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Perivenular Capillary Rarefaction in Diabetic Retinopathy

Interdevice Characterization and Association to Clinical Staging

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Purpose: Geometric perfusion deficit (GPD) is a newly described OCT angiography (OCTA) parameter identifying the total area of presumed retinal ischemia. The aim of our study is to characterize differences in GPD and other common quantitative OCTA parameters between macular full field, perivenular zones, and periarteriolar zones for each clinical stage of nonproliferative diabetic retinopathy (DR) and to assess the influence of ultrahigh-speed acquisition and averaging on the described differences.

Design: Prospective observational study.

Participants: Forty-nine patients, including 11 (22.4%) with no sign of DR, 12 (24.5%) with mild DR, 13 (26.5%) with moderate DR, and 13 (26.5%) with severe DR. Patients with diabetic macular edema, proliferative DR, media opacity, head tremor, and overlapping retinal diseases or systemic diseases influencing OCTA were excluded.

Methods: OCT angiography was performed 3 times for each patient: 1 using Solix Fullrange single volume (V1) mode, 1 using Solix Fullrange 4 volumes mode with automatically averaged scan (V4), and 1 using AngioVue.

Main Outcome Measures: Full macular, periarteriolar, and perivenular perfusion density (PD), vessel length density (VLD), vessel density index, and GPD for both the superficial capillary plexus (SCP) and deep capillary plexus (DCP).

Results: In patients showing no sign of DR, PD and VLD were significantly lower in the perivenular area in both the DCP and SCP using V1 and V4, whereas GPD was significantly higher in the perivenular zone in the DCP and SCP with all 3 devices. In patients with mild DR, all 3 measurements (PD, VLD, and GPD) were significantly different in the perivenular zone with all 3 devices. In patients with moderate DR, PD and VLD were lower in the DCP and SCP when measured with V1 and V4. Moreover, GPD was higher in the perivenular zone in the DCP with all 3 devices, whereas only V4 detected a difference in the SCP. In severe DR, only V4 detected a lower PD and VLD and a higher GPD in the DCP of the perivenular zone. V4 also detected a higher GPD in the SCP.

Conclusions: Geometric perfusion deficit highlights prevalent perivenular location of macular capillary ischemia in all stages of DR. In severe DR patients, only averaging technology allows detection of the same finding.

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Diabetic microvasculopathy plays a pivotal role in development and progression of diabetic retinopathy (DR). Early diabetic microvascular dysfunction reduces retinal capillary blood flow prevalently due to the effect of functional alterations inducing vasoconstriction.[1,2] In parallel, accelerated production of reactive oxygen species[3] and abnormal signaling of Ang2-related pathways[4] leads to pericyte loss and formation of fragile dysfunctional acellular capillaries. The resulting microvascular damage leads to retinal nonperfusion and chronical hypoxia, causing ischemic damage not only to retinal cells but also to capillary walls, thus perpetuating the process. The advent of OCT angiography (OCTA) made it possible to detect capillary nonperfusion and alterations in shape and dimensions of the capillary free zone, starting from the earliest phases of DR.[5] It also allowed to characterize changes in retinal microvasculature related to the progression of the disease, so much that it has become an increasingly important clinical

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tool for prognostic evaluation in DR.[6] In fact, a decrease in macular perfusion density (PD) is correlated to an increased risk of DR progression and diabetic macular edema development. Moreover, PD measurements have been correlated to functional parameters such as visual acuity and microperimetric assessment of retinal sensitivity.[7] Geometric perfusion deficit (GPD) is a newly described[8] OCTA parameter identifying the total area of presumed retinal ischemia according to the underlying theory of oxygen diffusion. Nesper et al[9] reported how GPD is the most accurate among quantitative OCTA parameters for DR screening and detection of diabetic patients, warranting further ophthalmic evaluation. Recently, GPD calculated from OCTA images acquired with the averaging technique was found to be specifically higher in the perivenular area of patients affected by diabetes mellitus without DR compared with healthy subjects.[10] Nevertheless, the hypothesis itself of a deeper and earlier involvement of the perivenular zone in diabetic capillary ischemia compared with other macular regions has never been extensively analyzed in literature.

The aim of our study is to expand this area of research through characterization of differences in GPD and other common OCTA parameters between macular full field, perivenular zones, and periarteriolar zones for each clinical stage of nonproliferative DR. Moreover, we aim to assess the influence of ultrahigh-speed acquisition and averaging on the described differences.

Materials and Methods

Patients' Selection and Study Design

Patients diagnosed with diabetes mellitus and showing best-corrected visual acuity $>$ 20/60, spherical refractive error $< \pm$ 3 diopters and cylinder correction $<$ 2 diopters were prospectively recruited in the Ophthalmology Department of the Hôpital Intercommunal de Créteil (University Paris Est) from February 1, 2022 to May 5, 2022. Diabetic macular edema, proliferative DR, media opacity precluding the acquisition of high-quality images, head tremor, overlapping retinal diseases, glaucoma, and inherited retinal diseases were considered as exclusion criteria. Moreover, patients with previous stroke, vascular dementia, severe chronic kidney disease, or collagenopathies were excluded from the study because of the potential influence of these conditions on OCTA quantitative parameters.[11,12] Ophthalmic exclusion criteria were ruled out with the support of slit lamp examination and multimodal imaging acquired with Solix Fullrange OCT (Optovue Inc). Diabetic retinopathy staging was assessed for each eye by a senior retinal specialist relying on slit lamp fundus examination and wide field fundus photographs. As per the ETDRS extension of the modified Airlie House Classification,[13] eyes were categorized as showing either no DR, mild DR, moderate DR, or severe DR. The primary outcome of the study was to characterize the differences in quantitative OCTA parameters between full macular, perivenular zones, and periarteriolar zones for each DR stage. The secondary outcome was to assess the influence of ultrahigh-speed acquisition and averaging on the described differences. The study protocol was approved by the Fédération France Macula Ethics Committee and informed consent with complete explanation of the protocol was provided to all participants in accordance with the Declaration of Helsinki.

