). Among peripheral sectors, the inferior sector was significantly lower compared to the temporal one ( $p < 0.05$ , Table 2). A significantly lower central and temporal PD was found in moderate/severe retinopathy group and PDR group compared to control group and diabetics without retinopathy group ( $p < 0.001$ , Table 2).

DCP showed lower VLD in all peripheral sectors compared to the macular region (post hoc analysis  $p < 0.001$ ), with the lowest value detected in the inferior sectors if compared with the other retinal sectors ( $p < 0.05$  Table 3).

In particular, a significant lower central, temporal and inferior VLD was found in moderate/severe NPDR group and PDR group compared to control group and no DR group (Table 3).

#### Perfusion density and vessel length density analysis of CC

At CC level, perfusion density showed statistically significant differences between different groups and between retinal sectors (Table 2). The interaction between groups and retinal sectors was not statistically significant.

At CC level, inferior sector showed lower PD compared to central sector and temporal sector ( $p < 0.001$ , Table 2). In particular, a significant lower central PD was found in PDR group compared to control group and diabetics without

**Table 4** Correlation among central and peripheral retinal and choriocapillaris perfusion density in controls and diabetics with different stages of diabetic retinopathy

|                             | Control group | No DR or mild NPDR | Moderate, severe or PDR | Control group | No DR or mild NPDR | Moderate, severe or PDR | Control group | No DR or mild NPDR | Moderate, severe or PDR |
|-----------------------------|---------------|--------------------|-------------------------|---------------|--------------------|-------------------------|---------------|--------------------|-------------------------|
|                             | SCP superior  |                    |                         | SCP temporal  |                    |                         | SCP inferior  |                    |                         |
| <i>SCP central</i>          |               |                    |                         |               |                    |                         |               |                    |                         |
| Correlation coefficient (R) | -0.402        | 0.054              | -0.122                  | 0.213         | 0.318              | 0.239                   | -0.660        | -0.084             | 0.150                   |
| p value                     | 0.503         | 0.854              | 0.599                   | 0.730         | 0.267              | 0.296                   | 0.225         | 0.776              | 0.517                   |
|                             | DCP superior  |                    |                         | DCP temporal  |                    |                         | DCP inferior  |                    |                         |
| <i>DCP central</i>          |               |                    |                         |               |                    |                         |               |                    |                         |
| Correlation coefficient (R) | 0.568         | <b>0.682</b>       | <b>0.479</b>            | 0.723         | <b>0.918</b>       | <b>0.554</b>            | 0.450         | <b>0.711</b>       | 0.338                   |
| p value                     | 0.318         | <b>0.007</b>       | <b>0.028</b>            | 0.167         | <b>&lt;0.001</b>   | <b>0.009</b>            | 0.447         | <b>0.004</b>       | 0.134                   |
|                             | CC superior   |                    |                         | CC temporal   |                    |                         | CC inferior   |                    |                         |
| <i>CC central</i>           |               |                    |                         |               |                    |                         |               |                    |                         |
| Correlation coefficient (R) | -0.510        | <b>0.567</b>       | <b>0.642</b>            | 0.845         | <b>0.572</b>       | <b>0.592</b>            | -0.728        | <b>0.553</b>       | 0.216                   |
| p value                     | 0.380         | <b>0.034</b>       | <b>0.002</b>            | 0.072         | <b>0.033</b>       | <b>0.005</b>            | 0.163         | <b>0.040</b>       | 0.346                   |

Statistically significant values are shown in bold

DR diabetic retinopathy; NPDR non proliferative diabetic retinopathy; PDR proliferative diabetic retinopathy; SCP superficial capillary plexus; DCP deep capillary plexus; CC choriocapillaries

retinopathy group ( $p < 0.001$ , Table 2). Central VLD was significantly lower than all peripheral sectors ( $p < 0.001$ , Table 3).

### Correlation analysis of flow parameters between central and peripheral retina among groups

A significant positive correlation was found between macular area and periphery in terms of PD and VLD (Tables 4, 5).

In detail, PD of macular area showed in DCP and CC a positive correlation with temporal sectors and superior sectors in diabetics without retinopathy and in all DR stages, and with inferior sector in diabetics without retinopathy and in mild DR.

Vessel length density of macular area showed in DCP a positive correlation with temporal sectors and superior sectors in diabetics without retinopathy and in all DR stages, and with inferior sector in diabetics without retinopathy and in mild DR.

In order to prove the ability of the each image size and parameter to discriminate moderate, severe NPDR or PDR, we calculated the ROC curves (Fig. 2). Central and temporal sectors in all plexuses and for all sectors both central and peripheral of DCP were characterized by

high specificity and sensibility in predicting the disease worsening.

