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Relative blood flow in patients with retinal artery occlusions

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BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

RELATIVE BLOOD FLOW IN PATIENTS WITH RETINAL ARTERY OCCLUSIONS

by

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B.S., University of California, Los Angeles, 2017

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RELATIVE BLOOD FLOW IN PATIENTS WITH RETINAL ARTERY OCCLUSIONS

SHASHVAT PUROHIT

ABSTRACT

Background: Using laser speckle flowgraphy to calculate retinal blood flow, we sought to measure changes in optic nerve blood flow compared to the normal fellow eye (relative blood flow) and changes in vision in patients with central retinal artery occlusions of varying duration.

Methods: Laser speckle flowgraphy was used to measure optic nerve blood flow in eyes with central retinal artery occlusions and the normal fellow eye of patients seen at Beth Israel Deaconess Medical Center to calculate relative blood flow. Visual acuity was assessed monocularly using Snellen Charts in a standardized fashion.

Results: In healthy control patients (n=20), relative blood flow was calculated to be 1.02 (p= 0.6843), indicating no significant difference in blood flow between eyes. In patients with unilateral central retinal artery occlusions (n=7), relative blood flow was calculated to be 0.66 ± 0.13 (p < .001), indicating on average a 33 percent loss in blood flow through the optic nerve head. When comparing relative blood flow values of CRAO patients measured within one year of vision loss versus patients when measured after one year of reported vision loss, values of patients measured within one year were lower.

Conclusions and Relevance: Laser Speckle Flowgraphy has been shown to be a useful diagnostic tool that can reliably provide quantitative information on retinal blood flow. Results suggest that blood flow through the optic nerve head does return over time, presumably as the occlusion resolves or recanalizes. Longitudinal analysis determined a higher relative blood flow in patients one-year post incident versus within one year. However no statistically significant difference in visual acuity between these groups was found, indicating that return of blood flow is not associated with a return in visual acuity. Relative blood flow may be a useful measure of retinal perfusion in other retinal vascular disorders.

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LIST OF ABBREVIATIONS

AMD	Age-Related Macular Degeneration
ANOVA	Analysis of Variance
BRB	Blood Retina Barrier
BRVO	Branched Retinal Vein Occlusion
CF.....	Counting Fingers
CRA	Central Retinal Artery
CRAO	Central Retinal Artery Occlusion
CRV	Central Retinal Vein
CRVO	Central Retinal Vein Occlusion
CSME	Clinically Significant Macular Edema
DME.....	Diabetic Macular Edema
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	Fluorescein Angiography
HM.....	Hand Movement
INL	Inner Nuclear Layer
IOP.....	Intraocular Pressure
IPL.....	Inner Plexiform Layer
logMAR.....	Logarithm of the Minimum Angle of Resolution
LSFG.....	Laser Speckle Flowgraphy

MBR.....	Mean Blur Rate
NA-CRAO.....	Non-Arteritic Central Retinal Artery Occlusion
OCT.....	Optical Coherence Tomography
ONH.....	Optic Nerve Head
ONL.....	Outer Nuclear Layer
OPL.....	Outer Plexiform Layer
RBF.....	Relative Blood Flow
RPE.....	Retinal Pigment Epithelium
RVO.....	Retinal Vein Occlusion
TIA.....	Transient Ischemic Attack
tPA.....	Tissue Plasminogen Activator
VA.....	Visual Acuity
VEGF.....	Vascular Epithelial Growth Factor

INTRODUCTION

More than 600 million years ago, organisms developed the ability to respond to light. Approximately 60 million years later, during the Cambrian explosion, organisms began rapidly evolving and diversifying, and visual systems such as the eye first emerged (Lamb et al., 2007). The eye is fundamental to the processing and function of our visual systems, a major sensory modality, and disease or dysfunction can lead to a dramatic impact in the quality of life of individuals. Like any organ of the body, ischemia and anoxia can damage and destroy non-perfused tissues. This is of particular relevance when addressing the retina. The anatomy and physiology of the eye are paramount in understanding how disease can dramatically alter a patient's ability to perceive light stimuli.

Anatomy of the Eye

In humans, the eye is situated in the two bony orbits of the skull, which is formed from a conjoining of the articulation of the frontal bone, maxillary bone, lacrimal bone, nasal bone, ethmoid bone, zygomatic bone, sphenoid bone, and palatine bone of the skull (Wolff, 1976). Light first passes through the cornea of the eye, a dome shaped, transparent structure composed of highly ordered layers of collagen, and is ultimately responsible for approximately 80% of the refraction that occurs as light passes through

the eye. The cornea is continuous with the sclera, an opaque, white structure that constitutes the exterior of the remainder of the globe and protects the inner structures of the eye from the environment (Meek & Knupp, 2015). Light then passes through the anterior chamber of the eye through aqueous humor, a transparent, thin fluid which is encapsulated by the cornea anteriorly and the ciliary body, lens, and suspensory ligaments posteriorly. It then is refracted through the lens, which is suspended from the surrounding ciliary body via zonular fibers and allows for a degree of accommodation as it is malleable and changes shaped based on the contraction or relaxation of the ciliary muscle (Wolff, 1976). Directly posterior to the lens is the largest chamber of the eye, the vitreous chamber, which contains the vitreous humor, a viscous, gel-like substance. Light passes through the vitreous humor last before contacting the retina, a complex, ordered layer of cells that include photoreceptors, neurons which are directly stimulated by electromagnetic radiation in the visible light spectrum. The retina is situated above the choroid, a highly vascularized layer that provides nutrients and oxygen to approximately 85% of the eye (Zeiss et al., 2018). It is the retina that ultimately begins the complex process of translating light stimuli into the visual perception of our surroundings.

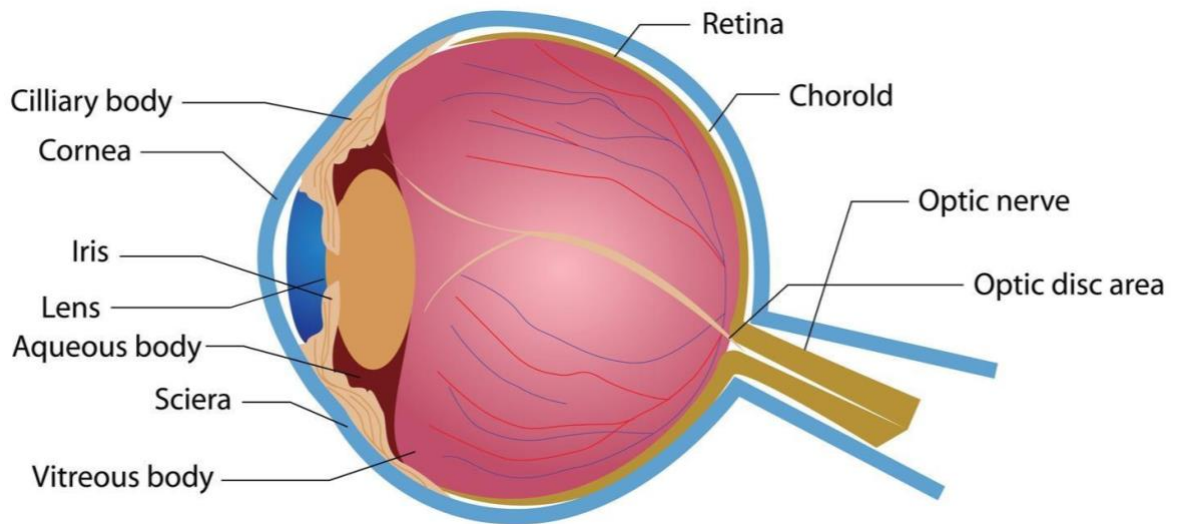


Figure 1: Major anatomical structures of the human eye. This illustrates a sagittal cross-sectional view of the human eye. Note that light passes through the eye beginning at with the cornea (left) and is refracted by both the cornea and the lens onto the retina (right), which surrounds the vitreous chamber.

The Retina

The retina is located between the vitreous humor of the posterior segment of the eye and the choroid, and is a complex and ordered series of epithelial, neuronal, and connective tissue. The retina is stratified into ten distinct layers, each containing distinct cell types with specific functions. Light passes through nine of the ten layers of the retina before coming in contact with the photoreceptors, which are located most posteriorly and in contact with the final layer, the retinal pigment epithelium. Light stimuli hyper-polarize photoreceptor cells by transforming the vitamin A derived

molecule 11-cis-retinal into all-trans-retinal through a radiation induced conformational change, which starts a complex signaling cascade that is continuously modified as it passes through the layers of the retina to ganglion cell layer. Ultimately the nerve fibers of the ganglion cells send signals through the optic disc and through the optic nerve for further processing in the thalamus and occipital lobe of the brain (Barrett et. al, 2019). Two distinct photoreceptor cell types are found in the mammalian retina; cone photoreceptors, which are used for sensing color and primarily utilized during photopic vision, and rod photoreceptors, which are utilized during scotopic vision conditions and are important in the detection of peripheral movement (Wolff, 1976). The macula of the retina is located centrally and is an area of particular importance, as it contains the highest density of cone photoreceptors and is responsible for high resolution, central visual acuity (VA). Located at the center of the macula is the fovea centralis, a depression in the overall thickness of the retina due to the displacement of non-photoreceptor retinal neurons. The displacement of non-photoreceptor neurons allows light to contact photoreceptors cells in an unobstructed fashion and contributes to the high acuity of central vision in the fovea. As cone density approaches between 200,000 to 300,000 cones per square millimeter and the photoreceptor to ganglion cell ratio approaches 1:1, which is thought to also contribute to the high VA in central vision (Mihailovic et. al., 2019). Directly peripheral to the fovea is the parafoveal and the perifoveal area. These areas together form the macula.

