STRUCTURE FUNCTION CORRELATION IN RETINAL ISCHAEMIA AND MACULAR OEDEMA

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Declaration

‘I, Piyali Sen, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that I have indicated them in the thesis.'

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Abstract

Diabetic retinopathy (DR) and retinal vein occlusion (RVO) are the two most prevalent retinal vascular diseases which affect macular perfusion, altering visual function.

Firstly, healthy eyes [eyes with no structural pathology identified on Optical coherence tomography (OCT) and OCT angiogram (OCTA)] were assessed for the foveal parameters to identify the reliability of structural markers before studying pathological changes. The white ethnic group had a smaller foveal avascular zone area and perimeter compared to the Asian and Afro-Caribbean (AFC) ethnic groups, respectively in our study. Retinal capillary loss is an irreversible complication of DR. We analysed patients with advanced DR and found higher parafoveal capillary vessel density (VD) at the level of superficial capillary plexus (SCP) was associated with better best corrected visual acuity (BCVA) and low luminance visual acuity (LLVA) while for radial peripapillary capillary plexus (RPCP) only VD of temporal sector was the predictor of LLVA. Disorganization of the retinal inner layers (DRIL), a potential biomarker of focal ischaemia affects LLVA more than BCVA. In a final adjusted model, the SCP density of the parafoveal area was the only parameter that most accurately ascertained BCVA and LLVA. In the next section of this thesis, functional assessment of the effect of treatment (anti-vascular endothelial growth factor [VEGF] versus laser) on active proliferative diabetic retinopathy (PDR) showed BCVA and LLVA are well correlated before and after treatment. Our findings suggest ten letters difference between BCVA-LLVA; however, this difference becomes more remarkable for a lower level of BCVA, suggesting more advanced ischaemia affects LLVA aggressively.

Next analysing, OCTA changes at the macula for PDR, and central retinal vein occlusion (CRVO), I found capillary loss was more pronounced in PDR than CRVO. The final chapter of the thesis looked at the baseline demographic and morphological changes in

CRVO patients to predict vision outcomes. Baseline retinal central subfield thickness (CST) up to 900 microns can be expected to improve by 2 or more lines on Early Treatment Diabetic Retinopathy Study **(**ETDRS) chart or achieve >70 letters visual acuity (VA) at 100 weeks of follow up and treatment. Age of the patient and vision at the time of diagnosis of CRVO and intact ellipsoid zone can predict BCVA at 100 weeks following initiation of treatment in CRVO eyes.

Impact statement

Prolonged diabetes affects central vision due to microangiopathy; however, anatomical alterations may not always be clinically apparent. Visual acuity may be normal in the presence of extensive microaneurysmal changes. Fluorescein angiography (FA) studies have shown macular dysfunction is closely related to the degree of perfusion loss and intraretinal exudation, which has been further confirmed with non-invasive imaging studies. Similar capillary changes are also observed in RVO. In both these pathologies, macular oedema (MO) and macular ischaemia are the leading causes of visual impairment. The introduction of anti-VEGF therapy has helped in treating MO more effectively; however, macular ischaemia continues to affect visual prognosis. In this thesis, I have looked at structural and functional biomarkers in PDR and CRVO patients, which can be helpful in future clinical trials targeting treatment for macular ischaemia.

When I assessed healthy eyes, I found that structural parameters vary between different ethnic groups and sex. Nearly half of our advanced DR patients maintained healthy foveal avascular zone (FAZ) size. This indicates that although such patients are expected to have significant peripheral perfusion deficit, the macula may not yet be severely affected. Functional test such as BCVA is the most widely used outcome measure to understand natural history and treatment outcome. In recent years LLVA has gained importance as a functional outcome measure, particularly in age-related macular degeneration (AMD). Patients with DR are known to have impaired rod photoreceptor function and impaired dark-adapted visual sensitivity. When OCTA-derived structural parameters like parafoveal and RPCP VD along with FAZ morphology were correlated with BCVA and LLVA in stable PDR, we found parafoveal superficial capillary density was the most reliable predictor of BCVA and LLVA. A positive relationship was noted between temporal RPCP vessel density and functional endpoints, BCVA and LLVA. This area is closest to

the parafoveal zone, indicating the importance of capillary integrity of the parafoveal macula.

To consider LLVA as a functional endpoint in trials involving PDR patients, we need to assess the effect of PDR management on LLVA. BCVA and LLVA correlated well before and after treatment for PDR, either with aflibercept or panretinal photocoagulation (PRP). My findings also suggest that longer follow-up is necessary to observe a change in LLVA. Stasis of blood flow secondary to increased intracapillary pressure is a common pathological pathway for PDR and CRVO. In this thesis, I found that structural alterations are more prominent in treated PDR eyes than in those with stable treated CRVO. This difference could be due to an early alteration in capillaries in diabetics and are not primarily related to the elevation of intracapillary pressure. We found that age, pre-treatment vision and gradable intact photoreceptor layer are predictors of VA outcome in MO due to CRVO. Severe MO in CRVO eyes is unlikely to achieve driving standard visual acuity or more than two-line improvement on ETDRS chart.

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Abbreviations

AIC	Akaike information criterion
AFC	Afro-Caribbeans
AOSLO	Adaptive optics scanning laser ophthalmoscope
AMD	Age-related macular degeneration
Anti-VEGF	Anti-vascular endothelial growth factor
BCVA	Best corrected visual acuity
BRVO	Branch retinal vein occlusion
CNP	Capillary non-perfusion
CST	Central subfield thickness
CVOS	Central Vein Occlusion Study
CMO	Cystoid macular oedema
DCP	Deep capillary plexus
DM	Diabetes mellitus
DMO	Diabetic macular oedema
DRN	Diabetic retinal neurodegeneration
DR	Diabetic retinopathy
DRT	Diffuse retinal thickening
DRIL	Disorganization of the retinal inner layer
ETDRS	Early Treatment Diabetic Retinopathy Study
EZ	Ellipsoid zone
ERM	Epiretinal membrane
ELM	External limiting membrane

FA	Fluorescein angiography
GCL	Ganglion cell layer
GA	Geographic atrophy
HRVO	Hemispheric retinal vein occlusion
HRF	Hyperreflective foci
INL	Inner nuclear layer
IQR	Interquartile range
IReST	International Reading Speed Texts
LLVA	Low luminance visual acuity
MCP	Middle capillary plexus
MO	Macular oedema
MNREAD	Minnesota low vision reading chart
mfERG	Multifocal electroretinography
OCTA	Optical coherence tomography angiogram
OCT	Optical coherence tomography
OR	Odds ratio
OPL	Outer plexiform layer
PRP	Panretinal photocoagulation
PCFZ	Perifoveal capillary-free zone
PVD	Posterior vitreous detachment
PRN	Pro re nata
PDR	Proliferative diabetic retinopathy
RPCP	Radial peripapillary capillary plexus
RNFL	Retinal nerve fibre layer

RPE	Retinal pigment epithelium
RVO	Retinal vein occlusion
SD	Standard deviation
SRD	Serous retinal detachment
SCP	Superficial capillary plexus
VD	Vessel density
VA	Visual acuity
VMT	Vitreomacular traction

Chapter 1. Introduction

This introductory chapter begins with description of macula in heathy eyes, macular blood supply and different layers of retina. The latter part of this chapter provides overview of pathophysiology of macular ischaemia and oedema, as these are the two most common complication noted in retinal vascular diseases, particularly PDR and CRVO. Here, I have also described the structural changes observed on common imaging platforms OCT, FA and OCTA in eyes affected by macular ischaemia or oedema.

1.1 Overview of normal macula

The macula lutea is a region of the posterior retina that measures approximately 5.5 mm in diameter and is exquisitely specialized for central and colour vision. The macula differs from the rest of the retina by presenting an exceptionally high density of neural elements and restricted blood supply. One of the unique features of the primate macula is the presence of xanthophylls, lutein (L), and zeaxanthin (Z), which provide its eponymous yellow colour. Together, these two xanthophylls are referred to as macular pigment. Macular pigment peaks in the central 1 - 2 degrees of the fovea and declines to optically negligible levels by 5 - 10 degrees radial eccentricity, along with shoulders or flanking peaks around 0.7 degrees eccentricity.

1.1.1 Fovea

The fovea is the central region of macula, which is characterized by a high density of cone photoreceptors, absence of retinal vasculature, and dominance of Midget pathways arising from these cones. Fovea constitutes 0.02% of the total area of the retina and 0.3% of the cones. The fovea has a margin, a downward slope, and a floor known as the *foveola.* The centre of the fovea contains the foveal pit (1). This area of the retina is responsible for accurate visual acuity. The wall of the foveal pit, described as a slope,

represents a transition from the cone-dominated avascular foveola to a rod-dominated vascular parafovea. Ganglion cell, inner plexiform, and inner nuclear layers form the slope surrounding the foveal pit (2). Foveola is 350 microns in diameter and 100 microns thick and only comprises cell body of cones (density more than 200,000/mm^2) and Muller glial cell processes (3, 4). It is important to note that short wavelength sensitive cones, rods, ganglion cells, and all inner nuclear layer neurons are absent from the foveola. Rod cells start to appear from the fovea. The thickness of the foveal wall is 275 - 410 μm on the temporal side and 220 - 350 μm on the nasal side (5). Histologically, fovea consist of ganglion cell layer (GCL) and quite thick outer plexiform layer. Here, the cone axons form elongated fibres of Henle as well as includes the synaptic pedicles of foveal cones.

An increasing rod: cone ratio and ganglion cells characterize the parafovea, with a relatively low density of retinal vessels. The outermost region, the perifovea, acts as a transition zone between the highly specialized macula and the peripheral retina. The greater than average densities of cones and ganglion cells sets this zone apart from the retinal periphery. A high rod: cone ratio (rod: cone ratio, 33 - 130:1), along with dense retinal vasculature, are two features common to the perifovea and the peripheral retina.

Fovea is a capillary-free region of the retina, surrounded by a terminal capillary ring (6, 7). Photoreceptors of this region are supplied by underlying choroidal circulation (8). Due to the importance of the fovea in maintaining central vision, histopathological and several imaging techniques have been used to measure FAZ over the last few decades.

In 1875, Nettleship was the first to demonstrate the absence of capillaries in the central retina. This capillary free zone dimension was 0.6 mm in the horizontal and 0.4 mm in the vertical diameter (9).

Since then, several techniques have been adopted to study the morphology of FAZ. This can be categorised into anatomical, psychophysical, and angiographic.

Anatomical

Bligard et al used trypsin digestion of retinal tissue to assess the diameter of the avascular zone. FAZ size ranged from 0.12 mm to 1.2 mm (0.4 - 4.0 degrees) assuming the eye's nodal point is 17 mm in front of the fovea (10).

Psychophysical

The psychophysical method is based on the "Flying corpuscles" concept, which are leucocytes present in the retinal capillaries. These leucocytes are visualized when the retina is illuminated with a blue light of wavelength around 430 nm. Leucocytes are not visible at the retina's centre around fixation, and this area estimates the FAZ area. Bird and Weale transilluminated the sclera with light from a Xenon arc (150-W) to observe retinal vasculature (11). The image thus obtained was 1.5 mm in length. A white diffusing screen illuminated with an incandescent lamp was placed before the observer. The measurements obtained from this process were the angular thickness of the fine vessels and the main retinal vessels near the optic disc, compared with black lines of known thickness observed at a fixed distance. All six participants noticed capillaries passing across the fixation area joining larger blood vessels along with a network of capillaries spread across the retina. The avascular zone size calculated in this study was 0.125 to 0.5 mm (0.42-1.68 degrees) with a mean of 0.2 mm (0.67 degrees). This study suggested that although the central retina appears capillary-free, fine capillaries traverse the avascular zone and have the least potential to affect visual acuity. Histopathologists do not identify them, nor do FA due to loss of such vessels during tissue processing or the inability of dye to reach such small calibre vessels.

Bradley et al used the technique of high-contrast entoptic viewing of the retinal vasculature to assess the FAZ in thirty-four eyes. The two methods adopted by this group involved: ‘reduction of the circular Maxwellian view field stop until it appeared to coincide with the edge of the FAZ, and tracing of the boundary of the FAZ with a point source. In doing so a mean area of 0.42 mm^2 and an elliptical shape of FAZ were established (12).

The foveal avascular zone was measured using blue light illuminator on an optical bench and video monitor on twenty-two healthy subjects. In one of the two methods, the subjects had to indicate when leucocyte activity stopped and reappeared as the field size was decreased and increased respectively. On the other, subjects had to match the size of the leucocyte-free area with a white circle superimposed on the field. The foveal avascular zone value was quantified in degrees. The mean FAZ size for all subjects was 2.57 degrees in the first method, whereas, with second one, a smaller value of 2.04 degrees was reported (13).

Adaptive optics scanning laser ophthalmoscope

Adaptive optics scanning laser ophthalmoscope (AOSLO) is a method which uses the speed of leucocytes through capillaries to image parafoveal capillaries. This non-invasive technique relies on the concept that blood cells move relative to their surrounding photoreceptors. Motion extraction was performed using multi-frame division videos, and videos of the parafoveal region were overlapped to create montage for FAZ analysis. The area of FAZ calculated from the quantification of 10 subjects was 0.323 ± 0.107 mm^2 (14). Adaptive optics scanning laser ophthalmoscope method has been used by Chui et al on thirty-two participants, where seven of them had both yes included. No statistical significance was found on FAZ area between the two eyes (paired *t*-test, $P = 0.09$). The average FAZ area reported by this group was 0.32 ± 0.160 (15).

Similarly, Dubis et al had set the plane of focus at the inner retina to capture reflected light from the capillaries around FAZ. Subjects were asked to fixate at different locations on the scanning beam, and multiple frames were combined to obtain a single image sequence. Foveal avascular zone area measurement was done on Matlab (Matlab; Mathworks, Natick, MA) using a custom semiautomated segmentation algorithm. They looked at 11 healthy subject eyes with AOSLO and described the mean (± SD) FAZ area as 0.44 ± 0.25 mm^2, with a range of 0.05 to 1.05 mm^2 (16). The FAZ area recorded in this study is smaller than described by other authors. This group suggested the reason for such observation being foveal pit size of extreme values.

Angiographic

Fluorescein Angiography

Fluorescein angiography involves intravenous dye administration and imaging for 10 to 30 minutes. Information like measurement of capillary free areas, patterns of dye leakage, pooling, and staining are obtained from two-dimensional image sets that allow for dynamic visualization of blood flow. The capillary free zone of the fovea was studied using FA in 1977 by Laatkainen. The mean diameter of the FAZ was reported to be 0.53mm for age group 10-39 years and 0.61 mm after 40 years. Although mostly FAZ was capillary-free few eyes had one or two capillaries crossing the FAZ. This difference is statistically significant (p less than 0.001)(17). A smaller FAZ of 0.35 sq mm was reported by Bresnick et al in an FA study of 20 healthy eyes with mean age of 44 years. However, for these eyes FAZ dimension seemed to decrease with age, but this was not statistically significant and could have resulted from a small sample size (18).

Wu et al described the capillary free zone in the normal macula in forty-five subjects using digital processing of FA images. The mean longest diameter was 0.88 +/- 0.16 mm (SD),

the horizontal diameter was 0.73 +/- 0.15 mm, and vertical diameter was 0.70 +/- 0.17 mm. The average area (0.43 +/- 0.16 mm^2) was like that suggested by Laatkainen and Bresnick. The FAZ area showed an increase in size with age (r = 0.383, P less than 0.005) (19).

Contrast-adjusted method to measure the FAZ in FA using HRA-2 on thirty-one healthy subjects from the Indian population was performed by John et al Subjects were of mean age of 29.7 years. Contrast enhancement was done using the GNU Image Manipulation Program (GIMP). The mean area of the FAZ calculated by the contrast-adjusted method was smaller (0.2753 ± 0.074 mm^2) compared to the conventional method (0.6241 ± 0.177 mm^2). This difference was statistically significant, with no correlation between the two methods (ICC = –0.002, *P* = 0.502) (20).

Fluorescein angiogram images captured using the scanning laser ophthalmoscope technique allow continuous recording of high-resolution retinal pictures. The average FAZ area in 21 healthy volunteers using this technique was 0.231 ± 0.06, which is less than previous studies, most likely due to mean age (26 ± 4 years) and the early time since dye injection used to visualise the contrast between the perifoveal network and the background (21). Arend et al used a similar scanning laser ophthalmoscope technique to capture and analyse FA images of 52 healthy subjects (mean age, 47±11 years). The mean FAZ area adjusted for refractive error of the participating eyes was 0.205±0.062 mm^2 (22).

Foveal avascular zone size described by Mansour et al, was 0.40 ± 0.559 mm^2 in participants aged between 39.4 ± 18.1 years. FAZ area calculation was adjusted for ocular refraction and magnification used by the fundus camera (23). FA investigation of 31 healthy eyes in the age group 48.42±17.5 years showed a mean FAZ area of 0.152±

0.086 mm^2. However, there was no significant correlation between age and size of FAZ.(24) Quantitative parameter, FAZ area seem to be highly variable in healthy eyes with normal vision and affected by capillaries intercepting avascular area, age, sex, race of the study population, refractive status of the eye and the magnification factor of the camera used. This affects comparison between different studies difficult as well.

Qualitative description FAZ in healthy eyes by FA shows it to be almost elliptical shape with a horizontal axis longer than the vertical axis. The margin of FAZ was found to be regular with mild undulation due to the presence of teeth and bays. The arterioles contributing to the terminal capillary ring appear branching at acute angles forming several rows of a single-layer capillary network between avascular area and terminal branching (18). This study also found capillaries entering FAZ in some eyes which breaks in the margin, which Laatikainen also recorded.

Optical Coherence Tomography Angiogram

Optical coherence tomography angiogram is a non-invasive imaging technique that helps us study the vascular map at different layers of retina. Foveal avascular zone has been extensively analysed in recent years due to the ease of quantification using OCT-A technology. The literature shows that normal FAZ varies based on age, sex, and ethnic origin.

Optical coherence tomography angiogram can reliably image the three capillary plexi superficial, intermediate, and deep capillary plexus (DCP) of the retina, situated at GCL, inner and outer boundary of INL respectively.

The superficial capillary plexus extends in the parafoveal region with a central FAZ surrounded by a continuous ring of interconnected capillaries. The blood flow in this layer

is observed to be homogenous throughout the entire scan. The DCP includes only capillary-sized vessels of smaller calibre than the superficial plexus. The DCP appears more complex with extensive homogenous branching of uniform size, and greater capillary density than the superficial plexus. Optical coherence tomography angiography imaging also shows multiple vertical anastomoses coming from the superficial plexus, branched to give rise to multiple deep radial capillaries in the deep plexus.

One of the earlier study analysing FAZ dimensions in 34 healthy eyes observed, area 0.27 ± 0.101 mm^2; perimeter 2.21 ± 0.451 mm at the level of superficial and that of deep FAZ, area 0.34 ± 0.116 mm^2; perimeter 2.50 ± 0.462 mm on Angiovue OCTA (25). Samara et al got similar result for FAZ area at superficial plexus. Their FAZ area in both plexuses was variable. The study group of 70 eyes of 67 patients with a mean superficial plexus FAZ of 0.266 mm^2 ± 0.097 mm^2, while the mean area of deep plexus FAZ was 0.459 mm^2 ± 0.227 mm^2 (26).

Coscas et al on the AngioVue OCT-A system used the automated non-flow area measurement option and at the SCP level, mean FAZ area was 0.28 ± 0.1 mm^2 and at DCP, mean FAZ area, 0.37 ± 0.12 mm^2 in the whole en face image. The mean age of this study population was 48.3 ± 17.5 years (27). They grouped their participants into three groups: 20 - 39 years old, 40 - 59 years old, and 60 years and older. The mean FAZ area was smaller ($P < 0.05$) in group 3 compared with groups 1 and 2 at the level of the SCP. There was no statistically significant difference for the mean FAZ in the three groups at the level of the DCP (27).

According to Iafe et al FAZ areas increased by 0.0014 mm^2 (0.63%) and 0.0011 mm^2 (0.20%) per year in the SCP and DCP, respectively. When age was considered in categories statistically significant difference was recorded for age 60–69 and subjects

aged 70–79 (*P* = 0.029) for SCP; for DCP VD, this was applicable for subjects aged 40–49 and 50–59 years of age (*P* = 0.033) as well as between subjects aged 60–69 and 70–79 years of age (*P* = 0.003). Their study population was 70 healthy individuals with mean age of mean 48 ± 20 years (28).

The superficial FAZ (0.258 mm^2 vs. 0.272 mm^2, *P* = 0.55) and deep FAZ (0.429 mm^2 vs. 0.480 mm^2, *P* = 0.13) were not significantly different between males and females, respectively. There was no correlation between superficial FAZ (r^2 = 0.01, *P* = 0.34) or deep FAZ (r^2 = 0.03, *P* = 0.13) and age (26).

In two hundred forty eyes of 120 healthy subjects, OCT-A was performed on swept source OCT. Superficial FAZ and deep FAZ areas were measured using their software package. The mean age of the subjects was 39.2 ± 17.4 years. The overall mean superficial FAZ size in women (0.297 ± 0.110 mm^2) was significantly larger (p = 0.002) than in men (0.254 ± 0.098 mm^2). Similarly, the overall mean deep FAZ in women (0.322 ± 0.111 mm^2) was significantly larger (p < 0.001) than in men (0.273 ± 0.099). Men did not show differences among the six age groups. In women, for both superficial FAZ and deep FAZ areas were larger, the younger subjects had a smaller FAZ size compared to > 50 years old group. Larger FAZ in women than men are relevant in extremes of age (29).

Ethnicity and sex

A study on healthy Indians showed that the mean FAZ area was 0.42±0.23mm^2 (30). In another study on the Chinese population, the mean FAZ area was 0.474 ± 0.172 mm^2. However, in that study, the authors had used Avanti (Optovue, Fremont, CA, USA) device, and the mean age of their patients was 36 years (31).

Guo et al measured FAZ manually in the Chinese population using Image J and compared the correlation between two reads. The FAZ area was 0.373 ± 0.109 for the first read and 0.377 ± 0.112 mm^2 for the second read (32).

The mean FAZ size in the Japanese population was 0.32 ± 0.11 mm^2 using swept-source OCTA. This study was conducted on 144 healthy eyes aged 10–79 years. Neither sex nor axial length proved to be a significant factor for the FAZ size. The authors showed that the most significant difference in FAZ size was in a group with a mean age of 43.6 years (FAZ area = 0.27 mm^2) and a group with a mean of 53.8 years (FAZ area = 0.34 mm^2) (33).

The size of the FAZ was 0.33 ± 0.012 and 0.28 ± 0.014 mm^2 (p = 0.0289), and the diameter was 2.43 ± 0.06 and 2.18 ± 0.07 mm (p = 0.0057) for Chinese and Caucasian populations, respectively. The whole ETDRS circle vessel length density was significantly different for Chinese (17.05 ± 0.24 mm/mm^2) and Caucasian (16.08 ± 0.43 mm/mm^2) populations. In addition, the outer ETDRS vessel length density was significantly different for Chinese (16.43 ± 0.42 mm/mm^2) and Caucasian (17.47 ± 0.24 mm/mm^2) populations, but the central 7.33 ± 1.68 versus 9.32 ± 1.54 mm/mm^2 for the Caucasian and Chinese and inner 16.14 ± 0.52 for Caucasian and 16.93 ± 0.27 mm/mm^2 for Chinese subjects' density was not.

Women were shown to have larger FAZ (0.310 ± 0.013 versus 0.301 ± 0.014 mm^2, p = 0.691), and no significant differences in the FAZ area were observed for men and women (34).

The above literature review strongly suggests FAZ is highly variable based on demography, imaging technique, and the device used.

1.1.2 Macular blood supply

The retina is supplied by dual circulation, the inner retina by the central retinal artery while the outer retina by the choroidal capillaries. The central retinal artery divides into superior and inferior branches as it leaves the optic disc and further bifurcates in temporal and nasal branches in the nerve fibre layer (35). These blood vessels further divide and sub-divide to form the capillary network.

The capillary meshwork spreads from the GCL through the inner nuclear layer. There are no vessels in the outer plexiform and outer nuclear layers. The inner retinal circulation does not anastomose with other vascular systems which leave the inner retina vulnerable to ischaemia. Trypsin digest histology studies suggest a diffuse arrangement. In contrast, whole-mount preparations demonstrated that the capillary bed is divided into two distinct laminae throughout most of the posterior retina. According to this two-level distribution, the superficial capillaries is situated at the level of nerve fibre and GCL, while the deep capillaries lie in the inner nuclear layer and form a more closely set meshwork than the superficial plexus (36). The deep capillary network consists of fine-calibre vessels ranging from 15 to 130 μm in diameter. The superficial capillaries have a slightly wider calibre, ranging from 16 to 150 μm in diameter. The volume of the deep capillary network is relatively constant at different locations of the posterior retina.

In contrast, the volume of the superficial capillary network varies dramatically and parallels the thickness of the nerve fibre layer rather than the total retinal thickness. Thus, in peripapillary retina, the vascularity is the greatest. Here, up to four different capillary layers are identifiable, even though the retinal thickness is comparable to that of the parafoveal crest (36). The capillary network thins into a single layer in the perifoveal region and at the ora serrata. Retinal capillaries are characterized by circumferentially

oriented endothelial cells joined by non-leaky tight junctions. Capillaries are surrounded by thick basal lamina, pericytes and astrocyte foot processes. There is less extravascular connective tissue around retinal vessels and no lymphatic vessels in the retina. The capillary-free zone of the fovea is delineated by the terminal arterioles and venules that form a delicate circular web of concentrically arranged channels. This part of the retina is avascular so that incident light rays are not interfered with by the moving red blood cells, and visual acuity reaches its maximum precision in the fixation area.

1.1.3 Normal layers of retina

Histological studies describe the human retina as having ten layers. Starting from the layer facing the vitreous nerve fibre layer is formed of the axons from the optic nerve; the GCL is made of large cell bodies with a dendritic tree, followed by the inner plexiform layer, which contains the interneuronal synapses. The inner nuclear layer is the second layer of cell bodies, these are from horizontal, bipolar, and amacrine cells and the outer plexiform layer is composed of synapses between rod spherules and cone pedicles with bipolar cell dendrites and horizontal cell processes. The outer nuclear layer contains the cell bodies of the photoreceptors. The external limiting membrane is the linear meeting point of junctional complexes between Muller cells and photoreceptors. The next layer is the ellipsoid portion of photoreceptors with dense pacing of mitochondria. Retinal pigment epithelium (RPE) comprises 3 and 4 million hexagonal cells organised in a regular pattern structurally supported by pentalaminar Bruch’s membrane. Finally, the outermost layer is the choriocapillaris (37).

Optical coherence tomography is the most used non–invasive imaging modality to describe retinal layers. The first OCT imaging of the human fundus in vitro was performed in 1991 (38). This was a time domain OCT, with a resolution of 17 mm in air, and required

1.25 seconds to acquire an A-scan. They defined the nerve fibre layer as a hyperreflective band while the outer retina as hyporeflective. According to Swanson et al the retinal nerve fibre layer (RNFL) was highly reflective, and the photoreceptor layer had low reflectivity (39). With the improved resolution of OCT imaging, better visualization of outer retinal layers was possible. Drexler et al in 2001, reported visualization of three reflective bands, which they labelled as outer segments of the photoreceptors, RPE, and choriocapillaris (40). Zawadzki et al published an article using high-speed, high-resolution OCT imaging. With this technology, four distinct bands were visible in the outer retina. The innermost was thought to be the external limiting membrane. The band below was termed the connecting cilium, which is a specific anatomic structure in the border region between the inner and outer segments of the photoreceptors. External to this structure, the authors identified a new band they thought corresponded to Verhoeff's membrane, which itself has been described as representing the dense structures of the tight junctions between the RPE cells. The outermost band was defined to be the RPE (41).

The panel for International Nomenclature for Optical Coherence Tomography (IN • OCT) developed a consensus for the nomenclature to be used for retinal and choroidal layers and bands visible on spectral-domain optical coherence tomography (SD-OCT) images of a normal eye. The panellists were given a set of 3 individual, high-quality B-scan images from a normal eye that illustrated the various hyper- and hyporeflective bands and layers evident on OCT. Two B-scans were from the Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany) with an ART level of 100 frames, 1536 A-scans, and 30 wide. The third B-scan was obtained on the Cirrus HD-OCT (Zeiss Meditec Inc, Dublin, CA) using the HD Single Line Scan, composed of 1024 A-scans on a length of 9 mm, repeated 20 times. On each OCT B scan image, the panellists were asked to put a name

on each of the tagged band or feature. The table below describes each retinal layer along with brief description as proposed by IN • OCT panel (Table 1 and Figure 1)(42).

Table 1: Optical Coherence Tomography delineated layers as agreed by the International Nomenclature for Optical Coherence Tomography Panel along with their anatomical correlates.

	OCT description	**Nomenclature**	**Anatomic Correlates**
1.	Hyperreflective	Posterior cortical vitreous	
2.	Hyporeflective	Pre-retinal space	
3.	Hyperreflective	Nerve fibre layer	Axons of the optic nerve
4.	Hyporeflective	Ganglion cell layer	Retinal ganglion cells composed of large cell body with a dendritic tree (43).
5.	Hyperreflective	Inner plexiform layer	Complex zone of interneuronal synapses.
6.	Hyporeflective	Inner nuclear layer	Cell bodies of horizontal cells, bipolar cells, amacrine cells, interplexiform neurons, Müller cells, and sometimes displaced ganglion cells form the inner nuclear layer
7.	Hyperreflective	Outer plexiform layer	Outer plexiform layer comprised of synapse between rod spherules and cone pedicles with bipolar cell

			dendrites and horizontal cell processes (44).
8.	Hyporeflective band	Inner half: Henle's nerve fibre layer; outer half: outer nuclear layer	Henle's fibre layer is composed of the axons of photoreceptors while outer plexiform layer is consistent with the synapses between cone pedicles and rods spherules with dendrites of horizontal and bipolar cells.
9.	Hyperreflective	External limiting membrane	Linear meeting point of junctional complexes between Muller cells and photoreceptors (45).
10.	Hyporeflective	Myoid zone of the Photoreceptors	This low reflective zone of photoreceptors is characterised by sparsely packed density of mitochondria (46).
11.	Hyperreflective	Ellipsoid zone of the Photoreceptors	Ellipsoid portion of photoreceptors with dense pacing of mitochondria (46).
12.	Hyporeflective	Outer segments of the Photoreceptors	
13.	Hyperreflective	Cone interdigitation with	Interdigitation of the apical

		retinal pigment epithelium	processes of the retinal pigment epithelium with the tips of cone outer segments.
14.	Hyperreflective band	Retinal pigment epithelium/Bruch's membrane Complex	This band marks retinal pigment epithelium with potential contribution from Bruchs membrane and choriocapillaris.
15.	Layer with moderate reflectivity	Choriocapillaris	
16.	Mid choroidal layer of round or oval shaped hyperreflective profiles with hyporeflective cores	Sattler's layer	Medium sized choroidal vessels which are basically feeding arterioles to and draining venules from the choriocapillaris.
17.	Outer choroidal layer characterised by of round or oval shaped hyperreflective profiles with	Haller's layer	Larger outer oval choroidal vessel

	hyporeflective cores		
18.	Outer choroidal zone where ovoid profile ends into a homogenous zone of variable hyperreflectivity	Choroidal-scleral juncture	

Abbreviation: OCT – optical coherence tomography

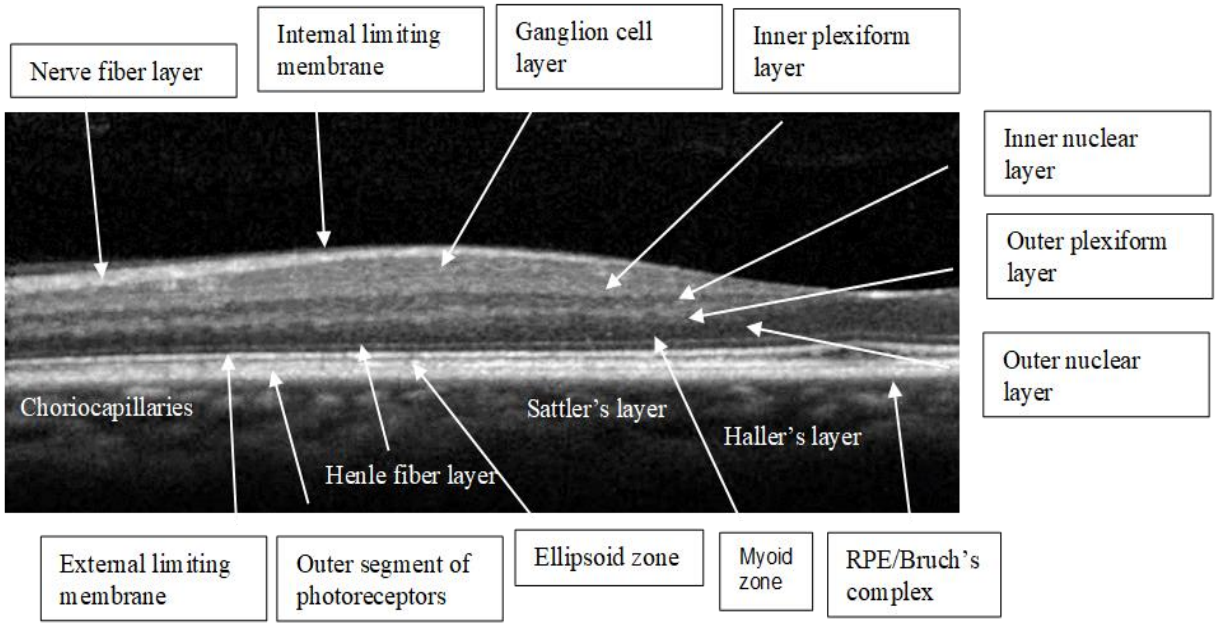

Figure 1: Spectral domain Optical coherence tomography (OCT) image of healthy retina showing normal anatomic landmarks (42).

1.2 Macular ischaemia and macular oedema

Ischaemia is a Greek Latin word that means an inadequate supply of blood to the affected tissue in order to meet the oxygen demand (47). As the hypoxia-induced damage to the retina progresses, small vessels in the macula may close off completely. This leads to ischaemia, depriving the macula of oxygen and nutrients.

Retinal ischaemia occurs when there is inadequate blood flow to meet cellular demands (48). Previous studies have proven that areas of the retina affected by ischaemia suffer from loss of ganglion cells. Outer retinal layers, including the photoreceptor cells, are also sensitive to a lack of perfusion. Morphological features considered predictors of poor visual acuity are external limiting membrane (ELM) disruption, ellipsoid zone (EZ) disruption, hyperreflective foci (HRF) and DRIL. In a small retrospective study of 13 eyes of diabetic patients without MO, nine eyes showed outer retinal layer disruption on SD-OCT that matched with areas of enlargement of the foveal avascular zone and macular capillary nonperfusion (49). Across different grades of DR, macular ischaemia is most prevalent in eyes with PDR (77.2%) (50). In CRVO patients, the three most important risk factors for ischaemia were duration of less than 1month, visual acuity of worse than 20/200, and five to nine-disc areas of nonperfusion, according to Central Vein Occlusion Study (CVOS) group (51).

Vascular endothelial cells maintain smooth microcirculation and play a crucial role in normal microcirculation. Vascular endothelial cells prevent thrombosis by producing vasodilator and vasoconstrictor mediators such as nitric oxide, prostaglandin I2, and endothelin. Chronic stress to the endothelium can happen due to hypertension, diabetes mellitus, and hyperlipidemia, which are potential causes of arteriosclerosis. In diabetics, loss of capillary endothelial cells and pericytes have been noted by histological studies.

Retinal vascular endothelial cells form a tight junction with each other, which acts as an inner blood–retina barrier, and preserves the retina's normal health. Hence, vascular endothelial injury and subsequent blood–retina barrier breakdown induces vascular hyperpermeability, which is responsible for MO. Vascular endothelial growth factor increases vessel permeability by increasing the phosphorylation of tight junction proteins (52). Macular ischaemia is identified by studying the FAZ and adjacent areas on multimodal imaging platforms such as FA, OCT and OCTA.