## Discussion

Our study evaluated the retinal and choriocapillaris flow features of diabetic patients without retinopathy or with different stage of DR comparing these data with flow features of eyes of healthy subjects in central and peripheral sectors using WFSS-OCTA depth-resolved scans.

The detailed analysis of retinal vasculature features is an important imaging biomarker in order to assess the retinal vascular damage related to diabetes [3].

Previous DR OCTA studies in diabetic patients had mainly focused their attention on retinal and choriocapillaris vasculature features of the macular area mainly due to the limited availability of widefield imaging devices [3].

Macular flow impairment of both retinal capillary plexuses and choriocapillaris has been demonstrated in diabetic patients with or without diabetic retinopathy related to retinopathy severity [19, 20].

In a prospective study evaluating patients with Type 1 DM without any signs of DR on the basis of fundus

**Table 5** Correlation among central and peripheral retinal and choriocapillaris vessel length density in controls and diabetics with different stages of diabetic retinopathy

|                             | Control group | No DR or mild NPDR | Moderate, severe or PDR | Control group | No DR or mild NPDR | Moderate, severe or PDR | Control group | No DR or mild NPDR | Moderate, severe or PDR |
|-----------------------------|---------------|--------------------|-------------------------|---------------|--------------------|-------------------------|---------------|--------------------|-------------------------|
|                             | SCP superior  |                    |                         | SCP temporal  |                    |                         | SCP inferior  |                    |                         |
| <i>SCP central</i>          |               |                    |                         |               |                    |                         |               |                    |                         |
| Correlation coefficient (R) | -0.468        | 0.046              | -0.094                  | 0.170         | 0.308              | 0.239                   | 0.456         | -0.067             | 0.177                   |
| p value                     | 0.426         | 0.876              | 0.686                   | 0.785         | 0.283              | 0.297                   | 0.189         | 0.820              | 0.443                   |
|                             | DCP superior  |                    |                         | DCP temporal  |                    |                         | DCP inferior  |                    |                         |
| <i>DCP central</i>          |               |                    |                         |               |                    |                         |               |                    |                         |
| Correlation coefficient (R) | 0.531         | <b>0.673</b>       | <b>0.482</b>            | 0.687         | <b>0.920</b>       | <b>0.555</b>            | 0.450         | <b>0.731</b>       | 0.344                   |
| p value                     | 0.357         | <b>0.008</b>       | <b>0.027</b>            | 0.200         | <b>&lt;0.001</b>   | <b>0.009</b>            | 0.447         | <b>0.003</b>       | 0.126                   |
|                             | CC superior   |                    |                         | CC temporal   |                    |                         | CC inferior   |                    |                         |
| <i>CC central</i>           |               |                    |                         |               |                    |                         |               |                    |                         |
| Correlation coefficient (R) | 0.076         | <b>0.537</b>       | 0.127                   | <b>0.906</b>  | <b>0.681</b>       | <b>0.539</b>            | -0.564        | <b>0.536</b>       | <b>0.526</b>            |
| p value                     | 0.904         | <b>0.048</b>       | 0.584                   | <b>0.034</b>  | <b>0.007</b>       | <b>0.012</b>            | 0.322         | <b>0.048</b>       | <b>0.014</b>            |

Statistically significant values are shown in bold

DR diabetic retinopathy; NPDR non proliferative diabetic retinopathy; PDR proliferative diabetic retinopathy; SCP superficial capillary plexus; DCP deep capillary plexus; CC choriocapillaries

biomicroscopy, Carnevali et al. [21] described early microvascular changes detectable with OCTA, such as alteration of the vessel density in perifoveal capillaries as a sign that precedes the enlargement and remodeling of FAZ.

In NPDR eyes, Borrelli et al. [22] described retinal perfusion reduction as well and found a strong association between diabetic choroidopathy and photoreceptor damage due to CC hypoperfusion and microvascular choroidal changes.

Nevertheless, the status of retinal periphery is considered to play an important role as predictor of diabetic retinopathy course [23, 24]. As already known, the risk of DR worsening is strongly associated with retinal peripheral lesions [14].

Recently, widefield OCTA studies evaluated microvasculature features of healthy patients and of patients with vascular diseases such as DR.

In particular, Hirano et al. reported a reduction in retinal perfusion both in superficial and deep capillary plexuses with worsening DR severity, using both small field and widefield scan sizes. Better prediction of DR was evidenced when using small scan sizes compared to widefield scans [25].

