Comparing Fundus Fluorescein Angiography (FFA) and Swept Source Optical Coherence Tomography Angiography (SS-OCTA) in the evaluation of diabetic macular perfusion

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Summary statement

This paper compares fundus fluorescein angiography and swept source optical coherence tomography angiography in the assessment of macular perfusion of diabetic patients.

Abstract:

Purpose: To compare fundus fluorescein angiography (FFA) and swept-source optical coherence tomography angiography (SS-OCTA) in the evaluation of macular perfusion in diabetic patients.

Methods: 41 eyes (21 diabetic patients) seen at Moorfields Eye Hospital (London) over a 1-month interval underwent color fundus photography, FFA and SS-OCTA imaging of the capillary superficial plexus (SP) using two different protocols: 3x3mm and 4.5x4.5mm. Quantitative assessment (FAZ diameters and area), qualitative analysis (macroscopic and microscopic levels) and ETDRS diabetic macular ischemia (DMI) grading were performed. Artifacts were recorded. Intraclass correlation coefficients (ICCs) and weighted kappa values were calculated.

Results: Mean(SD) FAZ area was 0.695(0.52)mm^2 on FFA, 0.627(0.54)mm^2 on SS-OCTA 3x3 and 0.701(0.54)mm^2 on SS-OCTA 4.5x4.5 protocol. ICCs showed good agreement between FFA and SS-OCTA for both vertical diameter and FAZ area measurements. The agreement between SS-OCTA 3x3 and 4.5x4.5 was good for all quantitative measurements. Weighted kappa for DMI grading showed low to fair agreement between FFA and SS-OCTA whereas the agreement was good between two different SS-OCTA protocols.

Conclusion: SS-OCTA is a reproducible technique in the assessment of macular perfusion in diabetic patients with special regards to FAZ analysis. The agreement with FFA is limited especially for DMI grading. FFA is more sensitive in identifying microaneurysms.

Introduction:

Diabetic retinopathy (DR) is one of the major causes of legal blindness worldwide[1] and the leading cause of vision loss in adults aged 20-74 years.[2] Diabetic macular ischemia (DMI) is one of the commonest findings in diabetic patients: Sim DA et al. demonstrated the presence of some degree of DMI in about 40% of cases of patients with DR, in a tertiary referral center.[3] DMI is characterized by an enlargement of the foveal avascular zone (FAZ) and the presence of parafoveal areas of capillary non-perfusion ("drop-out"). Multiple studies have shown the correlation between impaired foveal perfusion in diabetic patients and their visual acuity.[4,5] Furthermore, the progression of DMI has been linked to the potential deterioration of visual acuity,[3] thus the importance of its recognition in the assessment of patients affected by DR. Recently, microscopic imaging studies on rodents have demonstrated the breakdown of the outer blood retinal barrier induced by ischemia.[6]

The gold standard imaging technique for the assessment of macular perfusion is fundus fluorescein angiography (FFA). FFA involves the intravenous injection of a fluorescent dye followed by serial fundus photographs. The most common and less serious side effects of FFA are nausea, vomiting, yellow pigmentation of skin and urine. More serious adverse events include anaphylactoid reactions ranging from skin rash and itching to severe anaphylactic shock. An intrinsic limitation of the technique is related to the physical properties of the dye that can leak from pathologic blood vessels masking the underlying tissue fluorescence.[7]

Spectral domain optical coherence tomography angiography (SD-OCTA) is a non invasive technique that allows the visualization of blood vessels by

comparing the decorrelation signal between multiple sequential OCT B-scans taken at the same cross section.[8] The resulting decorrelation map is a reconstruction of the network of blood vessels with erythrocytes flowing into them. Three different vascular layers can be visualized: the superficial (SP) and deep (DP) capillary plexes and the choriocapillaris. OCTA does not involve the use of a dye, making it theoretically risk-free and independent from the physical properties of fluorescein. Extravasal leakage is often present in patients with DR due to the loss of pericytes and subsequent increased vascular permeability. Previous work by Bradley PD et al. demonstrated significant agreement between OCTA and FFA in the assessment of macular perfusion in subjects with DR.[9]