Procedures and Instruments

Each patient underwent best-corrected visual acuity assessment, Goldman applanation tonometry, and OCT examination. OCT angiography was performed 3 times for each patient: 1 using Solix Fullrange (Optovue Inc) single volume (V1) mode, 1 using Solix Fullrange 4 volumes mode with automatically averaged scan (V4), and 1 using AngioVue (Optovue Inc). Both Solix Fullrange OCT and AngioVue are integrated with motion correction technology software for automatic correction of distortions in all directions after volume acquisition and 3-dimensional Projection Artifact Removal for artifact removal from the B-scan volume.[14] This strategy was adopted to study the differences in quantitative parameters between standard speed spectral domain-OCT (AngioVue, 70 000 A-scans per second) and ultrahigh-speed acquisitions with averaging (Solix V4 mode, 120 000 A-scans per second + 4 volumes averaging) and without averaging (Solix V1 mode, 120 000 A-scans per second). All 3 acquisitions consisted of 3 $\times$ 3 mm volumes centered on the fovea and were performed by the same operator in the same light conditions. An interval of 5 minutes between each acquisition was planned to improve the patients' compliance. Segmentation was adjusted so as to consider the vascular net between the inner margin of the ganglion cell layer and the outer margin of the inner plexiform layer as part of the superficial capillary plexus (SCP) and the vascular net between the outer margin of the inner plexiform layer and the outer margin of the outer nuclear layer as part of the deep capillary plexus (DCP).[15–17]

Image Processing and Outcome Measures

Outcome measures of the study were full macular, periarteriolar, and perivenular PD, vessel length density (VLD), vessel density index (VDI), and GPD for both the SCP and DCP. For initial binarization and cropping of segmented OCTA en face images, an open-source image processing software (ImageJ, National Institutes of Health) was used while calculation of quantitative OCTA parameters (outcome measures) and creation of perivenular and periarteriolar regions of interest (ROIs) was performed with MatLab software (Mathworks). The creation of images for full macular analysis was performed by setting a global threshold of 65 for both SCP and DCP images[18] and subsequently applying a Mexican hat filter to reduce the influence of background noise on quantitative parameters calculation. Full macular PD, defined as the percentage area of the angiogram occupied by blood vessels, was calculated as the area of the total angiogram occupied by white pixels in binarized thresholded images. Skeletonization of the images and calculation of VLD (defined as the total length of the perfused vasculature divided by the total number of pixels in the analyzed area on the skeletonized image) was then performed.[19] The VDI, which represents the average vessel caliber, was calculated by dividing the total vessel area in the binarized image by the total vessel length in the skeletonized image. Geometric perfusion deficit was calculated as the total area of avascular (black) pixels with a minimum distance of 30 μm from the nearest vascular (white) pixel.[8] The periarteriolar and perivenular ROI creation process featured a first step in which B-scan OCTA was used for detection of arterioles and venules. This allowed for further splitting of the SCP and DCP via horizontal multiplanar segmentation with a previously described method.[20] The process thus created 2 different en face slices for both the SCP and DCP: 1 at the level of (and containing only) arterioles and 1 at the level of (and containing only) venules. Arterioles and venules were then isolated, raising the global threshold to 85