In our work, we analyzed the different topographical distribution of retinal and choriocapillaris flow of the central retina and peripheral retina in different sectors using widefield OCTA scans in normal healthy subjects, in diabetics without DR or diabetics with DR at different stages. In

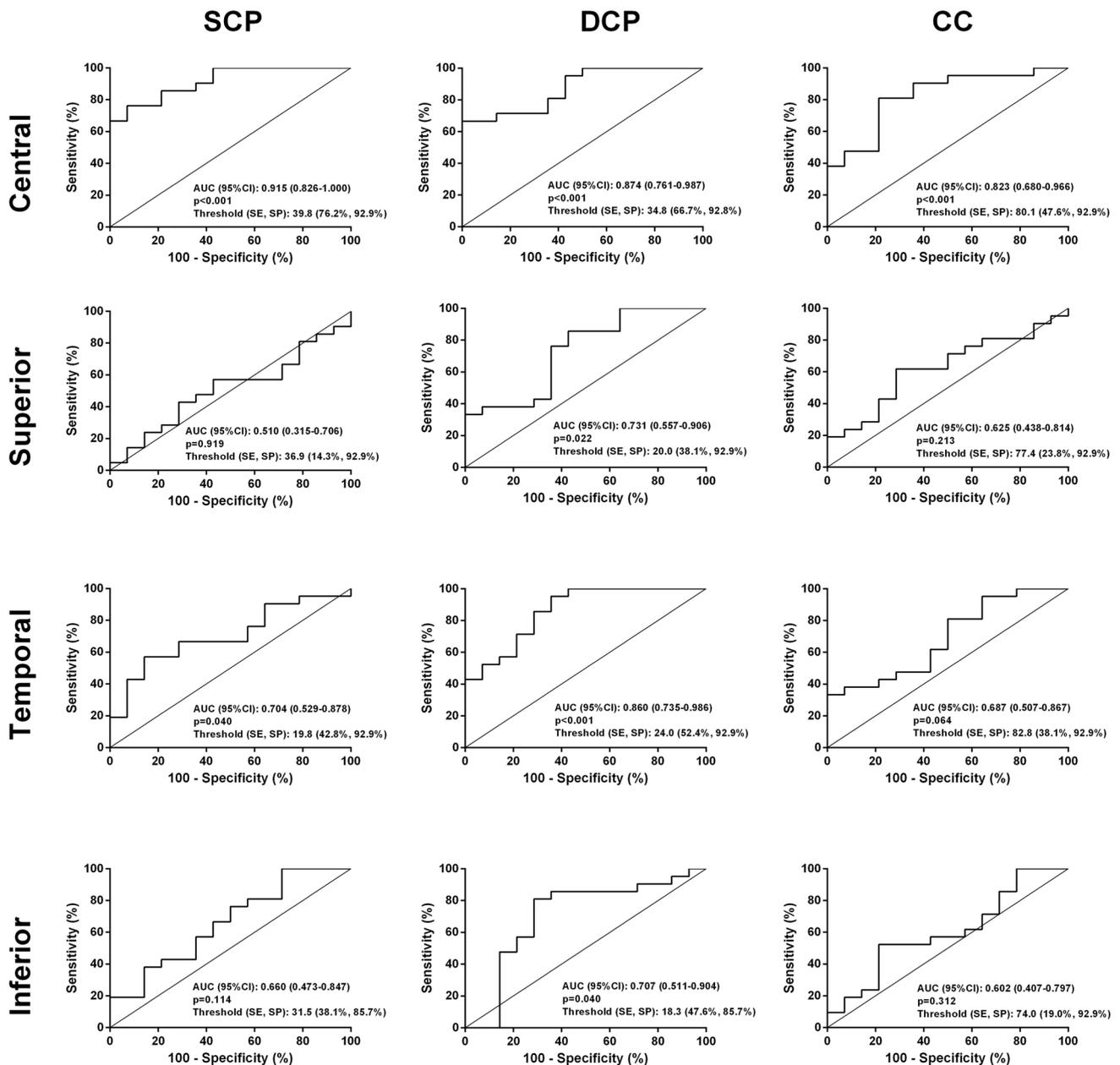
particular, we analyzed perfusion density and vessel length density of both retinal plexuses and choriocapillaris.

The vessel length density reflects a one-dimensional quantification of the retinal vasculature (length) and perfusion density a two-dimensional (length and width) measure thus a decrease in PD could be either a reduction in retinal vessel caliber or a decrease in branching patterns [26].

In accordance with previous studies [19, 20], in our patients the most advanced DR stages (moderate/severe NPDR group and PDR group) showed a significant lower PD and VLD in the macular area both in SCP and DCP compared to normal subjects, diabetics without retinopathy and diabetics with mild DR. Central CC PD was significantly lower in PDR group compared to normal subjects and diabetics without retinopathy.

Among peripheral sectors, the temporal one showed lower PD and VLD at DCP level in the most advanced DR stages compared to normal controls, diabetics without DR and mild DR.

In general, considering the relationship between PD and VLD the contemporary impairment of these two parameters for both retinal plexuses and choriocapillaris observed in our study at increasing stage of DR are probably related to a decrease in branching patterns due to capillary drop out.



