Comparison of Widefield OCT angiography features between severe non-proliferative and proliferative diabetic retinopathy

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Supplementary material 1 – Algorithmic steps

We extracted for each eye a full version and a binarized version of each the 4x4 and the 10x10 images.
We originally had the software analyse a cropped central 4x4mm section of the macula from the 10x10mm image to analyse the FAZ. However, we noted that the separate 4x4mm image was of higher quality, so we used both sizes for our analysis.
First, we had the software analyse the patient's 10x10mm image in binary and complete forms. Then we also analysed the 4x4mm binary image and its full image. The semiautomatic approach was used to define the centre of the fovea on both 4x4 and 10x10mm images. The optic disc area and eventual artefacts were manually segmented and excluded for qualitative analysis.
We analysed four main characteristics of the images:

- **Average Percentage of Skeletonised Capillary Vessels**: extent of the smaller capillary vessel network that covers the retina, excluding areas taken up by larger vessels and the FAZ after binarization and skeletonisation.
- **Mean Capillary Intensity**: capillary blood flow assessment by measuring the intensity of the small vessel/capillary pixels. We analysed the capillary vasculature's mean intensity (brightness scale: 0-black to 255-white) on the 10x10mm image. We also calculated the percentage of capillaries and the percentage of skeletonised capillaries from the 10x10mm image. Finally, we calculated the number (if any) of ischemic areas and the mean of the ischemic areas. We applied the threshold to remove low-intensity areas and applied morphological reconstruction (the area around the ischemic area). All intensity values described in this article are defined by average intensity of pixel values of the entity analysed.
- **FAZ (Foveal avscular area)**, and ischemic areas around the FAZ, the periFAZ (1/4mm out from the FAZ) and the paraFAZ (1/4mm out from the periFAZ) on the 4x4mm image. We calculated the vessel ratio and vessel intensity of the periFAZ and the paraFAZ. We also calculated the area of the FAZ and the circularity (perimetry) of the FAZ.
- **Mean Vessel Intensity**: represents the average intensity of the larger segmented vessels. this measure is the only discrete indicator of the status of the larger vessels in our study of 10x10 and 4x4mm images. It allows for some form of representation of all clinically recognisable anatomic features in resulting algorithms.

Supplementary material 2

Sample size calculation

We calculated sample size for this study for key metric mean capillary intensity. Mean capillary intensity of a normal patient from a previous study was 98.69, with σ= 6.23 difference in Normal (group 1) versus Diabetic with retinopathy (group 2) suggests clinically important difference in this metric of 5. Using sample size calculation, we obtained a result of 24. Our objective of recruitment was then 25 patients in each group minimum.

$$k = \frac{n_2}{n_1} = 1$$

$$n_1 = \frac{(\sigma_1^2 + \frac{\sigma_2^2}{k})(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2}{\Delta^2} = \frac{(6.23^2 + \frac{6.23^2}{1})(1.96 + 0.84)^2}{5^2}$$

$$n_1 = 24$$
$$n_2 = k * n_1 = 24$$

Where:
$\Delta = |\mu_2 - \mu_1|$ = absolute difference between two means
σ_1, σ_2 = variance of mean of group 1 and 2
n_1 = sample size for group 1
n_2 = sample size for group 2
α = probability of type I error (0.05)
β = probability of type II error (0.2)
z = critical Z value for a given α or β
k = ratio of sample size for group 2 to group 1

Manual analysis for software validation

We performed manual segmentation with the MATLAB software on the ultra-widefield OCTAs. Artefacts were cropped and disc area segmented. Ischemic areas were segmented between second orders vessels. Segmentation was performed by a research fellow with more than five years of experience in Ophthalmology (Figure 1-S2).

For each image we calculated the disc area and the area of total image in disc areas. For neovascularization and ischemia assessment, we calculated the number of zones, average zone area in disc area, and the average distance from the optic disc. We also calculated the average intensity of ischemic areas and the mean intensity of a control vessel.

We first present here the analysis including all the mosaic images we obtained: Tables 1 and 2 show the baseline characteristics of the groups and Table 3 shows the metrics obtained.

Secondly, we present the results of the manual analysis only for patients who had good enough quality imaging to be included in the semi-automated mosaic analysis: the baselines characteristics are presented in the main article and Table 4 shows the metrics obtained.