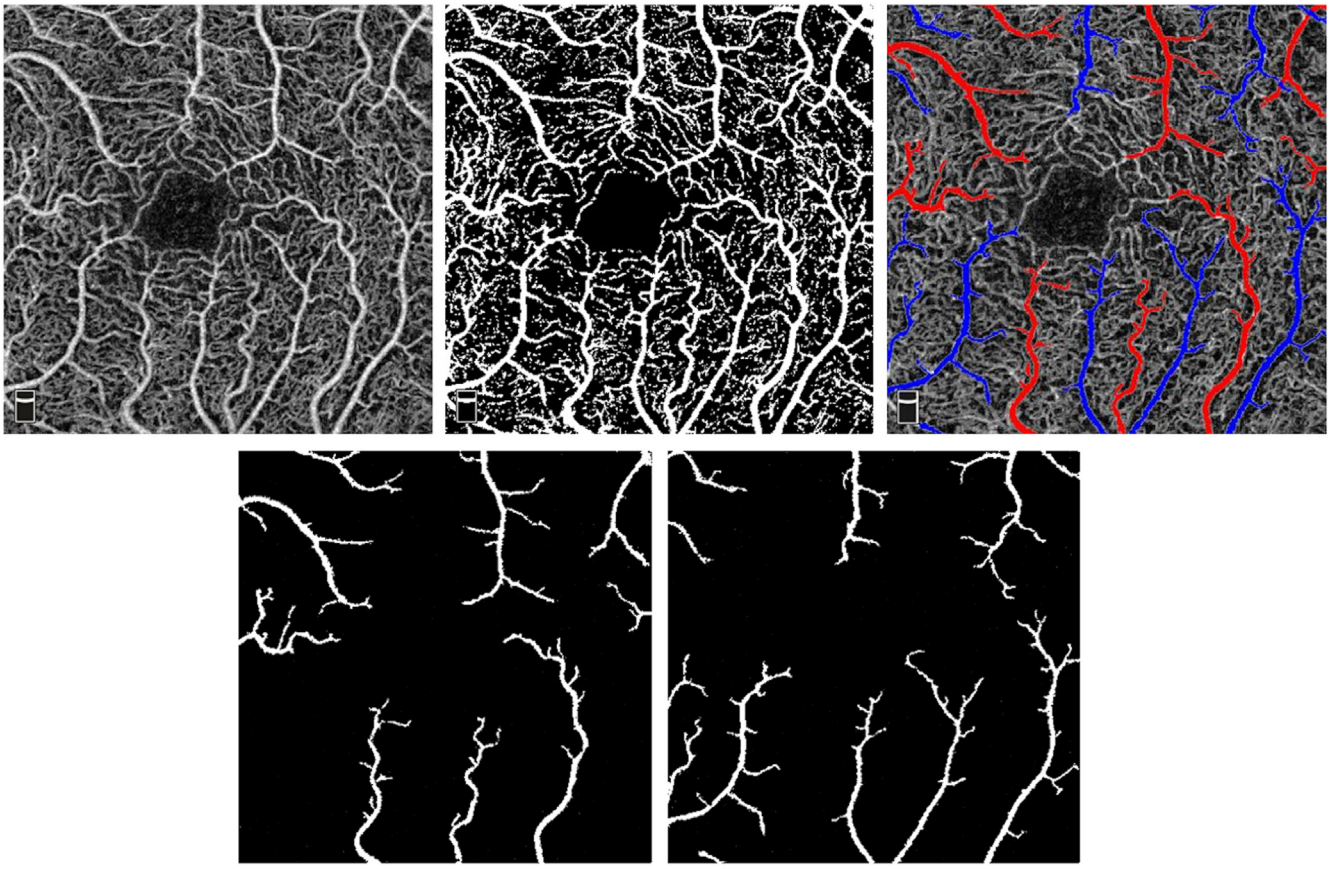

Figure 1. Upper left: Raw fovea centered 3 × 3 mm OCT angiography (OCTA) of the superficial capillary plexus of a patient from the moderate diabetic retinopathy subgroup. Acquired using Solix single volume mode. Upper middle: Binarized and thresholded version of upper left image (used for perfusion density [PD] and geometric PD calculation of full macular data). Upper right: Raw OCTA with arterioles highlighted in red and venules highlighted in blue. Lower left: Thresholded arterioles used for arteriolar region of interest (ROI) calculation. Lower right: Thresholded venules used for venular ROI calculation.
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and subsequently applying automatic local thresholding with Otsu's method for more accurate delineation (Fig 1).[21,22] The images of isolated arterioles and venules were then superimposed on the full macula en face OCTA of the SCP and DCP to highlight the position of larger diameter vessels on the original angiogram (Fig 1).

A ROI including all pixels located within 100 μm from respectively arterioles' and venules' outlines was created. Perfusion density, VLD, VDI, and GPD were subsequently calculated for periarteriolar and perivenular ROIs as previously described for full macula images. Periarteriolar and perivenular GPD are shown in Figure 2.

Statistical Analysis

Statistical analysis was performed using SPSS software v.26.0 (IBM SPSS Statistics). Normality of the distribution for quantitative variables was assessed with the Shapiro Wilk test. Quantitative variables were described using mean and standard deviation, whereas for qualitative variables, number over total and percentage were used. Comparison of each quantitative parameter as measured in the 3 different ROIs (primary outcome) was performed using 1-way analysis of covariance for independent samples using age as a covariate. The effect of the use of different OCT device on the measurements in the 3 ROIs (secondary outcome) was assessed with 2-way multivariate analysis of covariance. Agreement between quantitative measures resulting from the 3 OCT devices was assessed with intraclass correlation coefficient. A P value < 0.05 was considered as statistically significant.

Results

A total of 49 eyes of 49 patients was included in the analysis. Mean age of the study population was 61.35 ± 8.72 years, and 65.3% (32/49) were male subjects. Eleven eyes (22.4%) showed no sign of DR, 12 eyes (24.5%) showed mild DR, 13 eyes (26.5%) were affected by moderate DR, and 13 eyes (26.5%) by severe DR. Detailed characterization of generalities and ophthalmic data for each subgroup are provided in Table 1.

In the subgroup of patients showing no sign of DR, PD derived from high-speed acquisitions (V1 and V4) was significantly lower in the perivenular zone in both the DCP and SCP (respectively $P = 0.027$ and $P = 0.036$ for V1 and $P = 0.031$ and $P = 0.041$ for V4). By contrast, no significant difference was detected with acquisitions performed with AngioVue. The same results came from the analysis of