1.2.1 Changes in inner retinal layers on optical coherence tomography

Disorganisation of the retinal inner layers definition adapted from Sun et al describes it as areas of inner retina where the boundary between the inner nuclear layer (INL), outer plexiform layer (OPL), and the ganglion cell layer-inner plexiform layer (GCL-IPL) could not be identified on OCT (53). Histology studies have proven that neuronal degeneration and ganglion cell loss affects the eyes of diabetic patients (54). Neurodegeneration starts most likely before the onset of microvascular changes in the retina. In people with type 2 diabetes with minimal DR, thinning of RNFL, GCL and IPL was noted. Furthermore, electrophysiology studies support that neural change happens before vascular signs appear (55, 56). Localized areas of delayed implicit time on multifocal electroretinogram have been reported to be predictors of development of future retinopathy in corresponding retinal locations. Diabetic retinal neurodegeneration (DRN) involves neuronal cell apoptosis, reactive gliosis and reduced retinal neuronal function (57). Various studies have explored the underlying cause of DRIL. Although DRIL is thought to be the result of disruption of visual signal transmission between photoreceptors and ganglion cells, histologic studies need to support this theory. DRIL has been deemed a reliable predictor of areas of macular capillary non-perfusion (CNP); however, we must consider that not

all CNPs on FA show consequent areas of inner retinal disruption (58). A certain degree of perfusion loss may be necessary before it appears as DRIL on OCT imaging.

Disruption of EZ and ELM on OCT has been associated with DRIL (59). Optical coherence tomography angiography studies have correlated DRIL with areas of loss of superficial and deep vascular plexus flow and enlarged foveal avascular zone. On OCT, the central 1000 microns and up to 3000 microns area is analysed to assess the presence of DRIL.

Diabetes induced microvascular damage in the retina may represent structural deformation which is observed as DRIL on OCT. In PDR, fibrovascular membranes cause traction, which may subsequently impact distortion of the inner retinal layers. Vascular abnormality and mechanical stress can cause deformation or breakage of the synaptic junction between photoreceptors and retinal ganglion cells. DRIL could be the marker of damage to retinal cells such as Müller cells, bipolar cells, horizontal cells, and amacrine cells (53). Thus, its presence in retinal microvascular diseases affect VA.

Disorganisation of the retinal inner layers has been studied in different retinal conditions and post-operative outcome measures. Sun et al used DRIL to predict VA outcome in diabetic macular oedema (DMO). This group found that greater DRIL length at baseline correlated with worse baseline VA (point estimate, 0.04; 95% CI, 0.02-0.05 per 100 µm; P < .001). An increase in DRIL during 4 months was associated with VA worsening at 8 months (point estimate, 0.03; 95% CI, 0.02-0.05 per 100 µm; P < .001) (60). Considering 183 eyes of 123 diabetic patients in different stages of DR, the length of DRIL ($\beta = 0.24$, $P < 0.01$) was found to be a significant factor affecting visual acuity on multivariate analysis (61). Retrospective analysis of DMO eyes with good baseline VA showed DRIL (OR: 2.71, p = 0.026) was associated with a higher risk of VA loss ≥10 letters at 12 months of observation without any intervention (62).

A retrospective review of records of 147 patients 18 years or older with treatment-naive branch RVO (BRVO), central RVO (CRVO), or hemispheric RVO (HRVO), with a minimum of 12 months of follow-up treated with anti-VEGF injections showed; in BRVO group, baseline DRIL was associated with lower baseline ETDRS score (score of 66.7 for no DRIL vs 54.6 for DRIL, P = .002), absence of DRIL at baseline in the CRVO/HRVO group correlated with more significant VA gains at 6 months, adjusting for baseline VA (score change of 19.50 for no DRIL vs 12.72 for DRIL; P = 0.04). Furthermore, continued DRIL presence in BRVO was associated with less VA gain up to 6 months (P = .02), increasing DRIL scores in CRVO/HRVO were associated with reduced VA improvement at 6 months (P = .002) and 12 months (P < .001) (63). Yiu et al concluded that pretreatment poor baseline VA is associated with a higher percentage of DRIL (P < 0.0001) in eyes with MO due to retinal vein occlusion RVO. This study was based on an analysis of 202 participants during 15-month, phase 4 SHORE study comparing monthly versus pro re nata ranibizumab after 7 monthly doses in eyes with RVO with macular oedema (64). Hajdu et al compared group of patients with DR against RVO (BRVO and CRVO) and found no statistically significant difference in terms of the occurrence of DRIL (p = 0.54) (65).

1.2.2 Changes in foveal avascular zone and parafovea on fluorescein angiogram

Understanding of macular ischaemia is based on the foveal avascular zone and integrity of perifoveal capillary free areas. Early Treatment Diabetic Retinopathy Study report 11 is one of the earliest reports that standardised the FAZ grading scheme using the best available early-phase/ mid-phase stereoscopic FA photograph of diabetic retinopathy patients (66). The size of the FAZ is an estimate of its original diameter before any

damage to the surrounding capillary net. The diameter of FAZ varies from 0 to 1000 µm. Average FAZ size is considered around 500 to 600 µm. When the capillary outline was regular (round or oval), the grader was advised to compare its area with the areas included within the dashed and solid circles of the ETDRS proposed grid. Quantitative criteria were set as follows: grade 0 = size of FAZ less than the area within 300 µm radius circle (dashed); grade l =size of FAZ equals 300 µm radius circle; grade 2 = size of FAZ greater than 300 µm radius circle but less than 500 µm radius circle; grade 3 = size of FAZ greater than or equal to 500 µm radius circle; grade 8 = cannot grade. If the FAZ outline was severely destroyed and it was not possible to assess the original size of the FAZ, the grade was "cannot grade."

The outline of normal FAZ is smooth and symmetrical. In good quality angiograms with good capillary filling, it is usually easy to characterize the appearance of an abnormal capillary net; in such images, the dropout of nonperfused segments creates sharply angled bays, often with one or two prominent vessels crossing them. However, in many angiograms, quality and filling is insufficient to allow a reliable assessment of the FAZ outline. This characteristic is frequently ungradable, even when the size of the FAZ and the degree of capillary loss in the central subfield can be reliably assessed. The outline of the FAZ was graded as grade 0 = outline of the FAZ normal; grade 1 = questionable (outline not smoothly round or oval, but visible irregularities not abnormal); grade 2 = outline destroyed for less than one-half the original circumference; grade 3 = outline destroyed for one half or more of the original circumference, but some remnants remain; grade 4 = capillary outline destroyed; grade 8 = cannot grade. Following the above brief, the grading outcome for FAZ size showed complete agreement for 74% of eyes, and agreement within one-step for 89%; kappa was 0.509, unweighted, and 0.456, weighted.

FAZ outline grading was found to be less reproducible. Besides the above findings, the "cannot grade" option was chosen in 34% of all baseline gradings (66).

Bresnick et al concluded that macular ischaemia in diabetic retinopathy could range from mild capillary closure to extensive arteriolar and capillary obliteration in the posterior pole, indicating severe macular ischaemia. In many patients described in this report with DR, FA showed a widening of the normal perifoveal capillary-free zone (PCFZ) due to capillary closure. Some eyes had four to five times greater than the average area of the PCFZ, however, maintained visual acuity of 20/20 (67). Greater degrees of vaso-obliteration produce a larger decrement in VA, but here authors have mentioned that good visual function was retained in the presence of extensive retinal vascular occlusion. Another work by Bresnick suggested retinal capillary closure as the cause of FAZ enlargement in diabetic eyes. Among all the stages, PDR eyes had the greatest FAZ diameter (more than 1mm) (18).

Angiographic analysis in DR eyes showed that the contour of the FAZ in DR was irregular (29.2% of eyes with diabetic retinopathy vs 3.7% of control eyes) with scattered round microaneurysms. The qualitative changes in the FAZ outline included budding, prominent notching or interdigitations (68). Following ETDRS grading criteria, Conrath et al found FAZ size increases with increasing severity of retinopathy status. 61.5% of PDR eyes had outline one half or more broken compared to intact FAZ i.e. ETDRS grade 3, which was highest compared to other grades of retinopathy (69).

Sim et al quantified FAZ area and compared it with ETDRS DMI severity grade where upon median FAZ areas were 0.19 mm^2 (interquartile range [IQR], 0.13–0.25) in “none”; 0.25 mm^2 (IQR, 0.18–0.32) in “questionable”; 0.27 mm^2 (IQR, 0.19–0.38) in “mild”; 0.32 mm^2 (IQR, 0.25–0.54) in “moderate”; and 0.78 mm^2 (IQR: 0.60–1.32) in “severe” ETDRS-

DMI grades. Also, eyes with PDR (both treated and untreated) and severe non-proliferative diabetic retinopathy (NPDR) were found to have evidence of DMI in the majority of cases, PDR: 77.2%; severe NPDR: 59.7% (50).

In another study, Sim et al performed grading of DMI. Out of eighty-eight eyes graded for macular ischaemia, thirty-four eyes (38.6%) had none, 12 (13.6%) had questionable, 23 (26.1%) had mild, 6 (6.8%) had moderate, and 13 (14.8%) severe ETDRS-defined DMI grades. We know macular oedema affects the reliable grading of the outer retina. In this paper eyes with DMI without MO had retinal thinning in the outer retina (70).

1.2.3 Changes in foveal avascular zone and parafovea on optical coherence tomography angiography

Before the introduction of OCTA macular ischaemia was described using FA images. However, OCTA has advantage of providing depth resolved information on capillary non - perfusion and FAZ. Reduced VD has been observed in any stage of DR compared to normal eyes. Reductions in foveal VD with increasing severity of DR in superficial (47.72% vs. 47.25% vs. 45.5%, *P*-trend = 0.013) and deep plexuses (51.29 vs. 49.53% vs. 49.01%, *P*-trend = 0.007) was noted by Tsai et al, however after adjusting for demographic parameters deep plexus remained significant. Foveal avascular zone area also showed increase in size with advancing DR severity (71).

Reliability of OCTA to determine severity macular ischaemia was confirmed by La Mantia et al. They used traditional FA and Swept source (SS) OCTA to describe macular perfusion in eyes with variable DR severity. Macular ischaemia graded using ETDRS protocol for FAZ [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), and ungradable] along with other morphological features such as microaneurysms, intraretinal microvascular changes. In the majority of the cases, DMI was graded as ‘Moderate’, regardless of the imaging technique used. However, the collective number of patients with ‘Moderate’ and ‘Severe’ DMI was higher on SS-OCTA compared with FA. The degree of agreement of DMI grading was maximal between SS-OCTA 3 × 3 and 4.5 × 4.5 (0.654, [95% CI, 0.454–0.855]), however it was fair for FA and SS-OCTA 4.5 × 4.5, (0.236, [95% CI, 0.015–0.456]). Fluorescein angiography was better in demonstrating microaneurysms compared to OCTA 3x3 and 4.5 x 4.5 (97.5 % vs 56% and 65.8%) (72). A similar result for ischaemic diabetic maculopathy was suggested by Cennamo et al. Good agreement was noted for FAZ outline and capillary loss between OCTA and FA at the DCP level, and most eyes were DMI severity grade 3 on both imaging modalities (73).

1.2.4 Macular oedema: Optical coherence tomography (OCT) classification

Otani et al described three patterns of structural changes in DMO: diffuse retinal thickening (DRT), cystoid macular oedema (CMO) and serous retinal detachment (SRD) (74). Cystoid spaces are formed in the region of the outer plexiform layer, and the rosette appearance seen in late views on FA is caused by the anatomical structure of the plexiform layer. The orientation of the vertical Muller fibers determines the arrangement of the cystoid cavities.

In DMO, the most observed pattern is DRT (increased retinal thickness with areas of reduced intraretinal reflectivity) followed by CMO. Serous retinal detachment can be

described as a dark accumulation of subretinal fluid beneath the high reflective and dome-like elevation of detached retina. Diabetic tractional retinal detachment is recognized as the area of low signal underlying the highly reflective border of detached retina. Optical coherence tomography is of particular importance to detect MO in situations of obscured fundus viewing due to presence of vitritis.

Catier et al retrospectively reviewed OCT images from patients with DR and CRVO (75). They reported that MO was in the outer retinal layers in 92% of all cases. Serous retinal detachment was present in 37% of cases, and it was more frequent in central retina vein occlusion. Likewise, Spaide et al found majority of BRVO eyes (71.4%) had SRD involving the macula (76).

An epiretinal membrane is an avascular, fibrocellular membrane on the inner surface of the retina which can cause retinal thickening and CMO in retinal vascular diseases (BRVO and CRVO). The epiretinal membrane appears as a highly reflective band on OCT and can be well differentiated from comparatively less reflective posterior hyaloid. Wilkins et al analyzed 174 eyes and found two types of ERM attachment: focal attachment was seen in 26% of eyes and 67% of eyes had a globally attached ERM. Also, partial or complete posterior vitreous detachment (PVD) is observed in 80–95% of eyes with idiopathic ERM (77).

Vitreomacular traction (VMT) is identified as partial, anomalous, posterior vitreous detachment that causes anteroposterior traction on the macula in areas of residual vitreous adhesion. Vitreomacular traction causes retinal thickening. Based on SD-OCT, there are two subclasses of VMT: focal (=1500 μ) and broad (>1500 μ) adhesion. Koizumi et al showed that eyes with focal VMT have foveal cavitation, whereas eyes with broad VMT have more widespread CMO (78). Retinal schisis is splitting of inner retinal layers

which may mimic CMO, but this is commonly seen in patients with high myopia and posterior staphylomas.

Chapter 2. Review of visual function assessments

Best-corrected visual acuity (BCVA)

Visual acuity, the acuteness of vision, is a measure of the spatial resolving ability of the visual system under conditions of very high contrast. Best-corrected visual acuity (BCVA) and its change over time is the most commonly used endpoint to assess visual function in eye disease (79). The Snellen Eye chart has been used to measure vision for 100 years. The Food and Drug Administration and the National Institutes of Health's National Eye Institute symposium suggested Early Treatment Diabetic Retinopathy Study (ETDRS) BCVA protocol offers greater standardization of VA measurement in clinical trials. This method of VA assessment provides a "calculated visual acuity score" that can be used as a continuous variable for statistical analysis purposes (80). The Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart, also known as the "logMAR chart" is based on the Bailey-Lovie chart. This chart has 5 letters at each size, size progression at exactly 0.10 log units, and standardized spacing between letters and rows.

One of the reasons it gained popularity is that ETDRS specifies acuity in logarithm of the Minimum Angle of Resolution (LogMAR) and gives equal credit for each extra letter read accurately. ETDRS charts are read at 4 metres distance and use Solan letters. When scored in LogMAR, individual letters score 0.02 log units. Snellen chart has the disadvantage of poor reliability and repeatability (81, 82). The Food and Drug Administration recommends a change in BCVA of at least 15 letters relative to baseline as an accepted endpoint in clinical trials for macular oedema. Such a change corresponds to doubling of the visual angle and is considered as a moderate visual loss. Loss of six or more lines (greater than or equal to 30 letters) corresponds to a quadrupling of the visual

angle and is considered as severe decline in vision (83, 84). The reliability of the ETDRS chart depends on the baseline VA. For instance, in eyes with acuity better than 20/100, five or more letters change has a greater than 90% probability of being significant change, while those with worse than 20/100 requires ten letters or more change to achieve similar significance.

Some of the clinical trials using this measure of visual function, included diabetic retinopathy studies assessing efficacy of aflibercept, bevacizumab, and ranibizumab (85-87). However, there has been a recent trend for considering other less subjective parameters for functional endpoints. Sunness et al has demonstrated that when geographic atrophy (GA) was present in eyes with BCVA 20/50 or better, other features of visual function were considerably impaired. These included contrast sensitivity, low-luminance BCVA, and reading speed (88). Measurement of BCVA with a letter chart often provides insufficient information on the actual function of the retina due to foveal sparing and parafoveal scotoma in conditions like GA (89, 90). The mean change in number of letters from baseline at a particular time point, such as 12 months, is another possible endpoint; by doing so we may find a smaller change to be clinically meaningful for certain diseases of eye.

Low luminance vision testing

Low luminance vision testing is designed to address the problem of reduced vision in low illumination which is often faced by patients. Retinal pathologies affecting the DCP could reduce blood supply to the photoreceptors leading to decrease in low luminance acuity. Patients with early-stage DR may have impaired rod photoreceptor function and impaired dark-adapted visual sensitivity (91). Stockman and Sharpe explained that the visual acuity in a mesopic setting requires integrated cone function mediated by post-receptoral

pathways and a disruption of this would lead to a drop in LLVA (92). Reduction in both BCVA and LLVA could be secondary to mechanical disruption and disorientation of photoreceptors, thereby reducing spatial resolution at both illuminance levels. Possible ways of measuring LLVA, are by placing a neutral density filter in front of the study eye and asking the participant to read an illuminated letter chart, or by inserting a mesopic filter in front of the letter chart (93, 94). Low luminance deficit (the difference between normal and low luminance measures) has been indicated as a predictor of future visual acuity loss in GA (95).

Colour vision

Healthy retinal neurons, rods are responsible for scotopic function; whilst cones are used for photopic function. Highest density of cones is observed in fovea. Since colour vision is a cone mediated function, it can be used to monitor retinal pathology. There are three sub-types: the long- (L-), medium- (M-), and short- (S-)wavelength sensitive cones. The M and L cone subsystem has the ability to extract spatial detail from the visual scene (96). Hence, reduction in visual acuity in retinal diseases are often associated with acquired red-green colour deficiency (97). Neuro-inflammation is considered to play major role in diabetic retinopathy and subsequent colour vision defects. Earlier studies have identified increased levels of vascular endothelial growth factor (VEGF), interleukin (IL)-1 B, IL-6, IL-8 and tumour necrosis factor (TNF)-α in the vitreous of DMO patients and patients with PDR (98).

Gella et al found colour vision deficiency in Type 2 diabetics without presence of clinical retinopathy. They opined this could be due to neurodegenerative changes and unlikely of vascular etiology (99). A study conducted in eyes receiving dexamethasone implant for DMO showed loss of red-green and yellow - blue chromatic sensitivity at recruitment. Post

treatment red -green sensitivity improved while no recovery was noted for yellow -blue colour vision (98).

Contrast sensitivity

Measures of contrast sensitivity could be a better tool to track the progressive loss of vision associated with eye diseases (100, 101). Contrast sensitivity testing evolved because it was shown that those with AMD had poor contrast discrimination. Similarly, DR is associated with a loss of contrast sensitivity (102).

The Pelli-Robson test for contrast sensitivity comprises letters arranged into triplets, with two triplets per line; the intensity of the letter contrast decreases from one triplet to the next (103). The Pelli-Robson chart is considered the gold-standard contrast sensitivity measurement, but can be difficult to set up accurately and illuminate evenly, as well as to replicate at each visit (103). Additionally, the range of measurements is small, making it difficult to assess subtle differences between patients.

Later, the Mars Letter Contrast Sensitivity Test was developed to address the shortcomings of the Pelli-Robson chart, such as the viewing distance and large, impractical chart size (104). Unlike the Pelli-Robson chart, the intensity of the letter contrast decreases gradually over the course of the Mars chart, with the first letter displaying the highest contrast and the last letter displaying the lowest (103, 105). Notably, patients with low vision tend to have better repeatability when tested with the Mars chart versus the Pelli-Robson chart (103).

A more recent advancement in this category of testing is the Ora variable contrast flicker test (Ora-VCF™) (106). A diffuse light source is used to bleach most of the patient's cone photopigment, after which macular recovery is tracked using a variable contrast flickering

stimulus. The Ora-VCF has been previously used in GA patients where better and reliable outcome was achieved as compared to the Pelli-Robson chart (107).

However, contrast sensitivity may be a less clinically meaningful measure of vision for patients. In addition, it can be challenging to replicate the lighting conditions during each session, rendering accurate tracking of improvements difficult.

Microperimetry

Microperimetry works by combining fundus image with functional measures to detect retinal sensitivity. It can potentially map structure to function in the retina by measuring the minimum light intensity that patients can perceive when spots of light stimulate specific areas of the retina (79, 108-111). Microperimetry using the Macular Integrity Assessment (MAIA) and Nidek systems can accommodate patients with poor vision by using an enhanced fixation light. Microperimetry can track and display live fundus image even in patients who have unsteady or non-foveal fixation, which is vital for accurate measurements in patients with visual loss (110). Also, if the fixation characteristics change during disease progression, microperimetry is able quantify them. This makes microperimetry more appealing compared to conventional visual fields. In light condition micrometry estimates cone (photopic) or cone- rod (mesopic) function. Scotopic microperimetry usually requires 30 minutes of dark adaptation. According to dark adaptation curve for transition from light to dark condition, the initial rapid increase in sensitivity is from the cones followed by a plateau stage called the cone-rod break, after this sensitivity value is mainly from the rods under scotopic conditions (112). Although scotopic microperimetry can be quite time-consuming causing practical physiological constraint mesopic microperimetry is a quick and reliable alternative that requires minimal dark adaptation (about 10 minutes) (113, 114).

Interpretation of MP result should include location of the retinal area tested as topographical difference in densities of rods and cones varies according to eccentricity. Other challenges with microperimetry are lack of standardization among different devices and use of complex machinery requires a trained specialist, which may not be practical in all clinical situations. Clinical trial end points should be useful to patient and clinicians, it should be easily repeatable, feasible and affordable to perform. Microperimetry is known to be a lengthy procedure and requires in-depth knowledge for interpretation of the result.

Despite this, microperimetry remains an attractive endpoint for metrics of retinal sensitivity in conditions like GA, DMI and inherited retinal disorders, especially if short, custom-made programs can be developed (115).

Multifocal electroretinography

Multifocal electroretinography (mfERG) is one method of recording retinal responses to stimuli. During mfERG testing, patients view a fast sequence of images on a monitor while retinal response is recorded (116). There has been a study indicating this may not be a suitable method to identify ischemic areas in retina(117). Recording of bioelectrical responses from selective retinal areas enclosed between 0° and 20° from the fovea in intermediate AMD has shown early dysfunction of the foveal, and the parafoveal preganglionic elements.(118) Likewise, reduction in both amplitude and implicit time is noted on mfERG in Mactel type 2 (119).

Dark adaptometry

Like the Ora-VCF, dark adaptometry measures the length of time it takes for the retina to regain maximal sensitivity to low amounts of light after it has been exposed to bright light then returned to darkness (120-122). In diabetic retinopathy, dark adaptometry has shown

that rod recovery function degrades earlier than cone function (120). Time to recovery of baseline visual sensitivity after a period of photostress has also been used as a measure of macular function in elderly subjects (mean age 75 years) (106).

Again, dark adaptometry is a quantitative and reproducible measure of photoreceptor function; it is quite time-consuming (~30 minutes per eye) to be relevant daily clinical practice.

Reading speed

Reading speed is a functionally important measure of visual ability from the patient's perspective. Tests of reading speed, such as Minnesota low vision reading chart (MNREAD) and International Reading Speed Texts (IReST), may provide practical visual function endpoints that measure the impact of macular ischaemia on daily tasks (123-125).

In MNREAD and IReST, patients read text aloud while being timed by an investigator (123, 125). Minnesota low vision reading chart is a continuous reading acuity chart. After every three lines, the size of the text decreases by a certain font point number (123). Reading acuity, maximum reading speed and critical print size (smallest print that supports the maximum reading speed) can be determined via MNREAD (123). International Reading Speed Texts comprises 10 paragraphs of simple text (~132 words per paragraph) that are available in multiple languages. The simplicity of the words and equivalent availability in multiple languages makes IReST a robust tool, avoiding the confounding effects of education and nationality (125).

In 2013, Ian Bailey and Jan Lovie-Kitchin pointed out the importance of standardization in testing in clinical trials, which can be achieved by using same number of optotypes in

each row, a constant ratio of size progression, and the spacing between optotypes within rows and between rows being proportional to the optotype size (126). Best corrected visual acuity using ETDRS chart has been standardized through certification and centralized data review which supports accurate and consistent measurements. Hence, it remains as the most widely used functional endpoint in research.

Chapter 3. Literature review: central retinal vein occlusion (CRVO) and proliferative diabetic retinopathy (PDR)

3.1 Proliferative diabetic retinopathy

Diabetic retinopathy (DR) is the most common complication among working-age people who have diabetes. One-third of all people with diabetes are likely to have concurrent retinopathy (127). Proliferative diabetic retinopathy is characterised by of developing retinal or optic nerve neovascularisation in people with diabetes. Left untreated, PDR can result in a severe visual loss (128). Panretinal photocoagulation is an established treatment for this condition, but it destroys the peripheral retina to cause regression of the retinal and optic nerve neovascularisation (129). Repeated PRP sessions may be required to treat a recurrence of neovascularisation. They may be associated with visual function loss, such as peripheral field loss, impaired dark adaptation and reduced contrast sensitivity (130). Repeated intravitreal injections of anti-vascular endothelial growth factor (anti-VEGF) agents are as effective as PRP in a five-year follow-up study (131).These anti-VEGF agents modulate the disease process and cause regression of the neovascularisation, but there is a probability of disease recurrence when the therapy is stopped.

Both PRP and anti-VEGF agents stabilise visual acuity in PDR. Protocol S proved that treatment with anti-VEGF agents was non-inferior to PRP (131, 132). Five years data from Protocol S have concluded that severe vision loss or serious complications of PDR could be avoided with ranibizumab or PRP. The Clinical efficacy and mechanistic evaluation of aflibercept for proliferative diabetic retinopathy (CLARITY) study showed that aflibercept, an anti-VEGF agent, resulted in superior visual outcomes compared to PRP in eyes with treatment naïve PDR or active PDR after initial PRP (132). CLARITY

protocol suggested aflibercept injection every month for three months followed by pro-re-nata dosing to treat recurrences, reactivation or new onset neovascularisation over 52 weeks (133).

3.2 Predictors of visual acuity in proliferative diabetic retinopathy

Visual acuity tests such as Snellen and ETDRS are established tests of visual function (134). However, these are tested on high contrast charts and are affected by light scatter and wavefront aberrations. However, visual acuity is often not affected in PDR unless complications such as DMO and/or ischaemia, ERM, vitreous haemorrhage or tractional changes involve the fovea.

Mesopic visual acuity testing uses reduced contrast and is more translatable to real-world tasks such as driving at night (135). It has also been shown to better correlate with certain diseases than visual acuity (136, 137). Although mesopic visual acuity is also a foveal function, this pathway is more complex and less well understood. This pathway is influenced by both the photoreceptors and the post-receptoral pathway (92).

Hypoxia is the key driver of PDR. As retinopathy advances, the hypoxic stimulus from capillary drop-off causes the development of new blood vessels on the retina and /or the optic disc. As the rod photoreceptors require a significant amount of oxygen during dark adaptation, this imbalance between oxygen demand and supply may compromise the function of the rod photoreceptors. However, subjective impairment of night vision has been rarely reported by people with untreated PDR (138). Cone photoreceptors are also affected in diabetic retinopathy (139). It is unclear whether mesopic visual acuity may be a better test of visual function than photopic visual acuity in eyes with PDR.

Various factors have been identified in literature which affect visual acuity outcome in patients with DMO, since it is the most common cause worsening VA. Nature and duration

of macular oedema, OCT defined morphological features such as disruption of ELM, EZ, DRIL and HRF have known to affect VA in DR. Ninety four eyes of seventy three patients treated with intravitreal ranibizumab for macular oedema showed treatment response was significantly influenced by age (p = 0.003) and baseline BCVA (p = 0.001). Also, glycosylated hemoglobin (HbA1c) (p = 0.013) and proliferative diabetic retinopathy (PDR) (p = 0.019) were predisposing factors for visual outcome in the responders and non-responders, respectively (140). However, not much literature is available about VA predictors in advanced DR i.e. PDR without MO.

In PDR macular ischaemia plays an important role both in presence and absence of macular oedema in forecasting VA outcome. In Bezzina et al's paper following three loading doses of intravitreal bevacizumab eyes with PDR had a mean gain in central macular thickness of 16.86% (*P* = 0.001) quite reverse to the normal benefit of anti-VEGF treatment (141).

There is literature on long-term visual outcomes in DR after PRP. The Early Treatment Diabetic Retinopathy Study reported that 42% of patients had visual acuity of 20/20 or better in the better eye, and 84% had visual acuity of 20/40 or better in the better eye. When compared to baseline, 20% of patients had moderate vision loss in the better eye at follow-up, and only 1% had severe loss at 5 years (142). A study to identify VA outcome following PRP was done by Shimura et al in 64 patients with severe non-proliferative diabetic retinopathy or non-high-risk proliferative diabetic retinopathy whose VA was 20/20 or better. All these patients underwent PRP divided into four sessions. At 24 weeks, 84% patients-maintained pretreatment VA, VA initially decreased in three eyes but subsequently recovered to baseline during follow up and VA did not recover during follow-up in seven eyes. Also, parafoveal OCT thickness of >300μ had poorer VA outcome (143). Better pretreatment VA, younger age (p < 0.0001) has positive effect on treatment

outcome, while higher number of PRP sessions and vitrectomies to arrest disease progression has quite the opposite effect on VA (144).

In an OCTA-based study, 118 patients with previously treated quiescent PDR, FAZ area at the level of SCP and DCP were positively correlated with DM duration (β = 0.178, *P* = 0.047, β = 0.252, *P* = 0.045). Vessel length density, mainly in DCP, was associated with poor visual outcome (145).

3.3 Central Retinal Vein Occlusion

Diseases affecting either retinal arteries, veins and capillaries or combination can cause central vision loss. Retinal vasculature is an end arterial system, hence any hindrance in the path of flow increases venous and intra capillary pressure affecting the arterial blood flow to the area. The severity of its effect on occlusion of retinal veins depends on rapidity of onset, development of collateral vessel, and degree of obstruction. In case of mild obstruction, there is greater chance of complete resolution of fundus changes and recovery of visual acuity with early recanalization and development of collaterals. Increasing severity of occlusion causes extensive capillary endothelial damage, leading to cystoid degeneration and atrophy, which can persist even after renewal of normal venous pressure.

Central retinal vein occlusion happens due to obstruction of the venous outflow, at the site of the lamina cribrosa or posterior to it (146). Sites with maximal risk for obstruction are located at arteriovenous crossings or at the optic disc. Central retinal vein occlusion could be considered as non – ischemic when VA is better or equals 20/200 with mild to moderate macular oedema and no or <10-disc areas of non-perfusion recorded on angiography.

Based on systemic risk factors, Hayreh found significantly higher incidence of arterial hypertension and diabetes mellitus among ischemic CRVOs compared with nonischemic CRVOs (147). In the Multiethnic Study of Atherosclerosis, independent risk factors associated with RVOs were AHTN (Odds Ratio [OR]: 2.06), hypertriglyceridemia (OR: 1.98), and renal dysfunction (OR: 1.85). Presence of retinal AV nicking (OR: 4.01) and focal arteriolar narrowing (OR: 4.38) were also associated with the development of RVOs (148). Hayreh also steered a study on 535 patients and investigated associations of hematological abnormalities with various types of RVOs. Ischemic CRVOs showed a significantly higher prevalence of abnormal hematocrit, hemoglobin, and blood urea nitrogen than nonischemic CRVOs (149).

Visual morbidity and complications of CRVO occur mainly due to presence of MO, vitreous hemorrhage (VH), neovascularization, and neovascular glaucoma. During the last few decades, several new treatments have been introduced to address these complications. For instance, laser photocoagulation, medical therapy with anticoagulants, fibrinolytics and acetazolamide; and more recently, intravitreal injection of steroids or anti -VEGF agents (150-153).

Moore first reported vision outcome in CRVO eyes in 18 cases, he observed them for several years and majority had unfavorable visual acuity (154). Zegarra et al observed thirty-five patients with CRVO from one to eight years and described their visual prognosis under two clinical sub types favorable in venous stasis retinopathy and extremely poor in hemorrhagic retinopathy (155). One of the earlier studies on natural history has shown sixty-five percent of patients with initially good visual acuity (20/40 or better) maintained their visual acuity for 3 years follow of up. Patients with intermediate initial acuity (20/50-20/200) showed a variable outcome: 19% improved to better than 20/50, 44% stayed in the intermediate group, and 37% had final visual acuity worse than 20/200. Patients with

poor visual acuity at onset (<20/200) had an 80% chance of having a visual acuity less than 20/200 at final visit, whether perfused or non-perfused at baseline. This group suggested initial visual acuity could be a good predictor of long –term vision outcome in CRVO however, not for intermediate VA group. Furthermore, first 4 months is crucial for the rapid increase in non-perfusion (156).

Quinlan et al conducted retrospective study on 107 non-ischemic and 61 ischemic CRVO eyes. The criterion to define ischaemia was ≥5 disc-diameter area of capillary non-perfusion. They followed up patients for 6 months to 6 years (mean, 22 months). In non-ischemic CRVO eyes, the initial visual acuity varied from 20/15 to count fingers; among them, in eyes with initial visual acuity of 20/40 or better, 21 % had final visual acuity was 20/200 or less, and in those with initial visual acuity of 20/200 or less it was 20/200 or less in 88%. In this group, 15% improved by three or more lines from the baseline and 31% lost three or more lines. In all eyes with “ischaemic CRVO”, initial visual acuity was 20/100 or less; the final visual acuity was 20/200 or less in 93%, counting fingers or less in 54%, and hand motion or less in 36%. Thus, initial good visual acuity does not always indicate favorable outcome (157).

In a case series of 59 eyes with non-ischemic CRVO, with a minimum period of 1 year follow up (average 2.5 years), VA analysis showed an improvement by two or more lines in 15% eyes. Visual acuity in 56% of eyes remained stable and decreased in 29%. Conclusion from this study was non-ischemic CRVO frequently results in significant, permanent visual loss. They found that initial VA had no predictive value with respect to progression.

The Central Vein Occlusion Study Group described their results on VA in 714 eyes with CRVO. The criteria used to classify their cases into ischemic CRVO was the presence of

at least a 10-disc area of retinal nonperfusion (51). They divided their participant acuity 3 groups based on initial visual acuity: group 1 with visual acuity of 20/40 or better (29%), group 2 with acuity between 20/50 and 20/200 (43%), and group 3 with acuity worse than 20/200 (28%). The final visual acuity in group 1 was stable in 65% and deteriorated in 35% - in 10% to worse than 20/200; in group 2 it improved to better than 20/40 in 19%, stayed the same in 44% and deteriorated to worse than 20/200 in 37%; and in group 3 in 79% it was less than 20/200, in 19% improved to 20/50 to 20/200 and in 1% to 20/40. They concluded that “Visual acuity at baseline is a strong predictor of visual acuity at 3 years” for eyes in group 1 and 3 but a poor predictor for group 2. At onset, majority of non-ischemic CRVO patients present with VA of less than 20/40 while for ischemic CRVO this would be around 20/200.(158) The Standard Care vs. COrticosteroid for REtinal Vein Occlusion (SCORE)study observation arm (73 eyes) showed at 12 months follow-up, VA improved in 26%, remained the same in 19% and deteriorated in 55%. However, non-ischemic and ischemic eyes were not separately analysed here (159). Hayreh et al found 99% of ischaemic CRVO eyes mean age of 61±16 years who were seen within 3 months of onset of the condition had VA of 20/200 and worse while for non ischaemic CRVO 57% patients had initial VA of 20/40 and below. In eyes (non-ischemic) with initial visual acuity of 20/60 or better, 17% (95% confidence interval (CI): 13%, 23%) showed visual acuity deterioration at 3 months of follow-up, and 20% (95% CI: 15%, 28%) during the 2 to 5 years follow-up. While VA improvement was significant (p=0.03) during follow up for 20/70 or worse initial VA cohort. 32% (95% CI: 27%, 45%) showed visual acuity improvement at 3 months of follow-up, and 47% (95% CI: 40%, 63%) during the 2 to 5 years follow-up. Overall, the rate of VA improvement was better among non-ischaemic CRVO compared to ischaemic CRVO (154). Above evidence indicates that eyes with better baseline VA

has more chance of improvement in first 3 months with potential for further improvement in next few years.

Literature suggests mean decline in VA could range from 1 to 75 letters irrespective of ischaemia status at baseline. Cumulative information from studies have shown the mean improvement in VA ranges from 1.5 letters to 12.5 letters; however, none of the eyes could attain better than 20/40.(160) Information gathered from natural history studies show mean decrease in VA by 10 letters from baseline can happen at 6 months and 3 letters from baseline at 12 months, for non-ischemic CRVOs. For ischemic CRVOs, the mean decrease was more likely to be 15 letters from baseline at 6 months and 35 letters from baseline at 12 months or beyond (161).

Management of macular oedema in central retinal vein occlusion

Earliest proposed treatment of macular oedema in CRVO, to improve VA using macular grid photocoagulation did not show any difference between treated and untreated eyes in visual acuity at any point during the follow-up period (162).