Swept source technology in ophthalmic imaging has been investigated extensively since the first report in 2006.[10] It uses longer-wavelength infrared light compared to traditional spectral domain OCT. This enables for faster scans (100,000 A-scans per second) and increased tissue penetration with easier imaging through media opacities and improved visualization of the choroid. Numerous reports have been published on the applications of OCTA in patients with DR. In 2015, Ishibazawa et al. have published a pilot study on the OCTA features of DR, imaging microaneurysms, venous beading, new vessels and capillary drop-out.[11] Takase at al. have demonstrated the presence of macular circulation impairment on OCTA even before the development of retinopathy.[12] Agemy SA and colleagues have shown good correlation between OCTA images and the clinical features of DR.[13]

A swept source-OCTA (SS-OCTA) device is available from the Japanese company TOPCON [Topcon Corporation, Tokyo, Japan]: the DRI OCT Triton. While most reports in the literature have employed prototypes [14,15, 26] this is the

first commercially available SS-OCTA device. The DRI OCT Triton employs the OCTARA (OCTA ratio analysis),[16] a specific algorithm that according to the manufacturer increases detection sensitivity of retinal microvasculature.[17 18 19]

The aim of this study is to compare traditional FFA and SS-OCTA in the evaluation of macular perfusion in diabetic patients. To the best of our knowledge, this is the first report on this matter.

Methods:

In this retrospective observational study we aimed to systematically evaluate the quantitative and qualitative features of FFA and SS-OCTA images in the assessment of macular perfusion in diabetic patients.

The study location was the Medical Retina Service at Moorfields Eye Hospital, London. The period of study was from the 1st of January 2016 until the 28th of May 2016. Approval for data collection and analysis was obtained from the Institutional Review Board of Moorfields Eye Hospital, and adhered to the tenets set forth in the Declaration of Helsinki.

Patient demographics and clinical data were obtained from electronic medical records. Previous retinopathy or maculopathy treatments were recorded. Inclusion criteria were: history of diabetes mellitus with or without evidence of retinopathy and/or maculopathy. Exclusion criteria were: significant media opacity that obscured the view of the fundus and concurrent ocular conditions interfering with normal retinal blood flow (e.g. age-related macular degeneration, retinal vein or arterial occlusion, radiation retinopathy, sickle-cell retinopathy, idiopathic retinal teleangiectasia).

FFA and SS-OCTA images were acquired 1 week or within one month from each other.

Image acquisition

Color fundus photographs and FFA obtained with the Topcon TRC50IX [Topcon Corporation, Tokyo, Japan] were used for diabetic retinopathy severity grading.

SS-OCTA was performed with the Topcon DRI OCT Triton device [Topcon Corporation, Tokyo, Japan], using two different acquisition protocols: 3x3mm and 4.5x4.5mm, centered on the fovea (Fig.1). A web-based ophthalmic data management platform, IMAGEnet®6, was used for image processing.

Diabetic retinopathy grading

Grading of the retinopathy was based on the Early Treatment Diabetic Retinopathy Study (ETDRS) classification, endorsed in 2003 by the American Academy of Ophthalmology Guidelines Committee[20] and widely used in clinical trials.

FFA image analysis

Standard fluorescein angiograms were obtained from our digital database and they were analyzed quantitatively and qualitatively by a single examiner. The examiner was masked in that he could not pair each image with the corresponding patient.

Early-to-mid phase images were considered for the evaluation of macular perfusion, late frames were used to grade macular edema.

Quantitative analysis included measurement of maximum vertical and horizontal diameters (μm) as well as FAZ area (mm^2) for each fluorescein angiogram. The ImageJ suite, version 1.51a (available in the public domain at http://imagej.nih.gov) was used to analyze FFA images.

