Title: Optical coherence tomography angiography biomarkers that predict early response to anti-VEGF therapy in diabetic macular oedema

Short Title: DMO – OCTA biomarkers to predict early response

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Background

Diabetic retinopathy (DR) is a major cause of irreversible vision loss in the working-age population.[1]

The social and financial costs of vision loss due to DR are enormous. Large-scale epidemiologic studies have projected a sustained increase in the prevalence and incidence of diabetes mellitus over the ensuing decades and it is predicted that nearly 600 million people worldwide will suffer from this disease in the year 2035.[2]

Diabetic macular oedema (DMO) is the most common cause of vision loss associated with DR[3] and occurs in almost 7% of patients with diabetes mellitus.[4] There are several lines of evidence to suggest that intravitreal anti-vascular endothelial growth factor (anti-VEGF) injections should be used as first line treatment for DMO.[5] In many patients, anti-VEGF treatment results in significant improvements in visual acuity. However, **it is known that a subset of patients will maintain poor vision or continue to lose vision despite anti-VEGF therapy**. These patients are defined as non-responders or those that have suboptimal response to anti-VEGF therapy. A recent *post hoc* analysis of the Diabetic Retinopathy Clinical Research Network Protocol I study revealed that nearly 40% of eyes gain <5 letters after 3 months and approximately 50% of these maintain poorer long-term visual outcomes despite anti-VEGF therapy.[6] Currently, **there is very little knowledge about the clinical biomarkers that predict response to anti-VEGF therapy in the setting of DMO**. This information is urgently needed to better individualise treatment and optimise visual outcomes in DMO.

Pathophysiology of Diabetic Retinopathy and Diabetic Macular Oedema

The clinical signs that characterise DR are consequent to the time-dependent perturbation of retinal pericytes, inter-cellular proteins, muller cells, astrocytes and smooth muscle cells.[7] These changes occur in parallel and culminate in a spectrum of retinal manifestations including microaneurysm formation, capillary non-perfusion, haemorrhages, large vessel abnormalities and pathologic

neovascularisation. Some of these changes are illustrated in the histologic specimen prepared in our

laboratory (Figure 1).
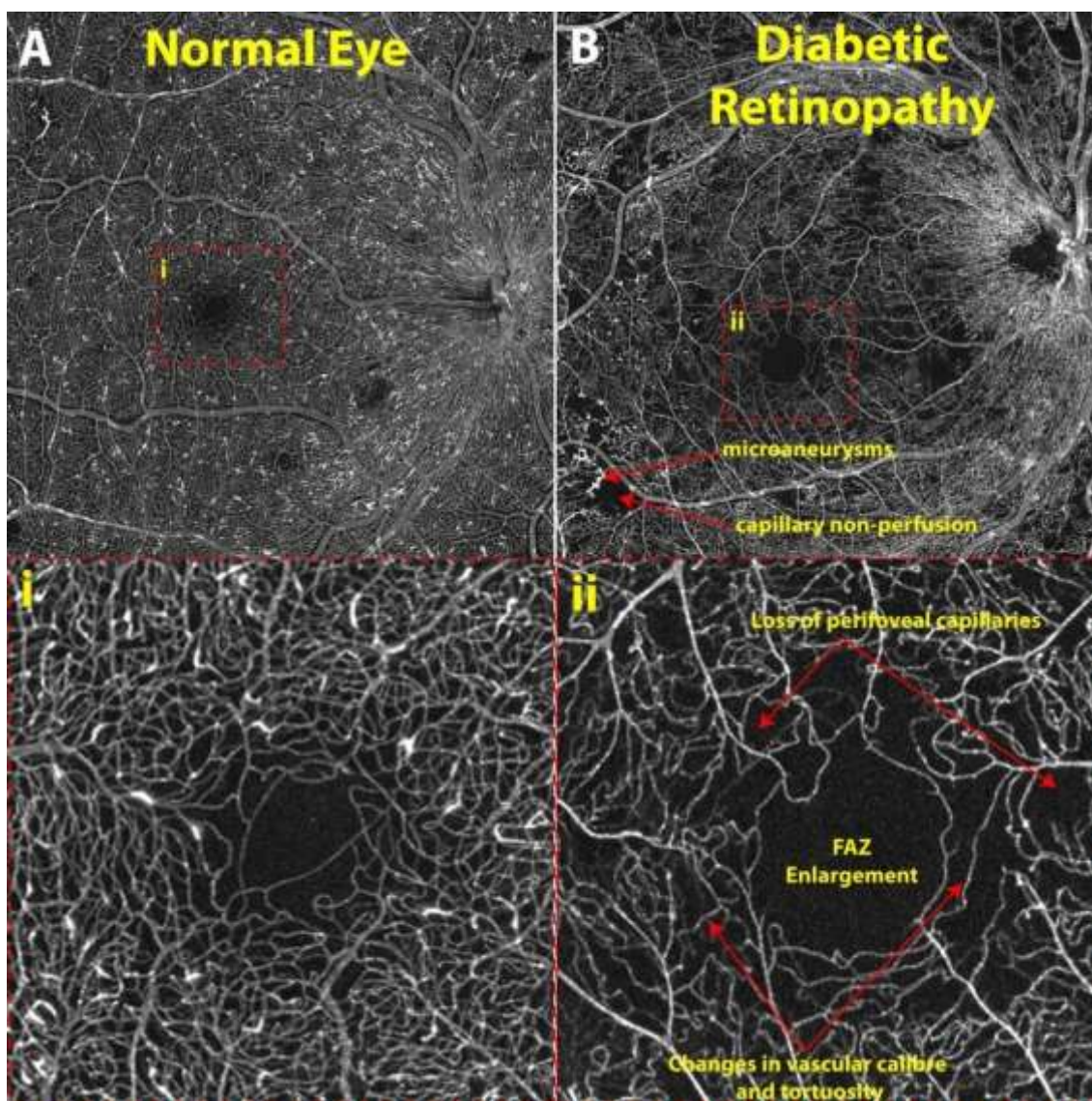
Figure 1 – Histology of the normal eye (A) and an eye with diabetic retinopathy (B). The