The primary paper on Dexamethasone (DEX) intravitreal implant in patients with macular edema due to CRVO or BRVO showed ≥15-letter improvement in BCVA from baseline was attained by 30% and 32% of patients 60 days after the first and second DEX implant, respectively at 12 months (163). Geneva study and allied study, where Ozurdex and an alternative dose of DEX implant (0.35mg) were compared to sham injection showed percentage of eyes with a > or =15-letter loss in BCVA was significantly lower in the DEX implant 0.7-mg group compared with sham at all follow-up visits (P< or =0.036). The mean BCVA improvement was greater in both DEX implant groups compared with sham at all follow-up visits (*P*≤0.006) (164).

According to the SCORE study group the percentage of participants with CRVO who gained visual acuity of 15 letters, or more was 26.5%, 25.6% and 6.8% for triamcinolone 1 mg, 4 mg, and sham, respectively (p=0.001 for 1 mg versus observation and for 4 mg versus observation). Participants loosing 15 letters, or more was nearly doubled in the sham group (44% at month 12) compared to 25% (1 mg) and 26% (4 mg) in the triamcinolone groups. This variability amongst treatment arms persisted at 24 months (48% [sham] versus 31% [1 mg] and 26% [4 mg]) (165).

The CRUISE (A Study of the Efficacy and Safety of Ranibizumab Injection in Patients with Macular Oedema Secondary to Central Retinal Vein Occlusion) was performed to assess the safety and efficacy of intravitreal ranibizumab to treat MO due to CRVO. The primary outcome for the CRUISE study was BCVA mean change at 12 months following recruitment in the trial. Eyes with macular oedema due to CRVO were randomised to receive 1:1:1 monthly injections of 0.3 mg or 0.5 mg ranibizumab or sham injections for 6 months, followed by further injections of ranibizumab if BCVA was ≤20/40 or central subfield thickness ≥250 μm. Patients treated with ranibizumab gained +13.9 letters (both groups 0.3/0.5 mg and 0.5/0.5 mg in parallel), and +7.3 letters, if treated 6 months with sham injections followed by ranibizumab 0.5 mg ($p<0.001$ for each ranibizumab group versus sham/0.5 mg group). Percentage of patients with a gain of 15 letters or more was highest in ranibizumab 0.5/0.5 mg (50.8%), versus 0.3/0.5 mg (47.0%) and sham/0.5 mg (33.1%) (166).

Three hundred and four participants from CRUISE study went into HORIZON study and who were monitored every 3 months and treated based on specific re-treatment criteria. After 12 months in this study mean visual acuity change was -5.2 (ranibizumab 0.3/0.5 mg), -4.1 (0.5/0.5 mg) and -4.2 (sham/0.5 mg) letters, respectively. The mean total

change in BCVA from baseline to month 24 were +8.2 (0.3/0.5 mg), +12 (0.5/0.5 mg), and +7.6 (sham/0.5 mg) letters (167).

Epstein et al observed that CRVO patients at 12 months' time point, treated with bevacizumab 1.25 mg every 6 weeks from baseline gained +16.1 letters, compared to +4.6 letters in those treated with sham injections for first 6 months followed by bevacizumab ($p<0.05$). Eyes with BCVA gain of more than 15 letters was 60% (bevacizumab) vs. 33.3% (sham/bevacizumab), respectively ($p<0.05$) (168).

The GALILEO [Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion] study was a randomised controlled trial to treat macular oedema due to CRVO, participants received 2-mg intravitreal aflibercept or sham injections every 4 weeks for 20 weeks from week 24 to 48, aflibercept group received aflibercept as required, and the sham group continued receiving sham injections. On completion of 52 weeks, the mean percentage of patients gaining 15 letters, or more was 60.2% in the aflibercept group and 32.4% in the sham group ($P = 0.0004$) and aflibercept arm patients had a significantly higher mean improvement in BCVA (+16.9 letters vs. +3.8 letters, respectively) with respect to sham group (169).

The COPERNICUS was a multicentre randomised controlled trial to evaluate intravitreal aflibercept injection for MO secondary to CRVO. Participants were randomised to receive 6 monthly injections of either 2 mg intravitreal aflibercept (IAI 2Q4) ($n = 115$) or sham ($n = 74$). All patients received 2 mg intravitreal aflibercept as needed [IAI 2Q4 + pro re nata (PRN) and sham + IAI PRN] from week 24 onwards until week 52 according to predefined criteria. The aflibercept 2 mg group gained +16.2 letters, compared to +3.8 letters in the sham/aflibercept ($p<0.001$) at week 52 and at week 100, +13.0 vs. +1.5 letters; $P<0.0001$ respectively. The percentage of patients with a gain of more than 15 letters was 55.3%

(aflibercept) and 30.1% (sham/aflibercept) at 52 weeks, respectively (p<0.001) while at 100 weeks this was 49.1% vs. 23.3% (P<0.001) (170, 171). Quarterly review and IAI PRN treatment was offered to all participants from week 52 to week 100 based on re treatment criteria which could reduce to every 4 weekly follow up if deemed necessary by clinician (171).

Anti vascular endothelial growth factor agents have introduced better prospects regarding visual prognosis in CRVO patients. However, not all eyes respond similarly to treatment. From the COPERNICUS study, we can say VA gain in the first year was more than in the second year. Also, the percentage of eyes gaining ≥15 letters dropped slightly in year two compared to the first year of treatment.

3.4 Predictors of visual acuity in central retinal vein occlusion

Standard Care vs. COrticosteroid for REtinal Vein Occlusion (SCORE) 2 study group looked at baseline parameters associated with 6 months VA outcome in RVO patients. They suggested younger patient age (odds ratio [OR], 0.95 per year of age; 95% CI, 0.93-0.98; P = .007) and lower baseline VA letter score (OR, 0.96 per letter; 95% CI, 0.94-0.98; P < .001) were associated with a 6-month VA gain of 15 letters or greater in eyes treated with bevacizumab or aflibercept. On subsequent analysis lower month 01 VA (p<0.001), higher age (p<0.001), absent EZ within CSF (p<0.04 and <0.01) and presence of subretinal fluid (p=0.01 and <0.001) were the variables associated with worse VA letter score at 6 and 12 months. Finally, when adjusted for demographics and SD OCT parameters, vision was the only significant factor for all visits (172).

Chan et al proposed increase in DRIL at 3 months' time point along with EZ disruption were only OCT morphological features that foretells worsening of VA over 1 years after adjusting for baseline VA (173).

A prospective study by Daien et al including ischaemic CRVO and non-ischaemic CRVO (total 50 patients) with MO was conducted to identify baseline pretreatment factors associated with VA changes. Patients were treated with monthly bevacizumab for 3 months followed by 3 months observation and PRN treatment for next 6months. Response to treatment was based on 3 categories: VA improvement with ≥15 ETDRS letters, a change in ETDRS letter score >0 to <15, and lack of response with a decrease in VA letter score. Factors strongly predicting gain in VA ≥15 ETDRS letters, were younger age with a mean of 56.0±9.9 years (p=0.002), a shorter pre-treatment symptomatic period with a median duration of 33.3±8.9 days (0.001), and a higher baseline VA (p=0.004) when compared to other two groups (174).

Twenty-four months retrospective analysis of 155 eyes (65 CRVO, 90 BRVO) treated with Dexamethasone intravitreal implant showed worse baseline BCVA was correlated with a greater final BCVA gain (p<0.001, unstandardized coefficient: 0.46, 95% CI: 0.25 - 0.68). They also found CRVO eyes with prior history of anti-VEGF treatment faced poor VA gain, p=0.006. This study also indicated range of VA change at 2 months can potentially predict 24 months VA outcome. 37 out of 67 patients (55.2%) who gained ≥10 letters at 2 months, had VA gain ≥10 letters at 24 months. Similarly, 34 out of 52 patients (65.4%) who gained <5 letters after 2 months persisted as poor responder throughout the follow-up.

The Study Evaluating Dosing Regimens for Treatment with Intravitreal Ranibizumab Injections in Subjects with Macular Edema following Retinal Vein Occlusion (SHORE) was a randomized control trial comparing monthly vs PRN treatment with ranibizumab in branch and central RVO. Retrospective analysis of SHORE study data (171 randomized patients) was done to understand the baseline predictors associated with early clinically significant VA improvement, defined by increase in BCVA of 15 or more ETDRS letters from baseline or improvement to a Snellen equivalent of 20/40 or better vision. Patients

presenting with better vision at the start of treatment shows earlier improvement to 20/40 or better vision. In patients with initial BCVA of 50 or more ETDRS letters (≈ Snellen equivalent: 20/100) the median time to first attain 20/40 or better vision was 32 days while those with ≤ 50 letters BCVA took 186 days to achieve the same (P < .0001). Secondly, patients with macular volume of greater than 9.99 mm^3 at baseline were earlier to achieve 20/40 or better vision (median of 36 days) compared to those less than 9.99 mm^3 (66days, p=0.02). Lastly, subretinal fluid on spectral-domain optical coherence tomography at baseline favored earlier accomplishment of 20/40 or better VA (p=0.03). Also, 15 or more ETDRS letters gainers within 3 months of treatment initiation were male patients (median 58 vs 92 days, p=0.03). Baseline BCVA ≤ 50 ETDRS letters required shorter median time to attain improvement of 15 or more ETDRS letters (p=0.57) (175).

In this chapter, I have discussed the existing literature on predictors of VA outcomes in PDR and CRVO eyes.

Chapter 4. Aims

The aims of my thesis were:

First, as OCTA is a new device to measure the flow of blood through the macular microvasculature, I assessed the normative value of FAZ parameters based on age and sex and ethnic origin. As it is already established that central retinal thickness varies with age, sex and ethnicity, the aim was also to look at the correlation of FAZ parameters on OCTA with retinal thickness on OCT to understand the correlation of neuronal and vascular parameters at the macula.

I then studied the changes in the microvasculature in severe NPDR and PDR on OCTA. These were also correlated to OCT parameters to evaluate the changes in neuronal and vascular changes at the macula in severe stages of retinopathy. As PDR also causes neovascularisation of the disc, I evaluated the OCTA parameters in the peripapillary area and compared these changes to that at the macula.

As CRVO also affects the microvasculature but is an acute condition compared to PDR, I then evaluated the similarities and differences in microvascular changes at the macula in PDR and CRVO.

Having studied the structural changes, I then correlated these vascular changes with visual function in PDR. I wanted to understand which of the structural changes were associated with BCVA and LLVA. I also studied the rank order of these association. I looked at the correlation between LLVA and BCVA in treatment naïve PDR patients and the effect of aflibercept and PRP on these visual function parameters. I then assessed whether these visual functions would change when fill-in PRP is done on eyes with previously PRP treated PDR eyes with active neovascularisation.

Lastly, I studied the relation of visual acuity and CST at presentation of CRVO and following treatment with anti-VEGF. I then evaluated the predictors of visual acuity outcome in macular oedema due to CRVO treated with anti-VEGF therapy.

Chapter 5. Methodology

This thesis includes data collected from three clinical studies:

1. DMI study (A prospective study of structure-function correlation in patients with diabetes with varying sizes of foveal avascular zone).

2. Post-hoc analysis of the CLARITY study (Clinical efficacy of intravitreal aflibercept versus panretinal photocoagulation for best corrected visual acuity in patients with proliferative diabetic retinopathy at 52 weeks): a multicentre, single-blinded, randomised, controlled, phase 2b, non-inferiority trial.

3. Post-hoc analysis of the LEAVO trial, which was a multicentre, prospective, 3-arm, double-masked, randomized, noninferiority trial comparing clinical effectiveness of intravitreal therapy with ranibizumab vs aflibercept vs bevacizumab for macular oedema secondary to central retinal vein occlusion.

Retrospective data was collected for the chapters 6 and 9. Details of study approval, participants and statistical analysis described in their respective chapters.

5.1 Informed consent

The participant invitation letter and participant information sheet were given to the patient before attending a screening or baseline visit for the DMI, CLARITY and LEAVO trials. The study investigator was responsible for giving the informed consent of each participant at the beginning of any trial procedure. Participants had the full opportunity to ask questions before signing the consent form—further details of study participants are described in respective chapters.

5.2 Details of study assessments

5.2.1 Best Corrected Visual Acuity

For participants included in chapters 7,8 and 9, refraction was carried out following predesigned protocol. The right eye was refracted first. The subject was seated at 4 metres from the chart. The fellow eye was covered with a pad and tape. To begin with, the visual acuity was assessed with the subjects' own spectacles or unaided if the subject does not have distance spectacles. Retinoscopy was then performed to estimate starting point for subjective refraction. BCVA was measured using ETDRS chart 1 for the right eye and chart 2 for the left eye. Each eye was tested at 4 metres initially. If a subject cannot read 20 letters or more at 4 metres, this test was repeated at 1 metre. In such a case, only the first six rows were attempted. The final VA score was the number of letters read correctly at 4 metres plus those read correctly at 1 metre. If the subject did not need to be tested at 1 metre, i.e., they read 20 or more letters at 4 metres; then the score was the number of letters read correctly at 4 metres, plus 30. If any subject could not read any letters on the ETDRS chart at 1 metre, then their ability to detect hand movements or light perception was measured.

5.2.2 Low Luminance Visual Acuity

After the BCVA testing, the same lenses were kept in the trial frame with the same lighting conditions as before. A neutral density filter was either held close to the trial frame or an ETDRS chart mounted type filter was used to decrease the luminance by 2.0 log units. This filter reduces luminance 100-fold. The vision measurements were repeated in the same manner as before, at 4m, re-instructing the subject as before. If less than 20 letters

were read correctly 1m test was performed. Low luminance deficit (LLD) was defined as the difference between BCVA and LLVA.

BCVA and LLVA measurements were performed following these steps for the prospective studies. For the retrospective studies visual acuity measurement involved subjects using their existing spectacles or if not using glasses, unaided BCVA was accepted.

5.2.3 Optical Coherence Tomography

The SD-OCT macular scans were performed on Spectralis (HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). It combines high-resolution SD-OCT with a scanning laser ophthalmoscope.

This device determines the retinal thickness by measuring the distance between the inner limiting membrane (ILM) and the inner boundary of the RPE in the line scans.

a. The standard macular scan was captured for chapter 6, 8, and 9 using 20x20-degree scan pattern, with 49 raster lines, separated by 120mm. Macular thickness map composed of the ETDRS rings measuring 1 mm (innermost ring or central sub-foveal thickness) and 3 mm (inner ring) in diameter with further sub-division into 4 equal zones. Thus, we had five retinal zones used for analyses (innermost 1mm (CST), and the surrounding nasal, temporal, superior and inferior zones).

b. Scan parameter used to assess retinal morphology for the LEAVO study (chapter 10) subjects was Spectralis macular raster with dimensions of 30 x 25 degrees and 31-line scans at 241µm spacing. To analyse DRIL, five horizontal OCT scans: one b-scan encompassing the fovea, two-line scans covering 500μm superior and 500μm inferior to the fovea was used. The definitions implemented for grading of OCT morphological features are described below.

ERM is a hyperreflective band overlying ILM, which could be present as adherent throughout or attached at focal points with the retina. Membranes which were fully attached was associated with macular pseudo hole, a difference in optical reflectivity between the membrane and retina, and/or a visible membrane tuft or edge (77). OCT-based definition of vitreo macular adhesion (VMA) and vitreomacular traction (VMT) was taken from The International Vitreomacular Traction Study (IVTS) Group. For the purpose of grading VMT was considered present if abnormal posterior vitreous detachment was associated with anatomic distortion of the fovea, which may include pseudocysts, macular schisis, cystoid macular edema (176). DRIL definition is adapted from Sun et al, areas of inner retina where the boundary between the GCL-IPL , inner nuclear layer and outer plexiform layer could not be identified is considered as DRIL present provided the average across central 5-line scan suggest ≥50% involvement (60). Macular oedema could be cystoid, diffuse or combination of both (mixed). Diffuse oedema categorizes any sponge-like retinal swelling with reduced intraretinal reflectivity. Intraretinal cysts were minimally reflective round spaces situated in neurosensory retina. Cysts sizes were quantified as small, <250 mm, medium ≥250mm and <500 mm, and large ≥500mm based on the greatest horizontal diameter (177). Previous literature suggests hyperreflective foci could be marker of inflammatory response in retina, degenerated photoreceptors, or future hard exudates (178). Following the European School for Advanced Studies in Ophthalmology classification we considered hyperreflective intraretinal spots present if > 30 spots are seen throughout any or all retinal layers. Integrity of ELM is important along with healthy photoreceptor layer with respect to visual acuity. ELM is the faint narrow line superior to the ellipsoid zone on OCT and was graded as intact if visible throughout the entire foveal line scan, not intact if disrupted or completely absent under high contrast settings. If ELM was not discernible due to shadowing from oedema, then graders identified it as absent

and partly visible as questionable. EZ, the photoreceptor ellipsoid is the third hyperreflective band.

c. Macular scan with dimensions of 20 x 20 degrees, 97-line scans at 60 μm spacing was used to capture central subfield and perifoveal retinal thickness map for the angiometric study in stable PDR patients (chapter 7). For this study, we looked for DRIL along 7-line scans (line 46 to 52) within central 1mm area and deemed as DRIL present if ≥50% of area was affected.

5.2.4 Optical Coherence Tomography Angiography

For chapter 6, I have collected data from Zeiss Angioplex OCTA. OCTA images captured using Zeiss Cirrus HD-OCT 5000 (Zeiss Meditec, Dublin, CA) with Angioplex uses optical microangiography (OMAG) algorithm to generate the OCTA images (179). The A scan rate for this machine is 68,000 scans per second and the central wavelength of light source 840nm. The 3x3 en face angiograms with superimposed ETDRS grid was used to estimate the VD at central 1mm and the nasal, superior, temporal, and inferior zones in the 3mm annular parafoveal area. The OCTA parameters collected directly from the in-built software were FAZ area, circularity, perimeter, and VD at the level superficial plexus. Images of poor quality indicated by OCTA of signal strength below 7 were excluded from analysis.

The RTVue XR Avanti OCTA device was used for study participants of chapter 7 to assess the OCT-A parameters at the macula and around the optic disc, it uses a light source at 840 nm, a bandwidth of 45 nm and an A-scan rate of 70 000 scans per second with axial resolution of 5 μm approximately. This system has the advantage of split-spectrum amplitude-decorrelation algorithm (SSADA) to reduce signal to noise ratio for both flow detection and microvascular network connectivity (180). Only images with good

quality signal strength index (SSI) > 50 on Optovue, were considered for analysis. Motion artefact corrected 3x3 mm^2 enface OCT-A centred on the fovea were analysed. The boundaries for SCP and DCP analysis were manually adjusted. The inner boundary of SCP was set at 3μm beneath the internal limiting membrane and outer boundary at 15μm beneath the inner plexiform layer, while the DCP enface image was segmented considering inner boundary at 15μm below the inner plexiform layer and an outer boundary at 70μm below the inner plexiform layer.

The FAZ measurement used in chapter 9, was manually calculated for both superficial and deep capillary plexus on ImageJ software (http://rsb.info.nih.gov/ij/index.html) using Angioplex images. The FAZ area and perimeter was ascertained using the formula: FAZ area in $pixel^2$ x 9 mm^2/ area of the whole image in $pixel^2$ (Figure 2). Vessel density was obtained by binarization of original image using the threshold obtained using Otsu's method, which minimizes the intra-class variance of the resulting vessel and background distributions (181). The VD metric was computed as ratio between vessel area and total image area and value obtained using Matlab 2019a.

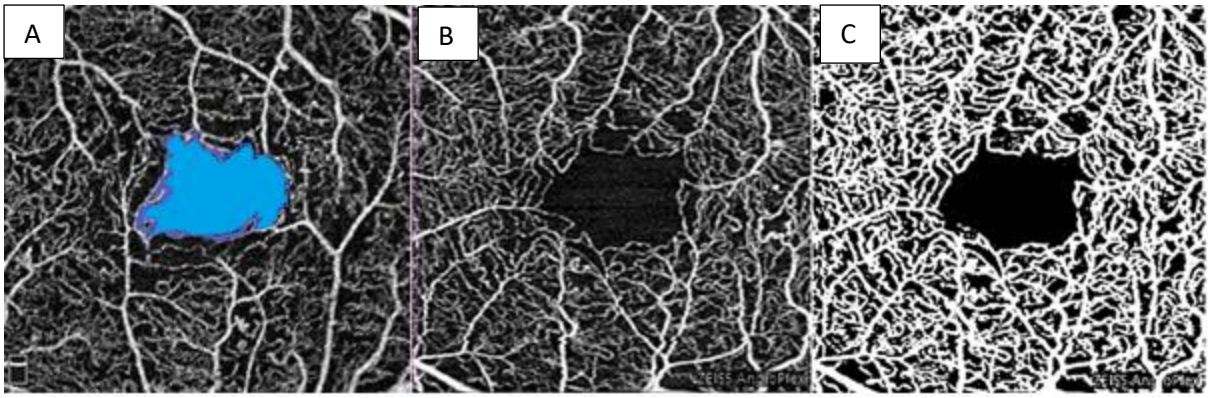

Figure 2: Zeiss Angioplex optical coherence tomography angiogram (OCTA) image of a stable diabetic retinopathy eye. A, Foveal Avascular Zone (FAZ) drawn here using manual tracing. B, superficial capillary plexus (SCP) original image, C, SCP of the same eye after binarization

Chapter 6. A study on variation in foveal avascular zone (FAZ) and central retinal thickness according to age, sex and ethnicity in healthy adult eyes.

6.1 Background

In the review of literature in section 1.1.1, I found that there are inconsistent results on the effect of age, sex, and ethnicity on FAZ. In addition, there was no data on the comparative OCTA parameters in the minor ethnic groups in the United Kingdom. They are mainly AFC and Asians. This study group has previously shown that macular thickness varies with age, sex and ethnicity (182). Therefore, it is also important to assess the relation between OCT and OCTA parameters in healthy macula.

6.2 Aims

The primary aim of this study was to assess age, sex, and ethnic variations in normal FAZ dimensions and explore if these changes correlate with differences in retinal thickness.

6.3 Methods

6.3.1 Study approval

This was a retrospective observational study performed at the Moorfields Eye Hospital approved by Moorfields Review Board (ROAD 17/013). Imaging was performed as part of standard clinic procedure. The study adhered to the tenets of the Declaration of Helsinki.

6.3.2 Study participants

Patients aged 20 years or above of White, AFC or Asian (Indian, Pakistani, Bangladeshi) ethnicities were included. Consecutive patients with at least one normal healthy eye who had OCT and OCTA done on the same day in the medical retina clinics at Moorfields Eye Hospital City Road London between September 2016 to December 2018 were included in the study. Eyes were considered healthy if no structural abnormality was identified on the OCT and OCTA images. Only one eye per patient was used for analysis. Exclusion criteria included all patients with other ethnicities, those with bilateral ocular pathology, glaucoma, diabetes and AMD patients and any findings suggestive of ocular pathology in the selected eye on ocular examination.

6.3.3 Study assessments

The demographic data, including age, sex, and ethnic profile, were extracted from the electronic patient records database. The OCT parameters were CST and retinal thickness of nasal, superior, temporal, and inferior zones of 3mm ETDRS circle. The OCT and OCT-A data was collected from Spectralis (HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) and Angioplex, Zeiss Cirrus HD-OCT 5000 (Zeiss Meditec, Dublin, CA), respectively. The OCTA parameters included the VD of nasal, superior, temporal, and inferior zones of the 3 mm ETDRS circle, VD of whole 3 mm circle, and VD of the inner 1mm circle. The Cirrus HD-OCT uses a light source of 840 nm wavelength to capture 68,000 A-scans per second. The inner boundary of the SCP was set at the ILM, and the outer boundary was set at the level between 70% of the thickness ILM and the outer plexiform layer (OPL). The macular OCT scan was captured using a 20x20-degree scan pattern, with 49 raster lines separated by 120 mm.

6.3.4 Statistical analysis

Normality of study data were assessed both graphically and by using the Pearson measure of kurtosis test. Categorical variables were summarised using n (%) and continuous variables were described by mean (SD) or Median (IQR). Continuous data for the macular parameters, obtained from OCTA and SD-OCT respectively were analysed using the Wilcoxon rank sum test (Mann Whitney) or the independent samples t- test based on the distribution of data. Correlations between the SD-OCT (retinal thickness) and OCTA (macular capillary) parameters by ethnic group, age and sex were assessed using Pearson's or Spearman's coefficient. Regression analysis was performed to assess the statistical significance of age, sex on macular thickness and OCTA parameters in each of the three ethnic groups. All statistical tests were two tailed ($\alpha=0.05$) and a p-value<0.05 was considered statistically significant. Statistical analysis was performed on SPSS version 25 statistical software package.

6.4 Results

One healthy eye of 110 individuals were included in this study. There were 47 Whites, 30 AFC and 33 Asians. The study sample consisted of 68 males and 42 females. The mean age ± SD of the Whites were 62.36±12.21 years, 56.47±11.36 years in AFC and 56.39±13.82 years in Asian (p=0.05).

Macular thickness

The macular thickness in the innermost 1mm ETDRS zone (CST) was significantly lesser in AFC (p=0.03) and Asian (p=0.01) compared to White eyes. Table 2 shows that the inner ETDRS zones were thinner in the Asians versus the Whites. Other than the CST, there were no significant difference in retinal thickness on any other zones between AFC

and Whites. There were also no differences in thickness in any of the 5 zones between AFC and Asians ($p>0.05$).

Foveal Avascular Zone Parameters

Like CST, the central SCP was significantly lesser in AFC ($p=0.02$) and Asian ($p=0.009$) compared to White eyes. However, there were no differences in any other VD measurements between the ethnic groups (Table 2). The FAZ circularity was similar in the 3 groups whereas compared to the Whites, but the FAZ area and FAZ perimeter were both significantly larger in AFC (area $p=0.001$, perimeter $p=0.006$) and Asian groups (area $p<0.001$, perimeter $p<0.001$).

Table 2: Retinal thickness and Macular angiometric parameters in the three ethnic groups

	White British N=47		Afro Caribbean (AFC) N=30		P value White British vs AFC	Asian N=33		P value White British vs Asian	AFC vs Asian P value
	Mean	Std. Deviation	Mean	Std. Deviation		Mean	Std. Deviation		
Age	62.36	12.21	56.47	11.36	0.05	56.39	13.82	0.05	0.76
% Female	24(51.06%)		8(26.67%)			10(30.3%)			
OCT characteristics									
CST	283.44	28.42	268.40	31.65	**0.03**	263.21	40.33	**0.01**	0.60
Nasal	336.79	25.93	337.80	20.43	0.98	327.58	34.78	0.09	0.63
Superior	337.70	18.47	335.47	20.49	0.55	328.67	28.25	**0.03**	0.82
Temporal	327.45	17.75	327.77	20.57	0.67	317.70	32.47	**0.007**	0.46
Inferior	332.55	27.19	336.70	24.29	0.39	322.33	33.96	0.05	0.14
OCTA characteristics									
SCP full	19.26	1.94	18.53	3.17	0.64	18.83	2.61	0.75	0.71
SCP inner	20.21	1.97	19.96	2.78	0.84	19.86	2.53	0.79	0.84
Central	11.26	3.82	9.06	3.76	**0.02**	9.04	3.44	**0.009**	0.86
Nasal	20.56	2.46	19.64	3.31	0.26	20.34	2.36	0.59	0.53
Superior	20.51	2.05	19.93	2.93	0.61	19.91	2.70	0.43	0.91
Temporal	20.22	2.18	19.44	3.22	0.47	19.94	3.08	0.87	0.29
Inferior	20.01	1.96	20.16	2.77	0.24	19.93	2.81	0.68	0.73
FAZ area	0.21	0.09	0.31	0.14	**0.001**	0.32	0.13	**<0.001**	0.87
FAZ perimeter	1.97	0.48	2.38	0.55	**0.006**	2.57	0.80	**<0.001**	0.56
FAZ circularity	0.63	0.09	0.66	0.06	0.23	0.61	0.12	0.50	0.13

Abbreviations: AFC, Afro Caribbeans; CST, central sub-field thickness; FAZ, foveal avascular zone; SCP, superior capillary plexus.

Sex Variations

The mean ages of male and female patients were 57.1 ± 12.2 and 61.9 ± 13.2 years respectively. However, no statistical difference was found in the CST or any of the surrounding zones except nasal zone (p=0.04). Comparable to CST, the SCP in the surrounding zones were similar between the sexes. The FAZ area was minimally larger in female compared to male but not statistically significant, FAZ perimeter and circularity was similar in both sexes (Table 3). When male and female subjects were compared in terms of CST, central SCP, FAZ area and circularity, adjusted for age and ethnic origin central SCP was statistically significant (p=0.02).

Intra ethnic correlations between OCT and OCTA metrics are described in Table 4. FAZ area and perimeter are negatively correlated with CST within whole cohort and among individual ethnic groups. Positive correlation was observed between SCP Centre and CST (p<0.01). Retinal thickness in 4 parafoveal sectors are well correlated with vessel densities in the corresponding sectors within the whole cohort. Likewise, good correlation was seen between CST superior and vessel densities in four quadrants in whole cohort as well as within each ethnic group (Table 4).

Imaging markers which were significant between the ethnic groups in Table 2, remained significant after adjustment for age when Caucasians were compared with AFC and Asians (Table 5), but none of the markers reached significance between AFC and Asians. Furthermore, when adjusted for age and ethnicity no significance was noted across all 4 parameters between male and female participants.

Table 3: Sex differences in retinal thickness and Foveal avascular zone (FAZ) parameters

Mean (SD) or Median (IQR)	Female (n=42)	Male (n=68)	p-value
Age	63.00 (20.25)	57.00 (18.5)	0.054
OCTA characteristics			
FAZ area [Mean (SD)]	0.27(0.13)	0.26(0.13)	0.668
FAZ perimeter [Mean (SD)]	2.26(0.57)	2.26(0.72)	0.945
FAZ circularity	0.64 (0.10)	0.65 (0.09)	0.383
SCP inner	20.00 (4.00)	21.00(3.00)	0.260
SCP full	19.00 (4.00)	20.00 (3.00)	0.271
Central [Mean (SD)]	9.28 (3.59)	10.44 (3.91)	0.122
Nasal	21.00 (3.25)	21.00 (3.00)	0.307
Superior	20.00 (4.00)	21.00 (3.00)	0.272
Temporal	21.00 (3.25)	21.00 (3.00)	0.723
Inferior	20.08 (4.00)	21.00 (3.00)	0.569
SSOCTA	10.00 (2.00)	10.00 (3.00)	0.334
OCT characteristics			
CST [Mean (SD)]	272.8 (27.5)	273.6 (37.9)	0.912
Nasal	333.5 (24.5)	342.5 (29.50)	0.044
Superior	332.5 (24.5)	337.5 (32.75)	0.500
Temporal	324.5 (23.0)	328.5 (26.25)	0.143
Inferior	330.5 (19.0)	337 (27.25)	0.104

Abbreviations: SD, standard deviation; IQR, inter quartile range; CST, central sub-field thickness; FAZ, foveal avascular zone; SCP, superior capillary plexus.

Table 4: Correlation between OCT and OCTA parameters within each ethnic group

	Whole cohort	**Afro-Caribbean**	**Asian**	**White**
CST				
FAZ area	-0.553***	-0.301*	-0.544***	-0.659***
FAZ perimeter	-0.506***	-0.314*	-0.395**	-0.622***
FAZ circularity	-0.112	0.023	0.02	-0.280*
SCP inner	0.043	-0.08	0.152	0.079
SCP full	0.161*	0.037	0.22	0.187
SCP central	0.569***	0.383**	0.475***	0.699***
Nasal				
SCP nasal	0.233**	0.328*	0.109	0.277*
SCP superior	0.273***	0.213	0.181	0.355**
SCP temporal	0.221**	0.324*	0.148	0.222
SCP inferior	0.182*	0.26	0.074	0.244*
Superior				
SCP nasal	0.319***	0.361*	0.359**	0.234
SCP superior	0.371***	0.390**	0.432**	0.295**
SCP temporal	0.391***	0.509***	0.397**	0.326**
SCP inferior	0.277***	0.370**	0.371**	0.143
Temporal				
SCP nasal	0.207**	0.094	0.370**	0.182
SCP superior	0.335***	0.371**	0.377**	0.304**
SCP temporal	0.303***	0.265	0.373**	0.297**
SCP inferior	0.207**	0.201	0.330*	0.121
Inferior				
SCP nasal	0.117	0.174	0.244	0.049
SCP superior	0.263***	0.399**	0.275	0.182
SCP temporal	0.212**	0.297	0.279	0.19
SCP inferior	0.157*	0.257	0.242	0.073

*** $p<0.01$, ** $p<0.05$, * $p<0.1$*

Abbreviations: CST, central sub-field thickness; FAZ, foveal avascular zone; SCP, superior capillary plexus.

Table 5: Multivariable analysis of imaging parameters

	Ethnic differences (adjusted for age)						Sex (adjusted for age and ethnicity)	
	White(ref) vs AFC		White(ref) vs Asian		Asian(ref) vs AFC			
	Mean difference (95% CI)	p-value	Mean difference (95% CI)	p-value	Mean difference (95% CI)	p-value	Mean difference (95% CI)	p-value
CST*	-17.13 (-32.79, -1.47)	0.032	-19.29(-34.21, -4.38)	0.012	2.17(-14.02,18.35)	0.791	9.14(-3.91,22.19)	0.168
Central VD	-2.73(-4.45, -0.99)	0.002	-2.69(-4.37, -1.01)	0.002	-0.04(-1.85,1.77)	0.966	1.69(0.23,3.16)	0.023
FAZ area	0.11(0.059,0.17)	<0.001	0.12(0.063,0.17)	<0.001	-0.002(-0.06,0.06)	0.943	-0.04(-0.08,0.01)	0.108
FAZ perimeter	0.45(0.16,0.75)	0.003	0.64(0.35,0.92)	<0.001	-0.18(-0.49,0.12)	0.242	-0.11(-0.35,0.14)	0.401

*One participant was removed due to high cooks' distance value

Abbreviations: AFC, Afro-Caribbeans; CST, central sub-field thickness; FAZ, foveal avascular zone; SCP, superior capillary plexus; VD, vessel density.

6.5 Discussion

In this study, I compared retinal CST and macular VD and FAZ morphology in White Caucasians, AFC, and Asians. Caucasians have higher CST and SCP with relatively smaller FAZ area and perimeter compared to the AFC and Asians. However, these parameters were not significantly different when AFC were compared with Asians. These observations hold true even after age adjustment, although not so when adjusted for sex. Our results also suggest CST and FAZ area and perimeter are negatively correlated while a positive correlation exists between retinal thickness and VD of individual sectors of ETDRS inner ring.

We did not conduct repeatability studies. However, FAZ area measurements using Angioplex showed excellent repeatability and reproducibility, thus making it a reliable device to assess such parameters. Repeatability was proven by Guo et al who measured FAZ in Chinese population using Image J (32). Their FAZ area was slightly higher than our Asian cohort (0.32±13 vs 0.373 ± 0.109) which could be because they only included participants of only Chinese ethnicity. However, the Chinese population analysed by Wylegala et al (0.33 ± 0.012 mm^2) showed similar FAZ area (0.32±0.13) as our Asian cohort (34).

Anegondi et al studied eyes of people of Indian origin using the Zeiss Cirrus 5000 and showed that the mean FAZ area was 0.35 ± 0.05 mm^2, which is similar to our cohort of Asian subjects (0.32±0.13 mm^2) (183). Yu et al used Avanti (Optovue, Fremont, CA, USA) device, for participants with mean age of 36 years and their study found the mean FAZ area to be 0.474 ± 0.172 mm^2 in a Chinese population (31). Considering these studies

along with our present work the result highlights the fact that FAZ area is very variable in healthy eyes.

Our study did not find any significant difference in FAZ area when compared between male and female participants, and this was similar to what Samara et al suggested (184). Yu et al, in a Chinese population, showed that the FAZ area is larger in women than in men (man mean, 0.42 mm^2; woman mean, 0.52 mm^2) (31). Furthermore, their study did not show any differences in macular perfusion and VD between men and women, which corroborates our finding.

Wylegala et al showed the mean vessel length density in the central part of the ETDRS circle for the Caucasian and Chinese subjects (p = 0.3841) as well as for the inner 3 mm ring (p = 0.1610) were not significant (34). Amongst our study groups SCP VD of central 1mm zone was significant between Caucasians and Asians (p=0.009) but the same was not true for inner 3mm ring. The FAZ perimeter was smaller in Caucasian subjects (2.18 ± 0.07 mm) than in Chinese subjects (2.43 ± 0.06 mm, p = 0.0057). FAZ circularity was not significant between these two groups, p = 0.8197. Both perimeter and circularity results support our findings.