Layers of the Retina

The retina is comprised of ten distinct layers, nine of which light passes through before contacting the photoreceptors responsible for transforming the stimuli into electrical signals. The most superficial layer of the retina is the inner limiting membrane followed immediately by the nerve fiber layer, which contains the axons of the ganglion cells. The ganglion cell layer follows, which contains the cell bodies of the ganglion cells, which receive, integrate, and modify signals from the remaining layers of the retina before transmitting information through the optic nerve to the occipital lobe. The inner plexiform layer (IPL) contains synapses and connections between the ganglion cells and the cells of the inner nuclear layer (INL), which contains the cell bodies of bipolar cells, Müller cells, amacrine cells, and horizontal cells (Barrett et. al, 2019). These cells serve a number of functions including glial support, light-dark adaptation, and a number of complex signal integrations and modifications not yet fully understood in the current literature (Shekhar et. al., 2016). The outer plexiform layer (OPL) contains synapses between these cells and the photoreceptors, whose cell bodies are located in the outer nuclear layer (ONL). The outer limiting membrane separates the cells bodies of the photoreceptors from their outer and inner segments contained in the photoreceptor layer, where light signals are converted to electrical signals. Finally, the retinal pigment epithelium (RPE), a single layer of pigmented cells separates the retina from the choroid,

and serves a number of functions, including supplying nutrients to the photoreceptors and preserving the position of the retina (Zeiss et. al., 2018).

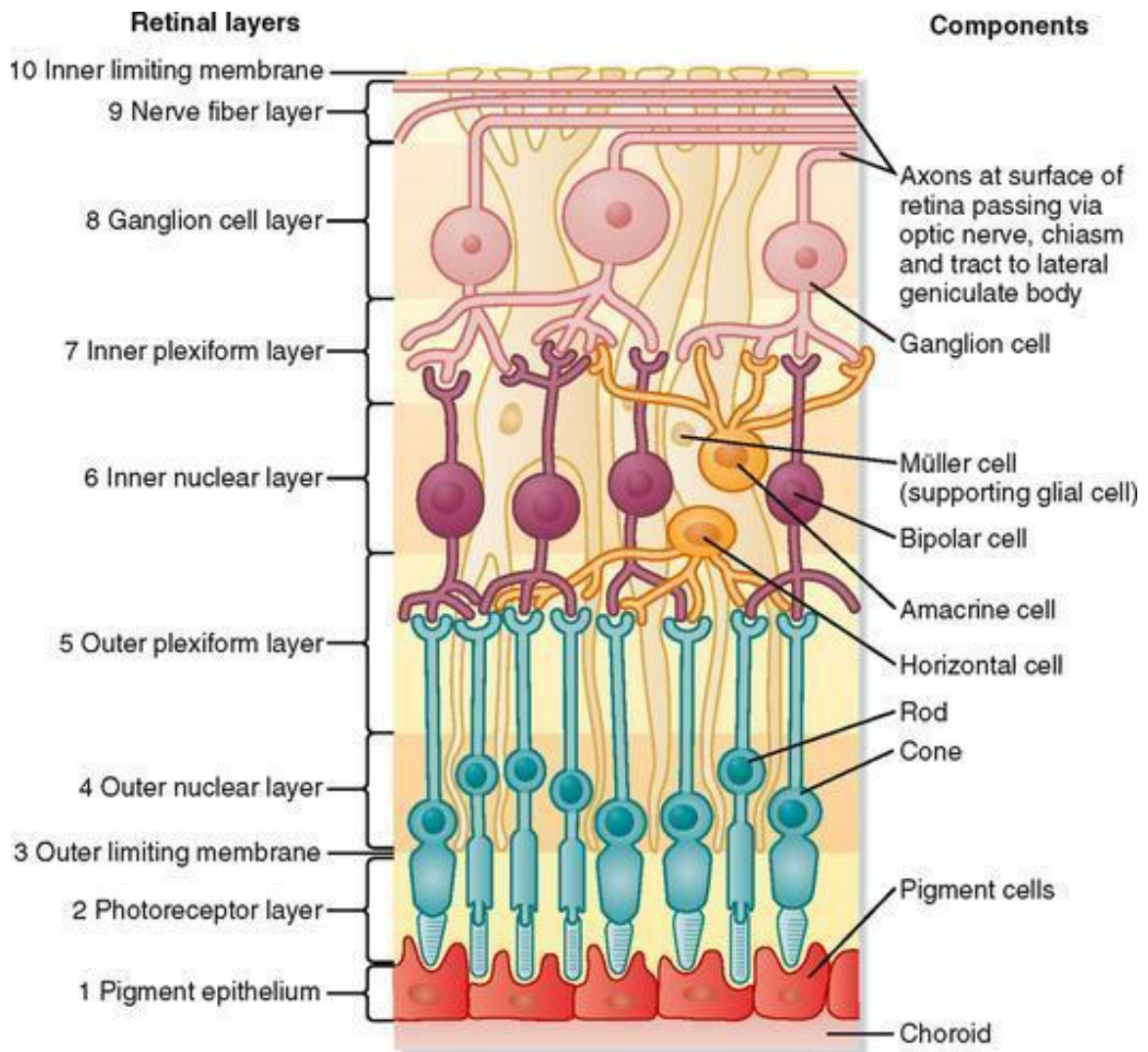


Figure 2: Layers of the retina and corresponding cells types. Light passes through nine of the ten layers of the retina before contacting the photoreceptor layer, which converts light stimuli into electrical signals, starting a cascade of signaling cascade that ultimately results in visual perception.

Vasculature Supplying the Retina

The retina receives the majority of its blood supply from two sources, the central retinal artery (CRA) and the choriocapillaris. The choriocapillaris supplies oxygen and nutrients to the outer segments of the retina, including the RPE and the photoreceptor layer. It supplies small, fenestrated capillaries in the inner portion of the choroid, which contains the highest blood flow per unit of tissue in the entire human body (Spencer & Frasier, 2013). The CRA is a branch of the ophthalmic artery of the internal carotid, and enters the globe of the eye through the optic nerve head (ONH) alongside the optic nerve, and is the sole supply of perfusion for the inner retinal layer of the macula in 75% of humans. The first division of the CRA are the superior and inferior branching arteries, which further subdivide into temporal and nasal branching arteries. In approximately 25% of humans, a cilioretinal artery emerges from the ONH as well, and supplies oxygen and nutrients to the macula (Varma et. al., 2013). Vessels emerging from the CRA do not anastomose with other vessels, therefore an occlusion of the CRA results in anoxia to the inner layers of the macula. These arteries supply two major capillary beds: the superficial capillary bed, which is located in the between the ganglion cell layer and the nerve fiber layer, and the deep capillary bed, which is situated in the INL. These capillary beds are not found in the foveal avascular zone, as they are displaced along with the inner layers of the retina (Mihailovic et. al., 2013). Similar to the brain, there is tight

regulation of fluid balance through these capillaries via the blood-retina-barrier (BRB), a tight meshwork of endothelia, tight junctions, basement membranes, and pericytes that regulate electrolyte balance and thus fluid movement into surrounding tissues. Disease and dysfunction of the BRB can lead to macular edema, as is commonly seen in diabetics. The capillaries from the CRA form tributaries to the branched retinal veins which then coalesce into the central retinal vein (CRV). The CRV exits the globe through the ONH directly adjacent to the CRA.

Central Retinal Vein Occlusions

Central Retinal Vein Occlusions (CRVOs) and Branched Retinal Vein Occlusions (BRVOs) are the most common occlusive injuries of the retina, and are the second most common vascular retinopathy after diabetic retinopathy (Karia, 2010). Globally, approximately 28 million individuals are affected by either CRVOs or BRVOs. They can be further subdivided into ischemic occlusions and non-ischemic occlusions, which present in clinically distinct ways. (RVOs) in general present with a combination of painless vision loss, vascular tortuosity, optic disc swelling, cotton wool spots, retinal hemorrhaging, and macular edema. Glaucoma and ocular hypertension are commonly diagnosed with RVOs, although the exact association between these conditions, whether causative or simple correlative, remains unclear (Hayreh, 2005). Risk factors for

RVOs include Virchow's Triad of thrombogenesis; hypercoagulability stasis, and endothelial damage. Underlying diseases that exacerbate or can cause any of these three classic factors are therefore also risk factors for the development of RVOs, such as diabetes mellitus, hypertension, atherosclerosis, and mutations affecting blood coagulability, such as prothrombin mutations (Janssen et. al. 2005).

Treatment of CRVOs

Treatment following the conformation of CRVOs and BRVOs is necessary to prevent further complications, such as neovascularization in the retina, cystoid macular edema, secondary neovascular glaucoma, and to is ultimately performed to preserve VA (Yeh et. al. 2005). Neovascularization often leads to the growth of tortuous, poorly formed vessels and ultimately, the breakdown of the BRB. This can exacerbate macular edema and lead to progressive deterioration of VA (Hykin et. al. 2019). Often intravitreal injections of corticosteroids or anti-vascular epithelial growth factor (anti-VEGF) drugs are used to prevent subsequent neovascularization caused by VEGF, which is released by endothelia in response to hypoxic conditions caused by ischemia. Panretinal photocoagulation has also been shown to be an effective intervention in preventing subsequent neovascularization secondary to RVOs, and is thought to also prevent the release of VEGF from the peripheral retina (Lui et. al., 2019). Other

treatment options explored include the inhalation of concentrated oxygen (hyperoxia) (Arroyo et al., 2021) to assist in resolving ischemia and subsequently inhibit the release of VEGF, and the inhalation of carbogen, a mixture of 95% oxygen and 5% carbon dioxide to promote vasodilation and restore perfusion (Pournaras et. al., 2004).

