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Retinal vascular blood flow in patients with retinal vein occlusions

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RETINAL VASCULAR BLOOD FLOW IN PATIENTS WITH RETINAL VEIN OCCLUSIONS

by

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ABSTRACT

Purpose: This study aims to quantify the retinal vascular blood flow in eyes affected by unilateral central retinal vein occlusions (CRVO) or branch retinal vein occlusions (BRVO). We created and explored a new, unitless metric for the severity of these diseases: relative blood flow (RBF). We then contextualized RBF in terms of patient demographics, ocular presentation and other systemic conditions, as well as explored its efficacy as a predictor of future outcomes.

Methods: Data was collected from 20 control subjects and 32 patients with clinically diagnosed retinal vein occlusions (15 CRVO and 17 BRVO). Laser speckle flowgraphy was then used to quantify retinal vascular blood flow in terms of mean blur rate, a metric shown to be highly heterogeneous between patients but fairly consistent in intra-patient repeated measurements over time. After confirming this and establishing a strong correlation between a healthy patient's two eyes, we used an RVO patient's fellow eye as a nondiseased expectation and presented relative blood flow as the ratio between their diseased and healthy eye. We then correlated this data with demographic variables and disease characteristics from patients' medical history.

Results: We found an average blood flow decrease of 26% in CRVO eyes relative to healthy eyes in the same patients and an average decrease of 7% in BRVO eyes. In CRVO, duration of occlusion, central macular thickness, intraocular pressure, diabetes, previous laser and injection treatments, and an injection within three months after blood flow measurement were significantly associated with relative blood flow. In BRVO, no demographic variables or disease characteristics were significantly associated with relative blood flow.

Conclusions: Relative blood flow represents a promising new, consistent and informative metric for quantifying the severity of unilateral retinal vein occlusions. With both descriptive and predictive properties in eyes with CRVO, future work should explore its great potential.

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LIST OF ABBREVIATIONS

BCVA	Best-corrected visual acuity
BRAO	Branch retinal artery occlusion
BRVO	Branch retinal vein occlusion
CME	Cystoid macular edema
CMT	Central macular thickness
CRAO	Central retinal artery occlusion
CRVO	Central retinal vein occlusion
ETDRS	Early treatment diabetic retinopathy study
FA	Fluorescein angiography
IOP	Intraocular pressure
LogMAR	Logarithm of minimum angle of resolution
LSFG	Laser speckle flowgraphy
MBR	Mean blur rate
OCT	Optical coherence tomography
OCTA	Optical coherence tomography-angiography
OD	Oculus dextrus (right eye)
ONH	Optic nerve head
OS	Oculus sinister (left eye)
PDR	Proliferative diabetic retinopathy
RAO	Retinal artery occlusion
RB	Rubber band

RBF	Relative blood flow
RMSE	Root-mean-squared-error
RPE	Retinal pigment epithelium
RVO	Retinal vein occlusion
SD-OCT	Spectral domain optical coherence tomography
tPA	Tissue plasminogen activator
VA	Visual acuity
VEGF	Vascular endothelial growth factor

INTRODUCTION

The eyes are required for the sensory modality of vision and play an important role in interpersonal interaction and communication. Diseases of the eye can therefore have a profound effect on quality of life. Vision can be impaired by any condition decreasing or preventing the passage of light toward the back of the eye or impairing the conversion of light into neural signals for processing by the brain. The structure of the eye is fundamental to its function (Batterbury & Murphy, 2019).

Anatomy of the Eye

The tissue architecture of the eye serves to transmit and focus light (Figure 1). Two structures, the cornea and conjunctiva, are in direct contact with the environment and protect the eye from mechanical or chemical trauma. The cornea is transparent because of its molecular architecture: there are no blood vessels, thin-diameter collagen is laid out in a highly regular fashion, and water is actively transported out of the tissue (Meek & Knupp, 2015). The anterior chamber, iris, pupil, and posterior chamber lie behind the cornea, and these components are in contact with the aqueous fluid. The lens separates the aqueous fluid from the vitreous, which contains a gel-like fluid that occupies a majority of the eye. Light travels through these elements to arrive at its final destination located in the posterior aspect of the eye, the retina (Kolb, 2007).