topologic characteristics of the macula in the two specimens are provided in insets I and ii.

FAZ = foveal avascular zone.

The macula is a highly specialised area of the retina that, for poorly understood reasons, is prone to oedema. Diabetic macular oedema is due to the breakdown of the blood-retina-barrier (BRB). **The most important molecule to mediate breakdown of the BRB in DR is VEGF** which is upregulated during states of hyperglycemia, protein kinase C activation and advanced glycation end product production.

VEGF receptors are predominantly expressed by endothelial cells in the retinal circulation and are upregulated in the diabetic eye.[8] In organ systems outside the eye, the concentration of VEGF and VEGF receptor expression is correlated with the morphologic properties of the microcirculation.[9] For example, in colorectal cancer specimens, VEGF concentration is associated with microvessel diameter and microvessel counts.[9] Similarly, in the retina, **regional changes in VEGF concentration is expected to modulate quantifiable measures of the macular circulation such as the size of the foveal avascular zone (FAZ), the integrity of the terminal foveal capillary ring, loss of perifoveal capillaries and changes in vascular tortuosity index** (Figure 1 Insets). Importantly, these vascular changes may serve as an indicator that significant changes in VEGF receptor concentration and distribution are occurring within the diabetic eye and may predict response to anti-VEGF therapy.

**Retinal Vascular Imaging in the Clinical Setting**

Optical coherence tomography (OCT) techniques are widely used in the management of diabetic maculopathy.[10] OCT technology has evolved over the past 10 years such that the axial resolving power of most commercial systems is close to 6 μm. OCT therefore allows evaluation of fine retina anatomic detail but in the context of diabetic maculopathy, OCT provides greater information about the consequences of regional BRB breakdown rather than identifying individuals that are predisposed to DMO. For example, anatomic endpoints as seen on OCT that are commonly evaluated in diabetic clinical trials include OCT-derived retinal thickness measurements, number/changes in the number of intraretinal cysts and attenuation/loss of the external limiting membrane and ellipsoid zone.[11] It is widely agreed that structural OCT B scans provide little

information about retinal microvascular anatomy. Rather, these scans provide structural information pertaining to neuronal and glial organisation within the retina.

Fluorescein angiography (FA) is an invasive, dye-based technique for imaging the retinal circulation.

First described in 1961,[12] the technique of FA continues to be widely used for evaluating the diabetic retina. Major disadvantages of FA include its invasive nature, the wide array of adverse effects associated with dye administration and also the expense of this technique. Importantly, my previous work has shown that **FA is unable to clearly visualise the retinal circulation at the capillary level** and has adequate reproducibility for only visualising the large-order vessels of the retina. For this reason, FA is not a precise tool for stratifying those subjects that are at risk of DMO due to BRB breakdown at the capillary level. Similar to structural OCT, fluorescein angiography is a tool that is best suited for evaluating the consequences of BRB breakdown.