**Fig. 2** Receiver operating characteristic analyses comparing the accuracy of each widefield swept-source optical coherence tomography angiography image scan size and parameter in predicting the presence

PDR or moderate, severe NPDR. In each graph lists, the area under the ROC curve (AUC), 95% confidence interval, *p* value, specificity and sensitivity when the threshold is set to the indicated value

The greatest susceptibility of central and temporal sectors to microvascular or ischemic insults may be related to the anatomical vascular features of these regions [27–29].

The macular area has an increased metabolic demand due to high density of cone and ganglion cells with increased superficial capillary plexus density compared to periphery. The temporal retina is the thinnest anatomically in normal subjects with intermediate and deep capillary plexus

merging into a single layer approximately 6–7 mm temporal to the fovea [27–29].

In addition, a reduction in temporal retinal thickness has been reported in diabetic patients without retinopathy compared to normal controls related to loss or degeneration of glial cells [29].

Regional differences of retinal perfusion density both in the superficial and deep capillary plexus have been described

in normal eyes. Campbell et al. reported a higher vessel density of SCP in the peripapillary and macular regions (except for the foveal area) with decrease in the periphery and a constant density of the intermediate capillary plexus and DCP in the peripapillary and macular regions, merging in the periphery with slightly higher combined density than the single plexuses [29].

In our study, to avoid as possible inaccurate assessments of peripheral retina and particularly of the temporal retina, we considered a central circle of 5.5 mm with a radius of 2.75 mm and tangential midperiphery circles of 4.5 mm; therefore, analysis of retinal capillary plexuses and choriocapillaris was performed up to approximately 7 mm temporal to the fovea in order to avoid as much as possible that the merging of the intermediate and deep capillary plexuses could interfere with an accurate vessel density evaluation.

If automatic segmentation failed to include DCP/intermediate capillary plexus, manual editing was applied.

In accordance with previous data [19, 20], in our cohort, the vessel perfusion density showed differences depending on retinal regions in both healthy and diabetic cohorts. In detail, central area showed the highest PD and VLD both in SCP and DCP. Among peripheral sectors, the temporal one showed the lowest PD and VLD in the SCP, and in the DCP, the inferior sector was less perfused.

It is possible to hypothesize that the lower PD and VLD of the temporal sector in SCP could be related to the presence in the temporal area of the watershed of the temporal vascular major arcades.

Another aspect explored in diabetic retinopathy is the ischemic status of the retina and the relationship between central and peripheral ischemia in order to better understand the pathophysiology of the disease and the risk of disease progression.

Recent studies using ultra-widefield fluorescein angiography (FA) have reported an association between peripheral ischemic index and foveal avascular zone (FAZ) area showing a moderate correlation [30].

A relationship between the microvascular indices measured on OCTA such as FAZ area and vessel density and the presence and extent of peripheral non-perfusion in DR has also been assessed in a recent study [31].

Peripheral ischemia measured on FA was related to a significant increase in various FAZ metrics.

We evaluated the correlation between central and mid-peripheral OCTA metrics. A significant positive correlation was found between macular area and periphery in terms of PD and VLD of DCP and CC both in diabetics without DR and in diabetic with different stage of DR. The highest significance was found for the early DR stages (no DR and mild NPDR) between the central and temporal sector at the DCP level.

Several studies evaluated sensitivity and specificity of OCTA parameters in assessing the presence of DR using both small and widefield OCTA scan [32]. Hirano et al. [25] found high sensitivity and specificity of 3 × 3 mm OCTA images for predicting the presence/absence of DR compared to larger scan size mainly related to image quality. We found a good sensitivity and an excellent specificity in terms of prediction of disease worsening from the early stages (no DR and mild DR) to the advanced stages of DR (moderate, severe NPDR or PDR), especially for central and temporal sectors of all plexuses and for all sectors both central and peripheral of DCP. It has been suggested that DR-related microvasculature damage may begin around the macula thus the central sector would be expected to have the best predictive sensitivity for DR [25].

The results of our study confirm this hypothesis. Among the peripheral sectors, the temporal one at DCP level showed the highest sensitivity and specificity in predicting disease progression. It is possible to hypothesize that due to the anatomical characteristics this region is more prone to reflect disease progression.

This study has some limitations. When considering diabetic retinopathy stages, we combined in a single group moderate and severe NPDR to increase the sample size of this group. We studied only near/mid periphery because of the higher chance to have many shadowing artifacts likely caused by patient's eyelashes in the peripheral images thus excluding information from the peripheral retina.

In summary, our study demonstrated progressive impairment of retinal and choriocapillaris flow features both in central and peripheral retina at advancing DR severity with high correlation between central and peripheral retina. Moreover, both central and peripheral retina particularly at the DCP level show high sensitivity and specificity in predicting DR progression.

**Acknowledgements** None.

**Funding** None.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Ophthalmology Clinic, Department of Medicine and Science of Ageing) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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