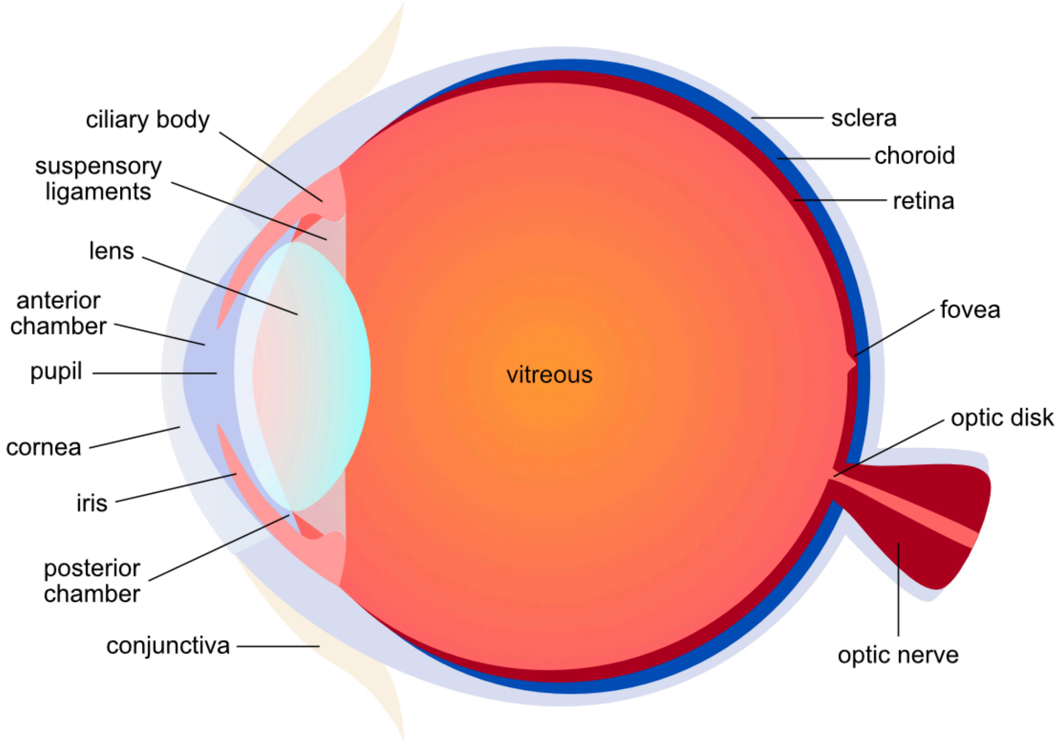


Figure 1. Gross anatomy of the eye. This graphic shows a cross-section of the eye, with light entering through the anterior aspect of the eye (on the left) where the cornea and the lens are located. Light traverses the vitreous and arrives at the retina in the posterior pole of the eye (on the right).

Structure of the Retina

Histologically, the retina is comprised of stratified layers of neural, connective, epithelial tissue. The neuronal cell layers are responsible for conversion of light into neural signals, which are conveyed to the brain via the optic nerve. Light must pass through the eight inner, anterior layers of the retina before it reaches the photoreceptor rod and cone cells (Figure 2). Electrical signals from photoreceptors are transmitted through the signal-conducting bipolar and amacrine cells to the ganglion cells. The axons of the ganglion cells travel toward the optic disk and eventually enter the optic nerve, bringing the signals to the brain. The ganglion cells and the photoreceptor cells form the inner and outer boundaries of the neurosensory retina, respectively (Kolb, 2012).