Chun et al conducted a study on RTVue-XR Avanti OCTA and, found that Black subjects had significantly lower foveal VD in SCP than white subjects (28.62 ± 5.44% vs 32.21 ± 4.08%, p = 0.011).(185) However, when considering parafoveal VD at SCP, there was no significant difference between Black and White subjects (55.01 ± 3.85% vs 55.70 ± 3.09%, p = 0.407). When the parafovea was divided into quadrants, there was no significant difference between the study groups. Black subjects had significantly larger FAZ than white subjects in the SCP (0.30 ± 0.11mm^2 vs 0.22 ± 0.07mm^2, p = 0.006. This study corroborates our findings in terms of fovea, parafovea and FAZ area.

Comparing 12 healthy Caucasians and 15 healthy African individuals Aurégan et al found mean FAZ area was 0.26 ± 0.008 mm^2 in the SCP in Caucasians versus 0.33 ± 0.08 mm^2 in the SCP (p = 0.01) in Africans using Triton swept-source OCTA. In the SCP, the mean VD was 40.5 ± 0.8% in Caucasians versus 34.3 ± 1% (p = 0.008) in Africans (p < 0.001). (186) Although we did not use swept source technology our group of AFC patients had larger FAZ, and lower superficial capillary plexus VD compared to Caucasians (p=0.001 and 0.02 respectively).

As described in the introduction chapter, foveal pit is the center of fovea surrounded by slope and consists of densely packed cone photoreceptors. Wagner-Schuman et al previously proved that the foveal depth and diameter were greater in black Africans than in white Caucasians while there is no significant difference in foveal pit slope (187). 43 women, 47 men underwent retinal imaging with spectral-domain OCT. Compared to Caucasians, the Africans and African Americans had reduced central subfield thickness (p<0.0001). Central subfield thickness was reduced in the women compared with the men (P<0.05). More recent study by Zouache et al found foveal pit to be wider and pit volume greater in Ghanaians compared to Caucasian. Ghanaians possess more flatter foveal depression. In age adjusted analysis, foveal depression was narrower in men as compared to women in both Ghanaian and Caucasian. Both these studies compared foveal morphology between participants of Caucasian and African ancestry (188).
Previous literature had verified that sex-based differences tend to include multiple ETDRS segments, while race-related differences were largely confined to the central subfield.(189) The FAZ area in both plexuses correlated inversely with central macular thickness (P < 0.0001)(184).

Females had a larger superficial (0.32 ± 0.11 mm^2 versus 0.23 ± 0.09 mm^2) and deep FAZ (0.40 ± 0.14 mm^2 versus 0.31 ± 0.10 mm^2) (*P* < 0.001) than males (190). The

central foveal thickness below the foveal pit was lower in eyes of Blacks compared to Asians (12 μm, P = 0.035) and White subjects (18 μm, P < .0001) (182).

In conclusion, our study as well as previous literature indicates lower VD in Black/African and Asian ethnicity. It is unclear whether these anatomical differences predispose them to a higher likelihood of retinal vascular diseases. Eyes of patients suffering diabetes have lower VD and greater FAZ size compared to normal eyes before the clinical onset of retinopathy. Similarly, RVO eyes also have reduced macular VD. More work is required to understand the complex relation of these OCTA and OCT metrics with future.

Chapter 7. Structure and functional correlation in severe NPDR and PDR

7.1 Background

Decreased retinal VD in the SCP and DCP is known to happen with increasing retinopathy severity status. FAZ size enlarges along with the decrease in parafoveal VD with the progression of DR (191). Previous literature has shown peripapillary capillary network is affected along with thinning of peripapillary RNFL in the early stages of DR (192).

In the study by Shaler et al, the diminished reading capacity in low luminance conditions in healthy eyes was highlighted (193). The possible reason is that rod response is slower than cone due to poor spatial and temporal characteristics of rod photoreceptors. Furthermore, hypoxia-induced damage, in all probability, affects rods more than cones due to their increased oxygen demand during dark adaptation. Thus, there is an unmet need to understand the morphological factors which influence LLVA along with BCVA in these patients.

7.2 Aims

1. Are the FAZ parameters better predictors of visual function than VD?

2. Are macular VD better predictors of BCVA and LLVA than RPCP VD?

3. Is DRIL a better predictor than FAZ area and perimeter in predicting BCVA and LLVA

7.3 Methods

7.3.1 Study approval

This is a prospective cross-sectional study where imaging and patient related data was collected from patients who consented for DMI study (A prospective study of structure-

function correlation in patients with diabetes with varying sizes of foveal avascular zone). The study adhered to the tenets of the Declaration of Helsinki. Study approved by the United Kingdom (UK) National Research Ethics Committee Service (19/NI/0030).

7.3.2 Study participants

Inclusion criteria for this study were severe non-proliferative diabetic retinopathy (NPDR), and stable treated PDR in type 1 or 2 diabetic patients with no treatment history in the past six months, and no active NV noted for six months prior to inclusion and BCVA 49 letters or better. Key exclusion criteria were clinical evidence of DMO involving the fovea (CST >300), laser scar at macula within a 3mm diameter area centred at fovea, any history of anti -VEGF treatment in the last six months and any ocular condition which in the opinion of the investigator might affect the retinopathy status or alter visual acuity during the study.

7.3.3 Study assessments

The study participants underwent baseline examination including visual acuity in ETDRS letters and low luminance visual acuity, slit lamp examination, Ultrawide field colour fundus image (Optomap 200Tx, Optos plc, Dunfermline, UK), spectral domain optical coherence tomography (SD-OCT) (Heidelberg Engineering) and OCT angiography, RTVue XR Avanti SD-OCT device (Optovue, Fremont, CA) using the prototype AngioVue OCTA software. The same OCT and OCTA device was used for each participant included in the study. Prospective study data from this device was collected from the inbuilt software generated metrics which included FAZ area at the default retina slab, vessel densities at the SCP and DCP level in the central 1 mm and 3 mm (which is parafoveal

2-mm-ring–shaped area surrounding the fovea) zones, as well as the RPCP small vessels (Figure 3).

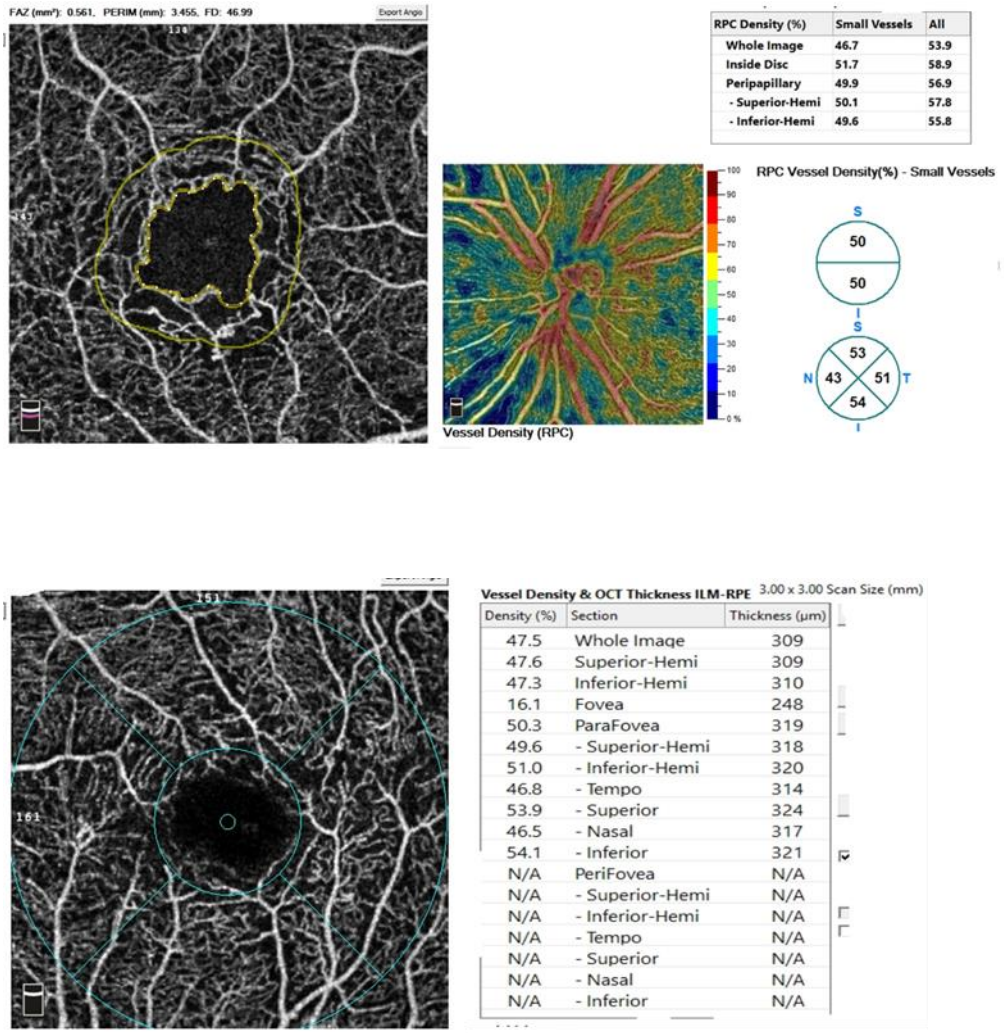

Figure 3: Optical coherence tomography angiography (OCTA) image of stable treated PDR eye. A. Enface image showing automatic demarcation of FAZ and software generated retinal peripapillary capillary plexus vessel density (VD) metrics. B. Enface OCTA image and ETDRS parafoveal 3mm ring

7.3.4 Statistical analyses

Univariate analysis was performed to explore associations between demographics (Age and Sex), clinical characteristics (HbA1c, diabetes duration), and OCT and OCTA metrics with continuous outcome variables BCVA and LLVA. Multivariable analysis was then performed, adjusting for age, sex, baseline hba1c level and diabetes duration. The generalized estimating equation method (GEE) was used in regression analysis (Gaussian with identity link), assuming an unstructured working correlation structure, to account for the correlation between the eyes of the same individual. The statistical significance was adjusted for the number of tests using the Bonferroni method. Parameters were compared using the GEE model selection criterion, quasi-likelihood information criterion (QIC), analogous to the Akaike information criterion (AIC) under quasi-likelihood theory as AIC cannot be directly applied. Akaike information criterion is based on maximum likelihood estimation while GEE is nonlikelihood based.(194) It is a criterion for model selection applicable to GEE models (195). All analysis was conducted in Stata version 15 (Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

7.4 Results

A total of 93 eyes (64 patients) with diabetes mellitus were eligible for the study. Table 6 describes the demographic, clinical, 3x3 image OCT angiography metrics at the macula and optic disc for all 93 eyes of 64 patients. All OCT-A SSIs were above 50. There were 40 (43.4%) eyes with FAZ area >0.5mm^2. 57 (89.1%) had HbA1c above 7%. 28 (30.1%) had evidence of DRIL, where 2 (2.2%) were in eyes with severe NPDR and 26 (28.0%) in patients with PDR.

Table 6: Baseline demographic and ocular characteristics

Demographic Characteristic	Results [Mean (SD) or n (%)
Age at inclusion in the study, years	56.81(13.21)
Age at diagnosis of DR, years	35.01(19.96)
Female	29 (45.3%)
Ethnicity	
Black	9 (14.1%)
Asian	14 (21.9%)
White	41 (64.1%)
DM type	
Type 1	27 (42.2%)
Type 2	37 (57.8%)
Duration of DM, years	21.8 (15.3)
HbA1c (%)	8.80 (1.58)
Ocular characteristics	
Central Subfield thickness (microns)	268.52 (37.93)
DRIL (%)	28 (30.1%)
Lens status	
Clear lens	25 (26.9%)
Pseudophakia	30 (32.3%)
Visually insignificant Cataract	38 (40.9%)
DR severity	
Severe NPDR	12 (12.9%)
Stable treated PDR	81 (87.1%)
3x3 Image Metrics	
FAZ area, mm^2	0.49 (0.27)
FAZ perimeter, mm	3.15 (1.09)
SCP (whole), (%)	36.77 (5.05)
DCP (whole), (%)	41.87 (4.81)

Superficial Parafovea, (%)	38.41 (5.76)
Deep Parafovea, (%)	43.16 (5.05)
Optic disc metrics (vessel density)	
Nasal, (%)	41.19 (5.26)
Superior, (%)	47.11 (5.95)
Temporal, (%)	48.89 (4.98)
Inferior, (%)	48 (5.23)
Radial Peripapillary, (%)	45.97 (4.44)

Abbreviations: DM, Diabetic Mellites; HbA1c, Haemoglobin A1c or glycated haemoglobin; PDR, Proliferative diabetic retinopathy; FAZ; Foveal avascular zone; SCP.

The correlation of structural changes on OCT and OCT-A with visual function tests in PDR

The mean BCVA was 75.43 (9.85) letters, and 17 participants (18.3%) had BCVA between 50 and <70 letters.

The range of BCVA varied from 40 to 89 letters, and LLVA varied from 35 to 85. In eyes with BCVA <70 letters, the mean LLVA was 51.8 (SD 8.3) letters, and in those 70 letters or more, the mean LLVA was 70.5 (SD 7.6).

Factors associated with BCVA, Gaussian GEE models with identity link

GEE models were used to assess the relationship between OCT and OCTA metrics for continuous BCVA, presented in table 7. No demographic or clinical characteristic was found to be associated with BCVA, in both univariate and multivariable analyses. In univariate analysis, all 3x3 metrics except FAZ area, and FAZ perimeter had a significant positive association with BCVA. For the optic disc metrics, temporal VD was found to be associated with BCVA in univariate analysis.

All 3x3 image metrics except FAZ area and FAZ perimeter, had a significant positive association with BCVA after adjusting for HbA1c, sex, disease duration and age (uncorrected for multiple testing). Every 5%-point increase in whole SCP VD was associated with a 4.35 higher BCVA in ETDRS letters (β=0.87/1%-point increase in SCP; 95% CI, 0.54-1.21; p<0.001) in the adjusted analysis. DCP VD (whole) was also found to be associated with BCVA (β=0.72/1%-point increase;95% CI,0.40-1.04; p<0.001). Every 5%-point increase in superficial parafovea was associated with a 4.1 letter increase in BCVA (95% CI; 2.7-5.50; p<0.001). For the optic disc metrics, after adjustment, temporal and radial peripapillary were found to be associated with BCVA. Every 5%-point increase

in temporal was associated with a 2.35 letter increase in BCVA, ETDRS letters (β=0.47 letters/1%-point increase; 95% CI, 0.12-0.82; p=0.008). For radial peripapillary region, participants with higher RPCP VD were presented with higher BCVA, where every 5%-point increase was associated with a 2.3 letter increase in BCVA (β=0.46 letters/1%-point increase; 95% CI,0.06-0.86; p=0.025). The presence of DRIL was found to be associated in both univariate and multivariable analysis, where participant eyes with DRIL were on average 6.65 letters lower than eyes without DRIL (adjusted β=-6.65; 95% CI, -10.84- -2.46; p=0.002). Following Bonferroni correction, all optic disc metrics and DRIL did not reach statistical significance in univariate analysis. In the adjusted analysis, all optic disc metrics did not reach statistical significance. DRIL was not identified in the univariate analysis but was found to be associated with BCVA in the adjusted analysis following a Bonferroni correction (p=0.002).

Table 7: Best corrected visual acuity vs OCT/OCTA metrics; GEE model (Gaussian with identity link)

N=92 [a] **Parameter**	**Univariate analysis**	**Adjusted analysis** [b]
Standard model		
HbA1c, %	-0.60(-1.51,0.31);p=0.199	-0.50 (-1.40,0.41);p=0.280
Sex Male	3.05 (-0.73,6.83);p=0.114	3.69 (-0.09,7.46);p=0.056
Age, years	-0.13 (-0.29,0.03);p=0.113	-0.16(-0.32,0.002);p=0.054
Disease (DM) duration, years	0.003 (-0.11,0.12);p=0.954	0.02(-0.10,0.13);p=0.770
3x3 Image Metrics		
FAZ area, per 0.1 mm^2 increase	-0.79 (-1.64,0.06); p=0.069	-0.83 (-1.71,0.05); p=0.064
FAZ perimeter, per 1 mm increase	-1.68 (-3.59,0.23); p=0.085	-1.80 (-3.72,0.12); p=0.066
3x3 Image Metrics, Vessel Density (%)		
SCP (whole)	**0.89 (0.55,1.23); p<0.001**	**0.87(0.54,1.21); p<0.001**
DCP (whole)	**0.72 (0.40,1.04); p<0.001**	**0.73 (0.38,1.08); p<0.001**
Superficial Parafovea	**0.82(0.54,1.10); p<0.001**	**0.81(0.54,1.09); p<0.001**
Deep Parafovea	**0.70(0.40,1.00);p<0.001**	**0.71 (0.41,1.02);p<0.001**
Optic disc metrics (vessel density %)		
Nasal sector of RPCP	0.35(-0.06,0.76);p=0.092	0.32 (-0.08,0.73);p=0.115
Superior sector of RPCP	0.15(-0.16,0.47);p=0.335	0.27 (-0.04,0.59);p=0.087
Temporal sector of RPCP	0.48(0.11,0.85);p=0.011	0.47 (0.12,0.82);p=0.008
Inferior sector of RPCP	0.15(-0.22,0.53);p=0.416	0.23 (-0.10,0.57);p=0.171
RPCP	0.41(-0.03,0.85);p=0.067	0.46 (0.06,0.86);p=0.025
Other OCT		
Absence of DRIL Presence of DRIL	Ref -5.40(-9.67,-1.12);p=0.013	Ref **-6.65(-10.84,-2.46);p=0.002**

Coefficients expressed as per 1 unit increase for continuous variables unless otherwise specified

[a] Single observation was omitted from the analysis as they were below 3SD of the mean visual acuity (BCVA or LLVA)

[b] Models were adjusted for age, duration, hba1c, type of diabetes and sex

Bonferroni adjusted significance level set to 0.003 for 16 tests, with variables that reached statistical significance bolded.

Abbreviations: DM, diabetes mellitus; FAZ- Foveal Avascular Zone; OCT- Optical coherence tomography; SCP- superficial capillary plexus; DCP- deep capillary plexus; RPCP- radial peripapillary capillary plexus; DRIL- Disorganisation of the retinal inner layers

Factors associated with LLVA, Gaussian GEE models with identity link

Table 8 presents the associations between the OCT/OCTA metrics and continuous LLVA for both univariate and following an adjustment for age, disease duration, sex and HbA1c. No demographic or clinical characteristic was found to be associated with LLVA. In univariate analysis all 3x3 metrics except FAZ area and FAZ perimeter were found to be positively associated with LLVA. The only optic disc metric associated with the outcome was temporal sector of RPCP (β=0.61/1%-point increase in temporal sector; 95% CI, 0.20-1.02; p=0.004).

In the adjusted analysis, temporal sector of radial peripapillary plexus was the only optic disc metric associated with the LLVA (β=0.60/1%-point increase; 95% CI, 0.22-0.99; p=0.002). Additionally, participant eyes with DRIL were on average 6.5 LLVA ETDRS letters less than participant eyes without DRIL (β =-6.54; 95% CI, -11.30, -1.78; p=0.007). All 3x3 metrics except FAZ area, FAZ perimeter were found to be associated with LLVA in the adjusted analysis.

Four variables were identified in the univariate analysis (table 8). None of the optic disc metrics and DRIL reached statistical significance in univariate analysis. In the adjusted analysis, three variables (SCP [whole] VD, superficial parafoveal capillary VD and temporal RPCP VD) were identified following a Bonferroni correction; other than that, DCP VD whole, deep parafoveal capillary plexus, and all other optic disc metrics did not reach statistical significance. DRIL was not significant in the univariate analysis following a Bonferroni correction (p=0.007).

Table 8: Low luminance visual acuity vs OCT/OCTA metrics; GEE model (Gaussian with identity link)

N=92 [a] **Parameter**	**Univariate analysis**	**Adjusted analysis** [b]
Standard model		
HbA1C, %	-0.90(-2.05,0.24);p=0.123	-0.93(-2.15,0.28);p=0.131
Sex Male	0.72(-3.57,5.00);p=0.743	1.14(-3.19,5.47);p=0.605
Age, years	-0.13(-0.31,0.04);p=0.140	-0.15(-0.33,0.03);p=0.100
Disease (DM) duration, years	0.002(-0.13,0.14);p=0.974	0.02(-0.11,0.16);p=0.724
3x3 Image Metrics, Vessel Density (%)		
FAZ area, per 0.1mm^2 increase	-0.70(-1.56,0.17);p=0.114	-0.78(-1.71,0.14);p=0.097
FAZ perimeter, per 1mm increase	-1.66 (-3.45,0.12);p=0.068	-1.74(-3.64,0.15);p=0.071
SCP (whole)	**0.93(0.55,1.31);p<0.001**	**0.89(0.50,1.28);p<0.001**
DCP (whole)	**0.61(0.29,0.92);p<0.001**	0.56(0.19,0.92);p=0.003
Superficial Parafovea	**0.84(0.52,1.16);p<0.001**	**0.83(0.50,1.16);p<0.001**
Deep Parafovea	**0.58(0.24,0.92);p=0.001**	0.55(0.17,0.92);p=0.004
Optic disc metrics (vessel density %)		
Nasal	0.24(-0.19,0.67);p=0.274	0.22(-0.21,0.65);p=0.316
Superior	0.06(-0.34,0.46);p=0.781	0.15(-0.29,0.59);p=0.506
Temporal	0.61(0.20,1.02);p=0.004	**0.60(0.22,0.99);p=0.002**
Inferior	0.12(-0.26,0.50);p=0.531	0.17(-0.21,0.55);p=0.378
Radial Peripapillary	0.33(-0.18,0.84);p=0.205	0.37(-0.12,0.86);p=0.137
Other OCT		
Absence of DRIL	Ref	Ref

Presence of DRIL	-5.67(-10.11,-1.23);p=0.012	-6.54(-11.30,-1.78);p=0.007

Coefficients expressed as per 1 unit increase for continuous variables unless otherwise specified.
[a] Single observation was omitted from the analysis as they were below 3SD of the mean visual acuity (BCVA or LLVA)

[b] Models were adjusted for age, duration, hba1c, type of diabetes and sex

Bonferroni adjusted significance level set to 0.003 for 16 tests, with variables that reached statistical significance bolded.

Abbreviations: DM, diabetes mellitus; FAZ- Foveal Avascular Zone; OCT- Optical coherence tomography; SCP- superficial capillary plexus; DCP- deep capillary plexus; RPCP- radial peripapillary capillary plexus; DRIL- Disorganisation of the retinal inner layers

Ranking variables for visual acuity by QIC

QIC was assessed in the variables that were statistically significant following an adjustment for the standard parameters, these are ranked by QIC in descending order in table 9. The standard model for BCVA had a QIC of 7070.27. Under the unstructured correlation structure, we found that the model with superficial parafovea had the smallest QIC and therefore was identified as the most parsimonious model. The addition of temporal RPCP VD to the standard model led to the least parsimonious model, with the highest QIC of 6610.49. Temporal RPCP VD did not pass the significance threshold following a Bonferroni correction for BCVA. This means that the parameter most accurately determines BCVA is VD of superficial parafoveal capillary plexus, followed by the whole SCP and deep parafoveal capillary plexus.

Like BCVA, the best subset model for LLVA included superficial parafovea. The variable that was identified in the least parsimonious model was DCP (whole), which did not pass the significance threshold following a Bonferroni correction. Temporal RPCP VD was ranked as the better fitting model than models with deep parafovea or DCP (whole), unlike the models for BCVA. The presence of DRIL was ranked higher for LLVA than BCVA. This means that all these parameters contribute to the determination of BCVA and LLVA in varying degrees. The parameter most accurately determines LLVA is superficial parafoveal capillary plexus density, followed by whole SCP VD and DRIL. Figure 4 shows that the superficial parafoveal capillary plexus is highly correlated to SCP; the deep parafoveal capillary plexus is highly correlated to DCP; and BCVA and LLVA are also highly correlated. However, deep parafoveal capillary plexus determines BCVA more accurately and has a lesser impact on LLVA. On the contrary, DRIL affects LLVA more than its contribution to BCVA. Out of these parameters, DCP (whole), deep parafovea, SCP (whole) and superficial parafovea were found to be on average lower in the presence

of DRIL than when it is absent (figure 5) and these differences were statistically significant ($p<0.001$). No differences were found in the temporal sector of the peripapillary radial plexus in the presence or absence of DRIL.

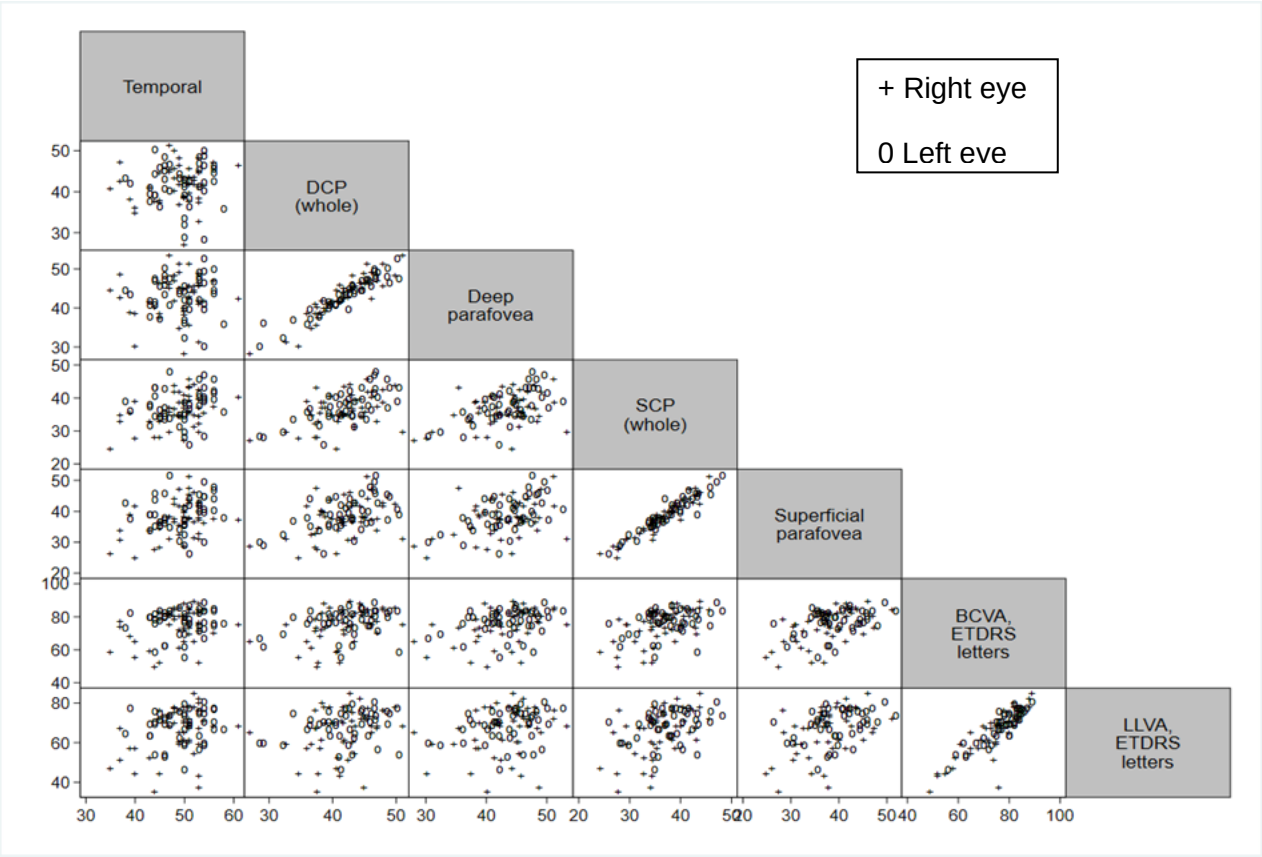

Figure 4: Correlation matrix of identified continuous parameters

Abbreviations: BCVA, best corrected visual acuity; LLVA, low luminance visual acuity; DCP, deep capillary plexus; SCP, superficial capillary plexus; ETDRS, Early Treatment Diabetic Retinopathy Study.

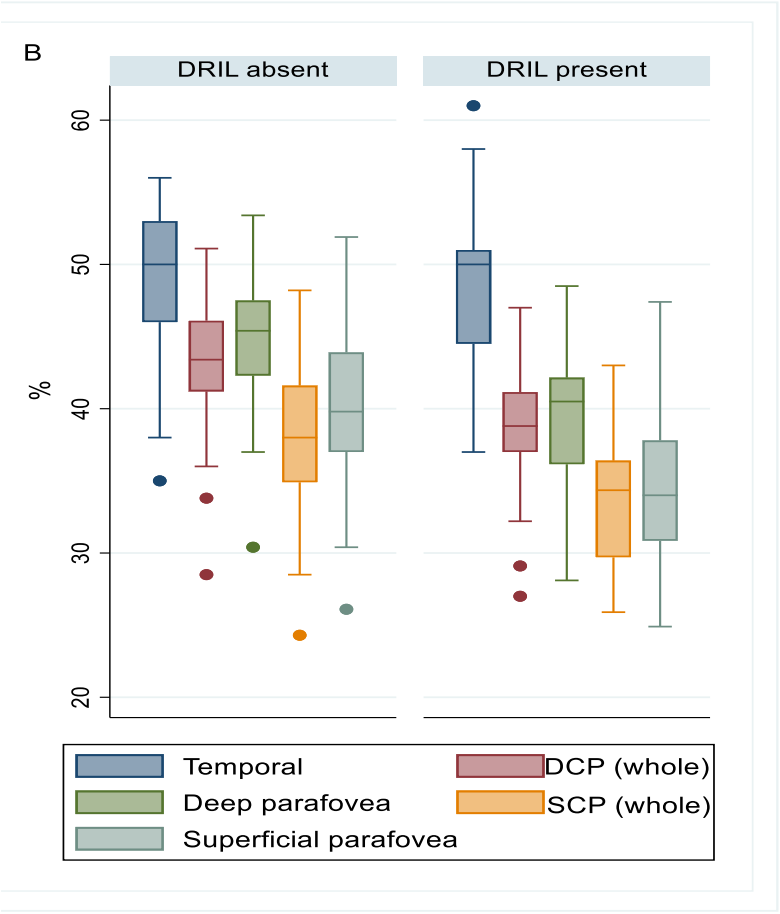

Figure 5: Average vessel density plot at superficial and deep capillary plexus for whole enface and parafoveal area was lower in eyes with Disorganisation of the retinal inner layers (DRIL)

Abbreviation: SCP, superficial capillary plexus; DCP, deep capillary plexus.

Table 9: QIC analysis of parameters deemed statistically significant in table 7 and 8

BCVA		**LLVA**	
Parameter	**QIC**	**Parameter**	**QIC**
Standard model	7070.27	Standard model	9456.18
+DRIL	6269.43	+DCP (whole)	8840.64
+DCP (whole)	6054.81	+Temporal	8710.26
+Deep parafovea	5967.11	+DRIL	8684.16
+SCP (whole)	5410.38	+SCP (whole)	7724.09
+Superficial parafovea	5215.71	+Superficial parafovea	7539.67

Abbreviations: BCVA, best corrected visual acuity; LLVA, low luminance visual acuity; QIC, quasi-likelihood information criterion; DCP, deep capillary plexus; SCP, superior capillary plexus.

7.5 Discussion

In this study, out of the 93 eyes studied, 40 (43.4%) eyes with FAZ area >0.5mm^2. This shows that the FAZ area can be normal in eyes with treated PDR. FAZ area was also not related to HbA1C. Similarly, DRIL is not a universal finding in severe NPDR and PDR patients. Only approximately a third of participants had DRIL. A subgroup of patients within the treated PDR cohort had macular ischaemia. Most PDR patients are likely to have areas of peripheral capillary non-perfusion, but they may not have macular ischaemia simultaneously. Sun et al found that FAZ size at SCP and DCP did not relate to DR progression (196). Park et al showed that FAZ is best distinguished at the middle capillary plexus (MCP) (197). Furthermore, DCP terminates well before the FAZ margin, giving a false idea of a bigger size at this capillary level. Hence, there remains a debate regarding the consistency of quantitative measurement of FAZ.

Previous studies based on FA described DMI as enlargement or disruption of the FAZ and capillary dropout in the parafoveal area. We also know that DCP was not captured by FA. Determining the FAZ area at different plexi may help us understand this condition better. We used RTVue software which considers retina slab encompassing SCP and DCP layers to define FAZ metrics.

Ghassemi et al proposed that the whole RPCP VD could be a reliable biomarker for the assessment of DR staging. Among the different subsegments of the optic disc, a decrease in temporal VD has been observed in PDR subjects (198). Sub-analysis of TIME-2b study, involving moderate, severe NPDR eyes with no DMO at baseline and treated with AKB-9778 suggested FAZ area, superior temporal peripapillary VD, and inferior temporal peripapillary VD were associated with increased odds of DR progression during 12 months of study period (199).

New trials are ongoing to find pharmacologic agents to arrest the progression of DMI (200). This study shows that it is unnecessary to target all PDR patients with such agents as only a third shows signs of DMI. Based on our findings, these agents targeting ischaemia are best used in eyes with severe NPDR or PDR with signs of decreased SCP, but long-term follow-up is required to observe conclusive changes.

The correlation of structural changes on OCT and OCT-A with visual function tests in PDR

For the study participants with previously treated stable PDR and severe NPDR, I found that higher VD at superficial and DCP is suggestive of better BCVA in univariate and demography-adjusted analysis. Similarly, significant positive association was also noted for radial peripapillary and temporal RPC VD and BCVA. Although higher FAZ area and perimeter indicate poorer BCVA, this relation did not reach significance. In the model adjusted for age, duration, hba1c, type of diabetes and sex, DRIL was a robust predictor of BCVA (p=0.002).

When considering LLVA in this cohort, we see a similar trend where VD metrics at both SCP and DCP appear to relate at a statistically significant level both before and after adjustment for demography. However, only SCP and temporal RPCP VD remained significant following the Bonferroni adjustment. It is interesting to note that DRIL did not show similar significant relation with LLVA like BCVA. The best model explaining BCVA and LLVA was superficial parafoveal VD. In addition, DRIL affects LLVA more than its contribution to BCVA. Vessel area and length density at both SCP and DCP correlated with VA in diabetic eyes, according to Samara et al (201). Parafoveal VD at the level of SCP can be a good biomarker to predict VA, irrespective of the stage of DR (202). In literature, some studies have indicated VD at SCP, while others suggested VD at DCP or both to be a predictor of vision. The importance of DCP in providing for the oxygen

requirement of photoreceptors and the outer retina has been highlighted in DMI-affected eyes (203). In our opinion, perfusion from DCP is more active when choriocapillaris perfusion is compromised.

Visual acuity is the acuteness of vision, a measure of the spatial resolving ability of the visual system under conditions of very high contrast. VA is the standard visual function test and the most frequently used indicator of spatial vision in clinical practice and research studies. Visual acuity may be considered as a three-stage hierarchical sequence. The first stage of the visual process involves the detection of contrast. The second stage resolves that contrast into different elements by distinguishing various strokes, which comprise a letter or a symbol. Finally, the third stage involves identifying a particular arrangement, such as a Snellen alphabet or symbols. Each stage has its inherent limitations and the limitations of the previous stage. The stage involving identification or recognition stage is tested universally in the field of ophthalmology.

LLVA is a measure of mesopic function, predominantly cone-mediated in reduced illumination. Stockman and Sharpe explained that the visual acuity in a mesopic setting requires integrated cone function mediated by post-receptoral pathways, and a disruption of this would lead to a drop in LLVA (92). Adaptive optics analysis of perifoveal cone photoreceptor in Type 1 diabetics with no DR showed lower cone density ($p<0.05$), except for the temporal meridian at 2-and-4 degrees eccentricity. Marked asymmetry in cone photoreceptor density was also noted between the eyes of diabetic and healthy individuals (204). We can say there is definite importance of considering LLVA as an outcome measure while studying DR eyes.

According to Brown et al, increasing the luminance of the VA chart is associated with a parallel increase in VA; however, this increase in VA is more significant in healthy subjects when compared with age-related macular degeneration (AMD) patients at all the tested

levels of luminance (205). Mostly, studies have been conducted using LLVA as an outcome measure in intermediate and dry AMD (Table 10).