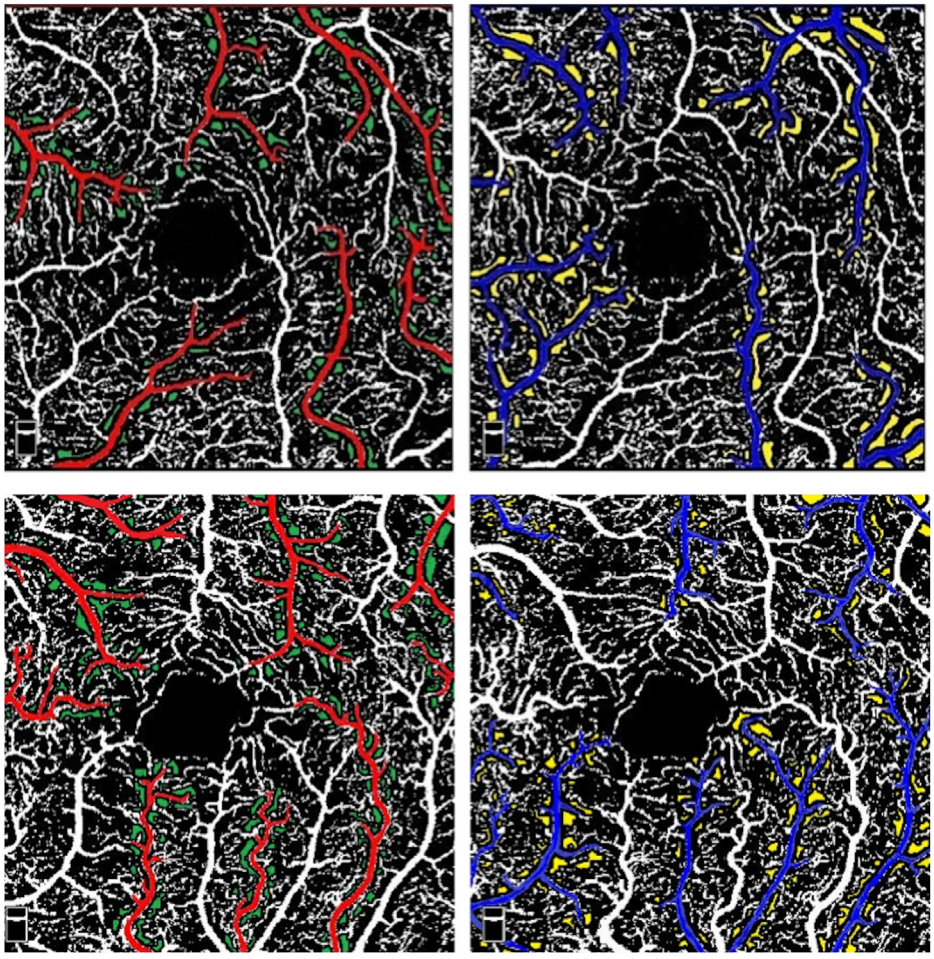

Figure 2. Binarized thresholded 3 × 3 mm en face OCT angiography of the superficial capillary plexus of a patient from the mild diabetic retinopathy (DR) (upper panels) and moderate DR (lower panels) study subgroups acquired using Solix single volume mode. Left image shows arterioles highlighted in red and geometric perfusion density (GPD) in the arteriolar region of interest (ROI) (within 100 mm from every arteriolar pixel) highlighted in green. In the upper panels showing a case of mild DR, the periarteriolar GPD calculated for this image is 11.80%. Right image shows venules highlighted in blue and GPD in the venular ROI (within 100 mm from every venular pixel) highlighted in yellow; perivenular GPD calculated for this image is 12.91%. In the lower panels showing a case of moderate DR, the periarteriolar GPD calculated for this image is 13.34%. Right image shows venules highlighted in blue and GPD in the venular ROI (within 100 mm from every venular pixel) highlighted in yellow; perivenular GPD calculated for this image is 13.61%.

VLD, because it was significantly lower in the perivenular zone when assessed with V1 and V4, but it showed no difference when assessed with AngioVue (Table 2). Geometric perfusion deficit was significantly lower in the perivenular zone both in the DCP and SCP regardless of the device used (significant difference with all 3 devices). Solix V1 was the device that reported the highest degree of difference between zones in this subgroup of patients.

Table 1. Characterization of General Anamnestic Data and Ophthalmic Details for Each Study Subgroup

	No DR (n = 11)	Mild DR (n = 12)	Moderate DR (n = 13)	Severe DR (n = 13)	P
Age (y)	59.17 ± 10.58	62.94 ± 10.24	63.80 ± 10.52	60.71 ± 12.13	0.63
Male sex	7/11 (63.6%)	8/12 (66.7%)	9/13 (69.3%)	8/13 (61.5%)	0.55
BCVA (logMAR)	0.03 ± 0.01	0.07 ± 0.02	0.15 ± 0.04	0.33 ± 0.10	0.048
SE (D)	0.52 ± 0.23	0.77 ± 0.31	−0.24 ± 0.40	−0.50 ± 0.65	0.71
IOP (mmHg)	14.52 ± 2.61	15.07 ± 2.91	15.84 ± 4.10	17.01 ± 3.96	0.89

BCVA = best-corrected visual acuity; D = diopter; DR = diabetic retinopathy; IOP = intraocular pressure; logMAR = logarithm of the minimum angle of resolution; SE= spherical equivalent.

Table 2. Comparison of Full Macular, Periarteriolar, and Perivenular PD, VLD, VDI, and GPD in the SCP and DCP of Patients with No Signs of DR or Mild DR