Qualitative analysis was performed at two different levels:

Macroscopic level: all images were classified based on standard reference photos from the ETDRS DMI grading system with special regards to FAZ alterations.[21] According to this classification, a specific score is assigned as follows: absent (no alteration of the capillary outline), questionable (outline not smoothly round or oval, but visible irregularities, not definitely abnormal), mild (outline definitely destroyed for less than one half the original circumference), moderate (outline destroyed for one half or more of the original circumference, but some remnants remain), severe (capillary outline completely destroyed), ungradable.

Macroscopic analysis also included stratifying each image into one of four quality categories (excellent, good, poor but gradable, ungradable) based on the presence of image artifacts and their potential to limit detailed description of angiographic findings.

Microscopic level: the macular area only was screened for specific angiographic features of diabetic retinopathy (microaneurysms, vascular loops, intraretinal microvascular abnormalities or IRMAs) and maculopathy (capillary drop-out).

SS-OCTA image analysis

Images obtained from the IMAGEnet®6 database were analyzed from a quantitative and qualitative point of view by a single masked examiner. En face

SS-OCTA images were generated for the SP only, to allow for better comparison with corresponding FFA images. Manual segmentation was performed for each scan.

Quantitative analysis was performed using the ImageJ suite scaling each image with a built in plugin. The standard SS-OCTA image format imported from IMAGEnet®6 was 320x320 pixels regardless of the scanning protocol used (3x3 or 4.5x4.5). ImageJ was instructed to scale the image proportionally from pixels to mm. Systematic measurements of both maximum vertical and horizontal diameters (μm), as well as FAZ area (mm^2), were included in the assessment using the “straight line” and “freehand” tools respectively.

Qualitative analysis was performed as previously described for FFA both at macroscopic and microscopic levels. Figure 2 shows an example of microscopic diabetic retinal changes on corresponding FFA and SS-OCTA images.

Image artifacts were recorded and these included: white lines, vessel displacement, gap defects and projection artifacts (Fig. 3).

Statistical analysis

Descriptive statistics were used to summarize clinical characteristics. Normal distribution was assessed using the Kolmorov Smirnow test. Kruskal-Wallis test was performed to compare non-normal distributed variables among three groups. The intraclass correlation coefficient (ICC) and its 95% confidence interval (CI) were used to assess the agreement between FFA and SS-OCTA in pairwise for continuous variables. Intraclass correlation coefficient (range: 0–1) was accepted as good agreement ICC 0.75-1, moderate agreement ICC 0.5-

0.75 and poor agreement ICC<0.5. [22] The agreement between imaging techniques was also assessed by Bland-Altman plots.

Weighted kappa test was used to assess the agreement of methods for ordinal variables in pairwise. In order to assess the interobserver agreement weighted kappa values were used and 0.00-0.20 showed low agreement, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 good agreement, 0.81-1.00 very good agreement.[23] A p value of <0.05 was considered statistically significant. Statistical analyses were performed using the MedCalc13 software (Mariakerke, Belgium).

Results:

Patient demographics and clinical characteristics

Patient demographics and clinical characteristics are summarized in Table 1. 41 eyes (21 patients) met the inclusion criteria. Mean patient age was 63.8 years (range, 37-81). The male:female ratio was 15:6. In 18 cases (43.7%) DR was diagnosed as non-proliferative whereas proliferative diabetic retinopathy (PDR) was evident in 22 eyes (53.6%). DR was absent in just 1 case. DR did not require any form of treatment in 41.4% of our cohort (17 eyes). 24 eyes (58.5%) underwent one or more sessions of panretinal photocoagulation due to the presence of proliferative disease and in 3 cases (7.3%) pars plana vitrectomy was performed to treat the complications of PDR.

More than 70% of eyes were affected by any degree of diabetic macular edema (DME). However, in 43.9% of eyes, no treatments were performed.

Interval between FFA and SS-OCTA imaging

The interval between two imaging techniques was less than one week in the majority of cases (30 eyes – 73.1%). In the remaining 11 cases SS-OCTA was performed within 1 month from FFA.