FAZ parameters in this study cohort did not add value in determining BCVA and LLVA. In the study subjects, temporal RPC shows a significant positive association with VA in normal and low-lighting conditions. This association could be due to the proximity of temporal RPC to the macula, which is the primary location of posterior pole ischemic changes in chronic vasculopathies like DR. Jia et al have found that relatively dense RPC spreads as far as 5.5 mm from the disc centre (206). Furthermore, the temporal peripapillary sector is least interfered by the emanating optic disc large vessels.

The strengths of this study are all subjects were imaged using the same OCT and OCTA device prospectively, and structural parameters were derived from the automated software, which avoids subjective reliability. None of the participants had macular oedema, which could potentially affect retina segmentation. The limitation of the study is the small sample size. Also, I looked at a single time point. It will be interesting to understand how these OCT-A parameters affect LLVA and BCVA over a longitudinal study period.

Table 10: Studies which have investigated low luminance visual acuity in age-related macular degeneration

Studies	Inclusion criteria	Outcome
Wu et al (207)	179 AMD, 26 controls (drusen larger than 63 μm, and any AMD pigmentary anomalies; drusenoid pigment epithelial detachments and non-foveal GA)	BCVA, LLVA, and central retinal sensitivity were reduced significantly for all AMD except drusen with 63 -125 microns. LLD was not significantly different from control participants in all groups ($P \geq 0.073$), except in the non-foveal GA group ($P = 0.008$). A significant positive relationship between central retinal sensitivity and LLD ($R = 0.613$; $P < 0.001$), but not BCVA.
Sunness et al (95)	91 participants with GA from AMD, 1 eye who completed a 2-year study examination.	The baseline low-luminance deficit (LLD) in VA was a strong predictor of subsequent VA loss for all levels of baseline VA. Within the good baseline VA group, the relative risk (RR) of 3-line loss for the worse LLD group compared with the better LLD group was 2.88 (95% confidence interval [CI], 1.13–7.35).
Grewal et al (208)	50 participants were recruited into the study in these groups: healthy ageing (n = 11), intermediate AMD (iAMD) with no subretinal drusenoid deposits (SDD) (n = 17), iAMD with SDD (n = 11) and non-foveal atrophic AMD (n = 11)	BCVA and LLVA scores were significantly reduced in the atrophic AMD group ($p < 0.0001$ and $p = 0.00016$, respectively) and in patients with SDD ($p = 0.028$ and $p = 0.045$, respectively) compared to healthy participant.

Chandramohan et al (209)	This prospective study enrolled 30 subjects at a single site (8 controls, 8 early AMD, and 12 intermediate AMD).	Compared with controls, patients with intermediate AMD showed significant deficits on best-corrected visual acuity, LLVA, low luminance deficit.
LEAD study report (210)	292 individuals with bilateral large drusen in the (LEAD) study underwent best-corrected visual acuity (BCVA), LLVA and microperimetry testing, as well as multimodal imaging to detect late (neovascular or atrophic) AMD onset.	The area under the receiver operating characteristic curve (AUC) for detecting neovascular and atrophic AMD onset using was not significantly different for LLVA (AUC=0.71 and 0.56 respectively) and microperimetry (AUC=0.82 and 0.62 respectively) compared to BCVA (AUC=0.57 and 0.56 respectively; P≥0.126 for all).
Puell et al (211)	22 subjects with early AMD and 28 healthy control subjects. Inclusion criteria included a photopic HC-VA of 20/25 or better	Mean mesopic distance HC-VA and LC-VA were significantly worse 0.1 logMAR and 0.28 logMAR, respectively) in the early AMD group than in the control group. Sensitivity and specificity were significantly greater for mesopic LC-VA than for mesopic HC-VA, AUC 0.94 ± 0.030 and 0.76 ± 0.067, respectively.
Cocce et al (212)	80 patients with AMD age-related eye disease study (AREDS) stage 2 (N=33) and stage 3 (N=47) and 21 age-matched, normal controls. 2 log unit ND filter used	Their study demonstrates that standard LLVA was significantly depressed between the more severe cases of AMD (AREDS 3) and normal controls but that this difference did not exist between

		the early cases (AREDS 2) and normal.

Abbreviations: BCVA, best corrected visual acuity; LLVA, low luminance visual acuity; AMD, age-related macular degeneration; AUC, area under the curve; SDD, subretinal drusenoid deposits; iAMD, intermediate age-related macular degeneration; ND, neutral density filter.

Chapter 8. The relation between Best Corrected Visual Acuity and Low Luminance Visual Acuity in Proliferative Diabetic Retinopathy eyes

8.1 Background

Visual acuity is the most established test of visual function (213). It is tested on high-contrast charts and is affected by light scatter and wavefront aberrations. In PDR, VA remains unaffected unless complications such as DMO and ischaemia, epiretinal membrane, vitreous haemorrhage or tractional changes involve the fovea.

Mesopic visual acuity is more translatable to real-world tasks such as driving at night (214). It has also been shown to correlate better with diseases like central serous retinopathy and GA than visual acuity (136, 137). There is debate on whether mesopic vision is rod or cone function or both are involved. Rod function may be particularly affected in PDR as the rod photoreceptors require a significant amount of oxygen during dark adaptation; this imbalance between oxygen demand and supply may compromise the function of the rod photoreceptors. The difference between BCVA and LLVA is the low luminance deficit (LLD). We do not know if LLVA or LLD is also a predictor of disease worsening in PDR and whether there is a dissociation between BCVA and LLVA in PDR.

It is also unknown whether LLD is uniformly affected in all PDR eyes or whether it is dependent on the baseline LLVA. Similarly, a change in LLVA and LLD before and after panretinal photocoagulation may reveal the impact of destroying the peripheral photoreceptors by PRP. Recently studies have shown anti-VEGF can treat active PDR. These agents only modulate the VEGF levels in the retina without destroying the photoreceptors.

We hypothesized that mesopic visual acuity (LLVA) may be more affected than photopic visual acuity (BCVA) in PDR. Additionally, LLVA may also be more impaired after PRP than anti-VEGF therapy, but these effects may vary by baseline LLD.

8.2 Aims

(1) What is the correlation between LLVA and BCVA in treatment naïve PDR patients?

(2) Is the dissociation between BCVA and LLVA (BCVA-LLVA = LLD) constant or does it vary with BCVA or LLVA in treatment naïve PDR?

(3) In treatment naïve PDR patients, does the change in BCVA and LLVA with aflibercept therapy differ from those treated with PRP by 52 weeks?

(4) Does LLD differ in each type of treatment?

(5) In eyes, previously treated with PRP, does repeat PRP worsen LLVA more than BCVA over 52 weeks?

8.3 Methods

8.3.1 Study approval

This is a post-hoc analysis of the CLARITY study. Each participant provided informed consent and the trial was approved by the National Research Ethics Committee Service London, Southeast (14/LO/0203) (132).

8.3.2 Study participants

Patients with Type 1 or 2 diabetes with treatment naïve PDR or active PDR post initial PRP with BCVA of 54 or more ETDRS letters with sufficient media clarity were deemed eligible for the study. Key exclusion criteria included coexistent ocular pathology that can

affect visual acuity, macular oedema, dense vitreous haemorrhage, fibrovascular proliferation or tractional retinal detachment.

Patients were randomised to have either PRP or repeated intravitreal aflibercept 2mg/0.05 ml. Patients randomised to the aflibercept arm received mandated 3 loading injections and were then re-treated as and when necessary based on regression patterns of new vessels (133). Participants in the PRP arm had PRP at baseline and were re-assessed every 8 weeks and re-treated with PRP, if necessary.

8.3.3 Study assessments

The study participants underwent baseline examination including visual acuity in ETDRS letters and low luminance visual acuity, slit lamp examination, Ultrawide field colour fundus image, (Optomap 200Tx, Optos plc, Dunfermline, UK), spectral domain optical coherence tomography (SD OCT) (Heidelberg Engineering) in the CLARITY study. I used the ETDRS BCVA and LLVA data collected in the study to answer the research questions:

8.3.4 Statistical analysis

All study parameters are reported as a percentage for categorical, mean and standard deviation for continuous variables. Independent t –test or nonparametric Mann Whitney U test was used to compare between groups based on the normality of data. P values of <0.05 were considered statistically significant. Spearman Correlation coefficients were reported to show the rank association between BCVA and LLVA in various treatment subgroups reported with 95% CI's. Statistical analysis was performed using Microsoft Excel version 14.0 (2010 Microsoft Corporation) and Stata version 15.1 (Stata Corp 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC).

8.4 Results

Relation between BCVA and LLVA in PDR eyes.

The flow diagram (figure 6) shows the CLARITY study groups that were evaluated in this post-hoc analysis. Treatment naïve subjects with complete data available at baseline and week 52 (n=110) were included to evaluate the correlation of BCVA and LLVA. This group was then assessed for the change in BCVA, LLVA and LLD following treatment with either aflibercept monotherapy (n=54) or PRP (n=56). The previously treated PRP group was assessed to understand the change in BCVA, LLVA and LLD and their correlations following repeat PRP (n=48).

The baseline characteristics of all treatment naïve (n=110) eyes with active PDR are described in Table 11. The average LLD was 11.79 (SD 6.08) letters, but eyes with better baseline BCVA had lower LLD compared to eyes with poorer baseline BCVA. This LLD pattern is related more to the variations in LLVA rather than BCVA. Participants with BCVA less than 84 letters (median or lower 50%) had SD LLVA of 8.78 compared to participants with BCVA greater than or equal to 84 letters where LLVA SD was 5.58 ($p<0.001$; test for equality of variances). Similarly, in the upper 75% of BCVA (greater than 88 ETDRS letters) SD for LLVA was 4.19 compared to LLVA SD in the lower 75% which was 9.32 ($p<0.001$). The same applied for the lower 25% (LLVA SD 10.4 vs 7.19; p-value=0.01).

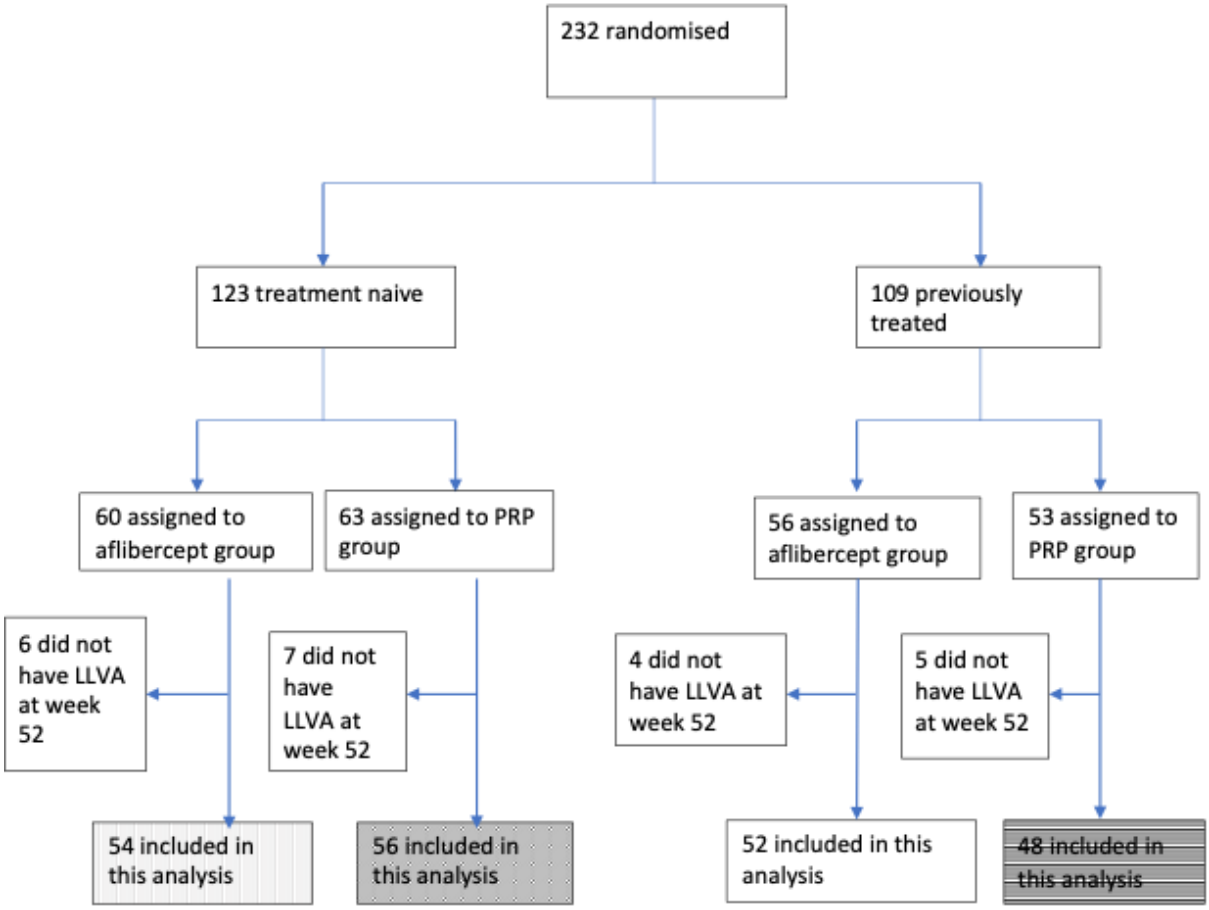

Figure 6: Flow chart showing distribution of participants in the study based on treatment arms

Table 11: Demographic characteristics of treatment naïve eyes at baseline

Baseline	**Treatment naïve (n=110)**	**Repeated PRP group (n=48)**
Age Mean ±SD	54.37 (14.56)	57.71 (12.04)
Age categories	N (%)	N (%)
30-50 years	50 (45.45%)	11 (22.92%)
51-70 years	45 (40.91%)	30 (62.5%)
71-80 years	15 (13.64%)	7 (14.58%)
Male	67 (60.91 %)	34 (70.83%)
Female	43 (39.09%)	14 (29.17%)
T1DM	55 (50.00%)	21 (43.75%)
T2DM	55 (50.00%)	27 (56.25%)
Eyes with NVD+/-NVE	36 (32.73%)	19 (39.58%)
Eyes with NVE only	72 (65.45%)	28 (58.33%)
Pseudophakic	12 (10.91 %)	6 (12.5%)
Phakic	98 (89.09%)	42 (87.5%)
Mean BCVA (SD)	82.94 (7.59)	81.48 (7.65)
Median BCVA (IQR)	84 (80-88)	82 (77-86.75)
Mean LLVA (SD)	71.15 (9.94)	67.88 (7.52)
Median LLVA (IQR)	73 (66-78)	68 (64.25-73)
Mean LLD (SD)	11.79 (6.08)	13.60(5.51)
Median LLD (IQR)	11 (7-15)	14 (10-16.75)
LLD 1-5 letters	13 (11.81%)	3(6.25%)
LLD 6-10 letters	38 (34.55%)	11(22.92%)
LLD 11-14 letters	30 (27.27%)	12(25.00%)
LLD 15-19 letters	21 (19.09%)	17(35.42%)
LLD 20 or more letters	8 (7.27%)	5(10.42%)
Proportion > mean + 2SD	2 (1.82%)	2(4.17%)

Abbreviations: BCVA, best corrected visual acuity; LLVA, low luminance visual acuity, DM, diabetes mellitus; NVD, new vessel disc; NVE, new vessel elsewhere; SD, standard deviation; IQR, inter quartile range; LLD, low luminance deficit.

The LLD was not influenced by any other parameters. There was no difference in LLD between eyes with only NVE (mean LLD 11.8 (SD 6.1) and eyes with NVD (mean 11.7 [SD 6.2] letters). Mean LLD was also similar in pseudophakic versus phakic eyes [mean 11.3 (SD 4.7) and 11.8 (SD 6.2)]. There was also no age-related change in LLD. The mean LLD categorised by age groups <60 years, 60-69 years, 70-79 years and 80 years or older was 16 (SD 6.1), 14 (SD 7.7), 12.6 (SD 8.1) and 10.3 (SD 3.1) respectively.

The correlations between BCVA and LLVA in treatment naïve eyes are shown in figure 7. The Spearman's correlation was 0.756 (95% CI: 0.662 to 0.826). These visual functions are highly correlated although more variations were observed with LLVA in lower levels of BCVA.

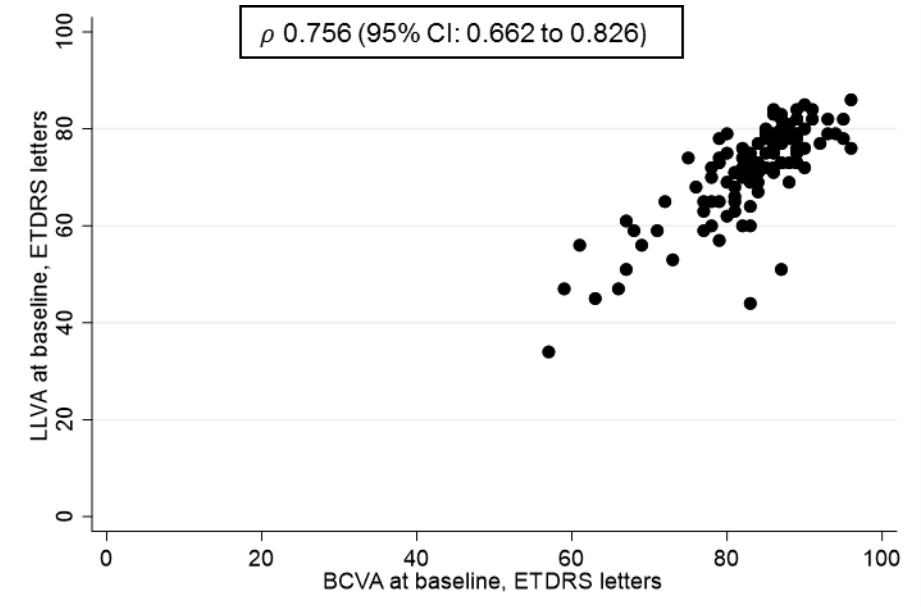

Figure 7: The relation between BCVA and LLVA in treatment naïve PDR eyes

Abbreviations: BCVA, best corrected visual acuity; LLVA, low luminance visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study.

Effect of treatment on BCVA and LLVA

In the aflibercept treated group (n=54), the mean BCVA at baseline was 82.4 (SD 8.03) letters and mean LLVA was 71.7 (SD 10.20) letters. The mean LLD at baseline was 10.8 (SD 4.95) letters.

In the 56 treatment naïve eyes that underwent PRP, the mean BCVA and LLVA at baseline were 83.4 (SD 7.17) and 70.6 (SD 9.76) letters respectively. Mean LLD at baseline was 12.8 (SD 6.89) letters.

In the aflibercept treated group, the mean BCVA at 52 weeks was 84.6 (SD 11.1) letters and mean LLVA was 72.1 (SD 11.9) letters. The mean LLD was 12.5 (SD 5) letters. The mean change in BCVA at 52 weeks after treatment with aflibercept was +2.1 (SD 6.05) letters and the mean change in LLVA was +0.39 (SD 5.6) letters. The mean change in LLD after aflibercept was 1.72(SD 5.83).

Fifty-six treatment naïve eyes underwent PRP and were also followed up for 52 weeks. Mean BCVA and LLVA at 52 weeks were 80.9 (SD 8.6) and 68.8 (SD 11.7) letters respectively. Mean LLD at 52 weeks was 12.1 (SD 6.7) letters. The mean change in BCVA from baseline at 52 weeks after PRP was -2.5 (SD 4.9) letters and the mean change in LLVA was -1.9 (SD 8.7) letters. The mean change in LLD following PRP was -0.64 (SD 7.39).

The individual patient-level response to treatment with aflibercept and PRP in treatment-naïve eyes are shown in Figure 8. When we compared the response between treatment arms, BCVA change was found to be statistically significant ($p<0.001$) but LLVA was not ($p=0.11$). The mean change in LLD between treatment arms in treatment naïve eyes did not reach statistical significance ($p=0.06$; independent t-test).

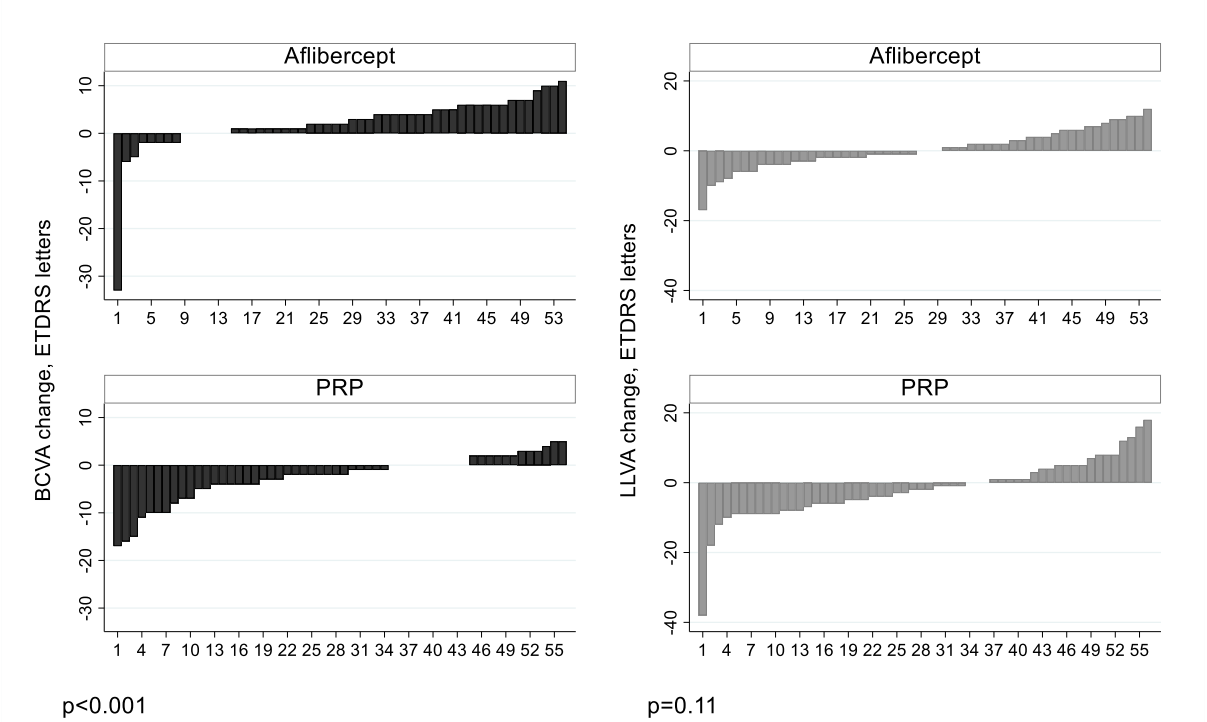

Figure 8: Shows the participants' level of change in BCVA and LLVA in treatment naïve patients at 52 weeks

Abbreviations: BCVA, best corrected visual acuity; LLVA, low luminance visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study; PRP, panretinal photocoagulation.

Table 12 shows the categorical distribution of LLD in both treatment arms. Most participants in both arms were within ± 5 letters of the mean difference of 12 letters observed before treatment. The proportion of participants with LLD > mean + 2SD in the aflibercept arm was 4 (7.41%) and PRP was 3 (5.36%).

Effect of repeat PRP in previously PRP treated eyes on BCVA, LLVA and LLD

The mean baseline BCVA of the 48 participants who had repeat PRP in the study was 80.8 (SD 7.5) letters and mean LLVA was 67.3 (SD 8.3) letters. Mean BCVA at week 52 was 77.9 (SD 10.2) letters and mean LLVA was 63.1 (SD 13.7) letters, whilst mean LLD was 14.8 (SD 6.9) letters. The mean change in BCVA from baseline at 52 weeks after repeat PRP was -3.6 (SD 8.8) letters and the mean change in LLVA was -4.77(SD 11.99) letters. There was no significant difference in LLD in this group between baseline and after repeat PRP at 52 weeks.

Correlation between mean change in BCVA and LLVA in treatment naïve and previously treated groups:

Figure 9 shows the correlation of mean change in BCVA and mean change in LLVA at 52 weeks in the treatment naïve aflibercept arm, in the treatment naïve PRP arm and in the repeat PRP arm. There was a statistically significant correlation between change in BCVA and change in LLVA in treatment naïve eyes receiving PRP and eyes that had repeat PRP ($p=0.001$, $p<0.001$ for treatment naïve and repeat PRP eyes). The spearman correlation coefficient for aflibercept eyes was not statistically significant ($p=0.073$). Spearman's correlation coefficients(ρ) presented with 95% CI's. Removing the single observation with high drop in BCVA and LLVA in aflibercept arm, spearman's correlation coefficient reduces to 0.198(95% CI: -0.076 to 0.445) on 53 observations.

Table 12: LLD difference between treatment naive arms at 52 weeks

	Aflibercept (n=54)	**PRP (n=56)**	**p-value**
Mean LLD (SD)	12.48 (4.96)	12.14 (6.69)	0.76[a]
LLD 1-5 letters	1 (1.9%)	9 (16.1%)	
LLD 6-10 letters	22 (40.7%)	14 (25%)	
LLD 11-14 letters	18 (33.3%)	19 (33.9%)	
LLD 15-19 letters	8 (14.8%)	7 (12.5%)	
LLD 20 or more letters	5 (9.3%)	7 (12.5%)	0.07[b]

a independent sample t-test; b Fishers exact test

Abbreviations: LLD, low luminance deficit; SD, standard deviation; PRP, panretinal photocoagulation

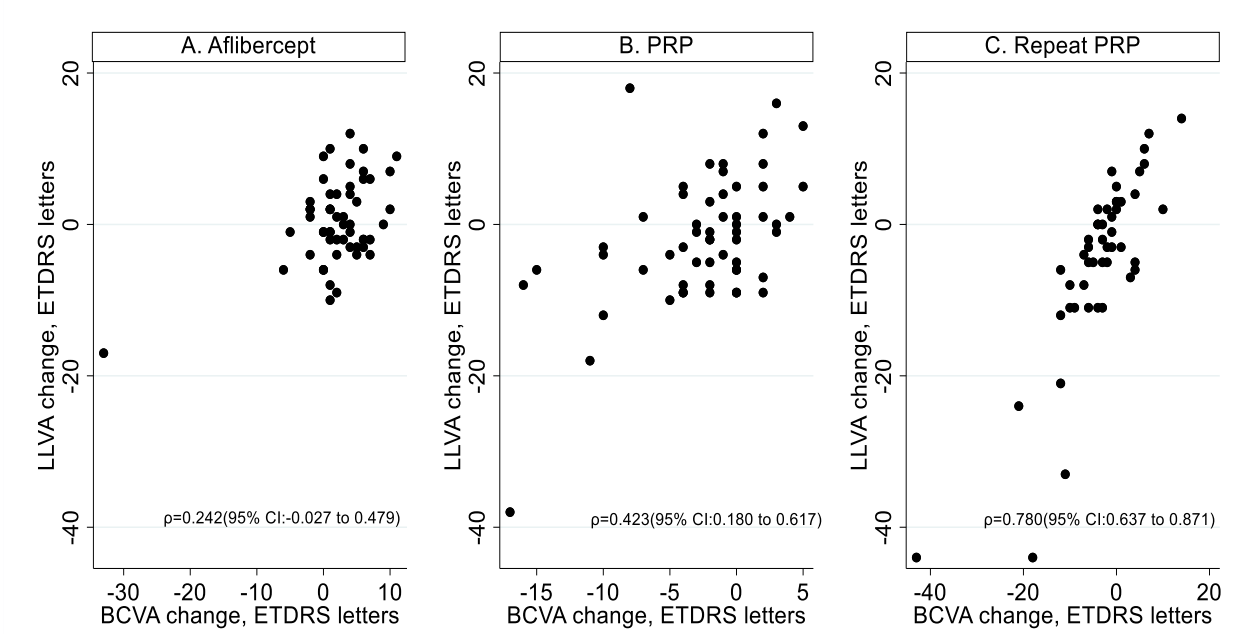

Figure 9: The correlation between change in BCVA and LLVA based on treatment arms. A) aflibercept arm; B) PRP arm in treatment naïve eyes and C) Repeat PRP. B and C statistically significant

Abbreviations: BCVA, best corrected visual acuity; LLVA, low luminance visual acuity; PRP, panretinal photocoagulation.

Despite the correlations shown in figure 9, eyes with smaller changes in BCVA than the median showed more variability in LLVA change compared to those who experienced higher gains in BCVA. For example, for repeat PRP eyes, BCVA letter change of less than -3 letters (median or 50%) lead to a SD of LLVA letter change of 6.07 while in eyes with more than or equal to -3 letter BCVA change i.e., higher decline in BCVA, the SD of LLVA change was 13.43 ($p<0.001$, test for equality of variances). For treatment naïve eye that had PRP, the SD in eyes with less than -2 (median or 50%) letter BCVA change was 10.43 whereas eyes with greater than or equal to -2 letter BCVA change was 6.64 ($p=0.02$, test for equality of variances).

For treatment naïve eyes that had aflibercept, SD of LLVA letter change in eyes with less than 2 BCVA letter change was 6.11 and SD in eyes with greater than or equal to 2 letter BCVA change was 4.99 ($p=0.2960$; test for equality of variances).

8.5 Discussions

In this post-hoc analysis of the CLARITY trial, we found that LLVA in treatment naïve eyes is, on average, two lines lower than BCVA when all other variables are kept constant. Simplistically, these observations can be explained by the fact that both BCVA and LLVA are foveal cone-mediated visual functions. Thus, the linear difference is due to the decrease in luminance. However, the mesopic pathway is more complex and we observed that although these visual functions are highly correlated, the difference between BCVA and LLVA (LLD) is more marked in lower levels of BCVA, and a more variable LLVA drives this at lower levels of BCVA. Stockman et al showed that mesopic vision is governed by several factors, such as rod-cone interactions, their retinal distributions, their integrated responses and the post-receptoral pathways controlling the rod and cone signals (92). This dissociation between BCVA and LLVA at lower levels of BCVA suggests that such a pathway may be more vulnerable than BCVA to neuronal and

ischaemic changes in PDR. A faster but less sensitive rod pathway is also active at mesopic levels. We also showed that although the change in BCVA was superior in the aflibercept arm compared to PRP, the changes in LLVA were not significant between these two treatment arms in treatment naïve eyes. These findings show that the mesopic pathway is likely to be slower to recover. We also showed that changes in BCVA did not correlate with changes in LLVA. Although the correlations were significant in the PRP arms, the change in LLVA in lower levels of change in BCVA is also more variable than those with higher gains in BCVA. These substantiate the above findings that the vulnerable mesopic pathway seems to have a predictable response to either aflibercept or PRP. As our study duration was only 52 weeks, these findings also suggest that the LLVA pathway is unlikely to show rapid responses if used as a clinical trial outcome.

When these observations are compared to the results of LLVA in studies on intermediate age-related macular degeneration (AMD) and GA, we can postulate a few points. Firstly, Sunness et al found that LLD worsens with worsening BCVA (88). They also showed that the LLD in eyes with GA with good BCVA was about 4.6 lines compared to those with drusen, only 2.2 lines. Therefore, in addition to BCVA, retinal atrophy may have affected the mesopic pathway (LLVA) more profoundly than BCVA.

Other studies in AMD also substantiate these findings. Wu et al classified AMD severity levels into six groups and reported that LLD was significantly different only in the non-foveal GA group (215). Furthermore, in a cross-sectional study, Cocce et al showed no differences in BCVA, LLVA and LLD between control, early and intermediate AMD as the BCVA eligibility criteria of the study was Snellen 20/50 or better. The mean BCVA in each group was more than 80 letters (216). Another study that evaluated the factors that determined LLD in AMD reported that LLD is affected by age, BCVA, AMD severity, reticular pseudodrusen and sub foveal choroidal thinning in AMD patients (217). Of these

factors, only age and BCVA apply to this study, but we found no age-related change of LLD in our study cohort. In our study, only BCVA was related to LLVA. Our baseline inclusion criteria were limited to 54 ETDRS Letters (Snellen 20/80) or better. Similar dissociations between LLVA and BCVA have also been observed in central serous chorioretinopathy and macular telangiectasia type 2 compared to controls. (136, 218). These observations suggest that LLVA and LLD may not be disease specific changes and the integrity of the mesopic pathway may predominantly determine the changes.

Clinical trials on interventions in GA have shown that the average LLD is>20 letters, while for PDR, it is about 12 letters (219-222). In addition, an LLD of 20 or more letters is a risk factor for disease progression (221). However, in PDR, our study shows that an LLD of 20 or more letters is rare. Therefore, such a clinical trial outcome is not feasible in PDR. Moreover, our study shows that the mean LLVA and LLD changes post PRP or aflibercept are minimal and may not be appropriate to be used as clinical outcome measures for future PDR trials.

The strengths of this study are the large sample size, the BCVA and LLVA measurements were protocol driven assessments, and the retention rate at 52 weeks was high. This is the first study on the effects of PDR and its treatment on LLVA.

The limitation of this study was that OCT-angiography measurements were not done, so the effect of diabetic macular ischaemia on BCVA and LLVA cannot be directly derived from this analysis. The correlation between BCVA and the size of the FAZ is low to moderate, so BCVA is not an ideal outcome measure for trials on diabetic macular ischaemia. Studies on the effects of increasing macular ischaemia on LLVA need to be done to assess the rate of deterioration of LLVA compared to BCVA in this condition. The study cohort also did not include people with poor visual acuity as our BCVA cut-off was 54 letters to avoid eyes with no potential for BCVA improvement. Future research on

LLVA in eyes with macular ischaemia confirmed by optical coherence tomography angiography is necessary to confirm or refute our postulation that the post-receptoral pathway may be more affected than the foveal cones in PDR and DMI.

Chapter 9. A study on microvascular changes comparing PDR and CRVO

9.1 Background

Proliferative diabetic retinopathy and CRVO are the two most common retinal vasculopathy with similar complications affecting VA. A rise in retinal VEGF levels due to hypoxia is the main culprit for such complications, most importantly macular oedema and ischaemia. We hypothesize that ischaemic damage due to these two conditions may affect the macular microvasculature differently due to different aetiopathogenesis. Optical coherence tomography and OCTA are reliable, non-invasive tools to assess structural changes in both pathologies.

9.2 Aims

The study aimed to compare macular structural changes in PDR and CRVO using OCT and OCTA imaging.

9.3 Methods

9.3.1 Study approval

Imaging performed during standard clinic visit has been used retrospectively for this study. The study approved by Moorfields Eye Hospital review board (CA20/RE/04–602). The study adhered to the tenets of the Declaration of Helsinki.

9.3.2 Study participants

Eyes with treated stable PDR or non- ischaemic CRVO with no macular oedema were included. Such PDR did not have treatment laser or Anti-VEGF in the last 6 months prior

to inclusion in the study. Exclusion criteria were eyes with any co-existent pathology and those with significant macular haemorrhages that can mask the changes in the microvasculature.

9.3.3 Study assessments

I compared the Spectral domain OCT (Heidelberg Engineering, Germany) and OCT-A of consecutive patients with CRVO and PDR in the electronic database from June 2017 to September 2018.

9.3.4 Statistical analysis

All study parameters are reported as mean and standard deviation. Independent-samples t or Mann-Whitney U test was used to compare between groups. Linear regression analysis was used to study the strength of relationship of these markers with either retinal pathology. P values of <0.05 were considered as statistically significant. Statistical analysis was performed with SPSS software version 22 (SPSS, Inc., Chicago, IL, USA).

9.4 Results

A total of 59 eyes were included 29 eyes with stable PDR and 30 eyes with CRVO. Table 13 shows the differences between these two study groups. The mean VD in both the superficial and DCP were significantly reduced and proportion of DRIL were higher in PDR compared to CRVO. Although FAZ areas were no different between the 2 conditions, FAZ perimeter was significantly higher in PDR patients (p=0.04). The outer retinal change represented by integrity of EZ were similar in PDR and CRVO eyes. Regression analysis showed FAZ perimeter is estimated to be 0.52 mm (95% CI 0.33,

1.005) higher in PDR eyes than CRVO. Vessel density of superficial plexus is expected to be 10% (95% CI 5.6,14.1%) and that of deep plexus 5% (95% CI 0.7,9.3%) higher in CRVO eyes.