			No DR			
			Full Macular	*Periarteriolar*	*Perivenular*	P
PD (%)	SCP	AngioVue	49.53 ± 2.87	48.66 ± 2.73	48.31 ± 2.71	0.34
		V1	47.48 ± 2.83	47.03 ± 2.61	43.98 ± 1.72	0.036
		V4	46.89 ± 2.91	46.74 ± 2.73	44.04 ± 1.85	0.041
	DCP	AngioVue	41.19 ± 2.01	40.67 ± 1.19	39.26 ± 1.33	0.64
		V1	39.55 ± 2.12	39.03 ± 1.82	36.95 ± 1.75	0.027
		V4	38.33 ± 1.95	38.04 ± 1.66	36.13 ± 1.68	0.031
VLD	SCP	AngioVue	5.18 ± 0.17	5.14 ± 0.18	5.13 ± 0.16	0.71
		V1	5.09 ± 0.25	5.01 ± 0.14	4.71 ± 0.10	0.029
		V4	4.97 ± 0.26	4.91 ± 0.18	4.75 ± 0.14	0.037
	DCP	AngioVue	4.80 ± 0.18	4.76 ± 0.15	4.70 ± 0.11	0.042
		V1	4.57 ± 0.21	4.49 ± 0.26	4.32 ± 0.15	0.028
		V4	4.40 ± 0.19	4.35 ± 0.11	4.23 ± 0.13	0.039
VDI	SCP	AngioVue	9.56 ± 0.41	9.46 ± 0.37	9.42 ± 0.33	0.21
		V1	9.32 ± 0.24	9.38 ± 0.21	9.33 ± 0.23	0.81
		V4	9.43 ± 0.30	9.51 ± 0.27	9.27 ± 0.21	0.020
	DCP	AngioVue	8.58 ± 0.20	8.54 ± 0.19	8.35 ± 0.23	0.025
		V1	8.65 ± 0.21	8.69 ± 0.22	8.55 ± 0.24	0.043
		V4	8.71 ± 0.19	8.74 ± 0.17	8.54 ± 0.18	0.017
GPD (%)	SCP	AngioVue	10.52 ± 0.29	10.64 ± 0.30	10.95 ± 0.33	0.042
		V1	10.64 ± 0.26	10.70 ± 0.22	11.30 ± 0.24	0.029
		V4	10.69 ± 0.20	10.73 ± 0.18	11.45 ± 0.19	0.024
	DCP	AngioVue	7.81 ± 0.32	7.93 ± 0.34	8.27 ± 0.21	0.046
		V1	8.03 ± 0.22	8.07 ± 0.25	8.53 ± 0.20	0.018
		V4	8.12 ± 0.20	8.11 ± 0.18	8.67 ± 0.19	0.020
			Mild DR			
			Full Macular	*Periarteriolar*	*Perivenular*	P
PD (%)	SCP	AngioVue	46.63 ± 2.14	46.02 ± 2.11	44.41 ± 2.08	0.035
		V1	46.11 ± 1.75	45.69 ± 1.64	43.65 ± 1.59	0.019
		V4	45.71 ± 1.68	45.21 ± 2.68	43.21 ± 1.51	0.037
	DCP	AngioVue	38.61 ± 1.34	38.33 ± 1.49	35.91 ± 1.38	0.023
		V1	37.68 ± 1.45	37.31 ± 1.62	34.85 ± 1.14	0.011
		V4	37.02 ± 1.23	36.81 ± 1.03	34.16 ± 1.28	0.026
VLD	SCP	AngioVue	5.05 ± 0.15	4.99 ± 0.16	4.57 ± 0.11	0.041
		V1	5.02 ± 0.14	4.98 ± 0.14	4.34 ± 0.10	0.013
		V4	4.96 ± 0.16	4.88 ± 0.19	4.45 ± 0.12	0.028
	DCP	AngioVue	4.51 ± 0.15	4.31 ± 0.16	3.99 ± 0.14	0.030
		V1	4.29 ± 0.22	4.19 ± 0.19	3.87 ± 0.09	0.005
		V4	4.15 ± 0.17	4.09 ± 0.18	3.86 ± 0.12	0.016
VDI	SCP	AngioVue	9.23 ± 0.29	9.22 ± 0.28	9.71 ± 0.26	0.004
		V1	9.18 ± 0.17	9.17 ± 0.16	10.05 ± 0.11	0.001
		V4	9.21 ± 0.21	9.26 ± 0.20	9.71 ± 0.21	0.004
	DCP	AngioVue	8.56 ± 0.19	8.89 ± 0.19	9.00 ± 0.18	0.011
		V1	8.78 ± 0.17	8.90 ± 0.18	9.00 ± 0.17	0.029
		V4	8.92 ± 0.14	9.00 ± 0.13	8.84 ± 0.14	0.18
GPD (%)	SCP	AngioVue	11.90 ± 0.37	12.08 ± 0.36	12.41 ± 0.30	0.040
		V1	12.05 ± 0.29	12.08 ± 0.26	12.71 ± 0.23	0.016
		V4	12.10 ± 0.17	12.21 ± 0.18	12.92 ± 0.20	0.006
	DCP	AngioVue	9.97 ± 0.46	10.02 ± 0.41	10.36 ± 0.40	0.048
		V1	10.05 ± 0.30	10.12 ± 0.27	10.92 ± 0.25	0.019
		V4	10.12 ± 0.18	10.18 ± 0.20	11.01 ± 0.19	0.005

DCP = deep capillary plexus; DR = diabetic retinopathy; GPD = geometric perfusion density; PD = perfusion density; SCP = superficial capillary plexus; VDI = vessel diameter index; VLD = vessel length density; V1 = single volume; V4 = 4 volumes mode with automatically averaged scan.

Agreement between devices in this group was good between Solix V1 and V4 (k = 0.81 for PD measurements and k = 0.85 for GPD measurements) and moderate between V1 and AngioVue (k = 0.71 for PD and k = 0.74 for GPD) and V4 and AngioVue (k = 0.70 for PD and k = 0.75 for GPD) (Fig 3). In the subgroup of patients showing mild DR, PD, VLD,