FAZ quantitative assessment

FAZ quantitative analysis was possible for 35 eyes (85.3%) on FFA, 38 eyes (92.6%) on SS-OCTA 3x3 and 37 eyes (90.2%) on SS-OCTA 4.5x4.5. Mean (SD) FAZ area was 0.695(0.526) mm^2 on FFA, 0.627(0.544) mm^2 on SS-OCTA 3x3 and 0.701(0.542) mm^2 on SS-OCTA 4.5x4.5.
Measurements of vertical and horizontal maximum FAZ diameters are shown in Table 2.

To evaluate maximum vertical diameter measurements of the FAZ, agreement of methods were analyzed in pairwise. Vertical measurement ICCs were significant between FFA and SS-OCTA 4.5X4.5 (0.822,[0.667-0.909],p<0.001), between FFA and SS-OCTA 3x3 (0.878,[0.768-0.938],p<0.001) and between SS-OCTA 3X3mm and SS-OCTA 4.5X4.5 (0.896,[0.807-0.946],p<0.001).

Horizontal FAZ measurements were evaluated with the same statistical method. ICCs were significant between FFA and SS-OCTA 4.5X4.5 (0.742,[0.534-0.865],p<0.001), between FFA and SS-OCTA 3x3 (0.595,[0.321-0.777],p<0.001) and between SS-OCTA 3X3 and SS-OCTA 4.5X4.5 (0.844,[0.715-0.917],p<0.001). The agreement between FFA and both SS-OCTA 3x3 and 4.5x4.5 was moderate, but it was good between two different SS-OCTA protocols.

Concerning total FAZ area, ICCs were significant for FFA and SS-OCTA 4.5X4.5 (0.885,[0.775-0.943],p<0.001), for FFA and SS-OCTA 3x3 (0.9,[0.806-

0.950],p<0.001), for SS-OCTA 3X3 and SS-OCTA 4.5X4.5 (0.933,[0.871-0.965],p<0.001). Figure 4 shows an example of multimodal analysis with FAZ area evaluation.

The above results were also demonstrated with Bland-Altman plots (Figures 5-7) assessing agreement for FAZ area measurement with three different techniques.

ETDRS DMI grading

In the majority of cases, DMI was classified as Moderate, regardless of the imaging technique used. However, the cumulative number of patients with Moderate and Severe DMI was higher on SS-OCTA compared to FFA. Table 3 summarizes DMI grading according to the ETDRS system.

Weighted kappa coefficient was used to measure the degree of agreement for the ordered assessment of DMI grading among methods by pairwise comparison. The degree of agreement of DMI grading between FFA and SS-OCTA 4.5X4.5 was fair (0.236,[95% CI, 0.015-0.456]), low (0.199,[95% CI,0.009-0.389]) between FFA and SS-OCTA 3X3 and good (0.654,[95% CI, 0.454-0.855]) between SS-OCTA 3X3 and 4.5X4.5.

Qualitative assessment

Qualitative macroscopic assessment was possible in all cases. In the vast majority of eyes, images were gradable both on FFA and SS-OCTA and they were considered of excellent or good quality in more than 70% of eyes on FFA and in more than 80% of cases on SS-OCTA. Images were deemed ungradable

in 6 (14.6%), 3 (7.3%) and 4 (9.7%) cases on FFA and SS-OCTA 3x3 and 4.5x4.5 respectively.

Qualitative microscopic analysis was possible for all FFA pictures whereas in 3 cases (SS-OCTA 3x3) and in 4 eyes (SS-OCTA 4.5x4.5) images were of poor quality, making it impossible to identify specific retinal diabetic changes. Microaneurysms and capillary drop-out were the commonest findings both on FFA and SS-OCTA (Table 4).

For all 41 eyes, SS-OCTA artifacts assessment was possible. White lines and vessel displacement were the commonest image artifacts both on 3x3 and 4.5x4.5 SS-OCTA protocols. Gap defects and projection artifacts were irrelevant. Results are summarized in Table 5.