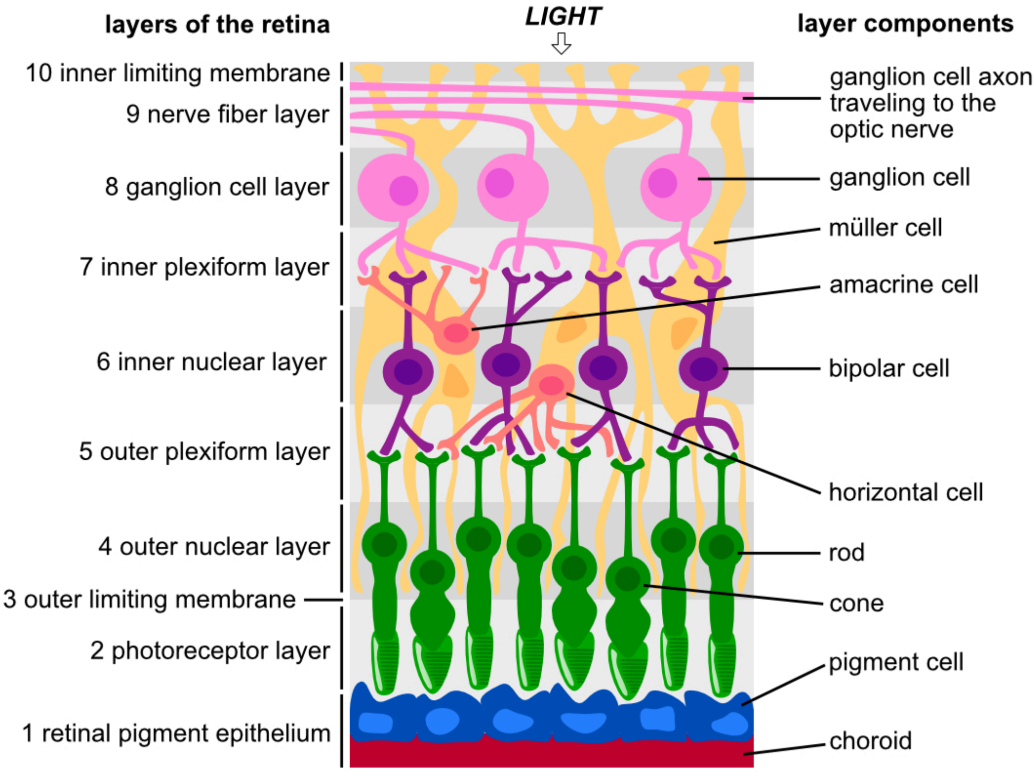


Figure 2. Schematic of the microscopic structure of the retina. There are ten distinct cell layers in the retina, with different populations of cells inhabiting the layers. Light enters the retina from the innermost (anterior) layer. In this schematic, the ganglion cell axons are traveling to the right toward the optic disk and nerve.

Function of the Retina

The retina creates sight. Among the previously mentioned neurosensory retina cells, the photoreceptor cells are the most important for vision. Both types convert light energy into electrical energy through structural and chemical intracellular changes. Photoreceptor rods are responsible for vision and recognition of movement in low-light conditions. In contrast, the photoreceptor cones function optimally in a bright environment and generate high-acuity color vision (Molday & Moritz, 2015).

The anatomical location of the photoreceptors impacts vision. Rods are located throughout the retina except in the foveola, while cones are localized to the macula and particularly concentrated in the fovea and foveola. The macula supports central vision, and the rest of the retina provides for peripheral vision. The optic disk, an entry and exit point for blood vessels and the optic nerve, is devoid of photoreceptors and therefore constitutes a physiological blind spot (Figure 3) (Purves et al., 2001).