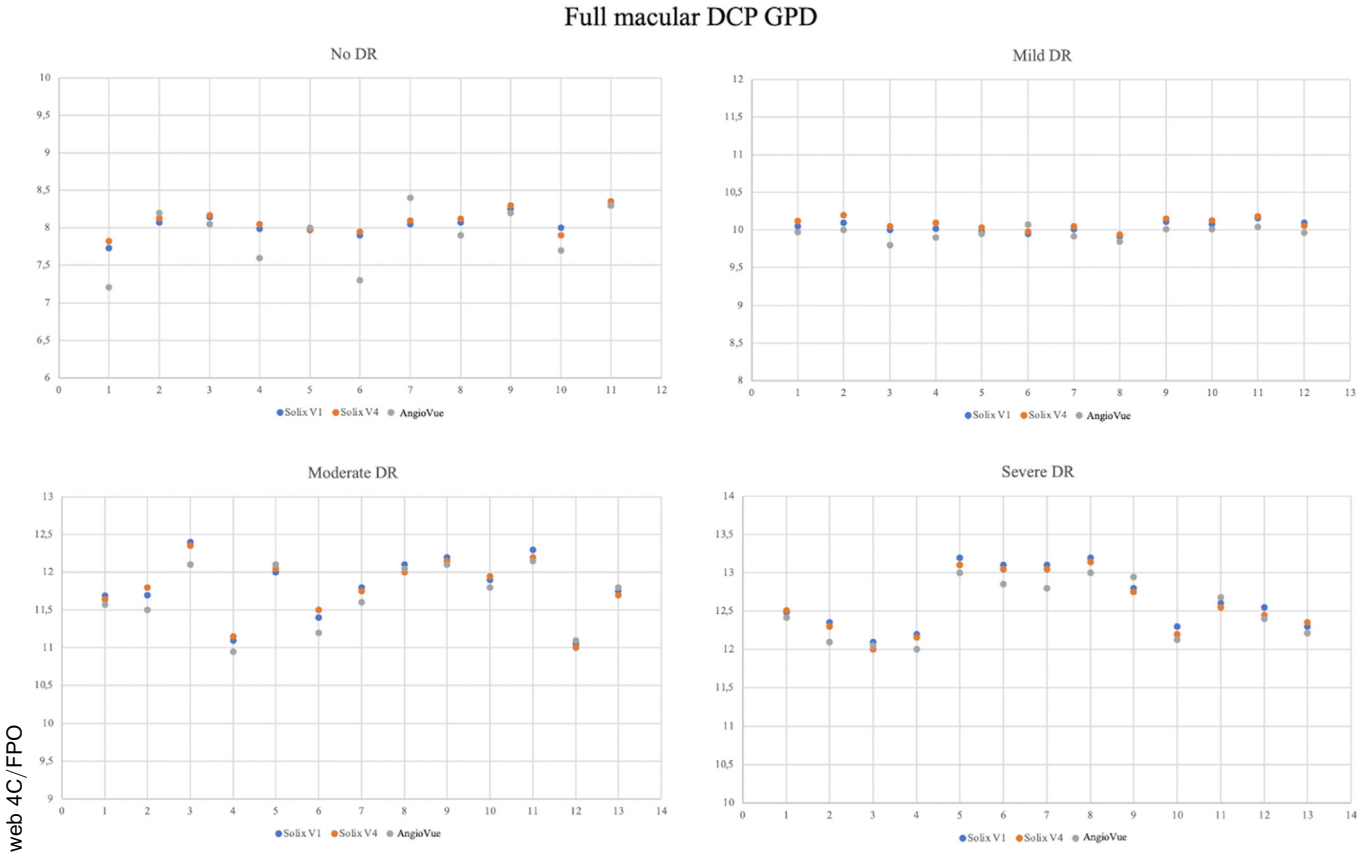

Figure 3. Full macular deep capillary plexus (DCP) geometric perfusion density (GPD) in diabetic patients with no diabetic retinopathy (DR) (upper left), mild DR (upper right), moderate DR (lower left), and severe DR (lower right). V1 = single volume; V4 = 4 volumes mode with automatically averaged scan.

VDI, and GPD were all significantly different between zones regardless of the device used. In fact, the perivenular zone showed lower PD, higher GPD, lower VLD, and higher VDI (see Table 2 for detailed description). Agreement between instruments was good between V1 and AngioVue (k = 0.80 for PD and k = 0.84 for GPD) and V4 and AngioVue (k = 0.78 for PD and k = 0.82 for GPD), and it was very good between V1 and V4 (k = 0.90 for PD and k = 0.91 for GPD).

In the subgroup of patients with moderate DR, PD and VLD were significantly lower in the perivenular zone in the DCP and SCP with both V1 and V4 while the difference was only borderline to statistical significance when acquisitions were made with AngioVue (respectively $P = 0.057$ and $P = 0.063$ for PD in the DCP and SCP and $P = 0.081$ and $P = 0.071$ for the DCP and SCP for VLD) (Table 3). Higher GPD in the perivenular zone was unanimously (all 3 devices) reported only in the DCP for this subgroup, whereas only V4 detected a higher perivenular GPD in the SCP ($P = 0.023$). Solix V1 and Solix V4 were the devices that detected the highest differences between zones, with Solix V4 being particularly performant in the detection of GPD differences. Agreement between the 3 instruments was good (k = 0.86 for PD and k = 0.88 for GPD).

In the subgroup of severe DR, only V4 detected a lower PD and VLD in the DCP in the perivenular zone compared with the other zones ($P = 0.040$ and $P = 0.049$ respectively). Similarly, it was the only one to detect a significantly higher VDI in the perivenular zone of the DCP ($P = 0.033$). Lastly, V4 detected a higher GPD in both the DCP ($P = 0.030$) and SCP ($P = 0.038$) in the perivenular zone, whereas V1 detected only a borderline higher perivenular GPD in the DCP ($P = 0.082$). Solix V4 was the instrument that detected the biggest differences between zones in this subgroup of patients. Agreement between devices was moderate (AngioVue versus V1 and V4) to good (V1 with V4). Table 3 reports detailed results for moderate and severe subgroups as concerns quantitative parameters.