The technique of OCT angiography (OCTA) is gaining increasing popularity for imaging the retinal circulation.[13] As OCTA can be readily incorporated into OCT machines that are currently used for structural retinal imaging, it is a tool that is becoming widely accessible to ophthalmologists
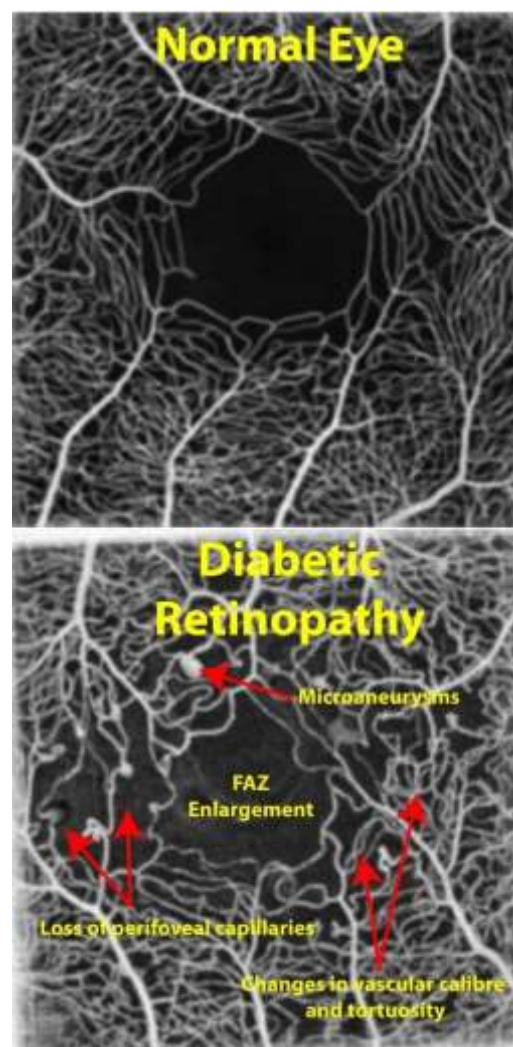
Figure 2 – Optical coherence tomography angiography images of a normal eye and an eye with diabetic retinopathy. The pertinent microvascular changes that characterise diabetic retinopathy are indicated on the right panel. FAZ = foveal avascular zone.

world-wide. Optical coherence tomographic angiography utilizes flow properties within a defined volume of tissue to visualize vascular structures and therefore obviates the need for dye administration. Other advantages of OCTA include its capability to resolve vascular information in the *en face* and cross sectional planes and the potential to stratify capillary plexuses relative to retinal depth. Importantly, OCTA is capable of resolving the retinal circulation at the capillary level (Figure 2) and can recognize macular vascular changes such as microaneurysm formation, perifoveal capillary loss and changes in the topologic properties of the foveal avascular zone (Figure 2).