Discussion:

In this study we compared the differences between FFA and SS-OCTA in the evaluation of macular perfusion of diabetic patients seen in a tertiary referral center over a 1-month interval.

FFA is at present the gold standard imaging technique in the evaluation, management and follow-up of patients affected by diabetic retinopathy and maculopathy, but due to its potentially serious side effects, it may be precluded in patients with multiple comorbidities. Over the last few years, new technologies have been evaluated as an alternative to FFA and, to date, OCTA has shown the biggest potential in this field, being non-invasive, easy to acquire and fast to perform. This is confirmed by the large number of peer-reviewed articles that have been published in the literature in recent years, showing big interest from the scientific community on this new technology. SD-OCTA

devices are so far the most widely studied, being the first ones that have become available in clinical practice. However, SS-OCTA is becoming increasingly popular thanks to its theoretical advantages (faster scans, deeper penetration through media opacities, better imaging of the choroid) compared to spectral domain technology.

To the best of our knowledge this is the first report systematically assessing the potential use of SS-OCTA as a non-invasive adjunct to traditional angiography in diabetic patients.

Our findings suggest that except for horizontal FAZ diameter measurement, where the agreement between FFA and SS-OCTA was moderate (ICCs 0.595, $p<0.001$ and 0.742,$p<0.001$ for FFA and SS-OCTA 3x3 and 4.5x4.5 respectively), the agreement was good in all other quantitative measurements (vertical FAZ diameter and FAZ area) regardless of the scan protocol used. However, ICCs were higher between SS-OCTA protocols in all quantitative measurements, confirming that this imaging technique has good reproducibility. The above conclusions, with respect to FAZ area measurement, are confirmed by Bland-Altman plots. We may conclude that SS-OCTA is useful in supporting quantitative analysis of the FAZ but, at present, it cannot replace traditional FFA.

Previous studies [9, 24] have shown good agreement between FFA and SD-OCTA in the evaluation of DMI using the ETDRS protocol. Our results are not consistent with these findings as weighted kappa showed low to fair agreement between FFA and SS-OCTA. We may speculate that the longer wavelength used by swept source technology influences the ability of the device to precisely identify the features of the SP. However, further studies are required to

establish whether SS-OCTA is more sensitive in analyzing the DP compared to the SP.

DMI grading on SS-OCTA showed again good agreement between the 3x3 and 4.5x4.5 protocols (weighted kappa 0.654, 95% CI), confirming the fact that SS-OCTA has good reproducibility between different analysis protocols.

Concerning qualitative macroscopic analysis, despite the presence of SS-OCTA artifacts in a non-negligible number of cases, the vast majority of images appeared to be gradable and about half of them were considered of excellent quality for interpretation both on FFA and SS-OCTA. We may conclude that image quality degradation and artifacts intrinsic to both imaging techniques do not interfere significantly with interpretation, making them suitable for a high volume clinic setting.

Microscopic assessment showed that microaneurysms and capillary drop-out were the commonest findings both on FFA and SS-OCTA. FFA identified more microaneurysms than SS-OCTA and these results are consistent with other reports published in the literature. [25, 26] In this respect, we may conclude that traditional FFA is more sensitive in identifying early signs of DR though, especially in the very early stages of the disease, the clinician is unlikely to offer the patient an invasive test involving intravenous injection of a dye. Conversely, SS-OCTA could be virtually performed in all diabetic patients without fear of exposing them to potentially serious adverse events.

Capillary drop-out was another common finding both on FFA and SS-OCTA and this is most likely due to a selection bias in our patient cohort. Most of the eyes enrolled in our study were affected by PDR, thus the underlying advanced retinal pathology resulted in an impaired blood supply often affecting the macular area.