Central Retinal Artery Occlusions

Central Retinal Artery Occlusions (CRAOs) are a relatively rare occlusive injury, with an incidence rate of approximately 1.9/100,000 people in the United States (Modi et al., 2017). CRAOs are akin to a stroke of the eye, as the CRA is the supply of nutrients and oxygen to the inner layers of the retina, particularly surrounding the macula. CRAOs present clinically as sudden, painless loss of vision in the affected eye. The central retinal artery is the primary and major blood supply to the inner retinal layers of the retina, therefore central retinal artery occlusions often result in severe decrease in VA in affected eyes (Chen et al., 2013). In animal models, irreversible damage to inner retinal layers from ischemia and anoxia occurred 240 minutes post-occlusion (Hayreh et al., 2004). Risk factors for CRAOs are identical to risk factors for strokes and cardiovascular disease, and include age, arterial hypertension, diabetes mellitus, carotid artery disease, coronary artery disease, transient ischemic attacks (TIAs) or cerebral vascular accidents, and smoking tobacco (Pielen et al., 2015). Along with sudden, painless loss in vision, the

most common associated signs upon examination of CRAO affected retinas include retinal opacity in the posterior pole (58%), cherry-red spot (90%), cattle trucking (19%), retinal arterial attenuation (32%), and optic disk edema (22%) and pallor (39%) (Scott, 2017). Currently, there are no established treatments that alter the course of this disease; management of CRAOs focus primarily on the prevention of subsequent neovascularization and edema (Lee et al., 2013) (Chronopolous & Schutz, 2019).

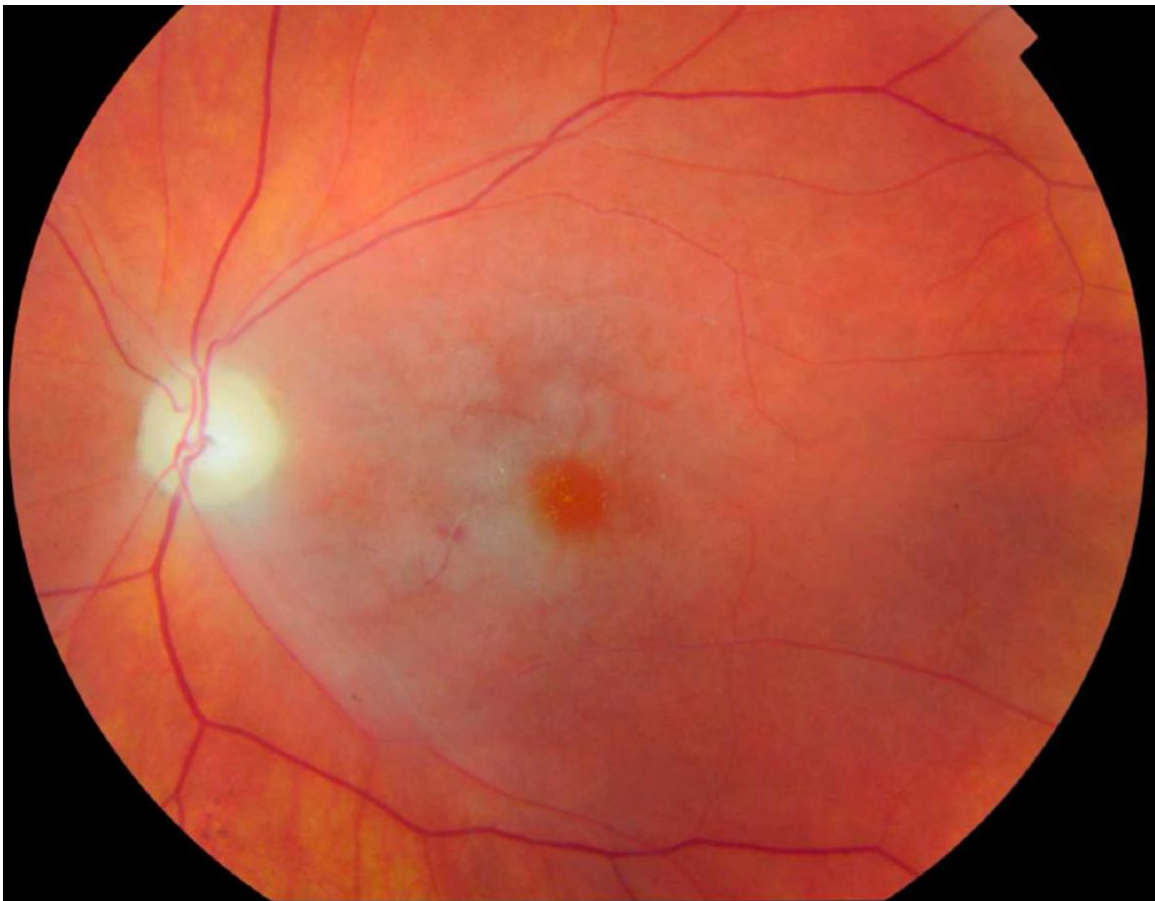


Figure 3: Fundus Photo of Early CRAO. Note the opacity surrounding the fovea associated with edema due to tissue damage stemming from anoxia. The central cherry red spot occurs in the avascular zone of the fovea, where the inner layer of the retina as well as the capillary beds are displaced.

In animal models, inhibition of platelet aggregating factors using ticlopidine has been shown to decrease the rate at which retinal vein occlusions are artificially induced, suggesting that these compounds may be useful as prophylactic agents against vascular occlusions (Arroyo et. al, 2001). The use of thrombolytic agents to resolve CRAOs have been associated with greater complications than benefits (Hakim & Hakim, 2019).

CRAOs can be categorized further clinically into four subgroups: non-arteritic CRAOs (NA-CRAOs), transient NA-CRAOs, NA-CRAOs with cilioretinal artery sparing, and arteritic CRAOs (Hayreh, 2018). NA-CRAOs are the most common and account for 2/3 of all cases. They are the result of atherosclerotic disease, and occur due to blockage of the CRA by platelet fibrin, calcium, or cholesterol embolus. Transient NA-CRAOs are analogous to transient ischemic attacks, and have the best prognosis for preservation of visual acuity out of the 4 subgroups of CRAOs. They account for 15% of all CRAOs, and are thought to be a result of serotonin induced vasospasms dislodging atherosclerotic plaques. NA-CRAOs with cilioretinal artery sparing can occur in the roughly 25% of humans that receive perfusion to the macula from both the CRA and the cilioretinal artery, and in these cases, the macula is largely spared due to the dual blood supply (Hayreh, 2018). Finally, arteritic CRAOs account for approximately 4% of CRAOs and are most often caused by occlusion secondary to giant cell arteritis.

Treatment of CRAOs

As mentioned previously, there is no single consensus on the treatment of CRAOs, and therefore multiple therapies have been proposed with mixed results. Non-invasive treatments proposed include hyperbaric oxygen which may improve oxygen delivery to the retina, possibly sparing the macula of the acute effects of anoxia. Carbogen inhalation has also been proposed as a potential vasodilator that in theory may help spontaneously resolve the occlusion. Ocular massage therapies may also be administered in an attempt to dislodge emboli, as well as globe massage (Farris & Waymack, 2021).

As CRAOs are analogous to strokes of the eye, the use of thrombolytic agents, particularly tissue plasminogen activator (tPA) has been utilized and studied as a possible intervention, although its efficacy is debated. TPA is a naturally occurring fibrinolytic agent found in endothelial cells that lyses clots by converting plasminogen to plasmin. Plasmin in turn enzymatically degrades blood proteins, including fibrin clots. While some studies have reported promising results in vision sparing when administering tPA, others have reported no changes in outcomes. Furthermore, the risk of hemorrhagic stroke when administering tPA as well as the costs associated with the necessary observation and post treatment evaluation suggest that tPA administration is not a practical intervention in the treatment of CRAOs. Ultimately the heterogeneity of

studies exploring the use of tPA in resolving CRAOs means that a consensus has not been reached, and it remains an area of potential future exploration (Bouisse et. al., 2007).

Other therapies proposed in treated CRAOs are aimed at reducing intraocular pressure in an attempt to dislodge the emboli. This includes the administration of intravenous agents such as acetazolamide, a carbonic anhydrase inhibitor which in turn would lower the production of aqueous humor in the anterior chamber and therefore lower intraocular pressure (IOP). Topical antiglaucoma drops have also been proposed through similar mechanisms of action, as well as intravenous mannitol. Like other proposed therapies, results have been mixed (Cugatti et. al., 2013).

Preventative treatments for CRAOs include the monitoring and maintenance of a healthy diet and lifestyle aimed at reducing the risk of atherosclerosis. This includes regular exercise, a diet with a low glycemic index that is comprised primarily of fruits, vegetables, grains, low-fat or nonfat dairy products, fish, legumes, poultry, and lean meats, polyunsaturated fatty acids. Smoking cessation is also an important aspect of lowering risk factors associated with CRAOs due to the propensity endothelial damage caused by inhaled toxins (Mehta et. al., 2017). Hyperhomocystemia is another recognized risk factor for CRAOs through induced dysfunction of endothelial cells, and therefore diets rich in folate, B6, and B12 are recommended as a preventative measure. Finally, hypercholesterolemia has been shown to play a major role in the formation of

atherosclerotic plaques, therefore preventative measures against CRAOs include the monitoring and appropriate interventions necessary to keep circulating low density lipoproteins at appropriate levels (Varma et. al., 2013).

Other Retinopathies Affecting Retinal Vasculature

In developed countries, retinopathies affecting retinal vasculature are the leading cause of blindness (Brand, 2012). The three most common retinopathies of vasculature in the United States in order are diabetic macular edema (DME), CRVOs (previously discussed), and age-related macular degeneration (AMD).

(1) Diabetic Macular Edema

The Early Treatment Diabetic Retinopathy Study (ETDRS) defines Diabetic Macular Edema (DME) as retinal thickening or the presence of hard exudates within 1 disk diameter of the macula. The term clinically significant macular edema (CSME) is used to describe macular edema that is characterized by any or a combination of the following: edema is within 500 microns of the center of the macula, hard exudates are found within 500 microns of the center of the macula and is associated with retinal thickening, or retinal thickening is present in at least one disk diameter on the retina within one disk diameter of the center of the macula (ETDRS Study Research Group, 1987). DME is the most common retinopathy affecting vasculature in the United States,

and approximately 75,000 new cases are reported annually. In diabetics, DME is the leading cause of vision loss and blindness. While the exact pathogenesis of DME is unclear, it is widely accepted to stem from microvasculature injury and the disruption of the BRB, which eventually leads to an accumulation of fluid in between the layers of the retina (Bresnick, 1986). DME is generally a chronic condition, and results in vision loss in 33% of individuals left untreated for a period of 3 or more years. The rate reported vision loss increases with patient age as well as duration of disease.