My previous work has shown that OCTA is capable of providing greater detail regarding the retinal capillary circulation than FA (Figure 3).[16] For the above reasons, OCTA appears to be a valuable tool
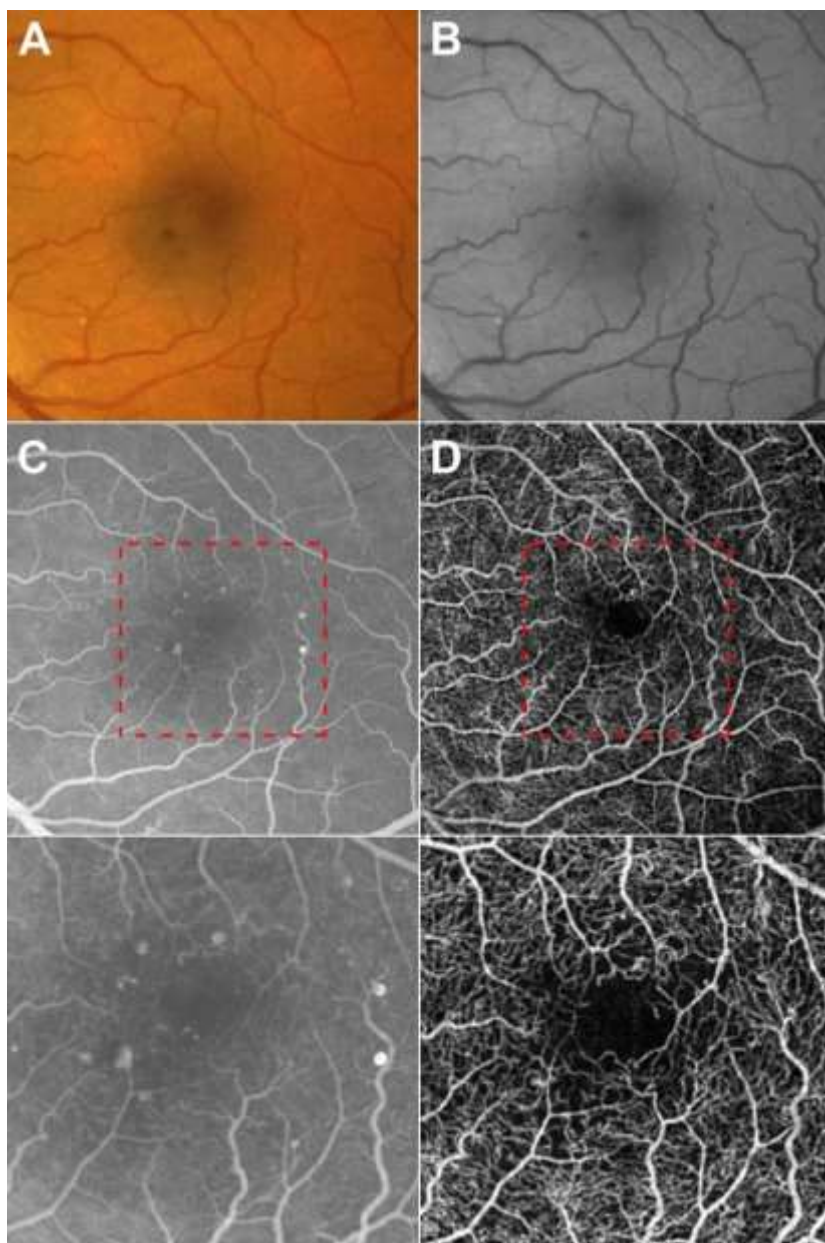
Figure 3 – A comparison of retinal vascular information as seen on colour imaging (A), red-free imaging (B), fluorescein angiography (C) and optical coherence tomography angiography (D). Magnified views of the macular circulation are provided in insets in the last panel. Note that optical coherence tomography angiography provides the greatest amount of detail regarding the macular capillary circulation. Images were acquired from the same eye on the same day.

for precisely understanding the vasogenic mechanisms that underlie DMO and, by extension, delineating anti-VEGF responders and non-responders in the setting of DMO.

Role of OCTA in Diabetic Macular Oedema

A plethora of descriptive studies have shown that OCTA is capable of resolving the topologic characteristics of retinal capillary beds.[13, 17, 18] These studies have also shown that OCTA is a safe imaging device that is well tolerated by patients and can be used to monitor response to DMO therapy. However, little is known about the OCTA biomarkers that predict response to anti-VEGF therapy. **As the target of anti-VEGF agents include VEGF receptors expressed by retinal capillary endothelia, it is plausible that the morphologic properties of retinal capillary beds will be intrinsically correlated, if not associated, with anti-VEGF response in DMO**. The basis of this postulation is the wide variation in the quantitative properties of the macular circulation in patients with DMO. In figure 4, I have illustrated the morphology of the central macular circulation in 9 of my diabetic patients that were recently diagnosed with DMO. It is readily apparent that there is great variation in perifoveal capillary density, foveal avascular zone size, microaneurysm number and distribution between eyes. These vascular characteristics are expected to alter VEGF receptor concentration and distribution in the macula and in turn modulate response to anti-VEGF treatment. To date, such a hypothesis has never been explored.

This proposal aims to define OCTA biomarkers that predict response to anti-VEGF therapy in the setting of DMO. **The over-riding hypotheses of this proposal are as follows:**

1. **The topologic characteristics of the macular circulation as seen on OCTA is useful for refining the subset of DMO patients that will/will-not respond to anti-VEGF therapy.**