								Shapiro-Wilk		Tests			
	Group	N	Mean	Median	SD	Minimum	Maximum	W	p	Test	Statistic	df	p
Age	Proliferative	16	55.7	62	18.4	23	80	0.92	0.169	Student's t	0.455	49	0.651
	Severe	35	53.5	55	15.2	21	87	0.975	0.59				
Duration of diabetes	Proliferative	11	18.7	18	5.5	10	25	0.899	0.18	Student's t	0.622	41	0.538
	Severe	32	16.9	15	9.1	1	43	0.938	0.066				
HbA1c (last 4 months)	Proliferative	7	85.7	86	15.0	64	110	0.982	0.969	Student's t	0.542	19	0.594
	Severe	14	80.1	74	25.2	44	140	0.926	0.272				
BCVA	Proliferative	15	0.4	0.2	0.2	0.02	0.7	0.882	0.051	Mann-Whitney U	124		***0.003***
	Severe	35	0.2	0.1	0.2	-0.2	0.8	0.879	0.001				
4x4 quality	Proliferative	16	5.8	6	1.2	3	8	0.923	0.191	Mann-Whitney U	196.5		0.076
	Severe	35	6.4	6	0.9	5	8	0.873	< .001				
10x10 quality	Proliferative	16	6.3	6.5	1.1	4	8	0.919	0.161	Mann-Whitney U	196		0.073
	Severe	35	6.9	7	0.9	5	8	0.859	< .001				

Table 1: Further description of qualitative patients' characteristics and analysis – Ultra-widefield (N: Number of patients; SD: Standard deviation)

		Type			χ^2 Tests		
		Proliferative	Severe	Total	Value	df	p
Gender	F	9 (56.3%)	7 (20%)	16 (31.4%)	6.7	1	***0.01***
	M	7 (43.8%)	28 (80%)	35 (68.6%)			
	Total	16 (100%)	35 (100%)	51 (100%)			
Type of diabetes (1/2/O)	1	5 (33.3%)	10 (28.6%)	15 (30%)	0.113	1	0.736
	2	10 (66.7%)	25 (71.4%)	35 (70%)			
	Total	15 (100%)	35 (100%)	50 (100%)			
Associated cardiovascular disease	No	5 (31.3%)	9 (25.7%)	14 (27.5%)	2.68	2	0.262
	Yes	5 (31.3%)	19 (54.3%)	24 (47.1%)			
	Unknown	6 (37.5%)	7 (20%)	13 (25.5%)			
	Total	16 (100%)	35 (100%)	51 (100%)			
Confounders (cataract, media opacity…)	No	12 (75%)	30 (85.7%)	42 (82.4%)	0.867	1	0.352
	Yes	4 (25%)	5 (14.3%)	9 (17.6%)			
	Total	16 (100%)	35 (100%)	51 (100%)			

Table 2: Epidemiological description of patients' and scans' characteristics and analysis – Ultra-widefield

		Descriptives				Shapiro-Wilk		Independent Samples Test			
	Group	**N**	**Mean**	**Median**	**SD**	**W**	**p**		**Statistic**	**df**	**p-value**
Area of total image in disc areas	*Proliferative*	15	90.8	89.8	16.2	0.963	0.738	Mann-Whitney U	163		0.638
	Severe	24	96.4	90.1	23.8	0.898	***0.019***				
Disc area (DA) in pixels	*Proliferative*	14	21969.4	21236.5	3209.1	0.939	0.404	Student's t	0.313	36.0	0.756
	Severe	24	21570.9	21274.5	4074.7	0.982	0.929				
Mean Intensity Vessel Control	*Proliferative*	15	243.6	244.1	4.9	0.939	0.365	Student's t	-1.097	37.0	0.280
	Severe	24	245.1	245.7	3.9	0.918	0.053				
Number of ischemic zones	Proliferative	15	9.5	8.0	5.5	0.872	0.036	Mann-Whitney U	37.0		**< .001**
	Severe	24	3.1	3.0	2.4	0.929	0.092				
Average area of ischemic zones (DA)	Proliferative	15	1.3	1.1	1.0	0.895	0.080	Mann-Whitney U	91.0		**0.033**
	Severe	21	0.7	0.5	0.6	0.869	***0.009***				
Average distance ischemic zone from disc (in disc diameter (DD))	Proliferative	15	4.5	4.4	1.0	0.962	0.720	Student's t	1.892	34.0	0.067
	Severe	22	3.8	3.7	1.1	0.941	0.204				
Average intensity of ischemic areas (DA)	Proliferative	15	75.7	71.6	14.8	0.951	0.536	Student's t	2.189	34.0	0.036
	Severe	21	67.0	67.3	8.9	0.945	0.278				
Number of neovascular zones	Proliferative	15	1.9	1.0	1.3						
Average neovascular zones area (DA)	Proliferative	15	0.4	0.1	1.0						
Average neovascular zones distance from disc (DD)	Proliferative	15	3.7	3.6	0.9						

Table 3: Description and statistical analysis of the metrics obtained – Ultra-widefield manual segmentation (N: Number of patients; SD: Standard deviation)