Poorly managed hyperglycemia is the cardinal risk factor for the development of DME, as elevated glucose levels induce the formation of damaging oxidative free radicals and protein kinase C activation. This can lead to further induced injury via hypoxia, altered blood flow, ischemia, and inflammation. Injury to endothelia, hypoxia, and the breakdown of the BRB can induce neovascularization through activation of the VEGF pathway, which can in turn exacerbate underlying conditions and increase fluid deposition in the macula and periphery.

In patients 60 years of age and older, DME is also associated with a significantly higher rate of posterior vitreous detachments (PVDs) when compared to non-diabetic CSME. This is thought to be related to the accumulation of advanced glycation end products (AGEs) leading to increased cross linkages in collagen fibrils along with hyperglycemia induced liquefaction of the vitreous gel. Standard imaging methods used to confirm the presence of DME include fluorescein angiography (FA) and Optical

Coherence Tomography (OCT) to confirm the presence of vascular leakage and macular edema respectively (Nasrallah et. al., 1998).

Treatment of DME is similar to treatments of other vascular retinopathies. If CSME is not present, the recommended course of action is periodic and regular evaluation. In areas containing microaneurysms or diffuse ischemia, laser photocoagulation is recommended to prevent subsequent neovascularization and edema, though the exact criteria for which photocoagulation is recommended remains a point of debate (ETDRS Study Research Group, 1987). Intravitreal injections of corticosteroids or anti-VEGF compounds have been shown to significantly reduce vascular permeability and neovascularization in all vascular retinopathies and is therefore effective in treating the underlying causes of DME.

(2) Age Related Macular Degeneration

Age Related Macular Degeneration (AMD) is a progressive chronic disorder that affects the macula of older individuals, leading to a progressive loss in vision. AMD can be further subdivided into two groups, wet AMD and dry AMD, the former being characterized by the development of neovascularization. AMD prevalence is rare in individuals younger than 55 and is more common in individuals 75 years or older, and is globally the third leading cause of blindness before cataracts and glaucoma. While dry AMD is more common, wet AMD accounts for the vast majority of severe vision loss (Georghe et. al., 2015).

AMD begins through the deposition of lipids on Bruch's membrane, the innermost layer of the choroid, and is thought to be due to defects in the RPE's ability to clear cellular debris. The appearance of drusen, deposits of lipids, amyloid, complement factors, and other cellular debris is the earliest clinical sign of AMD, and appears as focal, white-yellow spots deep within the retina. Thickening and degeneration of Bruch's membrane following can result in a significant barrier for fluid transport and lead to ischemia. The ischemia can lead to the activation of VEGF pathways and lead to subsequent neovascularization, which leads to wet AMD (Pauleikhoff, 1990).

Confirming the diagnosis of AMD is usually done using OCT imaging and occasionally B-scans. To test for neovascularization in the retina, FA imaging is conducted. Wet AMD can also lead to choroidal neovascularization, in which case Indocyan- angiography is performed, as the dye is more suitable for the visualization of choriocapillary circulation than a fluorescein dye (Bowling and Kanski, 2015). Treatment for AMD involves preventing the development of wet AMD and neovascularization, and follows a similar pattern to the vascular retinopathies mentioned above (Al-Zammil & Yassin, 2017).

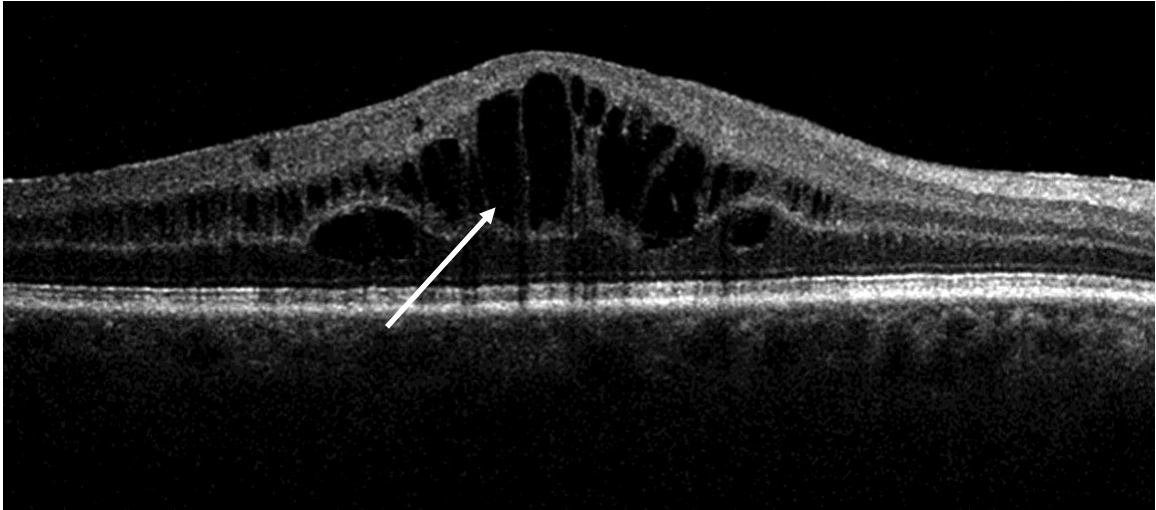


Figure 4: OCT Image of Clinically Significant Macular Edema. Note the fluid deposited between layers of the retina. Neovascularization secondary to a number of vascular retinopathies, including CRVO, DME, and wet AMD can lead to formation of CSME.

Diagnostic Imaging and CRAOs

Currently there are a number of imaging techniques that can be utilized to help accurately diagnose vascular occlusions affecting the retina, including the diagnosis of CRAOs. The two most commonly used imaging techniques are optical coherence tomography (OCT) and fluorescein angiography (FA), each with their respective strengths and limitations. A relatively newer imaging technique, laser speckle flowgraphy (LSFG) has shown promise in providing quantitative data that OCT imaging and FA imaging cannot provide.

(1) Optical Coherence Tomography

Optical Coherence Tomography is a non-invasive imaging technique that allows for the visualization of cross-sectional areas of tissue to certain depths. Compared to other medical imaging such as ultrasound and magnetic resonance imaging, OCT imaging has the added benefit of acquiring significantly higher resolution images. OCT imaging utilizes incident light of the near infrared wavelength, which can penetrate tissue several hundred microns in depth, which is split into a reference beam of known length and a central or probe beam which is directed through the cornea and lens onto the retina. These beams are then reflected back onto a photodetector, where they can constructively interfere, destructively interfere, or a mixture of the two, and is recorded by a signal processor. The signal processor records both the light intensity and the depth from which the light is reflected, thus creating a high-resolution depth profile of the retina in a fashion similar to ultrasound imaging techniques (Aumann et. al., 2019). OCT imaging allows for fast, accurate visualization of the layers of the retina, choroid, sclera, and posterior vitreous, and allow accurate diagnoses of a number of retinopathies, including retinal detachments, retinal holes, epiretinal membranes, and macular edema. SPECTRALIS OCT, introduced in 2006 by Heidelberg Engineering, combines confocal laser scanning ophthalmoscopy and OCT to allow for high resolution angiography imaging as well (Gaulino et. al., 2019). OCT and OCT-angiography are noninvasive techniques that can be used to confirm the presence of CRAOs and associated symptoms, however one

important limitation is that they cannot provide quantitative metrics relating to overall blood flow.

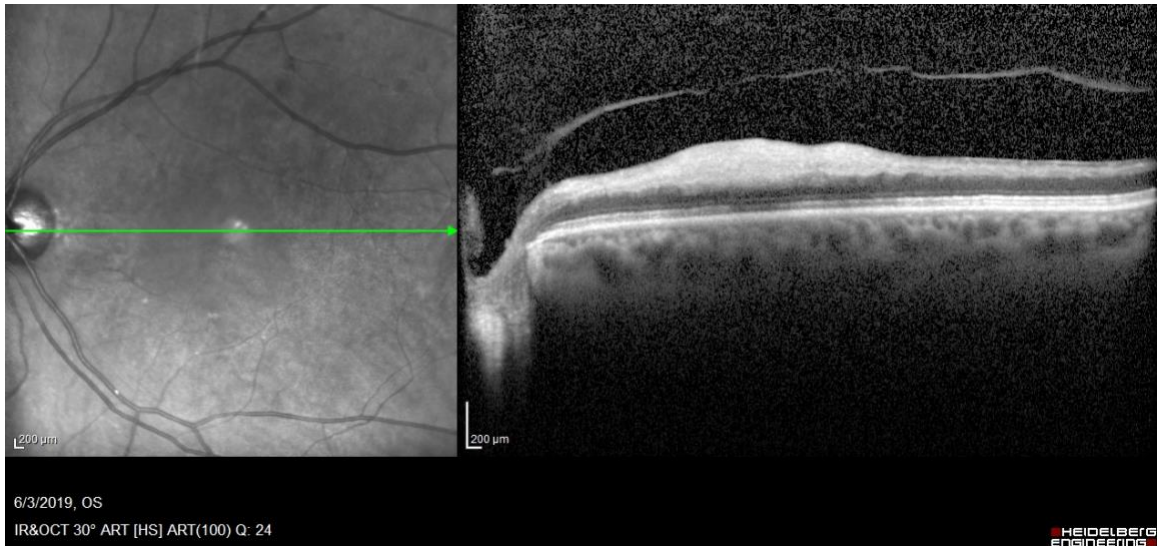


Figure 5: OCT Image of Acute CRAO. On the left, the green line demarcates the cross section corresponding to the image on the right. Note the hyperreflectivity of the inner layers of the retina. Hyperreflectivity and edema in the inner layers is associated with widespread cell death due to anoxia leading to the inner layers losing their normally ordered structure. This leads to hyperreflectivity in OCT images, and can be viewed as diffuse retinal opacity in fundus photography.