2. **OCTA has greater value than structural OCT or FA for predicting response to anti-VEGF therapy in DMO.**
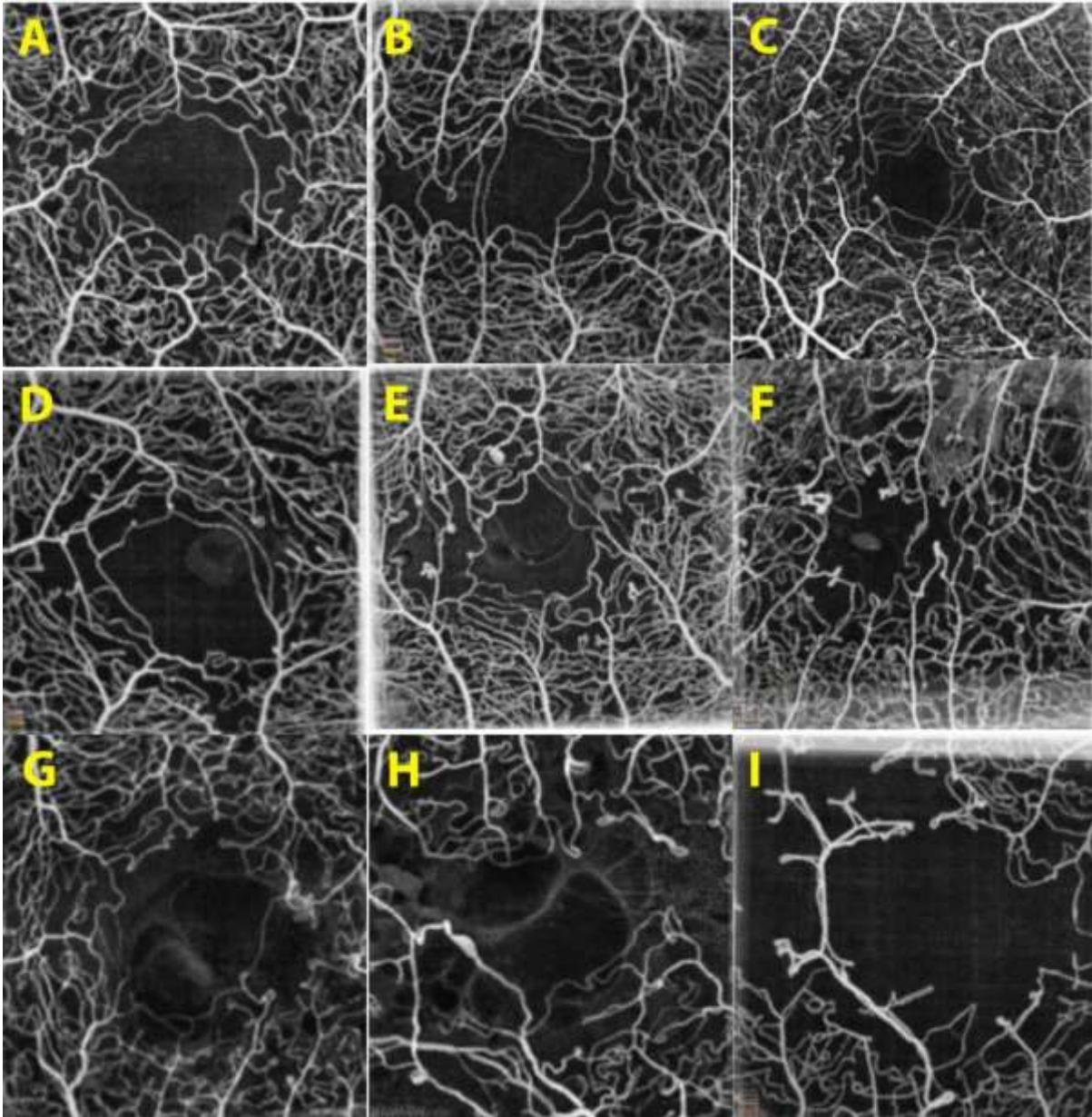
Figure 4 – The spectrum of macular microvascular changes due to diabetic retinopathy. Optical coherence tomography angiography images from 9 different patients with diabetic macular oedema are presented. Note the marked variation in macular vascular topology including perifoveal microvascular density, the area of the foveal avascular zone and number and distribution of microaneurysms.

Methods

FUNDING AMOUNT

$217,160 Australian dollars (Breakdown available upon request).

DURATION OF STUDY

2 years

STUDY PARTICIPANTS

Total of 50 eyes from 50 subjects with DMO (Sample number based on power calculation). Further analysis of the OCTA images and BCVA scores has suggested a need to increase the sample size again to accurately support the power calculation. We would also like to include subjects with baseline visual acuity between 0.0 to 0.3 logarithm of the minimum angle of resolution (original ethics application only included patients between 0.3 and 1.0 logarithm of the minimum angle of resolution) because there is increasing evidence to show that these subjects are at high risk of vision loss at 1 year without treatment (Ciulla et al. British journal of ophthalmology 2020 Apr 7;bjophthalmol-2020-315933 – real world analysis of 28658 patient eyes).