Figure legends

Figure 1- S2 : Examples of ultra-widefield image segmentation; On the left the original image and on the right the manually segmented image

	Group Descriptives					Shapiro-Wilk		Statistical tests				
	type	N	Mean	Median	SD	W	p	Test	H_a	Statistic	df	p-value
Mean vessel intensity	Proliferative	27	223.1	222.8	3.7	0.955	0.276	Student's t	$\mu_P < \mu_S$	-1.6607	71	*0.051*
	Severe	46	224.4	224.1	2.8	0.979	0.58					
Mean capillaries intensity	Proliferative	26	131.4	130.7	5.4	0.979	0.847	Student's t	$\mu_P < \mu_S$	0.547	66	*0.707*
	Severe	42	130.7	130.5	5.0	0.973	0.4					
Density of capillaries	Proliferative	28	42.5	42.7	2.6	0.947	0.168	Student's t	$\mu_P < \mu_S$	-0.1347	73	*0.447*
	Severe	47	42.6	43.0	2.3	0.973	0.337					
Density of skeletonised capillaries	Proliferative	28	8.4	8.4	0.6	0.958	0.308	Student's t	$\mu_P < \mu_S$	-0.616	73	*0.27*
	Severe	47	8.4	8.5	0.6	0.973	0.333					
Density of capillaries in peri FAZ	Proliferative	28	31.7	31.2	4.7	0.987	0.973	Student's t	$\mu_P < \mu_S$	-0.5365	73	*0.297*
	Severe	47	31.9	32.4	5.8	0.96	0.107					
Density of skeletonised capillaries in peri FAZ	Proliferative	28	7.9	7.8	1.2	0.982	0.885	Student's t	$\mu_P < \mu_S$	-0.7762	72	*0.22*
	Severe	46	8.2	8.1	1.4	0.989	0.936					
Intensity of capillaries in peri FAZ	Proliferative	27	95.8	94.7	16.8	0.966	0.516	Student's t	$\mu_P < \mu_S$	-1.3592	70	*0.089*
	Severe	47	100.4	99.2	13.8	0.987	0.874					
Density of capillaries in para FAZ	Proliferative	28	35.8	36.0	3.0	0.966	0.467	Student's t	$\mu_P < \mu_S$	-1.2503	72	*0.108*
	Severe	46	36.7	36.3	3.0	0.978	0.509					
Density of skeletonised capillaries in para FAZ	Proliferative	28	8.8	8.8	0.8	0.963	0.403	Student's t	$\mu_P < \mu_S$	-0.9053	72	*0.184*
	Severe	46	9.0	8.8	0.9	0.953	0.06					
Intensity of capillaries in para FAZ	Proliferative	25	120.5	119.9	10.2	0.923	0.06	Student's t	$\mu_P < \mu_S$	-1.2714	69	*0.104*
	Severe	46	123.4	122.8	8.8	0.988	0.917					
Density of capillaries in the 4x4mm image	Proliferative	28	39.2	39.5	1.8	0.952	0.219	Student's t	$\mu_P < \mu_S$	-0.4573	71	*0.324*
	Severe	45	39.4	39.6	1.8	0.984	0.791					
Intensity of capillaries in the 4x4mm image	Proliferative	26	134.7	136.2	7.4	0.655	**< .001**	Mann-Whitney U	$\mu_P < \mu_S$	593		*0.479*
	Severe	46	135.6	135.9	4.8	0.976	0.437					
Total areas of ischemia	Proliferative	26	810.4	391.5	1497.4	0.555	**< .001**	Mann-Whitney U	$\mu_P > \mu_S$	596		*0.512*
	Severe	46	1467.0	332.5	3250.5	0.492	**< .001**					

Number of ischemic areas	Proliferative	28	2.7	1	5.0	0.544	**< .001**	Mann-Whitney U	$\mu_P > \mu_S$	642	*0.428*
	Severe	47	2.6	1	4.0	0.699	**< .001**				
Area of FAZ	Proliferative	28	1847.2	1713	342.0	0.48	**< .001**	Mann-Whitney U	$\mu_P > \mu_S$	510	*0.94*
	Severe	46	1931.9	1749.5	479.1	0.506	**< .001**				
Perimeter of FAZ	Proliferative	28	0.9	1.0	0.2	0.637	**< .001**	Mann-Whitney U	$\mu_P > \mu_S$	524	*0.084*
	Severe	46	0.9	1.0	0.2	0.721	**< .001**				

Supplementary material 3 – Full analysis 4x4 and 10x10mm images (N: Number of patients; SD: Standard deviation; FAZ: Foveal avascular zone)