Table 13: Optical coherence tomography (OCT) and OCT-angiography derived measurements

	PDR Mean (SD) n=29	CRVO Mean (SD) n=30	p-value*
VA in ETDRS letters	78.41(8.13)	74.10 (14.50)	0.47
Age in years	52.86 (10.61)	56.4 (17.62)	0.36
SCP density, %	42.45 (8.86)	52.32 (7.67)	**0.001**
DCP density, %	48.14 (7.48)	53.11 (8.87)	**0.03**
FAZ SCP area (mm^2)	0.54 (0.22)	0.49 (0.23)	0.32
FAZ DCP area (mm^2)	0.62 (0.26)	0.79 (0.39)	0.05
FAZ perimeter	3.48 (1.12)	2.96 (0.70)	**0.04**
Proportion with DRIL	21 (72.41%)	10 (33.33%)	**0.002**
CST (µm)	249.10 (33.37)	253.17 (36.62)	0.41
Proportion with loss of integrity of ellipsoid layer	3 (10.34%)	6 (20%)	0.31
Patients received Anti-VEGF treatment	8	16	0.05

*Statistical comparison using Independent- samples t-test or Mann Whitney U test.

Abbreviations: CST, central sub-field thickness; FAZ, foveal avascular zone; SCP, superior capillary plexus; DCP, deep capillary plexus; anti-VEGF, anti-vascular endothelial growth factor, proliferative diabetic retinopathy (PDR) and central retinal vein occlusion (CRVO).

9.5 Discussion

Our study shows that stable treated PDR have more morphological changes due to ischaemia in both the superficial and deep plexi than non-ischaemic CRVO. Reductions of density of superficial and deep plexi have been shown to precede overt clinical diabetic retinopathy, highlighting the slow course of the capillary drop out (223). It usually takes years to develop PDR. The reduction in density of superficial plexus in PDR may also relate to higher proportions of DRIL, as a secondary neurodegenerative change. DRIL was less frequent in CRVO, it suggests that DRIL is more common in chronic ischaemia or there is a threshold for loss of superficial vascular density to appear as DRIL. CRVO is associated with capillary drop out even in eyes that are clinically diagnosed as non-ischaemic (224). Previous studies have shown that FAZ size increases with severity of diabetic retinopathy and CRVO eyes also have larger FAZ than healthy eyes (191, 225). However, the mean FAZ area was not different between the two conditions in our cohort. Tan et al suggested FAZ size may not be a reliable marker for foveal health as it is affected by inter-individual variation as well as axial length (6). Disruption of terminal capillary ring specified by FAZ perimeter would be better indicator of ischaemic damage when comparing microvascular conditions. In our study population, FAZ perimeter was larger among PDR eyes. One could argue that the two conditions are not comparable. However, both these conditions are end-stage of acute or chronic events and so comparing them is more valid than comparing CRVO to any severity of diabetic retinopathy or comparing PDR to ischaemic CRVO only. It is also very challenging to obtain good OCTA images in eyes with ischaemic CRVO. We conclude that although PDR and CRVO may result in severe capillary non-perfusion, there are differences in severity of microvascular changes, which may at least in part be explained mainly by the nature of onset of the conditions.

Chapter 10. Association between visual acuity and CST and prediction of visual acuity outcome in CRVO

10.1 Background

Macular oedema is a critical factor that impairs VA in CRVO patients. Previous reports have emphasised the association of certain MO morphological features with poor visual acuity. In section 3.4, I have discussed the existing literature on the predictors of VA outcomes in CRVO. Optical coherence tomography based macular morphological features such as subretinal fluid, loss of integrity of outer retinal layers DRIL, hyperreflective foci, large cystoid spaces and vitreoretinal interface abnormalities have been evaluated more commonly in DMO (177). In CRVO, the onset is more acute with a higher baseline MO. So far, studies have mostly looked at short-term outcomes of BCVA predictors in CRVO-related MO. This report describes analyses of visual outcomes in the LEAVO participants based on baseline visual acuity and Spectralis OCT parameters to better understand treatment benefits by 100 weeks. The LEAVO study is a randomised controlled prospective multicentre non -inferiority trial that compared three available anti-VEGF agents for MO due to CRVO (226).

10.2 Aims

The study aims to identify VA outcomes at 100 weeks in the LEAVO participants, including change in BCVA. The proportion of eyes with CRVO that gained 10-letters, (final BCVA-baseline BCVA>=10 letters) and those achieving BCVA>70 letters at 100 weeks.

10.3 Methods

This chapter of the thesis contains information from published article.

10.3.1 Study approval

The OCT data were collected from the LEAVO study that was conducted across 44 clinical sites throughout the United Kingdom. Each patient provided informed consent and the study was ethics approved (NRES Committee London - London Bridge, 04/09/2014, ref: 14/LO/1043).

10.3.2 Study participants

Patients deemed eligible for this study,

1. Subjects of either sex aged ≥ 18 years
2. Clinical diagnosis of centre-involving macular oedema (MO) due to CRVO
3. CRVO of ≤ 12 months duration
4. Best corrected visual acuity in the study eye ≥ 19 and ≤ 73 ETDRS letters (approximate Snellen VA 3/60 to VA 6/12)
5. Best corrected visual acuity in the non-study eye ≥ 14 ETDRS letters (approximate Snellen VA ≥ 2/60).
6. SD-OCT central subfield thickness (CST) > 320μm (Spectralis) predominantly due to MO secondary to CRVO in the study eye. See appendix 1 for equivalent CST value for alternative SD-OCT machines.
7. Media clarity, pupillary dilatation, and subject cooperation sufficient for adequate fundus imaging of the study eye
8. In cases of bilateral CRVO, if both eyes are potentially eligible, unless the patient prefers otherwise the worst seeing eye will be recruited.

Exclusion criteria were MO due to other ocular pathology including diabetic retinopathy, any eye condition affecting visual acuity during the study or intravitreal injection of

corticosteroids 90 days or anti-VEGF therapy 60 days prior to recruitment. Only one eye was included per patient as study eye. Each patient was randomised to receive one of the three anti-VEGF agents in the study eye: aflibercept [2.0mg /50µl], bevacizumab [1.25-mg /50µl] or ranibizumab [0.5mg /50µl]. The anti-VEGF agent and number of injections received by each patient were recorded. OCT gradings was performed without prior knowledge of the treatment arm of each patient.

10.3.3 Study assessments

The study participants underwent baseline examination, including visual acuity in ETDRS letters, slit lamp examination, ultrawide field colour fundus image (Optomap 200Tx, Optos plc, Dunfermline, UK), and OCT in the LEAVO study. Only participants who had Spectralis OCT (Heidelberg Engineering) throughout the study timelines were included as key grading features are better visualised with this device. The ETDRS BCVA were obtained from the data recorded for the LEAVO trial. Four retina fellows NP, SC, RR, and I graded the images. We graded all OCT images used in this study based on predesigned morphological definitions that I developed to answer the research question. Definition of grading parameters illustrated in section 5.2.3.

10.3.4 Statistical analysis

Baseline characteristics of the study population are reported as mean (SD) for continuous or % for categorical variables. Analysis was performed on the complete sample of observations available initially, at the univariate level to retain as many samples as possible for statistical power. Furthermore, covariates with less than 5% missing or ungradable were set to missing and excluded from the analysis, otherwise where there were more than 5% ungradable observations, these were modelled as a separate

category or a missing indicator (227). Correlations between BCVA and the continuous parameters, at baseline and at week 100 were investigated using Spearman's rank correlation coefficient (95% CI). The baseline characteristics and OCT parameters were analysed using linear and logistic regression for the continuous outcome and binary outcomes respectively. All analysis were adjusted for treatment type, age, baseline VA and disease duration. Variables with p-values >0.1 in the univariate adjusted analysis qualified for inclusion in the multivariable models. Explanatory power of the final models was interpreted using the R^2 index (Mcfaddens R^2 for logistic regression models). In sensitivity analysis, due to a small proportion of participants presenting with ischemic CRVO at baseline, we replicated all analysis after excluding these patients. All statistical analysis was performed in Stata version 16 (228).

10.4 Results

The randomised controlled trial LEAVO, involved 463 patients with macular oedema due to CRVO (153). 267 out of this total cohort who had Spectralis OCT data with good image quality available for baseline and week 100 were included for analysis (Figure 10). In this study cohort, 28 (10.49%) participants were recorded ischemic by examining clinician at baseline. Treatment received and other baseline characteristics are described in Table 14.

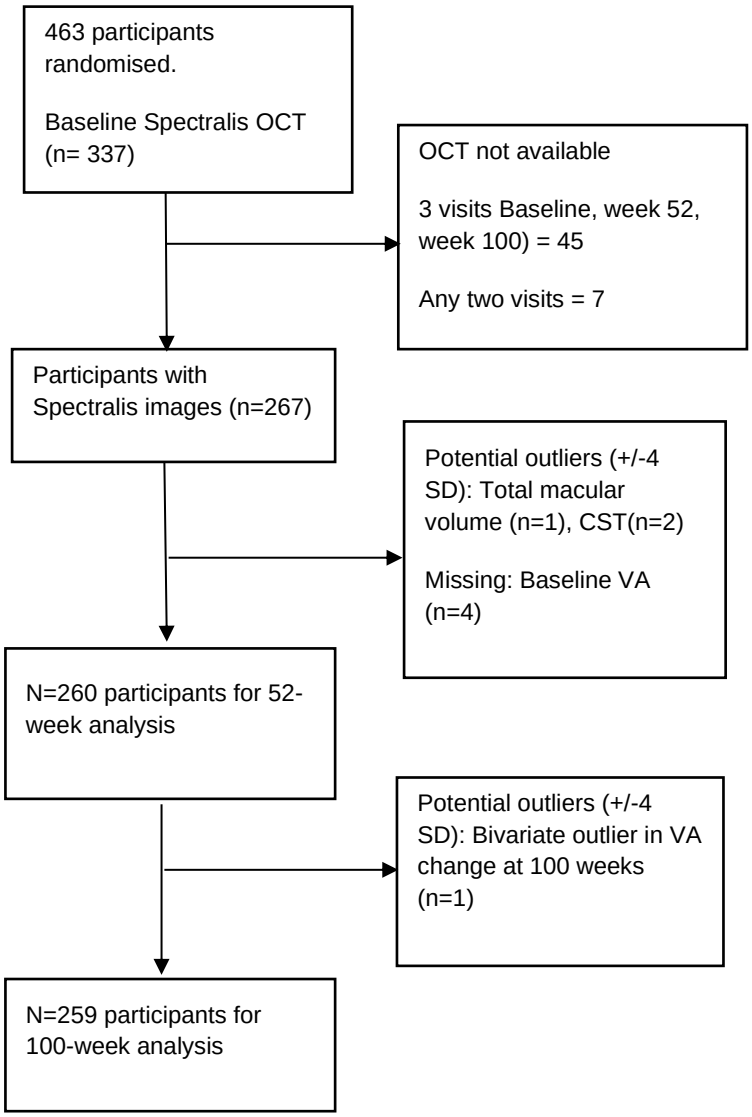

Figure 10: Flowchart showing participants' selection process from the LEAVO study

Abbreviations: VA, visual acuity; CST, central subfield thickness; OCT, optical coherence tomography

Table 14: Baseline characteristics and treatment received

Characteristics	**Total (n=267)**
Clinical characteristics at baseline and treatment over 100 weeks	
Age, Mean (SD), years.	68.6 (13.3)
<50 years (n %)	22 (8.2%)
50-74 years (n %)	145 (54.3%)
≥75 years (n %)	100 (37.5%)
Female (n %)	115 (43.1%)
Duration of CRVO at diagnosis	
median (IQR), months	0.93(0.37-1.83)
<1 month	143(53.6%)
>=1 month	124(46.4%)
Baseline BCVA, mean (SD)[a]	53.8(15.1)
>70 letters	28(10.5%)
55-70 letters	130 (48.7%)
37- 54 letters	64(24.0%)
<37 letters	45(16.9%)
Anti-VEGF agents (total participants)	
Ranibizumab	92 (34.5%)
Aflibercept	89(33.3%)
Bevacizumab	86(32.2%)
Mean number of injections (SD) by 100 weeks	
Ranibizumab	12.8(5.0)
Aflibercept	10.2(3.8)
Bevacizumab	12.5(5.4)
OCT parameters at baseline	
Baseline CST, mean (SD)[b]	723.1(226.9)
Total volume, mean (SD)[c]	12.96(2.93)
Proportion with macular edema N (%)	
Ungradable	1(0.4%)
Diffuse only	22(8.2%)
Cystoid only	208 (77.9%)

Mixed	36(13.5%)
Size of largest cyst in cystoid/mixed edema (n=244)	
Small (<250mm)	43/244 (17.6%)
Medium (≥250 to <500mm)	148/244 (60.6%)
Large ((≥500mm)	53/244 (21.7%)
Proportion with subretinal fluid, N (%)	
Absent	85 (31.8%)
Present	169 (63.3%)
Ungradable	13 (4.9%)
Proportion with VMIA, (ERM or VMT) N (%)	
No evidence	233 (94.33%)
Present	14(5.67%)
Ungradable	20(7.50%)
Ellipsoid zone, N (%)	
Intact	87 (32.6%)
Not intact	41 (34.8%)
Ungradable	139 (52.06%)
DRIL, N (%)	
Absent	144 (53.9%)
Presence	118 (44.2%)
Ungradable	5 (1.9%)
External limiting membrane (ELM), N (%)	
Intact	112(42.0%)
Not intact	24(9.0%)
Ungradable	131(49.1%)
Hyperreflective foci N (%)	
Absent	158(59.2%)
Present	109(40.8%)
Ungradable	0

Abbreviations: CRVO, central retinal vein occlusion; anti-VEGF – anti vascular endothelial growth factor; OCT, optical coherence tomography; BCVA, best corrected visual acuity; CST, central subfield thickness; SRD, subretinal detachment; DRIL, disorganisation of retinal inner layers; EZ, ellipsoid zone; ELM, external limiting membrane; VMIA,

vitreomacular interface abnormalities; ERM, epiretinal membrane; VMT, vitreomacular traction; SD, standard deviation

[a] 4 participants BCVA set to missing as they did not meet eligibility criteria / did not complete 4-meter tests despite having baseline BCVA <=19 / data entry error from the site;

[b] for CST data were missing for 3 participants at screening

[c] for total volume data were missing for 5 participants at screening

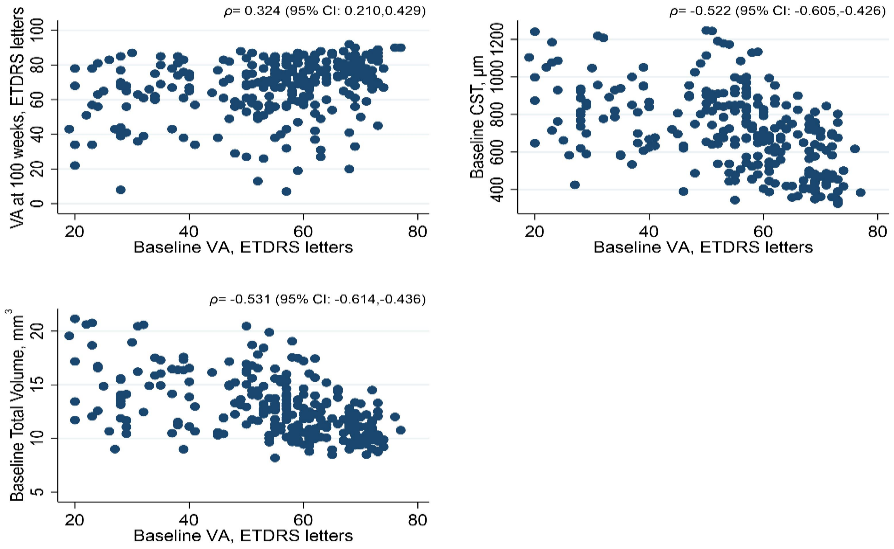

Figure 11: Scatter plots showing relationships between continuous variables BCVA, CST and total volume

Abbreviations: OCT, optical coherence tomography; VA, visual acuity; CST, central subfield thickness

Spearman correlation between baseline BCVA and BCVA change at week 100 as well as with CST, total macular volume is shown in Figure 11.

There were images which were ungradable or questionable for certain OCT parameters especially at baseline due shadow from overlying macular oedema. In such cases of unreliable EZ and ELM, a higher-than-average CST (ELM: mean, 823.6μm; SD, 173.2 and EZ: mean, 807.4μm; SD, 180.1), compared to CST in patients with these layers being intact at baseline (ELM: mean, 574μm; SD, 167.5, EZ: mean, 539μm; SD, 144) was observed.

Correlation between baseline parameters (demography and OCT defined) showed all were statistically significant except sub retinal detachment (Table 15).

Adjusted analyses were conducted on demography, baseline BCVA and ocular characteristics from screening visit OCT, shown in Table 16. We found that those eyes presented with lower BCVA at baseline were more likely to see larger gains in final BCVA (both by mean change and improving by 10 or more letters) but were less likely to reach a score >70 letters by 100 weeks. Furthermore, if baseline BCVA was better by 10 letters, it indicated 3.5 letters increase in absolute BCVA at 100 weeks ($p<0.001$), a 42% reduction in the odds of improving by 10 letters or more (95% CI: 28%-54%) and a 56% increase in the odds of reaching >70 letters at 100 weeks (95% CI:29%-89%). This observation was following adjustment of type of injection received. When adjusted for age and disease duration, result was similar; 3.6 (95% CI:2.3-5.0) letter drop in final BCVA; 44% (95% CI:29%-55%) reduction in odds of improving by 10 letters or more; 62% (95% CI:32%-97%) increase in odds of reaching >70 letters at 100 weeks.

Table 15: Showing p-values of intercorrelations for all baseline risk factors

	Age	**Sex**	**Duration of CRVO**	**BCVA**	**CST**	**Total Volume**	**SRD**	**DRIL**	**EZ**
Sex	0.91								
Disease duration	0.17	0.67							
BCVA	0.57	0.42	0.18						
CST	*0.005a*	0.77	0.30	*<0.001a*					
Total Volume	*0.002a*	0.82	0.83	*<0.001a*	*<0.001*				
SRD	0.29	0.38	0.55	0.33	*<0.001*	*0.001*			
DRIL	0.05	0.73	0.78	*<0.001*	*<0.001*	*<0.001*	0.37		
EZ	*<0.001*	0.81	0.47	*<0.001*	*<0.001*	*<0.001*	*0.004*	*<0.001*	
ELM	*0.002*	0.76	0.11	*<0.001*	*<0.001*	*<0.001*	0.16	*<0.001*	*<0.001*

Abbreviations: CRVO, central retinal vein occlusion; OCT, optical coherence tomography; BCVA, Best Corrected Visual Acuity; CST, Central subfield thickness; SRD, subretinal detachment; DRIL, Disorganisation of retinal inner layers; EZ, ellipsoid zone; ELM, external limiting membrane; Continuous vs continuous associations assessed by spearman's correlation test

Continuous vs categorical associations assessed by Kruskal-Wallis test.

Categorical vs categorical associations assessed by χ^2 test; fishers test used on those with >20% cells with expected count <5 Statistically significant p-values($p<0.05$) have been *italicized.*

[a] Negative (inverse) correlation (for Age vs; CST, spearman's correlation coefficient ρ=-0.21; Total volume, ρ= 0.20. For BCVA vs; CST, ρ = -0.52, Total volume, ρ= -0.53)

Likewise, adjusted analysis showed younger age was associated with a larger magnitude of treatment benefit on BCVA at 100 weeks (every year of older age associated with mean VA gains of -0.33(95% CI: -0.48, -0.19) at 100 weeks). Sex was not associated with visual acuity outcomes at 100 weeks. The adjusted R^2 for change in VA from baseline to 100 weeks was 15.2% for a model adjusting for these identified baseline factors (age, disease duration, baseline VA and treatment arm). For the binary outcomes, adjusted-Mcfadden's R^2 was 7.4% and 6.7%, corresponding to 10-letter gainers and those reaching visual acuity of 70 or more by 100 weeks.

Table 16: Relationship between demography and BCVA at baseline with various measures of visual acuity outcome at week 100

Patient characteristic	**Final BCVA at week 100[a]**		**BCVA improvement ≥10 letters**		**Final BCVA >70 letters**	
	Estimate (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Demography and baseline VA						
Age [b*†] <50 50-74 ≥75	 Ref -5.59(-12.84,1.66) -13.09(-20.57, -5.61)	 - 0.13 *0.001*	 Ref 0.78(0.25, 2.43) 0.38(0.12, 1.20)	 - 0.67 0.10	 Ref 0.24(0.07, 0.75) 0.15(0.04, 0.48)	 - *0.01* *0.001*
Age [b*‡ †] (linear)	-0.33(-0.48, -0.19)	*<0.001*	0.97(0.94, 0.99)	*0.004*	0.96(0.94, 0.98)	*0.001*
Disease duration [b]	-1.06(-2.06, -0.07)	*0.04*	0.90(0.78, 1.02)	0.11	0.89(0.78, 1.02)	0.10
Sex [b] Males Females	 Ref -1.59(-5.59,2.42)	 - 0.44	 Ref 0.98(0.57, 1.66)	 - 0.93	 Ref 1.08(0.64,1.80)	 - 0.78
BCVA, letters [b*‡ †] >70 55-70 37- 54 <37	 Ref -6.96(-13.69, -0.24) -12.76(-20.03, -5.50) -17.58(-25.50, -9.65)	 - 0.04 *0.001* *<0.001*	 Ref 2.03(0.87,4.73) 4.84(1.99,11.76) [c] -	 - 0.10 *0.001*	 Ref 0.65(0.26,1.62) 0.24(0.09,0.63) 0.14(0.05,0.41)	 - 0.36 *0.004* *<0.001*
BCVA [b*‡ †] (linear)	0.35(0.21,0.49)	*<0.001*	0.95(0.93,0.97)	*<0.001*	1.05(1.03,1.07)	*<0.001*

Abbreviations: BCVA, Best Corrected Visual Acuity; OR, Odds ratio.

[a] For baseline VA, the outcome should be interpreted as the final visual acuity at 100 weeks

[b] Adjusted for baseline VA and treatment arm

[c] Groups 37-54 and <37 was collapsed for outcome due to low numbers in group <37 letters that did not improve. 1 bivariate outlier was identified in VA change (67 letters drop) after truncating at 3SD and 3 further outliers identified and removed from CST and total volume.

Variables that remained significant following adjustment for total injection number indicated with * (continuous BCVA), ‡ (BCVA improvement ≥10 letters) and † (final VA>70 letters)

BCVA, best corrected visual Acuity

Baseline OCT characteristics and visual acuity outcome at 100 weeks

Among the OCT defined morphological characteristics, EZ and ELM changes were associated with ≥ 10 letters gain and final BCVA of >70 letters (EZ $p<0.001$ and $p=0.002$, ELM $p=0.04$ and $p=0.02$) after baseline BCVA, age, disease duration and treatment type were adjusted (Table 17). More subjects with baseline CST ≤ 500 microns achieved >70 letters of final VA, whereas majority of 10 letter gainers had ≥700 microns CST at the time of inclusion in the study (Figure 12).

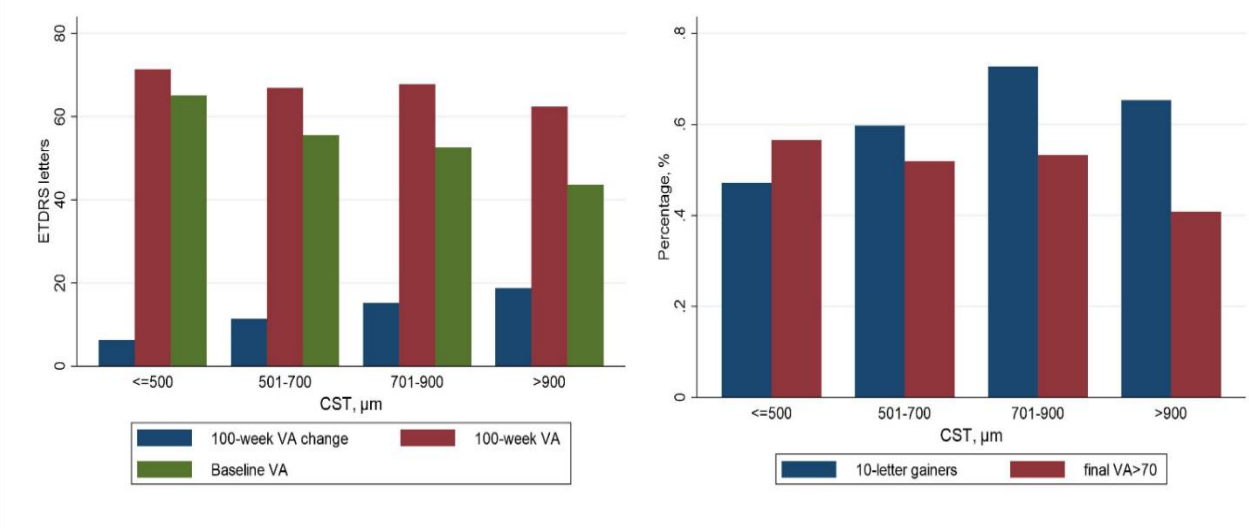

Figure 12: Effect of CST on visual acuity outcomes in CRVO eyes. The panel show bar plots for different vision outcomes across 4 subgroups of CST for a) change in VA, final VA and baseline VA and b) the proportion of 10-letter gainers and those reaching >70 letters final VA

Abbreviations: VA, Visual Acuity; CST, central subfield thickness.

Table 17: OCT characteristics defining visual acuity outcomes at 100 weeks

	Final BCVA at week 100[a]		**BCVA improvement ≥10 letters**		**Final BCVA >70 letters**	
	Estimate (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
OCT characteristics [d]						
CST, µm [e‡]	-0.01(-0.02,0.004)	0.18			1.00(0.999,1.002)	0.91
Volume, mm³ [e‡]	-0.18(-1.00,0.64)	0.66			1.02(0.91,1.15)	0.70
SRD[e]						
Absence	Ref	-	Ref	-	Ref	-
Presence	-1.36(-5.48,2.77)	0.52	0.86(0.47,1.58)	0.64	1.04(0.58,1.85)	0.80
DRIL[e]						
Absent	Ref	-	Ref	-	Ref	-
Present	-0.59(-4.78,3.60)	0.78	0.98(0.53,1.82)	0.96	1.06(0.58,1.93)	0.85
EZ [e*‡†]						
Intact	Ref	-	Ref	-	Ref	-
Not Intact	-15.90(-21.47, -10.33)	*<0.001*	0.18(0.07,0.47)	*<0.001*	0.20(0.07,0.56)	*0.002*
Ungradable/ Questionable	1.34(-3.02,5.69)	0.55	1.85(0.94,3.62)	0.08	2.26(1.14,4.48)	0.02
ELM [e*‡]						
Intact	Ref	-	Ref	-	Ref	-
Not Intact	-10.47(-17.36, -3.58)	*0.003*	0.32(0.11,0.92)	*0.04*	0.57(0.20,1.63)	0.29
Ungradable/ Questionable	2.98(-1.24,7.20)	0.17	1.65(0.89,3.06)	0.11	2.04(1.10,3.78)	*0.02*

[a] For baseline VA, the outcome should be interpreted as the final visual acuity at 100 weeks

[d] Showing only variables that were statistically significant at the 10% threshold (p<0.1).

[e] Adjusted for baseline VA, age, disease duration and treatment arm

Statistically significant p-values at the 5% threshold ($p<0.05$) have been italicized.

Variables that remained significant following adjustment for total injection number indicated with * (continuous BCVA), ‡ (BCVA improvement ≥10 letters) and † (final VA>70 letters)

Abbreviations: OCT; BCVA, best corrected visual Acuity; CST, Central subfield thickness; SRD, sub-retinal detachment; DRIL, Disorganisation of retinal inner layers; EZ, ellipsoid zone; ELM, external limiting membrane.

Multivariable analysis

Variables EZ, ELM, CST and total macular volume were used for the multivariable analysis for predicting gain of 10 or more letters, however after baseline adjustment of the demographic features and treatment, ELM was subsequently dropped. Figure 13 summarizes all variables that remained in the final multivariable models. On average for participants with non-intact EZ, the mean BCVA gains were 15.9 letters less, and 10-letter gains (OR=0.18, 95% CI: 0.07, 0.47) or achieving 70 letters by 100 weeks were less likely (OR=0.57,95% CI: 0.2-1.63). Age and baseline BCVA remained significantly associated in all models, however in this study disease duration was not found to be independently related to visual acuity when considering the OCT parameters. In this model, adjusted-R^2 for the change in BCVA over 2 years was 27.8% (vs 15% for a model adjusting for demographics, baseline VA and drug assignment only). For 10-letter gainers, Mcfaddens adjusted-R^2 increased to 17.4% (compared to 7.4%). For predicting final VA >70 letters, Mcfaddens adjusted-R^2 increased to 13.2% (vs 6.7%), where values 20%-40% indicate good-excellent fit (229).

Sensitivity analysis

Table 18 shows participants with ischemic versus non-ischemic CRVO at baseline, ischaemic CRVO participants on average had a lower baseline VA, higher baseline CST, higher total volume and greater gains in BCVA at 100 weeks. The proportion gaining 10 or more letters was consistently higher in the ischemic group at 100 weeks (78%). Table 19 shows the adjusted analysis restricted to demography, baseline BCVA and ocular characteristics from screening visit OCT against 100-week visual acuity outcomes after

excluding participants with ischemic CRVO at baseline. All variables that were statistically significant from the analysis on the total cohort remain significant at the 1% level despite the reduced sample size. In addition, visual acuity outcomes of ischemic CRVO defined in various ways in the whole LEAVO cohort as well in this study sample are described in Table 20.

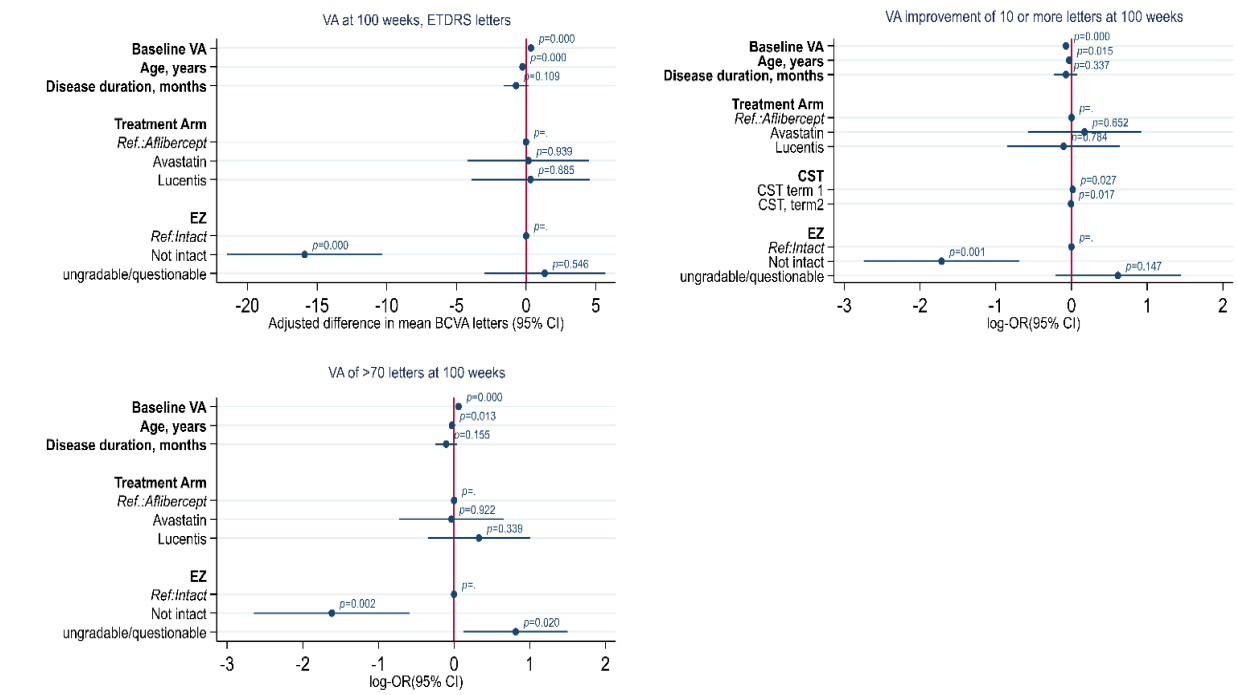

Figure 13: Forest plots from multivariable analysis of outcomes at week 100

Abbreviations: VA, Visual Acuity; EZ, ellipsoid zone; CST, central subfield thickness. Variables that passed the p<0.1 threshold in the univariate (adjusted) analysis were subsequently included in the multivariable models (ELM, EZ and CST). Backward elimination was carried out locking in control variables or confounders for precision regardless of statistical significance and setting the variable elimination threshold at p<0.05. In all models ELM was eliminated at the 5% level (not presented).

Table 18: Table comparing visual acuity outcomes in participants with ischemic vs non-ischemic CRVO at baseline

Characteristic		Non-ischemic (n=239)		Ischemic (n=28)	
		N	Median (IQR)/ Mean (SD) /N(%)	N	Median (IQR)/Mean (SD) / N(%)
Baseline VA		236		27	
Median (IQR)			58 (50-66.5)		37(28-52)
Mean (SD)			55.7(13.8)		41.3(15.6)
CST, µm		236		28	
Median (IQR)			695(537-852)		761(634-941.5)
Mean (SD)			714.9(221.4)		792.3(263.9)
Total volume, mm^3		236		26	
Median (IQR)			12.3(10.6-14.4)		14.1(11.5-16.5)
Mean (SD)			12.8(2.9)		14.2(3.0)
100-week	100-week VA	239		28	
Visual	Median (IQR)		71 (58-80)		68.5 (61.5-79)
Acuity	Mean (SD)		67.0(17.3)		65.7(17.5)
Outcomes	Change in VA	236		27	
	Median (IQR)		12.5(3-22)		24(12-39)
	Mean (SD)		11.5(19.1)		24.3(19.7)
	% 10-letter	236	142(60.2%)	27	21(77.8%)
	Gainers				
	% achieving >70	239	123(51.5%)	28	12(42.9%)
	Letters				

Abbreviations: VA, Visual acuity; CST, central-subfield thickness; SD, standard deviation; IQR inter quartile range.

Table 19: Visual acuity outcomes at 100 weeks, by demographic variables, baseline BCVA and OCT characteristics after excluding participants with ischaemic CRVO at baseline

	Final BCVA at week 100[a]		BCVA improvement		Final BCVA >70 letters	
Patient			≥10 letters			
Characteristics	Estimate (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
		Demography and baseline VA				
Age [b]						
<50	Ref	-	Ref	-	Ref	-
50-74	-5.92(-13.73,1.90)	0.14	0.66(0.19,2.27)	0.51	0.22(0.06,0.77)	0.02
≥75	-12.77(-20.80,-4.74)	0.002	0.34(0.10,1.18)	0.09	0.15(0.04,0.53)	0.003
Age [b] (linear)	-0.32(-0.47,-0.16)	<0.001	0.97(0.94,0.99)	0.005	0.97(0.95,0.99)	0.003
Disease duration [b]	-0.87(-1.96,0.22)	0.12	0.88(0.76,1.02)	0.09	0.93(0.81,1.07)	0.29
Sex [b]						
Males	Ref	-	Ref	-	Ref	-
Females	-1.56(-5.74,2.62)	0.46	0.95(0.55,1.66)	0.86	1.17(0.68,2.00)	0.57
BCVA, letters [b]						
>70	Ref	-	Ref	-	Ref	-
55-70	-7.10(-13.83,-0.36)	0.04	2.10(0.90,4.91)	0.09	0.63(0.25,1.55)	0.31
37- 54	-13.90(-21.31,-6.48)	<0.001	4.24(1.71,10.49)[c]	0.002	0.22(0.08,0.59)	0.003
<37	-16.66(-25.27,-8.05)	<0.001	-		0.16(0.05,0.52)	0.002
BCVA [b] (linear)	0.36(0.21,0.51)	<0.001	0.95(0.93,0.97)	<0.001	1.04(1.02,1.07)	<0.001
		OCT characteristics [d]				
CST, µm [e]	-0.008(-0.02,0.004)	0.17			1.00(1.00,1.002)	0.88
Volume, mm^3 [e]	-0.27(-1.16,0.62)	0.55			1.03(0.91,1.16)	0.69
SRD[e]						
Absence	Ref	-	Ref	-	Ref	-
Presence	-1.85(-6.23,2.53)	0.41	0.76(0.40,1.44)	0.40	0.98(0.54,1.80)	0.96
DRIL[e]						
Absent	Ref	-	Ref	-	Ref	-
Present	-0.60(-5.08,3.88)	0.79	0.92(0.49,1.75)	0.81	1.22(0.65,2.28)	0.53
EZ [e]						
Intact	Ref	-	Ref	-	Ref	-
Not Intact	-15.41(-21.31,-9.43)	<0.001	0.19(0.07,0.52)	0.001	0.21(0.07,0.60)	0.003
Ungradable /Questionable	0.47(-4.20,5.13)	0.84	1.74(0.87,3.51)	0.12	2.11(1.04,4.30)	0.04

ELM [e]						
Intact	Ref	-	Ref	-	Ref	-
Not Intact	-9.20(-16.68,-1.72)	0.02	0.39(0.13,1.15)	0.09	0.51(0.17,1.55)	0.24
Ungradable /Questionable	2.43(-2.08,6.94)	0.29	1.55(0.82,2.95)	0.18	2.17(1.14,4.15)	0.02

1 bivariate outlier was identified in VA change (67 letters drop) after truncating at 3SD and 3 further outliers identified and removed from CST and total volume

28 participants with ischemic CRVO at baseline were excluded

a For baseline VA, the outcome should be interpreted as the final visual acuity at 100 weeks

b Adjusted for baseline VA and treatment arm

c Groups 37-54 and <37 was collapsed for outcome due to low numbers in group <37 letters that did not improve.

d Showing only variables that were statistically significant at the 10% threshold ($p<0.1$). e Adjusted for baseline VA, age, disease duration and treatment arm

Abbreviations: OCT; BCVA, best corrected visual Acuity; CST, Central subfield thickness; SRD, sub-retinal detachment; DRIL, Disorganisation of retinal inner layers; EZ, ellipsoid zone; ELM, external limiting membrane.