Subjects will be enrolled in a prospective fashion through my clinics at the Lions Eye Institute

and Sir Charles Gairdner Hospital.

Inclusion criteria

Age ≥18 years.

Type 1 or 2 diabetes mellitus

Diabetic macular oedema (both treatment naïve and previously treated) causing vision loss,

with study eye BCVA measuring 0.0 to 1.0 logarithm of the minimum angle of resolution;

macular oedema defined clinically and by retinal thickness of <250 μm in the central

subfield.; and intraretinal or subretinal fluid seen on SD-OCT.

Exclusion criteria

Another concomitant ocular disease that causes macular oedema such as age-related

macular degeneration or retinal vein occlusion.

Another ocular condition that compromises visual acuity, except for the presence of

cataract.

Previous treatment with intraocular corticosteroids within the 6 months of the baseline visit.

ANTI-VEGF TREATMENT

All patients included in this study will be treated with intravitreal aflibercept therapy in the study eye.

Treatment will be initiated and maintained according to the DRCR Network treatment protocol for centre-involved diabetic macular oedema.

Six treatments will be given to each patient. Each treatment will be separated by an interval of 4 weeks with the exception of the 6th treatment that will be given between 4 to 8 weeks after the 5th treatment.

Baseline visit for this study is defined as the visit where the first intravitreal anti-VEGF treatment is administered.

Final visit for this study will be 4 weeks after the 6th intravitreal anti-VEGF treatment.

Anti-VEGF treatment for all subjects will be acquired through the PBS.

Anti-VEGF treatment will be administered under sterile conditions in a dedicated treatment room using conventional intravitreal injection protocols.

IMAGING AT BASELINE VISIT

All subjects included in this study will undergo the following imaging at baseline visit prior to the administration of intravitreal therapy:

Structural retinal imaging on the central macula with spectral domain optical coherence tomography: Heidelberg Spectralis, Heidelberg, Germany.

Retinal vascular imaging of the central macula (3x3 mm cube centred at the fovea) using optical coherence tomography angiography: Heidelberg Spectralis, Heidelberg, Germany and Optovue, Avanti.

Fluorescein angiography: Heidelberg Spectralis, Heidelberg, Germany for macular imaging and Optos, California for ultrawide field peripheral retinal imaging.

Colour photography (Canon fundus camera).

DATA ACQUISITION AT BASELINE VISIT

The following information will be attained from all patients included in this study:

Demographic data

Gender (Male or female)

Age (years)

Subtype of diabetes mellitus (Type 1 or 2)

Haemoglobin A_1C

Type of retinopathy (proliferative vs. non-proliferative)

Previous treatments for DMO

Smoking status (Yes or No).

Imaging data

Multimodal imaging data will be analysed with respect to a range of anatomic features as illustrated in Figure 5. This will include imaging features that have previously been shown to be associated with the clinical course of DMO.[19-21] Optical coherence tomographic angiography images will also be evaluated for a range of structural features,[18, 20] some of which are novel. The methodologies that will be used to quantify multimodal imaging data are described in the references. [19-21]

1. **Structural retinal imaging with spectral-domain optical coherence tomography**

   Presence of subretinal fluid

   Intraretinal cysts will be identified using previously determined OCT criteria and will be defined as the occurrence of round or oval hyporeflective spaces arranged in linear aggregates at the level of the inner nuclear or outer nuclear/Henle's fiber layers.

   Maximal cyst size in the outer nuclear layer (small <100 μm, large 100-200 μm or giant > 200 μm).

   Presence and length of disorganization of retinal inner layers (DRiL). Previously reported definitions of DRIL will be used. Disorganization of the retinal inner layers length will be

defined as the horizontal extent in microns for which any boundaries between the ganglion cell-inner plexiform layer complex, inner nuclear layer, and outer nuclear layer could not be identified. The horizontal extent of DRIL within each B scan image within the central 3mm of the fovea, circumscribed using the ETDRS grid, will be assessed. Average of measurements will be used to derive a global DRIL measurement for the eye.

Continuity of external limiting membrane (ELM) and ellipsoid zone (EZ). The central 3mm of the fovea, as circumscribed by the ETDRS grid, will be evaluated. Disruption of the EZ and ELM will be categorically graded as being present or absent. An absent grading will be denoted if there is any disruption to the ELM or EZ on OCT.

Presence of hyperrelective foci, as well as quantity (few, moderate or many).