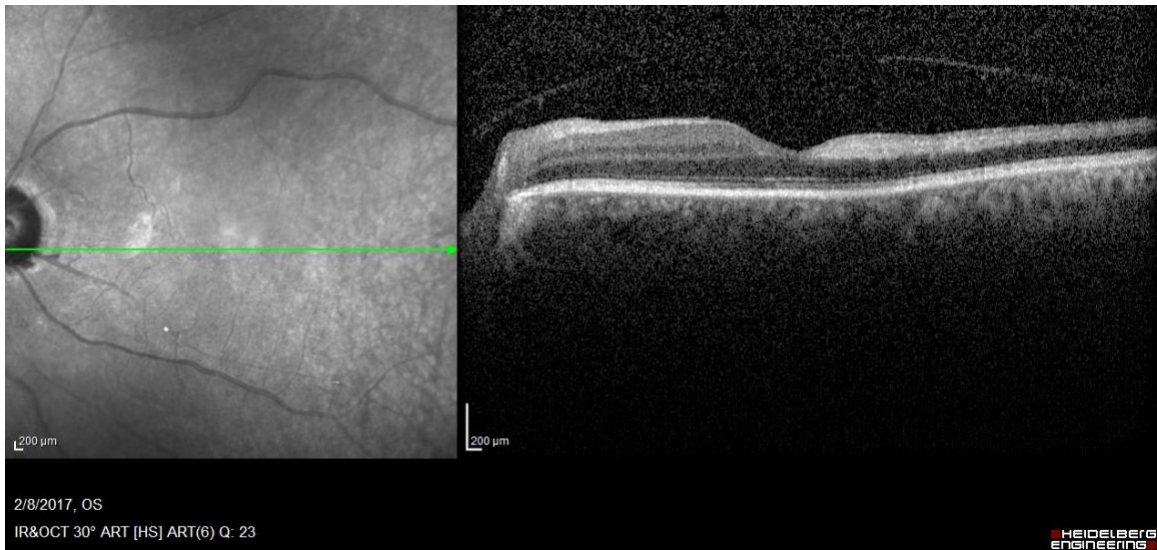


Figure 6: OCT Image of Late CRAO. On the left, the green line demarcates the cross section corresponding to the image on the right. This OCT scan was taken 69 days after reported vision loss. Note the depression of the inner retinal layers surrounding the fovea.

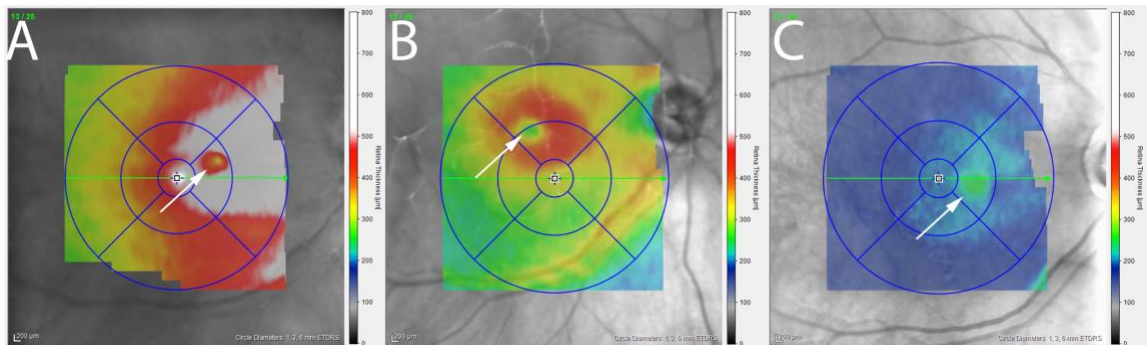


Figure 7: CRAO Central Thickness of Macula OCT Progression. Green areas represent healthy retinal thickness. Orange, red, and white areas show areas where retinal thickness is elevated due to edema. Blue areas represent areas of the retina that are thinner than what is considered normal. Panel A shows macular thickness as measured by OCT imaging approximately 2 days after reported vision loss. Edema is widespread, but spares the foveola (arrow) due to the lack of capillary beds. Panel B shows some regression of edema 14 days post incident. Panel C illustrates the regression of thickness from normal levels 69 days after CRAO as dead tissue is cleared. Early CRAOs present with marked edema and opacity surrounding the fovea due to ischemic necrosis of the inner layers of the retina. Gradually over time, this edema subsides as dead tissue is cleared, until finally, the overall thickness of the retina surrounding the fovea reduces to below average levels.

(2) Fluorescein Angiography (FA)

Fluorescein Angiography (FA) is an imaging technique first utilized in the 1960s, and allows for real time visualization and assessment of retinal vasculature. Fluorescent dye is first injected intravenously, typically in the patient's arm, while a fundus camera periodically takes photos of the patient's retina. Within seconds, the fluorescein dye begins to perfuse through the retinal vasculature, and its transit through has been noted to occur in six distinct phases. The first phase is the early choroidal flush, as the dye reaches choriocapillary circulation before entering the arteriolar circulation supplying the inner retina. This is followed by the early arteriolar and later arteriolar phase as the dye is transmitted through the CRA and branched arteries. The final three phases in order are the capillary phase, and the late and early venous phases, after which the dye is no longer detectable by fundus photography (Dollery et. al., 1962). The absence of dye through certain areas of retinal vasculature can confirm the presence of an occlusion such as a CRAO. Vascular leakage can be confirmed through the visualization of extravascular dye. Since its initial introduction, FA has been established as the gold standard in diagnosing certain diseases, such as macular choroidal neovascularization. Since its advent, advances in fundus camera technology have allowed for up to 200 degrees of horizontal view of the retina during FA imaging (Callaway & Mruthyunjaya, 2019), which can greatly assist in the visualization and diagnosing of peripheral retinopathies.

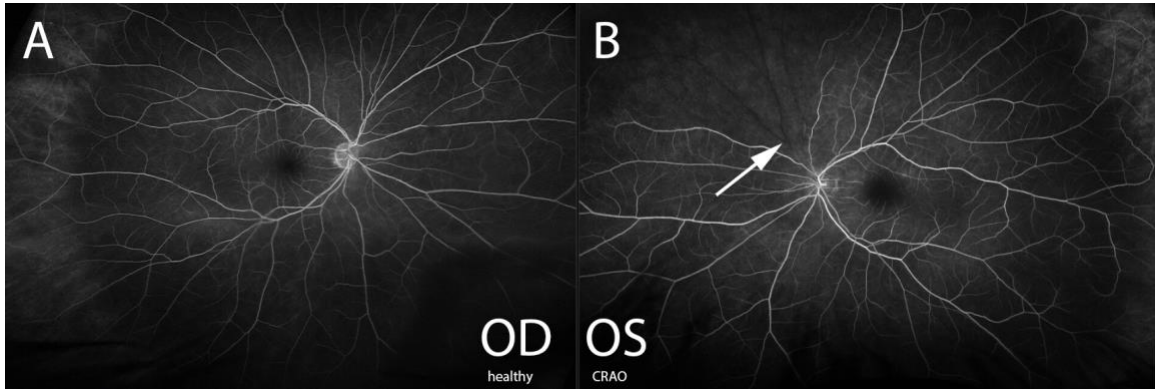


Figure 8: Fluorescein Angiography of CRAO. Panels A shows the healthy right eye of a patient. Panel B illustrates the findings of the left eye, confirming a CRAO. Note the absence of perfusing dye in the upper nasal quadrant of the vasculature in the FA image.

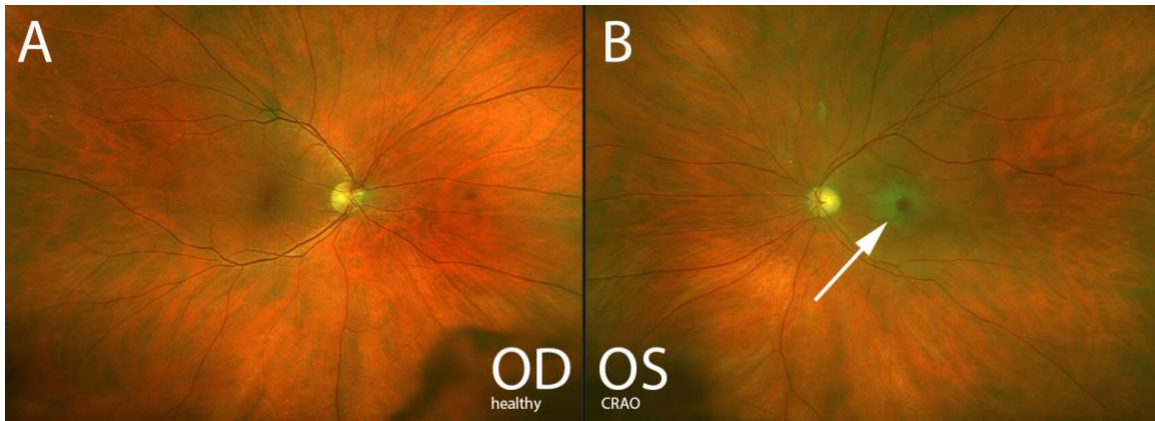


Figure 9: Fundus Photography corresponding with FA Imaging. Fundus photography of the healthy right (OD) yields unremarkable findings. Fundus photography of the left eye (OS) confirms cherry red spot and peripheral opacity commonly associated with CRAOs.

A major point of consideration when utilizing FA imaging is that, unlike OCT imaging and OCT angiographies, FA imaging is invasive as it requires an initial injection of fluorescent dye, most often as either a 5 ml bolus at 10% concentration or a 2.5 ml

bolus at 20% concentration. While side effects are typically uncommon, the potential for serious complications does exist. The most common reported side effect is nausea and vomiting, which is reported in approximately 5-10% of patients, and is much more prevalent in a patient's first injection of fluorescent dye. The rate of incidence drops to nearly 0% if further injections for multiple imaging sessions is conducted. In a retrospective study examining the effects of FA injection in patients, other rare but serious potential side effects included urticaria and skin allergic reactions, respiratory allergic reactions including laryngeal edema, hypotensive shock, fatal myocardial infarction and cardiac arrest, basilar artery ischemia, and pyrexia. However, the complications noted were exceedingly rare, occurring at a rate lower than 1:1000; and FA imaging overall is considered a safe and useful diagnostic tool (Hayreh et. al., 1974). Similar to OCT imaging and OCT angiography, a major limitation in assessing perfusion and blood flow using FA imaging is the lack of quantitative data on flow measurements.