Table 20: Visual outcome based on various definitions of ischaemic CRVO

Different definitions of ischaemia	**Baseline BCVA**	**Final BCVA**	**Change in BCVA at 100 weeks**
Clinician defined ischaemic CRVO in 463 patients – whole LEAVO cohort (n=56) Mean (SD) Median (IQR)	 44(16.3) 39(30-60)	 63.4(20.9) 69(56-77.5)	 20.6(24.4) 22.5(8.5-39)
Patients with RAPD in 267 patients included in this post-hoc analysis (n=25) Mean (SD) Median (IQR)	 42.4(15.1) 38.5(28-56)	 66.0(14.3) 68(58-77)	 24.6(19.4) 22(13-40)
Clinician defined in 267 patients in this post-hoc analysis (n=28) Mean (SD) Media (IQR)	 41.3(15.6) 37(28-52)	 65.7(17.5) 68.5(61.5-79)	 25.3(20.0) 26(12.5-39.5)

Abbreviations: BCVA- Best corrected visual acuity; CRVO- central retinal vein occlusion; RAPD- relative afferent pupillary defect; SD- Standard deviation; IQR- Inter quartile range.

10.5 Discussion

Unlike studies on DMO, quantitative OCT parameters at baseline do influence final BCVA with a CST of >900μm less likely to be associated with gains ≥10 letters compared to CST≤900μm. This difference from DMO is likely due to the more severity and acuteness of onset in CRVO. Although these eyes are also likely to present with poor vision, the finding that the potential for visual improvement of 10 or more letters in these eyes is limited suggests some element of irreversible damage. These eyes may indicate ischaemic component at onset, although the RAVE study showed that visual prognosis can be improved with anti – VEGF in pre proliferative ischaemic CRVO (230).

Among the eyes who gained 10 letters, eyes with CST ≥900μm have a decreasing probability of reaching >70 letters BCVA by 100 weeks. One might think this might be due to presence of eyes with clinically significant ischaemia, but this relation remains same after excluding ischaemic CRVO from this cohort.

The non-linear effect in terms of CST may be due to the ceiling effect of baseline VA seen in participants the lower extremity of CST, whereas participants with a moderately increased CST present with lower VA, therefore have greater room for improvement and are more likely to gain 10 or more letters by 100 weeks. Those with extremely high baseline CST may have irreversible structural changes or coexistent ischaemia and have a reduced odd of gaining 10 or more letters.

Predictors of visual acuity outcome in macular oedema due to CRVO treated with anti-VEGF therapy.

Our results suggest age of the patient at the time of diagnosis of CRVO, BCVA at initial presentation and an intact EZ were the predictors for final visual outcome at 100 weeks. Here we must consider EZ must be gradable to make this conclusion since during acute

stage of CRVO severe macular oedema precludes the reliability of grading of outer retina. There is more chance of reaching VA of > 70 letters at 100 weeks if they fall in the age group <75 years.

Previous CRVO studies found similar results regarding poor visual prognosis in older individuals (231, 232). The SCORE study evaluated triamcinolone for MO due to CRVO at 12 months and SCORE2 that treated CRVO and HRVO with monthly aflibercept or bevacizumab showed that younger age is more likely to gain 15 letters at 6 months (233, 234). Likewise, the SHORE study analysed predictors of visual acuity gains after 7 monthly intravitreal ranibizumab injections and also stated younger age is a good predictor of visual gain (64). These outcome from previous studies indicate that irrespective of treatment type, regimen or period of follow-up, older age is a poor visual prognostic indicator. The SCORE investigators postulated that photoreceptors in younger patients are more resilient to the acute insult of CRVO. Literature has already displayed younger age in DMO is associated with better visual prognosis, further supporting the hypothesis that a young retina more readily withstands the acute and chronic insults of MO (140).

Although duration of disease has been proposed as a prognostic indicator, we did not find duration of CRVO to be a favourable predictor of visual outcome probably because the majority (84%) of the study cohort were diagnosed with CRVO less than 3 months prior to randomization. The COPERNICUS and GALILEO studies showed that a CRVO diagnosis of 2 months or less had better visual outcomes compared to those diagnosed more than 2 months previously (169, 235).

Patients with lower baseline BCVA are more likely to gain 10 or more letters but eyes with baseline BCVA <55 letters are less likely to achieve >70 letters at 100 weeks. Baseline

BCVA is a known predictor of final visual outcome in several CRVO and DMO studies. The SHORE study looked at time to achieve 20/40 or better or 15-letter gain within 3 months of treatment while SCORE and SCORE2 evaluated predictors of 15 letter gainers at 12 and 6 months respectively (175). Baseline BCVA is a predictor of final visual outcome independent of drug used, treatment regimen followed, VA outcome measure or study period.

A definite intact EZ layer at baseline is a predictor of good final visual outcomes and a definite loss of baseline EZ integrity is a poor prognostic indicator. However, if the grading of EZ layer is questionable at baseline due to interference from severe macular oedema, it carries no predictive value.

Strengths of this study include 100-week on protocol-based follow-up and the predictors were adjusted for the type of anti-VEGF agents and the numbers of injections received.

A study limitation was that baseline angiographic macular nonperfusion status was not assessed. However, we found that DRIL, a surrogate marker of non-perfusion is not a predictor. A substantial proportion of eyes with CRVO related MO continued to be on anti-VEGF beyond 2 years and it is unknown if these predictors can be applied beyond two years of treatment as the LEAVO study final visit was at 100 weeks. The RAVE study shows that most eyes with ischemic CRVO deteriorate as soon as anti-VEGF therapy is withdrawn and so their course after stopping anti-VEGF therapy is different to eyes with non-ischaemic CRVO (230).

In conclusion, at presentation of MO due to CRVO, older age is a predictor of poor visual outcome, and lower BCVA predicts 10 letter gainers and higher gains in BCVA although it is a poor predictor of achieving >70 BCVA letters. An intact subfoveal ellipsoid zone predicts a BCVA letter score >70 at 100 weeks. This information is important to determine

appropriate and individualised anti-VEGF management plans up to 100 weeks for clinicians.

Chapter 11. Discussion

11.1 General discussion

The recent availability of OCTA has enabled us to study the correlation between macular structure and function in more detail. While previously, the structural assessment was mainly based on qualitative and quantitative changes of the retina observed on macular OCT scans and leakage and non-perfusion of retinal vessels on FA; OCTA has advanced our understanding of the macular microvasculature in healthy eyes and disease. With OCTA, we can quantitatively assess several metrics of the FAZ, superficial and deep vessels and peripapillary radial plexus. Compared to FA, OCTA also facilitates quantitative measurements of these vascular metrics because it only measures flow, so leakage does not confound the grading of these images.

As these are relatively novel parameters compared to OCT and FA, I first evaluated FAZ parameters on OCTA in a normal population because enlarged and irregular FAZ is currently the most important anatomical landmark that defines macular ischaemia. However, FAZ morphology is known to be variable in the literature.

I used the angioplex software of the Cirrus SD-OCTA device in healthy patients with normal fundus to explore the normative data of FAZ. Caucasian eyes had smaller FAZ with higher perifoveal capillary density compared to AFC and Asians. Although the exact reasons for these ethnic differences are not known, we have previously reported differences in OCT thickness between these ethnic groups too (182). These findings on OCT were substantiated in my study. The OCT and OCTA findings together in this study may suggest that these differences are due to genetic influence on anatomical splaying of the fovea forcing the microvasculature to be and the inner retina to be displaced laterally in minor ethnic groups. My findings on the correlation of OCTA vascular metrics

and macular thickness in the various subfields of OCT also show that it is insufficient to report only OCT retinal thickness when we describe anatomical changes. The corresponding OCTA metrics may have added value to understanding disease mechanisms. However, it is unclear whether these changes are relevant to susceptibility to diseases such as glaucoma and diabetic retinopathy in ethnic minority groups. As the correlation was not always linear and in the same gradient, it suggests that some retinal layers are more affected by changes in the microvasculature. This may be because the microvasculature is located in specific retinal layers. It may also explain why localised areas of the retina may be affected by the local loss of capillaries, as seen in DRIL and paramacular middle maculopathy. No correlation between FAZ parameters and age was found in the current study, substantiating a previous report (236).

As DR and CRVO are the most common conditions associated with macular ischaemia, peripheral capillary closure, and anterior and posterior segment neovascularisation, I then studied the changes in the SCP and DCP at the macula on OCTA in detail in both these conditions and related them to visual function.

In severe NPDR and PDR, structural changes in the macula identified using OCTA correlated with visual function. As previously reported, DRIL is an area of the disorganised inner retina due to focal ischaemia. I found that in eyes with DRIL, BCVA was 6.65 letters lower than those without DRIL. On the contrary, BCVA was better in patients with higher VD at SCP, DCP and temporal peripapillary capillary plexus. These findings are supported by previous studies on FA . A similar correlation of structural changes was observed with LLVA. When all the statistically significant structural parameters were analysed to identify the best-fit model to predict BCVA and LLVA, VD at the superficial parafoveal capillary plexus was the most accurate predictor. This analysis also highlighted DRIL to be a more reliable forecaster of LLVA. Likewise, Ghassemi et al

proposed parafoveal VD at SCP as an excellent biomarker to predict VA irrespective of the stage of DR (202). On the other hand, Dupas et al looked at a group of young type 1 diabetics with rapidly progressing DR treated with PRP and found VD at the deep capillary complex mainly drives VA outcome (237). They also highlighted the difficulty in delineating FAZ accurately in the presence of capillary dropouts extending to the adjacent vascular arcade. FAZ area and perimeter did not reach significance as a predictor of visual function in our study.

Assessing OCTA changes is not without challenges. Estimation of VD at DCP level can be affected by projection artefact; hence enface SCP image is more reliable for assessment of VD. Moreover, previous studies have used different software to calculate VD (238).

It is also interesting to note that although BCVA is the most widely accepted functional outcome, some untreated PDR patients have difficulty with driving and in environments with low luminance. Although visual acuity decreases with a reduction in luminance, we found that the correlation between BCVA and LLVA is only when the eye has good BCVA. In lower ranges of BCVA, LLVA is poorer than BCVA. LLVA and LLD have been extensively researched in early and intermediate AMD when BCVA is still near normal. Low LLVA is found in many patients with intermediate AMD, especially those with subretinal drusenoid deposits. Owsley et al proposed three possible physiological mechanisms; reduced LLVA could be due to compromised central foveal cone density or extrapolated effect from deranged rod-cone coupling in the parafoveal region or cone-to-cone circuits via horizontal and amacrine cells in the plexiform layers (239). In people with diabetes, cellular and extracellular alterations also cause changes in the inner retina, especially in eyes with macular ischaemia (240). It is unclear whether the same visual

circuits are affected in diabetic macular ischaemia, albeit due to different primary pathology.

I also found that LLVA does not respond to aflibercept therapy in PDR compared to BCVA. Therefore, LLVA and LLD are valuable additions to functional outcome measures in PDR. Over the last few decades, change in BCVA, particularly loss or gain of 15 or more ETDRS letters, has been adopted as a functional outcome in landmark trials and clinical practice. My thesis highlights the need to include minor BCVA changes and other structural and functional (i.e., LLVA) markers to identify functional deficits in early disease and track small changes in disease progression.

I also studied CRVO in detail because both PDR and CRVO are commonly associated with macular ischaemia. Previous reports have shown that DRIL in both PDR and CRVO is associated with poor visual prognosis (53, 241). In my study, eyes showed the presence of DRIL was higher in PDR. In addition, VD at both capillary plexi was lower in PDR than in CRVO eyes.

The inciting cause of both these conditions is different, although lead to macular ischaemia. We recognise that venous stasis is the primary cause triggering CRVO, but changes in retinal endothelial basement membrane, pericyte loss, and breakdown of the blood–retinal barrier are responsible for DR complications. Macular oedema occurs in both these conditions and may have a certain degree of underlying ischaemia, potentially affecting the visual outcome.

I then studied the factors that predict BCVA in CRVO. All eyes had MO at baseline and were treated with one of the anti-VEGF agents over two years. I found that age at diagnosis is an essential predictor of visual outcome. Lower baseline BCVA indicated a higher possibility of 10-letter gain following treatment initiation, although it is unlikely that

such eyes can achieve >70 BCVA letters. Similarly, eyes with severe MO (<900-micron central subfield thickness) were less likely to achieve more than 70 letters of vision even after robust treatment. Based on our findings, baseline BCVA, age and intact ellipsoid zone on OCT at the time of presentation diagnosis can guide clinicians to ascertain good post-treatment visual prognosis in eyes with CRVO.

11.2 Challenges

My thesis included both prospective and retrospective data. The benefits of using prospective data are that such studies were performed following a formally laid protocol, which guarantees higher reliability. Recruiting patients with diabetes into studies that do not offer treatment is always challenging. Due to the chronic nature of diabetes as a pathology, patients often fail to attend their hospital appointments. I faced an added challenge due to Coronavirus disease pandemic, which compelled vulnerable patients to undertake self-isolation. This has caused me to use retrospective data for some of the thesis.

I have shown here OCTA could be an indispensable tool in clinical practice. However, it is expensive, and the current outputs from OCTA have yet to be applied to our practice. Acquiring good-quality images and data is both time and labour-intensive. There is a steep learning curve, and image artefacts often affect diagnostic conclusions. Moreover, OCTA devices from different manufacturers use different segmentation boundaries of retinal layers and different thresholding methods to quantify imaging parameters. In research or clinics, the same OCTA device should be used to quantify sequential changes in vascular metrics. Moreover, poor central vision and media opacities affect image quality. For instance, I could not capture good OCTA images in patients with severe macular ischaemia due to this technical challenge.

Although I have suggested using LLVA in adjunct to BCVA, the assessment of LLVA needs to be standardised, including background luminance of the letter chart, neutral density filter used and duration of dark adaptation. In busy clinical practice, measuring both visual functions is only possible if future studies justify the need for LLVA as an additional test.

11.3 Conclusion and future works

In my thesis, I have addressed changes in macular microvasculature in two important and commonly encountered retinal conditions: CRVO and PDR. In both these conditions, MO and macular ischaemia can cause central vision loss. One should use FAZ morphology alone to assess ischaemia, and we should use multiple vascular metrics available on OCTA to provide more helpful information on the health of the macula and the visual function. However, large sample-size studies are required to evaluate the exact contribution of SCP and DCP on visual outcomes. Low luminance visual acuity is a novel area explored in my thesis in PDR. A recent report showed that FAZ area itself did not correlate with DMI, but cut-off values of FAZ area and deep vascular complex VD predicted mild vision loss (BCVA < 70 letters and LLVA < 70 letters) in sight-threatening DMI patients with visual acuity 54 letters or better (242). My study and this recent report justify the need to investigate the use of LLVA as a visual function outcome measure in treatment trials of PDR. More work is required to correlate peripheral ischaemia and OCTA metrics at the macula in both CRVO and PDR.

11.4 References

1. Hirsch J, Curcio CA. The spatial resolution capacity of human foveal retina. Vision research. 1989;29(9):1095-101.

2. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. Journal of comparative neurology. 1990;292(4):497-523.

3. Yamada E. Some Structural Features of the Fovea Centralis in the Human Retina. Archives of Ophthalmology. 1969;82(2):151-9.

4. Burris C, Klug K, Ngo IT, Sterling P, Schein S. How Müller glial cells in macaque fovea coat and isolate the synaptic terminals of cone photoreceptors. Journal of Comparative Neurology. 2002;453(1):100-11.

5. Duke-Elder S. The eye in evolution. System of Ophthal mology. 1958;1.

6. Tang FY, Ng DS, Lam A, Luk F, Wong R, Chan C, et al. Determinants of Quantitative Optical Coherence Tomography Angiography Metrics in Patients with Diabetes. Sci Rep. 2017;7(1):2575.

7. Provis JM, Sandercoe T, Hendrickson AE. Astrocytes and blood vessels define the foveal rim during primate retinal development. Investigative Ophthalmology & Visual Science. 2000;41(10):2827-36.

8. Delaey C, Van de Voorde J. Regulatory mechanisms in the retinal and choroidal circulation. Ophthalmic research. 2000;32(6):249-56.

9. Nettleship E. Note on retinal blood vessels of the yellow spot. Roy Lond Ophthalmol Hosp Rep. 1875;8:269.

10. Bligard E, De Venecia G, Wallow I, Bresnick G, Syrjala S. Aging changes of the parafoveolar vasculature: a trypsin digest study. Invest Ophthalmol Vis Sci. 1982;22(8).

11. Bird AC, Weale RA. On the retinal vasculature of the human fovea. Experimental eye research. 1974;19(5):409-17.

12. Bradley A, Applegate RA, Zeffren BS, van Heuven WAJ. Psychophysical measurement of the size and shape of the human foveal avascular zone. Ophthalmic and Physiological Optics. 1992;12(1):18-23.

13. Yap M, Gilchrist J, Weatherill J. Psychophysical measurement of the foveal avascular zone. Ophthalmic Physiol Opt. 1987;7(4):405-10.

14. Tam J, Martin JA, Roorda A. Noninvasive Visualization and Analysis of Parafoveal Capillaries in Humans. Investigative Ophthalmology & Visual Science. 2010;51(3):1691-8.

15. Chui TYP, VanNasdale DA, Elsner AE, Burns SA. The Association Between the Foveal Avascular Zone and Retinal Thickness. Investigative Ophthalmology & Visual Science. 2014;55(10):6870-7.

16. Dubis AM, Hansen BR, Cooper RF, Beringer J, Dubra A, Carroll J. Relationship between the Foveal Avascular Zone and Foveal Pit Morphology. Investigative Ophthalmology & Visual Science. 2012;53(3):1628-36.

17. Laatikainen L, Larinkari J. Capillary-free area of the fovea with advancing age. Invest Ophthalmol Vis Sci. 1977;16(12):1154-7.

18. Bresnick GH, Condit R, Syrjala S, Palta M, Groo A, Korth K. Abnormalities of the Foveal Avascular Zone in Diabetic Retinopathy. Archives of Ophthalmology. 1984;102(9):1286-93.

19. Wu LZ, Huang ZS, Wu DZ, Chan E. Characteristics of the capillary-free zone in the normal human macula. Jpn J Ophthalmol. 1985;29(4):406-11.

20. John D, Kuriakose T, Devasahayam S, Braganza A. Dimensions of the foveal avascular zone using the Heidelberg retinal angiogram-2 in normal eyes. Indian J Ophthalmol. 2011;59(1):9-11.

21. Wolf S, Arend O, Toonen H, Bertram B, Jung F, Reim M. Retinal Capillary Blood Flow Measurement with a Scanning Laser Ophthalmoscope Preliminary Results. Ophthalmology. 1991;98(6):996-1000.

22. Arend O, Wolf S, Harris A, Reim M. The Relationship of Macular Microcirculation to Visual Acuity in Diabetic Patients. Archives of Ophthalmology. 1995;113(5):610-4.

23. Mansour AM, Schachat A, Bodiford G, Haymond R. Foveal avascular zone in diabetes mellitus. Retina. 1993;13(2):125-8.

24. Conrath J, Giorgi R, Raccah D, Ridings B. Foveal avascular zone in diabetic retinopathy: quantitative vs qualitative assessment. Eye. 2005;19(3):322-6.

25. Shahlaee A, Pefkianaki M, Hsu J, Ho AC. Measurement of foveal avascular zone dimensions and its reliability in healthy eyes using optical coherence tomography angiography. American journal of ophthalmology. 2016;161:50-5. e1.

26. Samara WA, Say EAT, Khoo CTL, Higgins TP, Magrath G, Ferenczy S, et al. Correlation of foveal avascular zone size with foveal morphology in normal eyes using optical coherence tomography angiography. Retina. 2015;35(11):2188-95.

27. Coscas F, Sellam A, Glacet-Bernard A, Jung C, Goudot M, Miere A, et al. Normative Data for Vascular Density in Superficial and Deep Capillary Plexuses of Healthy Adults Assessed by Optical Coherence Tomography Angiography. Investigative Ophthalmology & Visual Science. 2016;57(9):OCT211-OCT23.

28. Iafe NA, Phasukkijwatana N, Chen X, Sarraf D. Retinal Capillary Density and Foveal Avascular Zone Area Are Age-Dependent: Quantitative Analysis Using Optical Coherence Tomography Angiography. Invest Ophthalmol Vis Sci. 2016;57(13):5780-7.

29. Gómez-Ulla F, Cutrin P, Santos P, Fernandez M, Abraldes M, Abalo-Lojo JM, et al. Age and gender influence on foveal avascular zone in healthy eyes. Exp Eye Res. 2019;189:107856.

30. Sodhi PK, Shaw E, Gautam A, Yadav A, R AT, Rao KC, et al. Evaluating the Quantitative Foveal Avascular Zone and Retino-Choroidal Vessel Density Using Optical Coherence Tomography Angiography in a Healthy Indian Population. Cureus. 2022;14(8):e27669.

31. Yu J, Jiang C, Wang X, Zhu L, Gu R, Xu H, et al. Macular perfusion in healthy Chinese: an optical coherence tomography angiogram study. Invest Ophthalmol Vis Sci. 2015;56(5):3212-7.

32. Guo J, She X, Liu X, Sun X. Repeatability and Reproducibility of Foveal Avascular Zone Area Measurements Using AngioPlex Spectral Domain Optical Coherence Tomography Angiography in Healthy Subjects. Ophthalmologica. 2017;237(1):21-8.

33. Fujiwara A, Morizane Y, Hosokawa M, Kimura S, Shiode Y, Hirano M, et al. Factors affecting foveal avascular zone in healthy eyes: An examination using swept-source optical coherence tomography angiography. Plos one. 2017;12(11):e0188572.

34. Wylęgała A, Wang L, Zhang S, Liu Z, Teper S, Wylęgała E. Comparison of foveal avascular zone and retinal vascular density in healthy Chinese and Caucasian adults. Acta Ophthalmol. 2020;98(4):e464-e9.

35. Hayreh SS. THE OPHTHALMIC ARTERY: III. BRANCHES. Br J Ophthalmol. 1962;46(4):212-47.

36. Iwasaki M, Inomata H. Relation between superficial capillaries and foveal structures in the human retina. Invest Ophthalmol Vis Sci. 1986;27(12):1698-705.

37. In: Kolb H, Fernandez E, Nelson R, editors. Webvision: The Organization of the Retina and Visual System. Salt Lake City (UT): University of Utah Health Sciences Center. Copyright: © 2022 Webvision . 1995.

38. Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, et al. Optical coherence tomography. science. 1991;254(5035):1178-81.

39. Swanson EA, Izatt JA, Lin CP, Fujimoto JG, Schuman JS, Hee MR, et al. In vivo retinal imaging by optical coherence tomography. Optics Letters. 1993;18(21):1864-6.

40. Drexler W, Morgner U, Ghanta RK, Kärtner FX, Schuman JS, Fujimoto JG. Ultrahigh-resolution ophthalmic optical coherence tomography. Nature medicine. 2001;7(4):502-7.

41. Zawadzki RJ, Jones SM, Olivier SS, Zhao M, Bower BA, Izatt JA, et al. Adaptive-optics optical coherence tomography for high-resolution and high-speed 3D retinal in vivo imaging. Opt Express. 2005;13(21):8532-46.

42. Staurenghi G, Sadda S, Chakravarthy U, Spaide RF. Proposed Lexicon for Anatomic Landmarks in Normal Posterior Segment Spectral-Domain Optical Coherence Tomography: The IN•OCT Consensus. Ophthalmology. 2014;121(8):1572-8.

43. Boycott BB, Wässle H. The morphological types of ganglion cells of the domestic cat's retina. J Physiol. 1974;240(2):397-419.

44. Remington LA. Chapter 4 - Retina. In: Remington LA, editor. Clinical Anatomy and Physiology of the Visual System (Third Edition). Saint Louis: Butterworth-Heinemann; 2012. p. 61-92.

45. Drexler W, Sattmann H, Hermann B, Ko TH, Stur M, Unterhuber A, et al. Enhanced visualization of macular pathology with the use of ultrahigh-resolution optical coherence tomography. Arch Ophthalmol. 2003;121(5):695-706.

46. Spaide RF, Curcio CA. Anatomical correlates to the bands seen in the outer retina by optical coherence tomography: Literature Review and Model. Retina. 2011;31(8):1609-19.

47. Sperelakis N. Defining ischaemia. BMJ. 1995;311(7010):890-1.

48. Osborne NN, Casson RJ, Wood JP, Chidlow G, Graham M, Melena J. Retinal ischemia: mechanisms of damage and potential therapeutic strategies. Progress in retinal and eye research. 2004;23(1):91-147.

49. Scarinci F, Jampol LM, Linsenmeier RA, Fawzi AA. Association of Diabetic Macular Nonperfusion With Outer Retinal Disruption on Optical Coherence Tomography. JAMA Ophthalmol. 2015;133(9):1036-44.

50. Sim DA, Keane PA, Zarranz-Ventura J, Fung S, Powner MB, Platteau E, et al. The Effects of Macular Ischemia on Visual Acuity in Diabetic Retinopathy. Investigative Ophthalmology & Visual Science. 2013;54(3):2353-60.

51. Baseline and early natural history report. The Central Vein Occlusion Study. Arch Ophthalmol. 1993;111(8):1087-95.

52. Noma H, Funatsu H, Yamasaki M, Tsukamoto H, Mimura T, Sone T, et al. Pathogenesis of macular edema with branch retinal vein occlusion and intraocular levels of vascular endothelial growth factor and interleukin-6. American journal of ophthalmology. 2005;140(2):256. e1-. e7.

53. Sun JK, Lin MM, Lammer J, Prager S, Sarangi R, Silva PS, et al. Disorganization of the retinal inner layers as a predictor of visual acuity in eyes with center-involved diabetic macular edema. JAMA Ophthalmol. 2014;132(11):1309-16.

54. Wolter JR. Color Atlas of Diabetic Retinopathy. SLACK Incorporated Thorofare, NJ; 1969. p. 55-.

55. Harrison WW, Bearse MA, Jr., Ng JS, Jewell NP, Barez S, Burger D, et al. Multifocal electroretinograms predict onset of diabetic retinopathy in adult patients with diabetes. Invest Ophthalmol Vis Sci. 2011;52(2):772-7.

56. Romero-Aroca P, Navarro-Gil R, Benejam G, Vizcarro M, Baget-Bernaldiz M. Differences in Multifocal Electroretinogram Study in Two Populations of Type 1 and

Type 2 Diabetes Mellitus Patients without Diabetic Retinopathy. J Clin Med. 2022;11(19).

57. Soni D, Sagar P, Takkar B. Diabetic retinal neurodegeneration as a form of diabetic retinopathy. International Ophthalmology. 2021;41(9):3223-48.

58. Nicholson L, Ramu J, Triantafyllopoulou I, Patrao NV, Comyn O, Hykin P, et al. Diagnostic accuracy of disorganization of the retinal inner layers in detecting macular capillary non-perfusion in diabetic retinopathy. Clin Exp Ophthalmol. 2015;43(8):735-41.

59. Das R, Spence G, Hogg RE, Stevenson M, Chakravarthy U. Disorganization of Inner Retina and Outer Retinal Morphology in Diabetic Macular Edema. JAMA Ophthalmol. 2018;136(2):202-8.

60. Sun JK, Lin MM, Lammer J, Prager S, Sarangi R, Silva PS, et al. Disorganization of the retinal inner layers as a predictor of visual acuity in eyes with center-involved diabetic macular edema. JAMA ophthalmology. 2014;132(11):1309-16.

61. Endo H, Kase S, Tanaka H, Takahashi M, Katsuta S, Suzuki Y, et al. Factors based on optical coherence tomography correlated with vision impairment in diabetic patients. Scientific Reports. 2021;11(1):3004.

62. Busch C, Okada M, Zur D, Fraser-Bell S, Rodríguez-Valdés PJ, Cebeci Z, et al. Baseline predictors for visual acuity loss during observation in diabetic macular oedema with good baseline visual acuity. Acta Ophthalmol. 2020;98(7):e801-e6.

63. Babiuch AS, Han M, Conti FF, Wai K, Silva FQ, Singh RP. Association of Disorganization of Retinal Inner Layers With Visual Acuity Response to Anti-Vascular Endothelial Growth Factor Therapy for Macular Edema Secondary to Retinal Vein Occlusion. JAMA Ophthalmol. 2019;137(1):38-46.

64. Yiu G, Welch RJ, Wang Y, Wang Z, Wang PW, Haskova Z. Spectral-Domain OCT Predictors of Visual Outcomes after Ranibizumab Treatment for Macular Edema Resulting from Retinal Vein Occlusion. Ophthalmol Retina. 2020;4(1):67-76.

65. Hajdu D, Told R, Angeli O, Weigert G, Pollreisz A, Schmidt-Erfurth U, et al. Identification of microvascular and morphological alterations in eyes with central retinal non-perfusion. PLoS One. 2020;15(11):e0241753.

66. Classification of diabetic retinopathy from fluorescein angiograms. ETDRS report number 11. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology. 1991;98(5 Suppl):807-22.

67. Bresnick GH, De Venecia G, Myers FL, Harris JA, Davis MD. Retinal Ischemia in Diabetic Retinopathy. Archives of Ophthalmology. 1975;93(12):1300-10.

68. Mansour AM, Schachat A, Bodiford G, Haymond R. Foveal avascular zone in diabetes mellitus. Retina. 1993;13(2):125-8.

69. Conrath J, Giorgi R, Raccah D, Ridings B. Foveal avascular zone in diabetic retinopathy: quantitative vs qualitative assessment. Eye (Lond). 2005;19(3):322-6.

70. Sim DA, Keane PA, Fung S, Karampelas M, Sadda SR, Fruttiger M, et al. Quantitative analysis of diabetic macular ischemia using optical coherence tomography. Invest Ophthalmol Vis Sci. 2014;55(1):417-23.

71. Tsai ASH, Gan ATL, Ting DSW, Wong CW, Teo KYC, Tan ACS, et al. DIABETIC MACULAR ISCHEMIA: Correlation of Retinal Vasculature Changes by Optical Coherence Tomography Angiography and Functional Deficit. Retina. 2020;40(11):2184-90.

72. La Mantia A, Kurt RA, Mejor S, Egan CA, Tufail A, Keane PA, et al. Comparing fundus fluorescein angiography and swept-source optical coherence tomography angiography in the evaluation of diabetic macular perfusion. Retina. 2019;39(5):926-37.

73. Cennamo G, Romano MR, Nicoletti G, Velotti N, de Crecchio G. Optical coherence tomography angiography versus fluorescein angiography in the diagnosis of ischaemic diabetic maculopathy. Acta Ophthalmol. 2017;95(1):e36-e42.

74. Otani T, Kishi S, Maruyama Y. Patterns of diabetic macular edema with optical coherence tomography. Am J Ophthalmol. 1999;127(6):688-93.

75. Catier A, Tadayoni R, Paques M, Erginay A, Haouchine B, Gaudric A, et al. Characterization of macular edema from various etiologies by optical coherence tomography. Am J Ophthalmol. 2005;140(2):200-6.

76. Spaide RF, Lee JK, Klancnik JK, Jr., Gross NE. Optical coherence tomography of branch retinal vein occlusion. Retina. 2003;23(3):343-7.

77. Wilkins JR, Puliafito CA, Hee MR, Duker JS, Reichel E, Coker JG, et al. Characterization of epiretinal membranes using optical coherence tomography. Ophthalmology. 1996;103(12):2142-51.

78. Koizumi H, Spaide RF, Fisher YL, Freund KB, Klancnik JM, Jr., Yannuzzi LA. Three-dimensional evaluation of vitreomacular traction and epiretinal membrane using spectral-domain optical coherence tomography. Am J Ophthalmol. 2008;145(3):509-17.

79. Scarinci F, Varano M, Parravano M. Retinal sensitivity loss correlates with deep capillary plexus impairment in diabetic macular ischemia. J Ophthalmol. 2019;2019:7589841.

80. Csaky KG, Richman EA, Ferris FL, 3rd. Report from the NEI/FDA Ophthalmic Clinical Trial Design and Endpoints Symposium. Invest Ophthalmol Vis Sci. 2008;49(2):479-89.

81. Kaiser PK. Prospective evaluation of visual acuity assessment: a comparison of snellen versus ETDRS charts in clinical practice (An AOS Thesis). Transactions of the American Ophthalmological Society. 2009;107:311-24.

82. Elliott DB. The good (logMAR), the bad (Snellen) and the ugly (BCVA, number of letters read) of visual acuity measurement. Ophthalmic Physiol Opt. 2016;36(4):355-8.

83. Csaky K, Ferris F, 3rd, Chew EY, Nair P, Cheetham JK, Duncan JL. Report from the NEI/FDA Endpoints workshop on age-related macular degeneration and inherited retinal diseases. Invest Ophthalmol Vis Sci. 2017;58(9):3456-63.

84. Varner P. Ophthalmic pharmaceutical clinical trials: design considerations. Clin Invest. 2015;5(5):457–75.

85. NCT03904056. ETDRS panretinal photocoagulation (PRP) combined with intravitreal ranibizumab (IVR) versus retinal photocoagulation targeted to ischemic retina combined with IVR for the treatment of proliferative diabetic retinopathy. Availabe at https://clinicaltrials.gov/ct2/show/NCT03904056. Accessed 5th May 2020.

86. NCT03246152. Evaluation of the effect of repeated intravitreal injections of anti-VEGF on macular perfusion in diabetic patients using optical coherence tomography angiography. Available at https://clinicaltrials.gov/ct2/show/NCT03246152. Accessed 5th May 2020.

87. NCT02432547. A phase IV randomised clinical trial of laser therapy for peripheral retinal ischaemia combined with intravitreal aflibercept (eylea®) versus intravitreal aflibercept monotherapy for diabetic macular oedema. Available at https://clinicaltrials.gov/ct2/show/NCT02432547. Accessed 5th May 2020.

88. Sunness JS, Rubin GS, Applegate CA, Bressler NM, Marsh MJ, Hawkins BS, et al. Visual function abnormalities and prognosis in eyes with age-related geographic atrophy of the macula and good visual acuity. Ophthalmology. 1997;104(10):1677-91.

89. Sadda SR, Chakravarthy U, Birch DG, Staurenghi G, Henry EC, Brittain C. Clinical Endpoints for the Study of Geographic Atrophy Secondary to Age-Related Macular Degeneration. Retina. 2016;36(10):1806-22.

90. Csaky KG, Patel PJ, Sepah YJ, Birch DG, Do DV, Ip MS, et al. Microperimetry for geographic atrophy secondary to age-related macular degeneration. Surv Ophthalmol. 2019;64(3):353-64.

91. Jackson GR, Scott IU, Quillen DA, Walter LE, Gardner TW. Inner retinal visual dysfunction is a sensitive marker of non-proliferative diabetic retinopathy. Br J Ophthalmol. 2012;96(5):699-703.

92. Stockman A, Sharpe LT. Into the twilight zone: the complexities of mesopic vision and luminous efficiency. Ophthalmic Physiol Opt. 2006;26(3):225-39.