OCT-derived measure of central retinal thickness. The central 1mm subfield thickness (CST) will be recorded from the retinal thickness ETDRS grid generated by Spectralis software (Heidelberg Engineering, Heidelberg, Germany).

2. **Fluorescein Angiography**

Focal vs. Diffuse pattern of angiographic leakage at the macula.

3. *Optical Coherence Tomography Angiography (OCTA). This will be performed at each visit including 1 week after the first treatment. OCTA images will be segmented into 3 slabs as follows: (1) All capillary plexuses as a single projection; (2) Superficial capillary plexus; (3) Deep capillary plexus. Each OCTA image will be assessed for the following features:*

Presence or absence of perifoveal capillary loss. This will be categorically graded as being present or absent.

Macular capillary density. Using ImagePro Plus image analysis software we will manually trace the capillaries in a 3x3 mm slab centre at the fovea and express macular density as the area of capillaries within a 3x3 mm area.

Number of microaneurysms. The number of microaneurysms within a 3x3mm slab will be manually counted.

Position of microaneurysms relative to the foveal avascular zone. The distance of microaneurysms from the terminal foveal capillary ring will be manually measured using ImagePro Plus software.

Integrity of the terminal foveal capillary ring (intact vs. disrupted). This will be categorically graded.

Area of the foveal avascular zone. Images from screen capture program integrated in the AngioView Software, representing the full thickness of the retina (inner and outer capillary networks combined), will be used for this analysis. Gain and contrast will be adjusted if necessary, using the brightness/contrast function, to allow clear delineation of the FAZ. The magic wand tool will then be used to manually demarcate the boundaries of the FAZ after the innermost capillaries in the fovea are identified. We will use a tolerance of 15 and repeatedly used the magic wand tool to select the FAZ. If the selection spills outside of the FAZ the excess selection will be trimmed using the lasso tool. If there are regions in the FAZ where pixel value exceeds the selected pixel value plus tolerance then they will not be selected by the magic wand tool. In these instances, the lasso tool will also be used to manually select pixel outliers. The area measurement function will then be used to determine the area of the FAZ in pixels^2 and this value will converted to μm^2 using scale conversion.

Presence of flow signals between retinal cysts. This will be categorically graded as being present or absent.

Tortuosity index of macular capillaries

DATA ACQUISITON AT THE FINAL VISIT

The following information will be attained from all patients at the final visit:

Best-corrected visual acuity (logMAR scale)

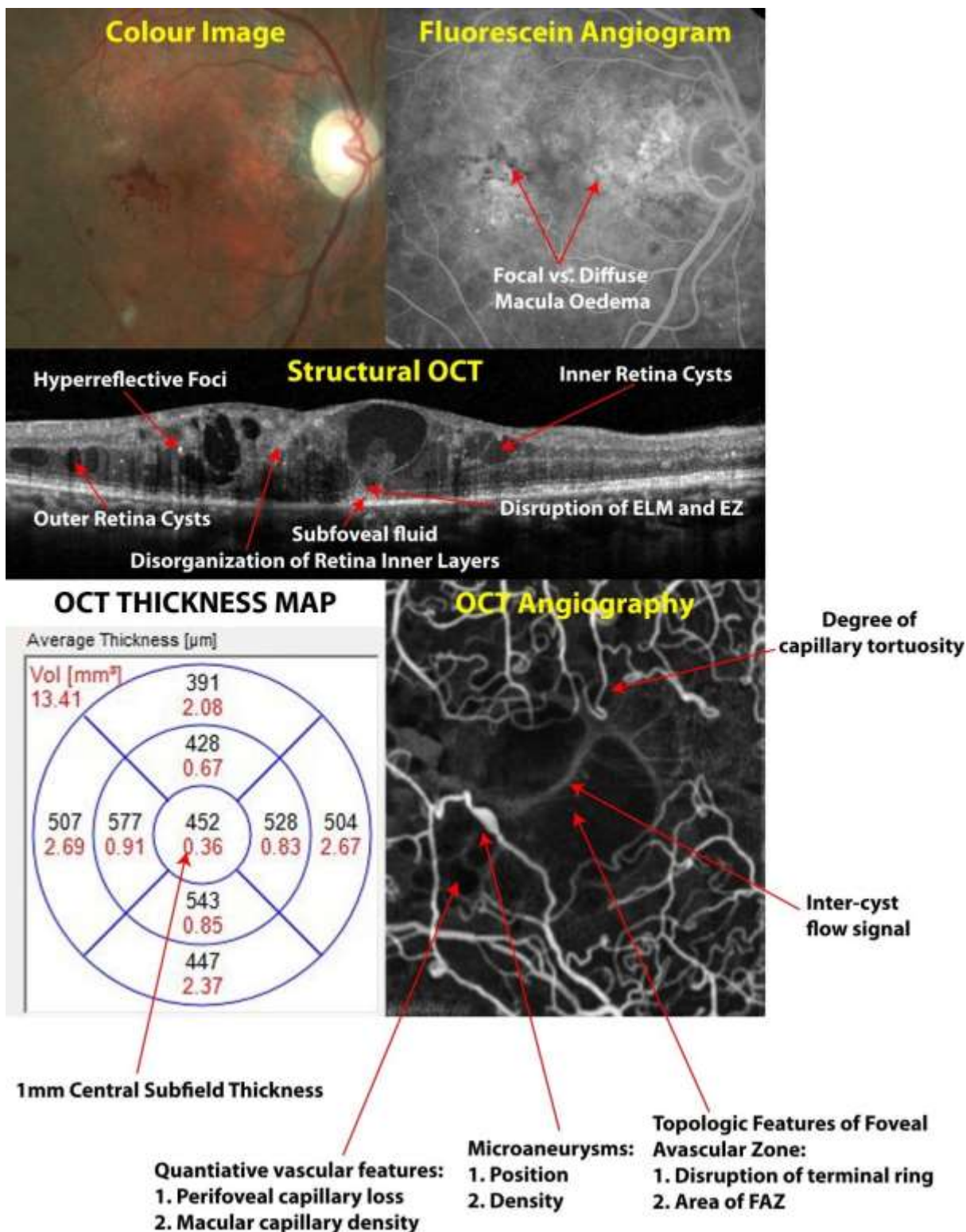OCT-derived measure of central retina thickness.