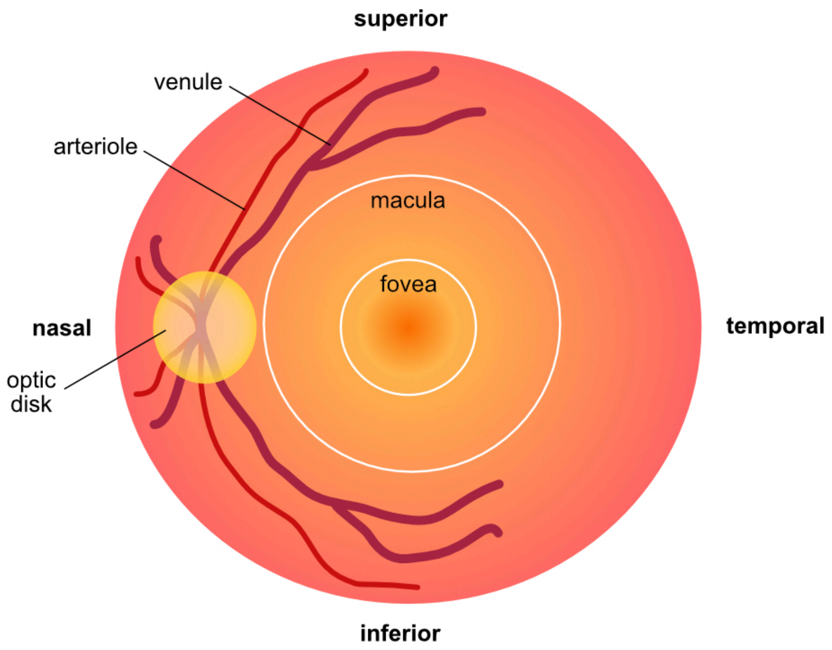


Figure 3. Fundus schematic of the left eye. The four main tributaries of the central retinal artery and vein are shown. In relation to venules, arterioles are generally smaller in diameter and have a lighter red color. The arterioles eventually branch into capillaries which coalesce back into venules (capillaries not shown). The optic disk is the area where the optic nerve and the central

retinal vessels enter and exit. The right eye fundus is an approximate mirror image of this schematic.

Histological examination reveals that the photoreceptor cells are anchored to retinal pigment epithelium (RPE) cells, which provide nutrients and phagocytose light-damaged cells. Because the photoreceptors comprise the outermost layer of the neurosensory retina, any loss of retinal transparency in the inner layers, due to a breach of the blood-retinal barrier, for example, can negatively affect vision. Photoreceptor cell physiology may also be impacted by a breakdown in the connection between the photoreceptor layer and the RPE. Provided the anterior elements of the eye transmit and focus light normally, proper functioning of the retina is paramount for good vision (Ivanova et al., 2019).

Retinal Vasculature

The retina receives blood from two sources derived from the ophthalmic artery. The network of capillaries within the choroid, or choriocapillaris, supplies the posterior, outer third of retinal layers and the foveola entirely. Choroidal vessels are bounded by fenestrated epithelium, so the RPE forms the outer blood-retinal barrier. The central retinal artery provides oxygen and nutrients to the anterior, inner two-thirds of the retina, from the nerve fiber layer to the outer plexiform layer. Despite its name, the central retinal artery is an arteriole which enters the eye from the optic nerve and branches into a capillary network that eventually coalesces into the central retinal vein. The central retinal vein exits the eye adjacent to the central retinal artery. Retinal capillaries are bounded by nonfenestrated epithelium with tight junctions, forming the inner blood-retinal barrier. The foveola is particularly vulnerable to harm because it relies on the choriocapillaris as its sole wellspring of nourishment and damage to this area will have a pronounced effect on central vision (Sun & Smith, 2018).

Retinal Vascular Occlusions

Retinal vascular occlusions occur when retinal arteries or veins become obstructed by compression or by a blood clot, also known as a thrombus. Virchow’s Triad is a set of conditions that increase the probability of thrombosis: stasis in blood flow, increase in blood coagulability, and injury to the endothelial lining of blood vessels. Systemic disease can also impact that likelihood of a retinal vascular occlusion from occurring and differ depending on the type of occlusion. There are four main categories of retinal vascular occlusions, determined by the vessel type and affected site: central and branch arterial occlusions, abbreviated CRAO and BRAO, and central and branch retinal vein occlusions, abbreviated CRVO and BRVO (Table 1). Arterial occlusions usually lead to more profound vision loss than venous occlusions (Bek, 2013).