93. Do DV, Nguyen QD, Mostafa SH, Bressler SB. Evaluation of visual acuity measurements with standard luminance compared with low luminance in eyes with geographic atrophy from age-related macular degeneration. Investigative Ophthalmology & Visual Science. 2015;56.

94. Brodnar Z, Shields AR, Dobrota S, Do DV. Low luminance deficits in retinal disease. Invest Ophthalmol Vis Sci. 2018;59.

95. Sunness JS, Rubin GS, Broman A, Applegate CA, Bressler NM, Hawkins BS. Low luminance visual dysfunction as a predictor of subsequent visual acuity loss from geographic atrophy in age-related macular degeneration. Ophthalmology. 2008;115(9):1480-8, 8.e1-2.

96. Jolly JK, Simunovic MP, Dubis AM, Josan AS, Robson AG, Bellini MP, et al. Structural and Functional Characteristics of Color Vision Changes in Choroideremia. Front Neurosci. 2021;15:729807.

97. Simunovic MP. Acquired color vision deficiency. Survey of ophthalmology. 2016;61(2):132-55.

98. Abdel-Hay A, Sivaprasad S, Subramanian A, Barbur JL. Acuity and colour vision changes post intravitreal dexamethasone implant injection in patients with diabetic macular oedema. PLoS One. 2018;13(6):e0199693.

99. Gella L, Raman R, Kulothungan V, Pal SS, Ganesan S, Sharma T. Impairment of colour vision in diabetes with no retinopathy: Sankara Nethralaya diabetic retinopathy epidemiology and molecular genetics study (SNDREAMS-II, report 3). PLoS One. 2015;10(6):e0129391.

100. Dorr M, Lesmes LA, Lu ZL, Bex PJ. Rapid and reliable assessment of the contrast sensitivity function on an iPad. Invest Ophthalmol Vis Sci. 2013;54(12):7266-73.

101. Della Sala S, Bertoni G, Somazzi L, Stubbe F, Wilkins AJ. Impaired contrast sensitivity in diabetic patients with and without retinopathy: a new technique for rapid assessment. Br J Ophthalmol. 1985;69(2):136-42.

102. Stavrou EP, Wood JM. Letter contrast sensitivity changes in early diabetic retinopathy. Clin Exp Optom. 2003;86(3):152-6.

103. Thayaparan K, Crossland MD, Rubin GS. Clinical assessment of two new contrast sensitivity charts. Br J Ophthalmol. 2007;91(6):749-52.

104. Arditi A. Improving the design of the letter contrast sensitivity test. Invest Ophthalmol Vis Sci. 2005;46(6):2225-9.

105. Latham K. The Mars contrast sensitivity chart. Available at: http://assets.markallengroup.com/article-images/image-library/147/uploads/importedimages/mars.pdf. Accessed July 13 2020. The Optician. 2007.

106. Rodriguez JD, Wallstrom G, Narayanan D, Welch D, Abelson MB. An Alternative Psychophysical Diagnostic Indicator of the Aging Eye. Journal of Ophthalmology. 2019;2019:2036192.

107. Ora-VCF Contrast test: Dry AMD Model. Available at: https://www.oraclinical.com/our-services/therapeutic-areas/posterior-segment/ora-vcf-contrast-test/. Accessed 13th July 2020.

108. Usman M. An Overview of Our Current Understanding of Diabetic Macular Ischemia (DMI). Cureus. 2018;10(7):e3064.

109. Pereira F, Godoy BR, Maia M, Regatieri CV. Microperimetry and OCT angiography evaluation of patients with ischemic diabetic macular edema treated with monthly intravitreal bevacizumab: a pilot study. Int J Retina Vitreous. 2019;5:24.

110. Acton JH, Greenstein VC. Fundus-driven perimetry (microperimetry) compared to conventional static automated perimetry: similarities, differences, and clinical applications. Can J Ophthalmol. 2013;48(5):358-63.

111. Tsai ASH, Gan ATL, Ting DSW, Wong CW, Teo KYC, Tan ACS, et al. Diabetic macular ischemia: Correlation of retinal vasculature changes by optical coherence tomography angiography and functional deficit. Retina. 2019;Publish Ahead of Print.

112. Yang Y, Dunbar H. Clinical Perspectives and Trends: Microperimetry as a Trial Endpoint in Retinal Disease. Ophthalmologica. 2021;244(5):418-50.

113. Corvi F, Pellegrini M, Belotti M, Bianchi C, Staurenghi G. Scotopic and fast mesopic microperimetry in eyes with drusen and reticular pseudodrusen. Retina. 2019;39(12):2378-83.

114. Han RC, Gray JM, Han J, Maclaren RE, Jolly JK. Optimisation of dark adaptation time required for mesopic microperimetry. Br J Ophthalmol. 2019;103(8):1092-8.

115. Edington M, Sachdev A, Morjaria R, Chong V. Structural-functional correlation in patients with diabetic macular edema. Retina. 2017;37(5):881-5.

116. Hood DC, Odel JG, Chen CS, Winn BJ. The multifocal electroretinogram. J Neuroophthalmol. 2003;23(3):225-35.

117. Tyrberg M, Ponjavic V, Lövestam-Adrian M. Multifocal electroretinogram (mfERG) in patients with diabetes mellitus and an enlarged foveal avascular zone (FAZ). Documenta Ophthalmologica. 2008;117(3):185-9.

118. Parisi V, Ziccardi L, Costanzo E, Tedeschi M, Barbano L, Manca D, et al. Macular Functional and Morphological Changes in Intermediate Age-Related Maculopathy. Invest Ophthalmol Vis Sci. 2020;61(5):11.

119. Narayanan R, Dave V, Rani PK, Chhablani J, Rao HB, Pappuru RR, et al. Multifocal electroretinography in type 2 idiopathic macular telangiectasia. Graefes Arch Clin Exp Ophthalmol. 2013;251(5):1311-8.

120. Bavinger JC, Dunbar GE, Stem MS, Blachley TS, Kwark L, Farsiu S, et al. The effects of diabetic retinopathy and pan-retinal photocoagulation on photoreceptor cell function as assessed by dark adaptometry. Invest Ophthalmol Vis Sci. 2016;57(1):208-17.

121. Kiser AK, Mladenovich D, Eshraghi F, Bourdeau D, Dagnelie G. Reliability and consistency of dark-adapted psychophysical measures in advanced eye disease. Invest Ophthalmol Vis Sci. 2006;47(1):444-52.

122. Jackson GR, Scott IU, Kim IK, Quillen DA, Iannaccone A, Edwards JG. Diagnostic sensitivity and specificity of dark adaptometry for detection of age-related macular degeneration. Invest Ophthalmol Vis Sci. 2014;55(3):1427-31.

123. Calabrese A, Cheong AM, Cheung SH, He Y, Kwon M, Mansfield JS, et al. Baseline MNREAD measures for normally sighted subjects from childhood to old Age. Invest Ophthalmol Vis Sci. 2016;57(8):3836-43.

124. Calabrese A, Owsley C, McGwin G, Legge GE. Development of a reading accessibility index using the MNREAD acuity chart. JAMA Ophthalmol. 2016;134(4):398-405.

125. Trauzettel-Klosinski S, Dietz K, Group IRS. Standardized assessment of reading performance: the New International Reading Speed Texts IReST. Invest Ophthalmol Vis Sci. 2012;53(9):5452-61.

126. Bailey IL, Lovie-Kitchin JE. Visual acuity testing. From the laboratory to the clinic. Vision Res. 2013;90:2-9.

127. Lee R, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. Eye Vis (Lond). 2015;2:17.

128. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet. 2010;376(9735):124-36.

129. Stefánsson E. The mechanism of retinal photocoagulation: How does the laser work. Euro Ophthalmol Rev. 2008;2(1):76-9.

130. Bailey C, Sparrow J, Grey R, Cheng H. The national diabetic retinopathy laser treatment audit III. Clinical outcomes. Eye. 1999;13(2):151-9.

131. Gross JG, Glassman AR, Liu D, Sun JK, Antoszyk AN, Baker CW, et al. Five-year outcomes of panretinal photocoagulation vs intravitreous ranibizumab for proliferative diabetic retinopathy: a randomized clinical trial. JAMA ophthalmology. 2018;136(10):1138-48.

132. Sivaprasad S, Prevost AT, Vasconcelos JC, Riddell A, Murphy C, Kelly J, et al. Clinical efficacy of intravitreal aflibercept versus panretinal photocoagulation for best

corrected visual acuity in patients with proliferative diabetic retinopathy at 52 weeks (CLARITY): a multicentre, single-blinded, randomised, controlled, phase 2b, non-inferiority trial. Lancet. 2017;389(10085):2193-203.

133. Sivaprasad S, Prevost AT, Bainbridge J, Edwards RT, Hopkins D, Kelly J, et al. Clinical efficacy and mechanistic evaluation of aflibercept for proliferative diabetic retinopathy (acronym CLARITY): a multicentre phase IIb randomised active-controlled clinical trial. BMJ open. 2015;5(9):e008405.

134. Wood LJ, Jolly JK, Buckley TM, Josan AS, MacLaren RE. Low luminance visual acuity as a clinical measure and clinical trial outcome measure: a scoping review. Ophthalmic Physiol Opt. 2021.

135. Kimlin JA, Black AA, Wood JM. Nighttime driving in older adults: effects of glare and association with mesopic visual function. Investigative Ophthalmology & Visual Science. 2017;58(5):2796-803.

136. Fujita K, Shinoda K, Matsumoto CS, Imamura Y, Mizutani Y, Tanaka E, et al. Low luminance visual acuity in patients with central serous chorioretinopathy. Clinical and Experimental Optometry. 2013;96(1):100-5.

137. Sunness JS, Rubin GS, Broman A, Applegate CA, Bressler NM, Hawkins BS. Low luminance visual dysfunction as a predictor of subsequent visual acuity loss from geographic atrophy in age-related macular degeneration. Ophthalmology. 2008;115(9):1480-8. e2.

138. Dhamdhere KP, Schneck ME, Bearse MA, Lam W, Barez S, Adams AJ. Assessment of macular function using the SKILL Card in adults with type 2 diabetes mellitus. Investigative ophthalmology & visual science. 2014;55(6):3368-74.

139. Cristescu I-E, Baltă F, Zăgrean L. Cone photoreceptor density in type I diabetic patients measured with an adaptive optics retinal camera. Romanian Journal of Ophthalmology. 2019;63(2):153.

140. Chen YP, Wu AL, Chuang CC, Chen SN. Factors influencing clinical outcomes in patients with diabetic macular edema treated with intravitreal ranibizumab: comparison between responder and non-responder cases. Sci Rep. 2019;9(1):10952.

141. Bezzina AD, Carbonaro F. Factors Predicting Treatment Response in Anti-Vascular Endothelial Growth Factor Naïve Diabetic Macular Edema Patients Treated with Intravitreal Bevacizumab. J Ocul Pharmacol Ther. 2019;35(10):551-7.

142. Chew EY, Ferris III FL, Csaky KG, Murphy RP, Agrón E, Thompson DJ, et al. The long-term effects of laser photocoagulation treatment in patients with diabetic retinopathy: the early treatment diabetic retinopathy follow-up study. Ophthalmology. 2003;110(9):1683-9.

143. Shimura M, Yasuda K, Nakazawa T, Tamai M. Visual dysfunction after panretinal photocoagulation in patients with severe diabetic retinopathy and good vision. Am J Ophthalmol. 2005;140(1):8-15.

144. Bek T, Erlandsen M. Visual prognosis after panretinal photocoagulation for proliferative diabetic retinopathy. Acta Ophthalmol Scand. 2006;84(1):16-20.

145. Kim J, Park IW, Kwon S. Factors predicting final visual outcome in quiescent proliferative diabetic retinopathy. Scientific Reports. 2020;10(1):17233.

146. Kida T, Flammer J, Konieczka K, Ikeda T. Retinal venous pressure is decreased after anti-VEGF therapy in patients with retinal vein occlusion-related macular edema. Graefes Arch Clin Exp Ophthalmol. 2021;259(7):1853-8.

147. Hayreh SS, Zimmerman B, McCarthy MJ, Podhajsky P. Systemic diseases associated with various types of retinal vein occlusion. Am J Ophthalmol. 2001;131(1):61-77.

148. Cheung N, Klein R, Wang JJ, Cotch MF, Islam AF, Klein BE, et al. Traditional and novel cardiovascular risk factors for retinal vein occlusion: the multiethnic study of atherosclerosis. Invest Ophthalmol Vis Sci. 2008;49(10):4297-302.

149. Hayreh SS, Zimmerman MB, Podhajsky P. Hematologic abnormalities associated with various types of retinal vein occlusion. Graefes Arch Clin Exp Ophthalmol. 2002;240(3):180-96.

150. Park CH, Jaffe GJ, Fekrat S. Intravitreal triamcinolone acetonide in eyes with cystoid macular edema associated with central retinal vein occlusion. American journal of ophthalmology. 2003;136(3):419-25.

151. Campochiaro PA, Brown DM, Awh CC, Lee SY, Gray S, Saroj N, et al. Sustained benefits from ranibizumab for macular edema following central retinal vein occlusion: twelve-month outcomes of a phase III study. Ophthalmology. 2011;118(10):2041-9.

152. Iturralde D, Spaide RF, Meyerle CB, Klancnik JM, Yannuzzi LA, Fisher YL, et al. Intravitreal bevacizumab (Avastin) treatment of macular edema in central retinal vein occlusion: a short-term study. Retina. 2006;26(3):279-84.

153. Hykin P, Prevost AT, Vasconcelos JC, Murphy C, Kelly J, Ramu J, et al. Clinical effectiveness of intravitreal therapy with ranibizumab vs aflibercept vs bevacizumab for macular edema secondary to central retinal vein occlusion: a randomized clinical trial. JAMA ophthalmology. 2019;137(11):1256-64.

154. Hayreh SS, Podhajsky PA, Zimmerman MB. Natural history of visual outcome in central retinal vein occlusion. Ophthalmology. 2011;118(1):119-33.e1-2.

155. Zegarra H, Gutman FA, Conforto J. The natural course of central retinal vein occlusion. Ophthalmology. 1979;86(11):1931-42.

156. Natural History and Clinical Management of Central Retinal Vein Occlusion. Archives of Ophthalmology. 1997;115(4):486-91.

157. Quinlan PM, Elman MJ, Bhatt AK, Mardesich P, Enger C. The natural course of central retinal vein occlusion. Am J Ophthalmol. 1990;110(2):118-23.

158. Natural history and clinical management of central retinal vein occlusion. The Central Vein Occlusion Study Group. Arch Ophthalmol. 1997;115(4):486-91.

159. Ip MS, Scott IU, VanVeldhuisen PC, Oden NL, Blodi BA, Fisher M, et al. A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with observation to treat vision loss associated with macular edema secondary to central retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 5. Arch Ophthalmol. 2009;127(9):1101-14.

160. Ramezani A, Entezari M, Moradian S, Tabatabaei H, Kadkhodaei S. Intravitreal triamcinolone for acute central retinal vein occlusion; a randomized clinical trial. Graefes Arch Clin Exp Ophthalmol. 2006;244(12):1601-6.

161. McIntosh RL, Rogers SL, Lim L, Cheung N, Wang JJ, Mitchell P, et al. Natural history of central retinal vein occlusion: an evidence-based systematic review. Ophthalmology. 2010;117(6):1113-23.e15.

162. Evaluation of grid pattern photocoagulation for macular edema in central vein occlusion. The Central Vein Occlusion Study Group M report. Ophthalmology. 1995;102(10):1425-33.

163. Haller JA, Bandello F, Belfort Jr R, Blumenkranz MS, Gillies M, Heier J, et al. Dexamethasone intravitreal implant in patients with macular edema related to branch or

central retinal vein occlusion: twelve-month study results. Ophthalmology. 2011;118(12):2453-60.

164. Haller JA, Bandello F, Belfort R, Jr., Blumenkranz MS, Gillies M, Heier J, et al. Randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with macular edema due to retinal vein occlusion. Ophthalmology. 2010;117(6):1134-46.e3.

165. Group* TSSR. A Randomized Trial Comparing the Efficacy and Safety of Intravitreal Triamcinolone With Observation to Treat Vision Loss Associated With Macular Edema Secondary to Central Retinal Vein Occlusion: The Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) Study Report 5. Archives of Ophthalmology. 2009;127(9):1101-14.

166. Campochiaro PA, Brown DM, Awh CC, Lee SY, Gray S, Saroj N, et al. Sustained benefits from ranibizumab for macular edema following central retinal vein occlusion: twelve-month outcomes of a phase III study. Ophthalmology. 2011;118(10):2041-9.

167. Heier JS, Campochiaro PA, Yau L, Li Z, Saroj N, Rubio RG, et al. Ranibizumab for macular edema due to retinal vein occlusions: long-term follow-up in the HORIZON trial. Ophthalmology. 2012;119(4):802-9.

168. Epstein DL, Algvere PV, von Wendt G, Seregard S, Kvanta A. Benefit from bevacizumab for macular edema in central retinal vein occlusion: twelve-month results of a prospective, randomized study. Ophthalmology. 2012;119(12):2587-91.

169. Korobelnik J-F, Holz FG, Roider J, Ogura Y, Simader C, Schmidt-Erfurth U, et al. Intravitreal aflibercept injection for macular edema resulting from central retinal vein occlusion: one-year results of the phase 3 GALILEO study. Ophthalmology. 2014;121(1):202-8.

170. Brown DM, Heier JS, Clark WL, Boyer DS, Vitti R, Berliner AJ, et al. Intravitreal aflibercept injection for macular edema secondary to central retinal vein occlusion: 1-

year results from the phase 3 COPERNICUS study. Am J Ophthalmol. 2013;155(3):429-37.e7.

171. Heier JS, Clark WL, Boyer DS, Brown DM, Vitti R, Berliner AJ, et al. Intravitreal aflibercept injection for macular edema due to central retinal vein occlusion: two-year results from the COPERNICUS study. Ophthalmology. 2014;121(7):1414-20.e1.

172. Etheridge T, Blodi B, Oden N, Van Veldhuisen P, Scott IU, Ip MS, et al. Spectral Domain Optical Coherence Tomography Predictors of Visual Acuity in the Study of COmparative Treatments for REtinal Vein Occlusion 2 (SCORE2). Ophthalmol Retina. 2020.

173. Chan EW, Eldeeb M, Sun V, Thomas D, Omar A, Kapusta MA, et al. Disorganization of Retinal Inner Layers and Ellipsoid Zone Disruption Predict Visual Outcomes in Central Retinal Vein Occlusion. Ophthalmol Retina. 2019;3(1):83-92.

174. Daien V, Navarre S, Fesler P, Vergely L, Villain M, Schneider C. Visual acuity outcome and predictive factors after bevacizumab for central retinal vein occlusion. European journal of ophthalmology. 2012;22(6):1013-8.

175. Lloyd Clark W, Liu M, Kitchens J, Wang PW, Haskova Z. Baseline characteristics associated with early visual acuity gains after ranibizumab treatment for retinal vein occlusion. BMC Ophthalmol. 2019;19(1):11.

176. Duker JS, Kaiser PK, Binder S, de Smet MD, Gaudric A, Reichel E, et al. The International Vitreomacular Traction Study Group classification of vitreomacular adhesion, traction, and macular hole. Ophthalmology. 2013;120(12):2611-9.

177. Panozzo G, Cicinelli MV, Augustin AJ, Battaglia Parodi M, Cunha-Vaz J, Guarnaccia G, et al. An optical coherence tomography-based grading of diabetic maculopathy proposed by an international expert panel: The European School for Advanced Studies in Ophthalmology classification. Eur J Ophthalmol. 2020;30(1):8-18.

178. Vujosevic S, Bini S, Torresin T, Berton M, Midena G, Parrozzani R, et al. HYPERREFLECTIVE RETINAL SPOTS IN NORMAL AND DIABETIC EYES: B-Scan and En Face Spectral Domain Optical Coherence Tomography Evaluation. Retina. 2017;37(6):1092-103.

179. DaCosta J, Bhatia D, Talks J. The use of optical coherence tomography angiography and optical coherence tomography to predict visual acuity in diabetic retinopathy. Eye (Lond). 2020;34(5):942-7.

180. Jia Y, Tan O, Tokayer J, Potsaid B, Wang Y, Liu JJ, et al. Split-spectrum amplitude-decorrelation angiography with optical coherence tomography. Opt Express. 2012;20(4):4710-25.

181. Otsu N. A Threshold Selection Method from Gray-Level Histograms. IEEE Transactions on Systems, Man, and Cybernetics. 1979;9(1):62-6.

182. Bafiq R, Mathew R, Pearce E, Abdel-Hey A, Richardson M, Bailey T, et al. Age, Sex, and Ethnic Variations in Inner and Outer Retinal and Choroidal Thickness on Spectral-Domain Optical Coherence Tomography. Am J Ophthalmol. 2015;160(5):1034-43.e1.

183. Anegondi N, Kshirsagar A, Mochi TB, Sinha Roy A. Quantitative Comparison of Retinal Vascular Features in Optical Coherence Tomography Angiography Images From Three Different Devices. Ophthalmic Surg Lasers Imaging Retina. 2018;49(7):488-96.

184. Samara WA, Say EA, Khoo CT, Higgins TP, Magrath G, Ferenczy S, et al. Correlation of foveal avascular zone size with foveal morphology in normal eyes using optical coherence tomography angiography. Retina. 2015;35(11):2188-95.

185. Chun LY, Silas MR, Dimitroyannis RC, Ho K, Skondra D. Differences in macular capillary parameters between healthy black and white subjects with Optical Coherence Tomography Angiography (OCTA). PLoS One. 2019;14(10):e0223142.

186. Giocanti-Aurégan A, Gazeau G, Hrarat L, Lévy V, Amari F, Bodaghi B, et al. Ethnic differences in normal retinal capillary density and foveal avascular zone measurements. Int Ophthalmol. 2020;40(11):3043-8.

187. Wagner-Schuman M, Dubis AM, Nordgren RN, Lei Y, Odell D, Chiao H, et al. Race- and sex-related differences in retinal thickness and foveal pit morphology. Invest Ophthalmol Vis Sci. 2011;52(1):625-34.

188. Zouache MA, Silvestri G, Amoaku WM, Silvestri V, Hubbard WC, Pappas C, et al. Comparison of the Morphology of the Foveal Pit Between African and Caucasian Populations. Transl Vis Sci Technol. 2020;9(5):24.

189. Kelty PJ, Payne JF, Trivedi RH, Kelty J, Bowie EM, Burger BM. Macular thickness assessment in healthy eyes based on ethnicity using Stratus OCT optical coherence tomography. Invest Ophthalmol Vis Sci. 2008;49(6):2668-72.

190. Ghassemi F, Mirshahi R, Bazvand F, Fadakar K, Faghihi H, Sabour S. The quantitative measurements of foveal avascular zone using optical coherence tomography angiography in normal volunteers. J Curr Ophthalmol. 2017;29(4):293-9.

191. Mastropasqua R, Toto L, Mastropasqua A, Aloia R, De Nicola C, Mattei PA, et al. Foveal avascular zone area and parafoveal vessel density measurements in different stages of diabetic retinopathy by optical coherence tomography angiography. Int J Ophthalmol. 2017;10(10):1545-51.

192. Rodrigues TM, Marques JP, Soares M, Dolan M-J, Melo P, Simão S, et al. Peripapillary neurovascular coupling in the early stages of diabetic retinopathy. Retina. 2019;39(12):2292-302.

193. Shlaer S. THE RELATION BETWEEN VISUAL ACUITY AND ILLUMINATION. J Gen Physiol. 1937;21(2):165-88.

194. Cui J. QIC Program and Model Selection in GEE Analyses. The Stata Journal. 2007;7(2):209-20.

195. Pan W. Akaike's information criterion in generalized estimating equations. Biometrics. 2001;57(1):120-5.

196. Sun Z, Tang F, Wong R, Lok J, Szeto SKH, Chan JCK, et al. OCT Angiography Metrics Predict Progression of Diabetic Retinopathy and Development of Diabetic Macular Edema: A Prospective Study. Ophthalmology. 2019;126(12):1675-84.

197. Park JJ, Soetikno BT, Fawzi AA. Characterization of the middle capillary plexus using optical coherence tomography angiography in healthy and diabetic eyes. Retina. 2016;36(11):2039-50.

198. Ghassemi F, Berijani S, Roohipoor R, Mohebbi M, Babeli A, Gholizadeh A, et al. Vascular density of optic nerve head in diabetic retinopathy using optical coherence tomography angiography. Int J Retina Vitreous. 2020;6(1):62.

199. Custo Greig E, Brigell M, Cao F, Levine ES, Peters K, Moult EM, et al. Macular and Peripapillary Optical Coherence Tomography Angiography Metrics Predict Progression in Diabetic Retinopathy: A Sub-analysis of TIME-2b Study Data. Am J Ophthalmol. 2020;219:66-76.

200. Chong V, Nguyen QD, Sepah Y, Giani A, Pearce E. HORNBILL: a phase I/IIa trial examining the safety, tolerability and early response of BI 764524 in patients with diabetic retinopathy and diabetic macular ischaemia-rationale, study design and protocol. Trials. 2022;23(1):669.

201. Samara WA, Shahlaee A, Adam MK, Khan MA, Chiang A, Maguire JI, et al. Quantification of Diabetic Macular Ischemia Using Optical Coherence Tomography

Angiography and Its Relationship with Visual Acuity. Ophthalmology. 2017;124(2):235-44.

202. Ghassemi F, Fadakar K, Berijani S, Babeli A, Gholizadeh A, Sabour S. Quantitative assessment of vascular density in diabetic retinopathy subtypes with optical coherence tomography angiography. BMC Ophthalmol. 2021;21(1):82.

203. Scarinci F, Nesper PL, Fawzi AA. Deep Retinal Capillary Nonperfusion Is Associated With Photoreceptor Disruption in Diabetic Macular Ischemia. American Journal of Ophthalmology. 2016;168:129-38.

204. Cristescu IE, Baltă F, Zăgrean L. Cone photoreceptor density in type I diabetic patients measured with an adaptive optics retinal camera. Rom J Ophthalmol. 2019;63(2):153-60.

205. Brown B, Kitchin JL. Dark adaptation and the acuity/luminance response in senile macular degeneration (SMD). Am J Optom Physiol Opt. 1983;60(8):645-50.

206. Jia Y, Simonett JM, Wang J, Hua X, Liu L, Hwang TS, et al. Wide-Field OCT Angiography Investigation of the Relationship Between Radial Peripapillary Capillary Plexus Density and Nerve Fiber Layer Thickness. Invest Ophthalmol Vis Sci. 2017;58(12):5188-94.

207. Wu Z, Ayton LN, Guymer RH, Luu CD. Low-Luminance Visual Acuity and Microperimetry in Age-Related Macular Degeneration. Ophthalmology. 2014;121(8):1612-9.

208. Grewal MK, Chandra S, Gurudas S, Rasheed R, Sen P, Menon D, et al. Functional clinical endpoints and their correlations in eyes with AMD with and without subretinal drusenoid deposits-a pilot study. Eye (Lond). 2021.

209. Chandramohan A, Stinnett SS, Petrowski JT, Schuman SG, Toth CA, Cousins SW, et al. Visual function measures in early and intermediate age-related macular degeneration. Retina. 2016;36(5):1021-31.

210. Wu Z, Luu CD, Hodgson LAB, Caruso E, Chen FK, Chakravarthy U, et al. Using Microperimetry and Low Luminance Visual Acuity to Detect the Onset of Late Age-Related Macular Degeneration: A LEAD Study Report. Retina. 2020.

211. Puell MC, Barrio AR, Palomo-Alvarez C, Gómez-Sanz FJ, Clement-Corral A, Pérez-Carrasco MJ. Impaired Mesopic Visual Acuity in Eyes with Early Age-Related Macular Degeneration. Investigative Ophthalmology & Visual Science. 2012;53(11):7310-4.

212. Cocce KJ, Stinnett SS, Luhmann UFO, Vajzovic L, Horne A, Schuman SG, et al. Visual Function Metrics in Early and Intermediate Dry Age-related Macular Degeneration for Use as Clinical Trial Endpoints. American Journal of Ophthalmology. 2018;189:127-38.

213. Hess R, Woo G. Vision through cataracts. Investigative Ophthalmology & Visual Science. 1978;17(5):428-35.

214. Plainis S, MURRAY IJ, CHARMAN WN. The role of retinal adaptation in night driving. Optometry and vision science. 2005;82(8):682-8.

215. Wu Z, Ayton LN, Luu CD, Guymer RH. Longitudinal changes in microperimetry and low luminance visual acuity in age-related macular degeneration. JAMA Ophthalmol. 2015;133(4):442-8.

216. Cocce KJ, Stinnett SS, Luhmann UFO, Vajzovic L, Horne A, Schuman SG, et al. Visual Function Metrics in Early and Intermediate Dry Age-related Macular Degeneration for Use as Clinical Trial Endpoints. Am J Ophthalmol. 2018;189:127-38.

217. Flamendorf J, Agrón E, Wong WT, Thompson D, Wiley HE, Doss EL, et al. Impairments in Dark Adaptation Are Associated with Age-Related Macular Degeneration Severity and Reticular Pseudodrusen. Ophthalmology. 2015;122(10):2053-62.

218. Müller S, Heeren TFC, Bonelli R, Fruttiger M, Charbel Issa P, Egan CA, et al. Contrast sensitivity and visual acuity under low light conditions in macular telangiectasia type 2. Br J Ophthalmol. 2019;103(3):398-403.

219. Rosenfeld PJ, Berger B, Reichel E, Danis RP, Gress A, Ye L, et al. A Randomized Phase 2 Study of an Anti–Amyloid β Monoclonal Antibody in Geographic Atrophy Secondary to Age-Related Macular Degeneration. Ophthalmology Retina. 2018;2(10):1028-40.

220. Yehoshua Z, de Amorim Garcia Filho CA, Nunes RP, Gregori G, Penha FM, Moshfeghi AA, et al. Systemic complement inhibition with eculizumab for geographic atrophy in age-related macular degeneration: the COMPLETE study. Ophthalmology. 2014;121(3):693-701.

221. Rosenfeld PJ, Dugel PU, Holz FG, Heier JS, Pearlman JA, Novack RL, et al. Emixustat hydrochloride for geographic atrophy secondary to age-related macular degeneration: a randomized clinical trial. Ophthalmology. 2018;125(10):1556-67.

222. Liao DS, Grossi FV, El Mehdi D, Gerber MR, Brown DM, Heier JS, et al. Complement C3 inhibitor pegcetacoplan for geographic atrophy secondary to age-related macular degeneration: A randomized phase 2 trial. Ophthalmology. 2020;127(2):186-95.

223. Dimitrova G, Chihara E, Takahashi H, Amano H, Okazaki K. Quantitative Retinal Optical Coherence Tomography Angiography in Patients With Diabetes Without Diabetic Retinopathy. Investigative Ophthalmology & Visual Science. 2017;58(1):190-6.

224. Karia N. Retinal vein occlusion: pathophysiology and treatment options. Clin Ophthalmol. 2010;4:809-16.

225. Tsai G, Banaee T, Conti FF, Singh RP. Optical Coherence Tomography Angiography in Eyes with Retinal Vein Occlusion. J Ophthalmic Vis Res. 2018;13(3):315-32.

226. Hykin P, Prevost AT, Vasconcelos JC, Murphy C, Kelly J, Ramu J, et al. Clinical Effectiveness of Intravitreal Therapy With Ranibizumab vs Aflibercept vs Bevacizumab for Macular Edema Secondary to Central Retinal Vein Occlusion: A Randomized Clinical Trial. JAMA Ophthalmol. 2019;137(11):1256-64.

227. White IR, Horton NJ, Carpenter J, statistics rim, social, Pocock SJ. Strategy for intention to treat analysis in randomised trials with missing outcome data. BMJ. 2011;342:d40.

228. StataCorp. Stata Statistical Software:Release 16. College Station, TX: StataCorp LLC; 2019.

229. McFadden D. Conditional Logit Analysis of Qualitative Choice Behavior. In: Zarembka P, Frontiers in Econometrics, editor.: Academic Press; 1973. p. 105-42.

230. Brown DM, Wykoff CC, Wong TP, Mariani AF, Croft DE, Schuetzle KL, et al. Ranibizumab in preproliferative (ischemic) central retinal vein occlusion: The Rubeosis Anti-VEGF (RAVE) Trial. Retina. 2014;34(9):1728-35.

231. Brogan K, Precup M, Rodger A, Young D, Gilmour DF. Pre-treatment clinical features in central retinal vein occlusion that predict visual outcome following intravitreal ranibizumab. BMC Ophthalmol. 2018;18(1):37.

232. Ach T, Hoeh AE, Schaal KB, Scheuerle AF, Dithmar S. Predictive factors for changes in macular edema in intravitreal bevacizumab therapy of retinal vein occlusion. Graefes Arch Clin Exp Ophthalmol. 2010;248(2):155-9.

233. Scott IU, VanVeldhuisen PC, Oden NL, Ip MS, Blodi BA, Hartnett ME, et al. Baseline predictors of visual acuity and retinal thickness outcomes in patients with retinal vein occlusion: Standard Care Versus COrticosteroid for REtinal Vein Occlusion Study report 10. Ophthalmology. 2011;118(2):345-52.

234. Scott IU, VanVeldhuisen PC, Ip MS, Blodi BA, Oden NL, King J, et al. Baseline Factors Associated With 6-Month Visual Acuity and Retinal Thickness Outcomes in Patients With Macular Edema Secondary to Central Retinal Vein Occlusion or Hemiretinal Vein Occlusion: SCORE2 Study Report 4. JAMA Ophthalmol. 2017;135(6):639-49.

235. Boyer D, Heier J, Brown DM, Clark WL, Vitti R, Berliner AJ, et al. Vascular endothelial growth factor Trap-Eye for macular edema secondary to central retinal vein occlusion: six-month results of the phase 3 COPERNICUS study. Ophthalmology. 2012;119(5):1024-32.

236. Tan CS, Lim LW, Chow VS, Chay IW, Tan S, Cheong KX, et al. Optical Coherence Tomography Angiography Evaluation of the Parafoveal Vasculature and Its Relationship With Ocular Factors. Invest Ophthalmol Vis Sci. 2016;57(9):Oct224-34.

237. Dupas B, Minvielle W, Bonnin S, Couturier A, Erginay A, Massin P, et al. Association Between Vessel Density and Visual Acuity in Patients With Diabetic Retinopathy and Poorly Controlled Type 1 Diabetes. JAMA Ophthalmol. 2018;136(7):721-8.

238. Pramil V, Levine ES, Waheed NK. Macular Vessel Density in Diabetic Retinopathy Patients: How Can We Accurately Measure and What Can It Tell Us? Clin Ophthalmol. 2021;15:1517-27.

239. Owsley C, Clark ME, Huisingh CE, Curcio CA, McGwin G, Jr. Visual Function in Older Eyes in Normal Macular Health: Association with Incident Early Age-Related Macular Degeneration 3 Years Later. Invest Ophthalmol Vis Sci. 2016;57(4):1782-9.

240. Tsilibary EC. Microvascular basement membranes in diabetes mellitus. J Pathol. 2003;200(4):537-46.

241. Mimouni M, Segev O, Dori D, Geffen N, Flores V, Segal O. Disorganization of the Retinal Inner Layers as a Predictor of Visual Acuity in Eyes With Macular Edema Secondary to Vein Occlusion. Am J Ophthalmol. 2017;182:160-7.

242. Tsai WS, Thottarath S, Gurudas S, Sen P, Pearce E, Giani A, et al. Correlation of Optical Coherence Tomography Angiography Characteristics with Visual Function to Define Vision-Threatening Diabetic Macular Ischemia. Diagnostics (Basel). 2022;12(5).

11.5 Publications

Karatsai E, Sen P, Gurudas S, Sivaprasad S. Low Luminance Visual Acuity and Low Luminance Deficit in Proliferative Diabetic Retinopathy. J Clin Med. 2021 Jan 19;10(2):358.

Sen P, Nunez do Rio JM, Bagchi A, Sivaprasad S. Differences in macular microvascular changes between eyes with central retinal vein occlusion and proliferative diabetic retinopathy. Eye (Lond). 2021 Nov;35(11):3170-3172.

Sen P, Gurudas S, Ramu J, Patrao N, Chandra S, Rasheed R, Nicholson L, Peto T, Sivaprasad S, Hykin P. Predictors of Visual Acuity Outcomes after Anti-Vascular Endothelial Growth Factor Treatment for Macular Edema Secondary to Central Retinal Vein Occlusion. Ophthalmol Retina. 2021 Nov;5(11):1115-1124.