Discussion

Diabetic retinopathy is the most common microvascular complication of diabetes, involving up to one-third of patients diagnosed with diabetes worldwide.[23,24] It accounts for 15% to 17% of total blindness in Europe and the United States, and its prevalence is expected to further increase within the next decade.[25] In this

Table 3. Comparison of Full Macular, Periarteriolar, and Perivenular PD, VLD, VDI, and GPD in SCP and DCP of Patients with Moderate DR or Severe DR

			Moderate DR			
			Full Macular	*Periarteriolar*	*Perivenular*	P
PD	SCP	AngioVue	45.38 ± 2.13	45.18 ± 2.09	44.40 ± 3.04	0.063
		V1	44.36 ± 1.94	44.17 ± 1.94	43.00 ± 1.24	0.041
		V4	44.16 ± 1.23	43.98 ± 1.28	42.90 ± 1.01	0.030
	DCP	AngioVue	36.49 ± 0.99	36.25 ± 0.98	35.81 ± 0.99	0.057
		V1	36.24 ± 1.03	36.15 ± 1.07	34.84 ± 1.06	0.038
		V4	36.17 ± 1.00	36.06 ± 0.96	34.97 ± 0.90	0.040
VLD	SCP	AngioVue	4.80 ± 0.31	4.79 ± 0.28	4.60 ± 0.31	0.071
		V1	4.84 ± 0.22	4.72 ± 0.20	4.35 ± 0.22	0.017
		V4	4.82 ± 0.24	4.71 ± 0.23	4.40 ± 0.24	0.038
	DCP	AngioVue	4.15 ± 0.21	4.12 ± 0.20	3.90 ± 0.19	0.081
		V1	4.11 ± 0.20	4.09 ± 0.18	3.72 ± 0.22	0.031
		V4	4.07 ± 0.19	4.01 ± 0.17	3.71 ± 0.19	0.028
VDI	SCP	AngioVue	9.45 ± 0.17	9.43 ± 0.15	9.65 ± 0.18	0.13
		V1	9.16 ± 0.14	9.35 ± 0.11	9.88 ± 0.13	0.026
		V4	9.16 ± 0.16	9.33 ± 0.17	9.75 ± 0.15	0.034
	DCP	AngioVue	8.79 ± 0.20	8.79 ± 0.22	9.18 ± 0.22	0.059
		V1	8.81 ± 0.15	8.83 ± 0.14	9.36 ± 0.13	0.042
		V4	8.88 ± 0.18	8.99 ± 0.17	9.43 ± 0.18	0.037
GPD	SCP	AngioVue	13.53 ± 0.33	13.42 ± 0.31	13.58 ± 0.30	0.25
		V1	13.66 ± 0.26	13.75 ± 0.22	13.90 ± 0.25	0.44
		V4	13.71 ± 0.21	13.88 ± 0.22	14.51 ± 0.24	0.023
	DCP	AngioVue	11.57 ± 0.44	11.68 ± 0.46	11.99 ± 0.48	0.041
		V1	11.69 ± 0.23	11.74 ± 0.25	12.08 ± 0.21	0.045
		V4	11.64 ± 0.20	11.80 ± 0.19	12.67 ± 0.18	0.013
			Severe DR			
			Full Macular	*Periarteriolar*	*Perivenular*	P
PD	SCP	AngioVue	44.33 ± 1.17	44.21 ± 1.15	44.04 ± 1.17	0.85
		V1	44.06 ± 1.68	44.01 ± 1.59	43.83 ± 1.44	0.64
		V4	43.72 ± 1.51	43.68 ± 1.66	43.31 ± 1.73	0.12
	DCP	AngioVue	35.92 ± 0.93	35.85 ± 0.81	35.20 ± 0.85	0.37
		V1	35.67 ± 1.48	35.63 ± 1.35	35.06 ± 1.28	0.45
		V4	35.69 ± 1.22	35.35 ± 1.10	34.05 ± 1.17	0.039
VLD	SCP	AngioVue	4.73 ± 0.21	4.69 ± 0.19	4.13 ± 0.18	0.51
		V1	4.59 ± 0.19	4.51 ± 0.18	4.06 ± 0.19	0.66
		V4	4.48 ± 0.18	4.53 ± 0.20	4.24 ± 0.17	0.73
	DCP	AngioVue	4.05 ± 0.07	3.95 ± 0.06	3.57 ± 0.07	0.53
		V1	3.98 ± 0.34	3.91 ± 0.28	3.78 ± 0.29	0.61
		V4	3.88 ± 0.28	3.92 ± 0.23	3.35 ± 0.19	0.049
VDI	SCP	AngioVue	9.37 ± 0.23	9.42 ± 0.21	10.66 ± 0.23	0.040
		V1	9.59 ± 0.18	9.75 ± 0.16	10.79 ± 0.16	0.038
		V4	9.75 ± 0.19	9.64 ± 0.18	10.21 ± 0.17	0.16
	DCP	AngioVue	8.86 ± 0.12	9.07 ± 0.15	9.85 ± 0.11	0.09
		V1	8.96 ± 0.17	9.11 ± 0.16	9.27 ± 0.17	0.25
		V4	9.19 ± 0.17	9.01 ± 0.18	10.16 ± 0.18	0.033
GPD	SCP	AngioVue	15.03 ± 0.30	15.10 ± 0.34	15.26 ± 0.29	0.59
		V1	15.11 ± 0.27	15.23 ± 0.30	15.31 ± 0.25	0.61
		V4	15.20 ± 0.19	15.36 ± 0.20	15.77 ± 0.21	0.038
	DCP	AngioVue	12.42 ± 0.40	12.68 ± 0.35	12.79 ± 0.38	0.78
		V1	12.49 ± 0.27	12.60 ± 0.29	12.83 ± 0.30	0.082
		V4	12.51 ± 0.29	12.79 ± 0.23	13.34 ± 0.27	0.030

DCP = deep capillary plexus; DR = diabetic retinopathy; GPD = geometric perfusion density; PD = perfusion density; SCP = superficial capillary plexus; VDI = vessel diameter index; VLD= vessel length density; V1 = single volume; V4 = 4 volumes mode with automatically averaged scan.