(3) Laser Speckle Flowgraphy (LSFG)

Laser speckle flowgraphy (LSFG) allows for the noninvasive, quantitative estimation of blood flow in the optic nerve head, choroid, retina and iris in vivo. (Orgul et al., 2010). LSFG imaging utilizes the LSFG-NAVI (Softcare Co., Ltd., Fukuoka, Japan), composed of a fundus camera, diode laser, infrared charged couple device (CCD), and CCD camera to calculate blur rate, which is an approximate reciprocal of the speckle contrast (Orgul et al., 2010). The laser speckle flowmetry phenomenon has been used in

other diagnostic techniques as well, when an optically rough surface is illuminated by incident light a speckle pattern is reflected based on consequent constructive and destructive interference of the incident wavelength. If surface is stationary, the speckle pattern remains constant, however if the object imaged is nonstationary, such as the case with erythrocyte movement through vasculature, the speckle pattern will produce a blur proportional to the magnitude of flow (Newman et al., 2010).

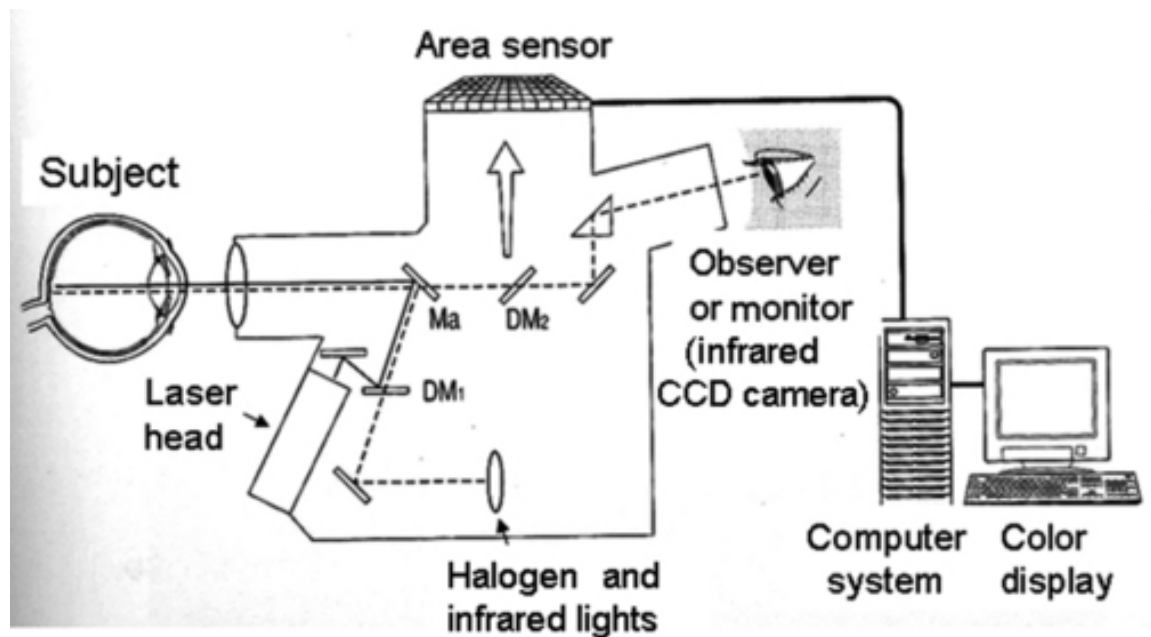


Figure 10: Schematic Diagram of LSFG System. Basic components of LSFG system highlighted include fundus camera, diode laser, infrared charged couple device (CCD), and CCD camera. Adapted from Orgul et al., 2010

Before beginning measurements, muscarinic eye drops such as tropicamide are administered to the patient to dilate the pupils. Alpha-adrenergic dilating drops such as phenylephrine are not recommended, as they may influence blood flow through areas

in question, including the ONH (Sugiyama et al. 1992). The subject is given 5 -10 minutes of rest before beginning of measurements to ensure blood pressure and heartbeat are stable and at approximately rest values and then instructed to position the eye in question in front of the fundus camera and to align their gaze on the internal fixation target. The operator begins by focusing the fundus camera onto the area in question, in this case the ONH. Once in focus, the operator can begin LSFG scanning, which continuously takes measurements of speckle blur for the duration of the exam (Sugiyama et al., 1992).

A major advantage of LSFG imaging when compared to more traditional imaging techniques such as FA and OCT is the ability to produce quantitative data that can then be utilized for interpatient and interpatient comparisons and statistical analyses. Mean Blur Rates (MBRs) as measured by LSFG scanning is the average blur rate calculated over the course of a single heartbeat. MBRs calculated by LSFG imaging vary significantly between patients, but show low interpatient variability over time (Seto et. al., 2020). Therefore, a more descriptive measurement, relative blood flow (RBF), can be calculated as a ratio of MBRs between patient's eyes, as this allows for the normalization of measurements and direct, unitless comparisons between and among patients.

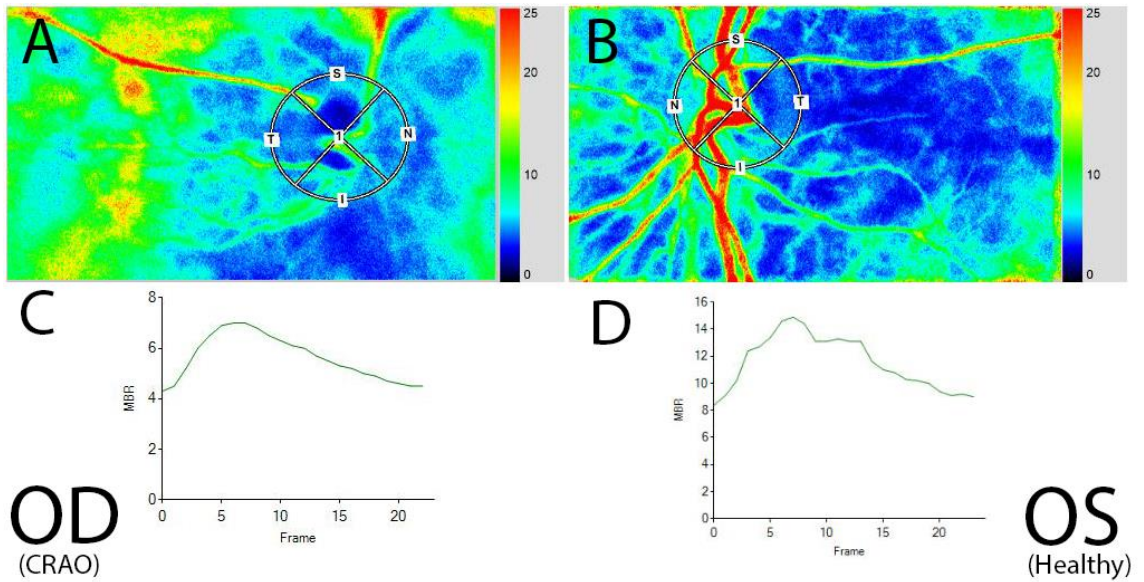


Figure 11: Laser Speckle Flowgraphy of Macula. Areas of high blood flow, including major vessels, are demarcated in red. Areas of low blood flow are demarcated in blue. Panel A illustrates the composite map of a CRAO affected right (OD) eye. The ONH area with labels corresponding to the superior (S), inferior (I), temporal (T), and nasal (N) quadrants are respectively highlighted. Panel B illustrates the composite map of the same patient's contralateral, healthy left (OS) eye. Panels C and D show the change in MBR calculated by the LSF system over the course of a single heartbeat.

SPECIFIC AIMS

The main goal of this study is to utilize the relatively new imaging instrument, LSFSG, to quantitatively describe the loss of perfusion through the ONH of eyes affected by CRAOs. Control patients with bilateral, healthy eyes were used to establish low intra-patient variability as well consistency in LSFSG measurements over time. By showing no significant difference in MBRs calculated between control patient eyes, these results allowed for the use of RBF as a unitless, ratio measurement of diseased eyes versus healthy eyes. RBF is therefore a useful quantitative measure of loss of perfusion that traditional imaging techniques such as OCT and FA are unable to provide. In order to explore the possibility of recanalization or spontaneous resolution of the occlusion, we divided patients into temporal subgroups to determine if RBF values differed significantly based on the time LSFSG scans were taken relative to the initial reported loss of vision. Lastly, statistical analysis was performed to determine if any meaningful return of vision was correlated to a return in perfusion over time.

METHODS

This study was a prospective observational analysis of retinal blood flow in patients with central retinal artery occlusions. It was approved by the BIDMC IRB. Participants included seven CRAO patients with healthy contralateral eyes from Beth Israel Deaconess Medical Center Longwood Eye Clinic. Patients with cataracts or underlying, confounding retinopathies affecting either eye were excluded from the study. Those who met inclusion criteria were invited to participate in the study. Twenty control patients with no ocular disease were recruited to establish low intra-patient variability in longitudinal LSFG MBR measurements and statistically nonsignificant differences between MBRs of these patients' eyes.

LSFG-NAVI (Softcare Co., Ltd., Fukuoka, Japan) imaging instrument was used to calculate frame blur rates of the ONH area for the duration of three separate heartbeats per eye per patient per session. Frame blur rates over the duration of a single heartbeat was averaged to calculate MBR per heartbeat. RBF was calculated in CRAO patients by dividing diseased eye's average MBR by the unaffected eye's average MBR. For control RBF values the average left eye MBR was divided by the average right eye MBR. The resulting numerical value represents a ratio of blood flows between eyes, therefore an RBF of 0.8 represents 80% blood flow in the affected eye when compared to the healthy eye.