Figure 5 – The retinal anatomic features that will be analysed and quantified at baseline visit using fluorescein angiography, structural optical coherence tomography and optical coherence tomography angiography are illustrated above. FAZ = Foveal avascular zone.

DEFINITION OF NON-RESPONSE OR TREATMENT FAILURE

Refractory DMO or non-response to anti-VEGF therapy will be defined as worsening of BCVA by 2 Early Treatment Diabetic Retinopathy Study lines or reduction of less than 10% of retinal thickness on SD-OCT measured 1 month after 3 anti-VEGF injections given at monthly intervals.

STATISTICAL ANALYSIS

Data will be summarized with descriptive statistics. Univariate regression models estimated using generalized estimating equations (GEE) will be fit using the above listed demographic and imaging data acquired at base line visit, each as a single predictor, and BCVA at the final visit as the outcome. A separate analysis will be performed using the same predictors and central retinal thickness at the final visit as the outcome. Results from univariate regression models will be used to create a final multivariate model in which VA and central retinal thickness measurement from the final visit will be the outcomes.

Significance of this work and my track record in the field

This proposal aims to answer a critical question in DMO management. A recent post hoc analysis of the DRCR Network Protocol I study showed that suboptimal BCVA response to anti-VEGF therapy is predictive of poorer long-term visual outcomes in DMO.[6] It still remains unclear why nearly 40% of eyes with DMO will have sub-optimal response to anti-VEGF therapy. This proposal outlines a robust framework for examining the anatomic factors, as seen on multimodal imaging, that may predict early response to anti-VEGF therapy. Conventional imaging measures in addition to novel OCTA biomarkers will be examined. The results of this report is expected to aid clinical management of DMO with greater precision and highlight patient subsets in which anti-VEGF therapy should be used as first-line therapy for DMO. It is also expected to shed new insights into why certain subsets of patients may demonstrate only a sub-optimal response to anti-VEGF therapy hence providing useful knowledge for the development of new therapies.

I have extensive clinical and laboratory-based research expertise in diabetic retinopathy. I worked for 2 years in New York City, USA with some of the leaders in the field of Retina and have a strong track record in OCTA. I was the first person to report the strong association between the topologic properties of the foveal avascular zone as determined using OCTA and visual acuity in diabetic retinopathy and retinal vein occlusion.[20] This work was published in the highest-ranked clinical journal in our field - *Ophthalmology*. The results of this work haves already been replicated by two independent studies by other groups.[22, 23] The work outlined in this proposal is a natural extension of this previous work. **My international standing in the field of retina is evidenced by my publication track record that includes over 80 peer-reviewed manuscripts.** I was also invited to author four chapters of the latest edition of '*The Retinal Atlas'* which is one of the most widely used textbook by retina specialists worldwide.

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