perspective, more and more accurate screening and prognostic tools are warranted to optimize the standard of care and reduce public cost of the disease. OCT angiography analysis of quantitative characteristics of the retinal capillary plexa has become an increasingly important prognostic tool for DR during the previous

decade, providing valuable in vivo assessment of retinal capillary nonperfusion, which is believed to be the common primum-movens of sight-threatening complications of the disease. The OCTA quantitative parameter that most directly indicates the amount of retina suffering from chronic ischemic condition is the newly described GPD, which identifies regions of retinal tissue that are inadequately perfused because of alterations in vessel geometry and capillary loss (e.g., vascular dropout, displacement, tortuosity), causing a distance of these regions from the closer capillary bed that exceeds the limit of oxygen diffusion. This limit, which is estimated to be almost 30 mm in the human retina, takes into account not only the ability of oxygen to diffuse within retinal tissue but also its consumption rate over the path by the highly metabolic demanding retinal cells.[8] In our study, GPD was found to be statistically significantly higher in the perivenular zone compared with the periarteriolar and full macular zones, this difference being particularly evident in the first clinical stages of the disease (diabetes without DR and mild DR) and, interestingly, prominent in the DCP. This is consistent to what was found by Nesper et al,[10] that demonstrated GPD to be compromised more prominently in the perivenular DCP in diabetic patients without DR compared with healthy controls. This characteristic pattern of primarily perivenular retinal ischemia was unanimously shown by all 3 used devices and involved both the SCP and DCP in the early stages of the disease. Moreover, it was also confirmed by the analysis of PD, VLD, and VDI, which were found to be significantly lower in the perivenular zone, even though not in all measurements. As concerns patients with moderate DR, the prevalent perivenular location was identified only in the DCP. Lastly, in patients with severe DR, only averaged acquisitions detected a difference between the perivenular zone and the rest of the macular field, and DCP was the most involved plexus.

Previous literature[26] reported how moderate and severe DR were characterized by less variability in DCP quantitative parameters and that later stages of nonproliferative DR were better distinguished by SCP perfusion parameters. Altogether, these findings suggest an early compromission of the DCP, which encounters a plateau of damage in later stages of the disease. The prevalent involvement of the DCP in the perivenular microvascular degeneration process is compatible with the fact that the DCP drains directly into venules and is therefore the first capillary bed to encounter impairment in the context of venular damage.[27–30] The reason for an earlier involvement of the DCP might be the higher susceptibility to ischemic damage due to its localization in a watershed zone, where the deep layer of the retinal circulation sits next to high oxygen requirements of the outer plexiform layer. In fact, evidence in the literature already demonstrated that the DCP is more susceptible to ischemic damage in diabetic patients.[6] Interestingly, in our study, all of the considered parameters (PD, VLD, VDI, and GPD) uniformly revealed DR capillary nonperfusion to be at first localized in the perivenular zone. Perfusion density is commonly used in clinical practice to monitor progression of DR because decrease in macular DCP PD is correlated to an increased risk of DR progression and a decrease in SCP PD is correlated to an increased risk of diabetic macular edema development.[31] In this context, closer attention to the perivenular decrease of PD might improve screening and monitoring performance compared with the measurement performed on the full macular OCTA. In our study, high-speed acquisition alone (represented by Solix V1) and, even more, high-speed acquisition coupled with averaging technique (represented by Solix V4) brought to an underestimation of PD and a parallel overestimation of GPD, which is coherent with what was found in the literature in other courts of diabetic patients.[32,33] Nevertheless, we detected a good agreement between high-speed techniques and moderate agreement of both with standard acquisition (AngioVue). Our results confirmed what was previously found by Crincoli et al,[32] since the higher differences between zones were detected by Solix V1 in early stages and Solix V4 in advanced stages of the disease, and this is compatible with the optimized performances demonstrated for each of the 2 acquisition modes in early and advanced DR, respectively.

Limitations of the study include the small sample size and the lack of an available fluorescein angiography examination to demonstrate functional perfusion defects in the same zones. Moreover, the novelty of the method and the lack of literature agreement on image processing technique of choice for en face OCTA quantitative analysis might represent an additional limitation. In conclusion, our results show that OCTA-assessed diabetic retinal ischemia is more pronounced in the perivenular zones in all stages of the disease, and that, in case of advanced stages of the disease, this difference can best be highlighted with averaging technology. We encourage further studies on the topic to confirm our findings.

Footnotes and Disclosures

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All authors have completed and submitted the ICMJE disclosures form.

The authors made the following disclosures:

A.M.: Consulting fees — Novartis; Honoraria — Novartis, Luneau; Travel expenses—Allergan.

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HUMAN SUBJECTS: Human subjects were included in this study. The Fédération France Macula Ethics Committee approved the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

No animal subjects were included in this study.

Author Contributions:

Conception and design: Crincoli, Colantuono, Miere, Zhao, Souied

Analysis and interpretation: Crincoli, Colantuono, Miere, Ferrara

Data collection: Crincoli, Colantuono, Zhao

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Overall responsibility:

Abbreviations and Acronyms:
DCP = deep capillary plexus; **DR** = diabetic retinopathy; **GPD** = geometric perfusion deficit; **OCTA** = OCT angiography; **PD** = perfusion density; **ROIs** = regions of interest; **SCP** = superficial capillary plexus; **VDI** = vessel density index; **VLD** = vessel length density; **V1** = single volume; **V4** = 4 volumes mode with automatically averaged scan.

Keywords:
Capillary ischemia, Diabetic retinopathy, Geometric perfusion density, OCT angiography.

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