Age	Sex	CRAO Status OD	CRAO Status OS
69	M	-	+
69	M	+	-
70	M	-	+
64	M	-	+
72	M	-	+
62	M	+	-
65	M	-	+

Table 1: Demographics of CRAO Patients. Seven patients (n=7) scanned with clinical history of unilateral CRAOs. Positive sign (+) indicates presence of CRAO.

VA was measured using the Snellen Chart, the most widespread diagnostic technique utilized in measuring VA (Ronquillo & Azzam, 2020) in standardized fashion, with normal vision defined as 20/20. Affected eyes were measured monocularly and results then converted to logarithm of minimum angle of resolution scale (logMAR) for statistical analysis.

Statistical analyses were conducted on measured variables to determine significance at an $\alpha = 0.05$. Analysis of variance (ANOVA) was used to compare inter-patient variability in MBR measurements and intra-patient MBR measurements, establishing the reliability of MBR and subsequently RBF as a quantitative measurement of blood flow. Welch's one-tailed t- tests were used to compare the RBF values of CRAO patients and control patients to determine statistical significance, as well as RBF values

of CRAO patients divided into groups one-year post-incident and one-year pre-incident. Welch's t -tests were also used to determine any statistically significant improvement in visual acuity between these groups of CRAO patients to determine if visual acuity and a return of blood flow hold correlation. All statistical analyses and figures were calculated and created using R Studio software (R Core Team, 2013).

RESULTS

MBRs of control patients demonstrated high variability in measured values between patients ($F_{19} = 14.60$, $P < 0.001$). Measurement in a single control patient showed no statistically significant difference in MBR values between eyes ($F_1 = 0.83$, $P = 0.37$) or in imaging sessions conducted on separate dates ($F_1 = 0.012$, $P = 0.91$). These results show high inter-patient variability in MBRs as measured by LSGF-NAVI, but low intra-patient variability, establishing reliability and consistency in blur measurements and allowing for the normalization of data using RBF to allow for ratio comparisons.



Figure 12: MBR Measurements in Healthy Eyes. MBRs of control patients demonstrated high variability in measured values between patients ($F_{19} = 14.60$, $P < 0.001$).

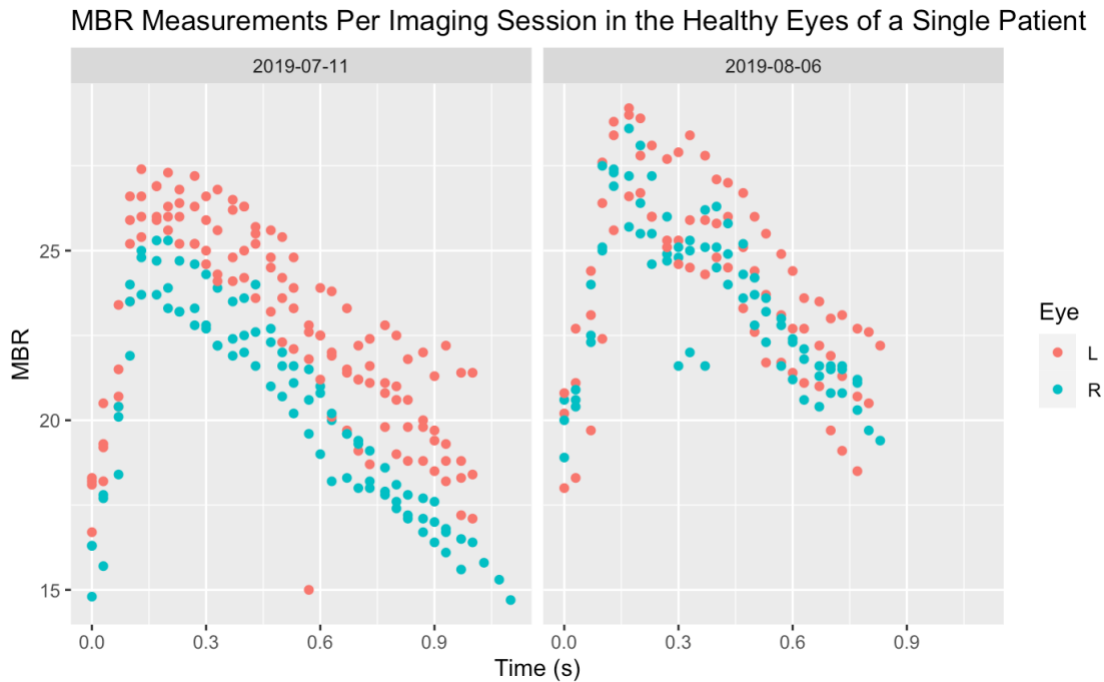


Figure 13: MBR Measurements in Two Separate Imaging Sessions in The Healthy Eyes of a Single Patient. Measurement in a single control patient showed no statistically significant difference in MBR values between eyes ($F_1 = 0.83$, $P = 0.37$) or in imaging sessions conducted on separate dates ($F_1 = 0.012$, $P = 0.91$). These results establish the reproducibility of LSFG measurements over time and validate RBF comparisons as a unitless ratio measure that can be used to compare

In control patients, RBF was calculated to be 1.02 ($P = 0.88$) indicating no significant difference in RBF between healthy eyes. In patients with unilateral CRAOs and healthy contralateral eyes, RBF was calculated to be 0.6650 ± 0.134 ($P < 0.001$), indicating on average a 33 percent loss in blood flow to the ONH in eyes with CRAOs.

Patients whose LSFG scans were taken within one year of reported vision loss had on average an RBF of 0.585, indicating on average a 42 percent loss in blood flow through the ONH. Patients whose LSFG scans were taken after one year of reported

vision loss had on average an RBF of 0.683, indicating on average a 31 percent loss in blood flow through the ONH.

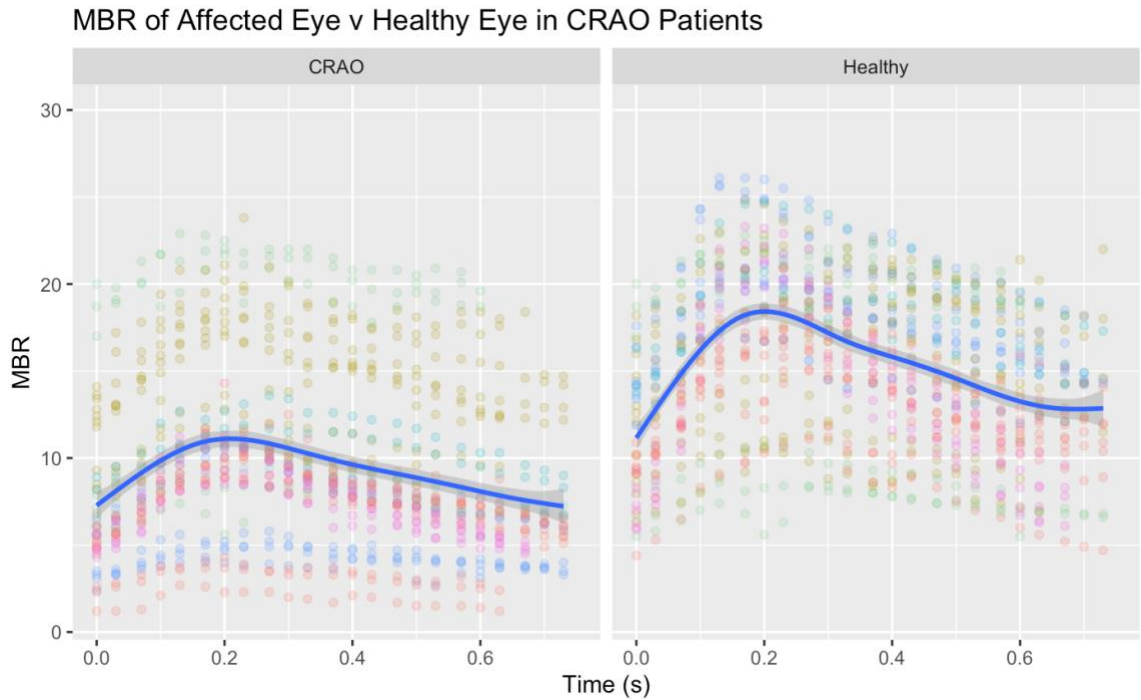


Figure 14: MBR of Affected Eye v Healthy Eye in CRAO Patients. Mean blur rates of affected eyes vs healthy eye of CRAO patients over the course of a heartbeat. Blur rates increase sharply at the initiation of each heartbeat and subside as erythrocytes diffuse into vasculature.