Table 1. Types of vascular occlusions in the retina and their causes

Disease	Affected Site	Etiology
Central Retinal Arterial Occlusion (CRAO)	Central retinal artery at or near the lamina cribrosa	- Embolism from carotid or cardiac vessel thrombi - Basement membrane thickening caused by atherosclerosis
Branch Retinal Arterial Occlusion (BRAO)	Smaller arterioles	
Central Retinal Vein Occlusion (CRVO)	Central retinal vein at or near the lamina cribrosa	- Thrombosis due to hypercoagulability states, endothelial damage, or stasis of blood flow - Compression of a venule crossing under an arteriole
Branch Retinal Vein Occlusion (BRVO)	Smaller venules, often at arteriovenous intersections	

(1) Pathogenesis of Retinal Arterial Occlusions (RAO)

Systemic conditions associated with retinal arterial occlusions include hypertension, ischemic heart disease, carotid occlusive disease, and diabetes mellitus. Hyperlipidemia and atherosclerosis are also associated with RAO (Hayreh et al., 2009). When considering Virchow’s Triad in the context of RAO pathogenesis, factors that affect vessels from outside the wall, within the wall, and in the lumen must be considered. Very high intraocular pressure can compress arterioles. Atherosclerosis and arteriosclerosis thicken vessel walls and can occlude the lumen. Excess white or red blood cells may decrease blood flow rate (Hayreh, 2011). The arterial circulation is vulnerable to embolism, in which an object traveling through the blood stream lodges in the lumen of an artery or arteriole and blocks it. Emboli are relevant to arterial occlusions because the cross-sectional diameter of the vessel lumen on the arterial side of circulation decreases progressively. Emboli have varying compositions: they can be calcific, fibrin and platelets, cholesterol, or, in the case of a myxoma, connective tissue. In some patients, the emboli may eventually pass through the vessel, allowing for resolution of vision loss (Yu et al., 2014; Cho et al., 2016).

(2) Pathogenesis of Retinal Vein Occlusions (RVO)

Due to the lower hydrostatic pressure within veins relative to arteries, the elements of Virchow's Triad that can cause RVO are slightly different from those that cause RAO. The systemic conditions associated with RVO relate to clotting tendency, where hyperviscosity states lead to higher chance of thrombus development. A meta-analysis of thrombophilic risk factors found an association between RVO and two conditions: hyperhomocysteinemia and presence of anticardiolipin antibodies (Janssen et al., 2005).

While CRVOs are due to obstruction at or near the lamina cribrosa, BRVOs often occur by compression at arteriovenous crossing points, where an arteriole passes anterior to the venule. Because an arteriole and venule can share a common adventitial sheath, any thickening of a sclerotic arteriole may cause compression of the paired venule (Duker, 1989; O'Mahoney, 2008). This leads to upstream venous dilation and turbulence of blood flow in the area. A change in the blood flow pattern at these sites can induce the formation of a thrombus (Christoffersen & Larsen, 1999). Elevated intraocular pressure (IOP) due to pre-existing glaucoma or ocular hypertension, is thought to exacerbate the compression of retinal venules and is associated with CRVO (Hayreh et al., 2004).

Vessel wall inflammation of veins, phlebitis, can also occlude the lumen. Phlebitis may arise from systemic conditions such as sarcoidosis, systemic lupus erythematosus, polyarteritis nodosa, and Behçet's disease, but there is little evidence showing that RVO is caused by these diseases. An observational case series on focal retinal phlebitis in three patients with BRVO could not attribute the RVO to a systemic predisposition for inflammation (Hoang et al., 2012). An older case series suggests that a physically compromised endothelium may result in increased permeability and leakage of fluid into the surrounding tissue (Clemett, 1974). More research is needed to clarify the role of endothelial inflammation in RVO.

(3) Treatments and Outcomes of Retinal Vascular Occlusions

A patient who presents with RAO should be evaluated for associated neurologic and cardiovascular conditions, so that treatment not only addresses the RAO but also the underlying systemic cause (Biousse et al., 2018). With complete CRAO, treatment must be within 12-48 hours after the onset of symptoms or permanent vision loss can occur (Pielen et al., 2015). Loss of vision is less pronounced with BRAO, as it affects a smaller area of the visual field (Yuzurihara & Iijima, 2004).