Image artifacts most frequently encountered on SS-OCTA were white lines and vessel displacement. In most cases, these were caused by patient eye movement during image capture and despite being the scans acquired by professional ophthalmic photographers, eye movement-related artifacts occurred in a significant number of eyes. Our experience suggests that even though swept source devices are based on fast imaging acquisition, motion artifacts are not completely absent, thus a faster technology could potentially reduce their occurrence.

Our study has one major strength: it is the first report that systematically compares two different imaging techniques, one of which introduced fairly recently, in the evaluation of macular perfusion and particularly the FAZ of diabetic patients. In addition to this, this is the first study that evaluated the potential of the 4.5x4.5 SS-OCTA analysis protocol.

This study, however, has several limitations, the first one being its retrospective nature. Another caveat is the small cohort size, limiting the statistical significance of our findings: large prospective studies are needed to confirm our encouraging results.

The selection bias represented by different baseline characteristics of the studied population, with special regards to macular edema and previous treatments, is another limitation of our study. A randomized design would exclude this bias.

Our arbitrary choice of taking into account only the SP on SS-OCTA further limits the significance of our findings and particularly our ability to detect early FAZ changes that may become visible in the DP before affecting the SP. However, recent evidence suggests that vessel density measurement of the DP may be influenced by the presence of coexisting macular edema due to the

interference with image segmentation[27 28] while the SP vessel density may be independent of the presence of cystoid edema.[29]

Technologic limitations of SS-OCTA, including increased variability with different axial lengths[30] and the impact of patient cooperation on image quality, introduce an intrinsic bias to our study that needs to be taken into account when drawing definitive conclusions.

In summary, SS-OCTA represents a useful, reliable and easy-to-use imaging technique in the assessment of macular perfusion in diabetic patients with special regards to FAZ analysis. Both 3x3 and 4.5x4.5 analysis protocols appear to be reproducible, however the agreement with traditional FFA remains limited especially in DMI grading. FFA is more sensitive in identifying retinal microaneurysms, but due to its potentially serious adverse events, it is not the ideal imaging technique for patients with early DR and SS-OCTA could eventually find its place in this niche. Based on our experience, overall image quality did not compromise SS-OCTA image interpretation.

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Figure legends

Figure 1. SS-OCTA imaging of the superficial capillary plexus in a patient with preserved macular perfusion. (**A**) 3x3mm protocol. (**B**) 4.5x4.5mm protocol.

Figure 2. Qualitative microscopic findings of DR on corresponding FFA and SS-OCTA images. (**A, D**) Cluster of microanuerysms. (**B, E**) Vascular loop. (**C, D**) Severe capillary drop-out.

Figure 3. SS-OCTA image artifacts (**A**) White lines (arrow) and vessel displacement (dashed arrow). (**B**) Gap defect (arrow). (**C**) Projection artifacts (arrows).

Figure 4. Multimodal analysis of an eye affected by mild non proliferative DR. (**A**) Color fundus photograph. (**B)** Mid phase FFA image demonstrating multiple clusters of microaneurysms and one area of early fluorescein leakage superotemporally to the fovea. (**C**) Swept source optical coherence tomography scan of the foveal region. Faint orange lines highlight layers of the SP segmented by the SS-OCTA device. (**D-F**) Analysis of FAZ area using ImageJ “freehand” tool on FFA, SS-OCTA 3x3 and 4.5x4.5 respectively.

Figure 5. Bland-Altman plot demonstrating the agreement between FFA and SS-OCTA 4.5x4.5 protocol. Mean difference between techniques, -0.05

mm^2, with the representation of the limits of agreement (dotted line) from -1,96s to +1.96s

Figure 6. Bland-Altman plot demonstrating the agreement between FFA and SS-OCTA 3x3 protocol. Mean difference between techniques, 0.01 mm^2, with the representation of the limits of agreement (dotted line) from -1,96s to +1.96s

Figure 7. Bland-Altman plot demonstrating the agreement between SS-OCTA 3x3 and 4.5x4.5 protocols. Mean difference between techniques, -0.06 mm^2, with the representation of the limits of agreement (dotted line) from -1,96s to +1.96s