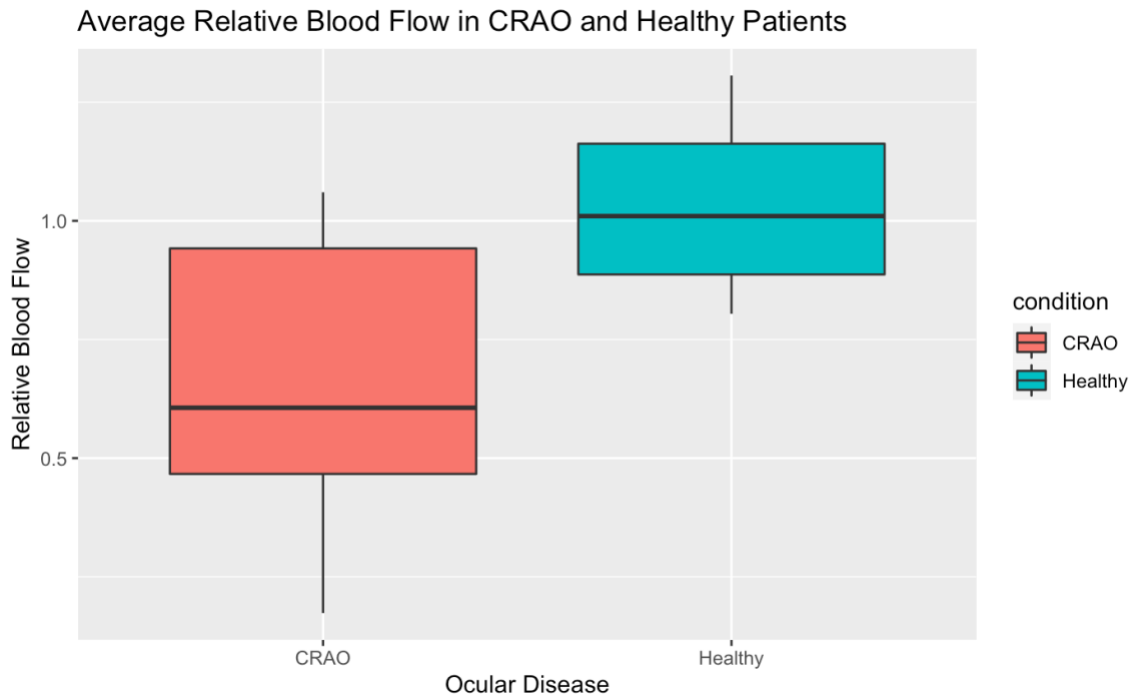


Figure 15: Average Relative Blood Flow in CRAO Patients vs Healthy Patients. In control patients, RBF was calculated to be 1.02 ($P = 0.88$) indicating no significant difference in RBF between healthy eyes. In CRAO patients, RBF was calculated to be 0.66 ± 0.13 ($P < 0.001$), indicating on average a 34 percent loss in blood flow to the ONH in eyes with CRAOs.

When comparing the RBF values measured in patients whose LSFG scans were taken within a year of their vision loss versus in patients whose LSFG scans were taken after a year of reported vision loss, patients whose scans were taken after one year had on average a 0.10 greater RBF through the ONH of the diseased eye. However, due to small sample size, this increase was not statistically significant at an $\alpha = 0.05$ ($P = 0.6519$).

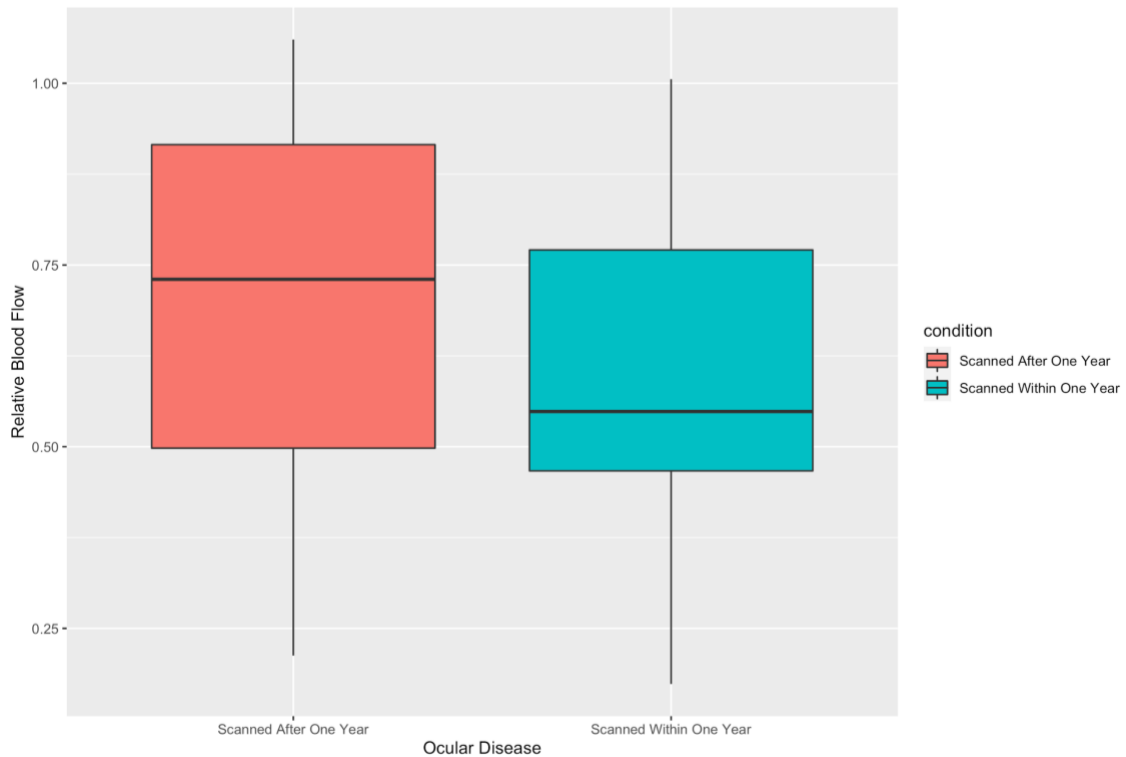


Figure 16: Average Relative Blood Flow in CRAO Patients Scanned After 1 Year of Reported Vision Loss vs. CRAO Patients Scanned Within 1 Year of Reported Vision Loss. Patients measured after one year have on average 0.10 greater RBF (0.683 v 0.585) which correlates to 10% greater blood flow through the ONH. However, due to small sample sizes, no statistical significance is associated with this increase at an $\alpha = 0.05$ ($p = 0.6519$).

Patients VA was measured the date of their first visit and every subsequent follow-up visit. Out of the seven patients measured, two did not return for follow-up visits, and therefore no data on VA one year after vision loss is reported. All patients measured had a VA of counting fingers (CF) or worse in their initial visit, with no significant improvement of vision in visits occurring after one year of reported vision loss. The average VA as measured by logMAR during initial visits was 2.07 ± 0.31 . VA measurements of the five patients measured after one year of reported vision loss all

yielded CF, for an average VA of 1.9. These values showed no significant difference in VA in patients when comparing VA on initial visit and VA one year after reported vision loss at an $\alpha = 0.05$ ($p = 0.257$).

Age	Sex	Visual Acuity (Snellen)	logMAR Conversion
69	M	CF	1.9
69	M	HM	2.3
70	M	CF	1.9
64	M	CF	1.9
72	M	CF	1.9
62	M	LP	2.7
65	M	CF	1.9

Table 2: Initial Visual Acuity of CRAO Pts. CRAO patients VA were measured within 48 hours of initial reported vision loss. All pts had VA of CF or worse, with an average VA of 2.07 ± 0.31 when converting Snellen Chart values to logMAR values.

Age	Sex	Visual Acuity (Snellen)	logMAR Conversion
69	M	N/A*	N/A
69	M	CF	1.9
70	M	CF	1.9
64	M	CF	1.9
72	M	CF	1.9
62	M	N/A*	N/A
65	M	CF	1.9

Table 3: Visual Acuity of CRAO Pts Measured After One Year of Reported Vision Loss. All patients measured had a VA of CF, or 1.9 when converted to logMAR values. Two patients did not follow-up one year after reported vision loss, and therefore no VA data was collected (N/A*).

DISCUSSION

Our results confirm a dramatic drop in blood flow due occlusion as well as a severe loss in visual acuity normally associated with CRAOs and are consistent with previous publications (Chen et al., 2013) (Hayreh et al, 2004) (Datillo et al., 2018). Ultimately, this study established and utilized RBF ratio calculations to provide quantitative data on the degree of ischemia caused by CRAOs. RBF was shown to be a reliable and quantitative measure of ischemia noninvasively and descriptively in ways that FA and OCT imaging alone cannot, and can serve as a diagnostic tool to further our understanding of blood flow as it relates to CRAOs.

RBF in the ONH was shown to be 33% lower on average in CRAO eyes, indicating a loss of a third of normal perfusion through the vasculature supplying the inner retina. To determine if perfusion in CRAO affected eyes returned over time, we subdivided RBF values based on the time LSFG scans were taken relative to when patients first reported vision loss. Patients scanned within one year of their reported vision loss had ten percent lower perfusion through the ONH when compared to patients scanned after one year of their reported vision loss . When comparing RBF values of scans taken within one year of initial vision loss to scans taken after one year after, RBF was significantly lower in patients scanned within one year. While these results did not yield statistical significance due to the limited sample size available (significant variation

within groups)?, they suggest that blood flow does return over time in CRAO patients, presumably as the occlusion resolves or re-canalizes. This increase in RBF was not correlated with a statistically significant increase in VA however, indicating that this loss in VA is permanent, consistent with the animal models describing massive irreversible retinal damage 240 minutes post-occlusion (Hayreh et al., 2004).

This study includes limitations. A significant limiting factor was the small sample size; an increase in which would provide more robust statistical power and decrease the possibility of Type II errors. Longitudinal analysis proved to be limited due to the duration of the study, future studies could continue these analyses over longer periods of time, recruit a larger population of patients, and further divide patients into temporal subgroups in order to further investigate the return of blood flow with time. While RBF does provide novel, qualitative data, it is a unitless ratio comparison that cannot be directly correlated with concrete measurements of flow, such as ml/sec. This stems from the fact that blur as measured by the LSFV-NAVI is correlated to a change in speckle pattern (Shiga et al, 2014). While blur is directly correlated with blood flow, there is no method of converting this measurement into traditional measurements of flow.

Further studies can expand on these findings by increasing sample size and longitudinal duration of examination and reexamination of patients affected not only by CRAOs, but other retinopathies that impact blood flow or vasculature in the retina. Studies can also be conducted to determine whether LSFV measurements can be used

as a diagnostic predictor of disease incident, possibly serving a diagnostic for intervention before CRAOs occur.

CONCLUSION

In conclusion, this study has demonstrated the reliability of LSFG measurements in providing qualitative data where currently diagnostics are limited, and RBF values calculated indicate a severe reduction in blood flow through the ONH in CRAO affected eyes. While it does appear that blood flow through the ONH returns over time, significant variation coupled with low sample sizes yielded results that were not statistically significant at an $\alpha = 0.05$, and therefore remains a point for future research interests. We found no statistically significant return of VA over time, consistent with the current understanding of the pathophysiology of CRAOs.

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