If the arterial blockage is resolved by therapy or spontaneously, it is possible to fully regain vision. When CRAO is caused by an embolus, treatments work toward dilating the arteries, increasing perfusion pressure, dissolving the clot, physically removing the embolus, preventing platelet activation, and reducing blood cell rigidity. One systematic review suggests that administration of oral pentoxifylline to make red blood cells more deformable or enhanced external counterpulsation to increase retinal perfusion are the most justifiable treatments for CRAO. Enhanced external counterpulsation has been applied to BRAO as well, though none of the therapies addressing RAO have been reliably proven (Fraser & Adams, 2009).

Similar to RAO, RVO treatments should take patients' systemic conditions into account. However, most studies support treatments that address the symptoms of RVO rather than the underlying causes, because the causal connections between many systemic conditions and RVO are tenuous. The treatments and outcomes of RVO will be discussed in further detail in following sections.

Clinical Presentation of Retinal Vein Occlusions (RVO)

RVO is a relatively common vision-impairing vascular eye disorder (Laouri et al., 2011). In 2015, the global prevalence of RVO in people aged 30-89 was estimated to be 0.77%, or about 28 million people. The prevalence of CRVO at that time was approximately 0.13% or 4.67 million individuals, while the prevalence of BRVO was about 0.64% or 23.38 million individuals. The pooled, cumulative incidence over the span of five years for both types of RVO was 0.86% and jumped to 1.63% over ten years. Age and prevalence show the strongest positive correlation, suggesting a link between advanced age and

RVO. There was no significant difference in prevalence between males and females (Song et al., 2019).

The first sign of retinal vein occlusion may be sudden, painless vision loss in an otherwise asymptomatic eye. RVO may also be diagnosed before vision loss occurs upon routine ophthalmic examination of the retina. In acute RVOs, fundoscopy reveals retinal hemorrhages and dilated tortuous veins. Retinal hemorrhages can be sparse or abundant and vary in shape from punctate to flame-shaped (Figure 4). Hemorrhages resolve over time, so they may not be present in older occlusions. Cystoid macular edema and optic disk edema may be observed in older RVO. The clinical manifestations of proliferative diabetic retinopathy (PDR) are similar to RVO; therefore, the patient should be assessed for diabetes during evaluation (Wong, 2010).

Figure 4. Fundus photograph of a branch retinal vein occlusion. Panel A: A widefield fundus photograph of a patient’s healthy, right eye (OD). Blood vessels are clearly visible, and the translucent light red patterning in the background is the choroidal vasculature. Note that the patient’s eyelashes appear as shadows

at the bottom of the photo. Panel B: A widefield fundus photograph of the same patient's left eye (OS), diagnosed with superior BRVO. Retinal veins appear dilated when compared to the right eye. There is minimal tortuosity, perhaps due to the milder nature of this particular BRVO. Vascular leakage can be seen above the fovea, in the superior quadrant of the macula. Both images were obtained with an Optos camera (Optos California, Dunfermline, UK).

Among RVO subtypes, CRVO results in the greatest severity of symptoms (Figure 5). CRVO affects all quadrants of the fundus (Wong, 2010). Almost 40 years ago, CRVO was designated as ischemic or nonischemic based primarily on the amount of nonperfusion visualized by fluorescein angiography (FA), a diagnostic imaging technique to be discussed in greater detail in the next section (Hayreh, 1983). Relative afferent pupillary defect is another indicator of ischemic CRVO (Servais et al., 1986). Ischemic CRVO reflects a different prognosis from nonischemic CRVO. Though macular edema may cause a decrease in visual acuity in both types, ocular neovascularization is a consequence of the former rather than the latter (Thomas et al., 2019).

BRVO results in milder symptoms than CRVO. BRVO affects only one quadrant of the fundus (Oztas et al., 2017). The negative effect of BRVO on retinal blood flow is attenuated by the presence of collateral vessels, which serve as alternate paths for blood flow. This is in contrast to CRVO, because the area surrounding the lamina cribrosa does not contain collateral channels to allow for the drainage of blood (Christoffersen & Larsen, 1999; Sakimoto et al., 2020).

Figure 5. Relative symptom severity scale of retinal vein occlusions. Fundoscopy reveals a greater area of retinal hemorrhages and dilated, tortuous vessels in CRVO relative to BRVO. Patients with CRVO usually present with greater vision loss than BRVO patients.

Diagnostic Imaging Techniques for Retinal Vein Occlusions

There are a multitude of diagnostic imaging techniques that ophthalmologists can use in the diagnosis of RVO. Fluorescein angiography and optical coherence tomography are among the more frequently used. This section will provide a brief overview of the benefits and limitations of pertinent imaging techniques.

(1) Fluorescein angiography (FA)

Since its development in the 1960s, FA has been invaluable to the assessment of retinal circulation (Novotny & Alvis, 1961). A fluorescent dye is injected into the arm of the patient, and, within seconds, the dye can be visualized in the retina. A fundus camera is used to take photographs at various time points following the injection of the dye. Recently, the advent of ultra-widefield fundus cameras (Optos California, Dunfermline, UK) has allowed for imaging of a greater field of view--up to 200 degrees horizontally (Callaway & Mruthyunjaya, 2019). FA provides a way of visualizing areas of vascular occlusion and leakage (Figure 6). However, it is an invasive procedure and can be difficult to repeat (Osamura et al., 2017). In some rare cases, the fluorescent dye can cause severe side effects (Stein & Parker, 1971).

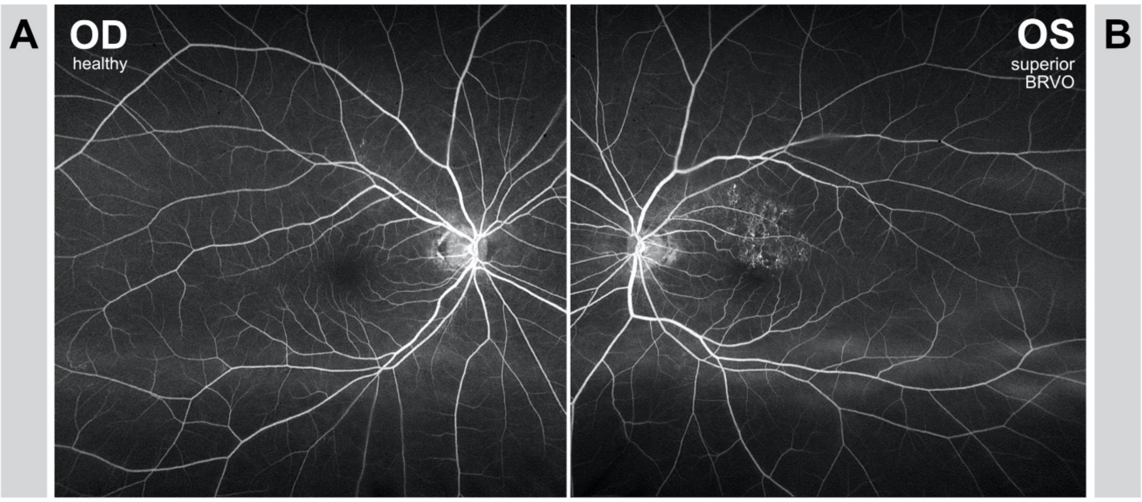


Figure 6. Fluorescein angiography of a branch retinal vein occlusion. Panel A: A widefield FA of a patient's healthy, right eye (OD). Blood vessels are clearly visible due to the presence of FA. Panel B: A widefield FA of the same patient's left eye (OS), diagnosed with superior BRVO. Vascular leakage can be seen above the fovea, in the superior quadrant of the macula. Both images were obtained with an Optos camera (Optos California, Dunfermline, UK).

(2) Optical Coherence Tomography (OCT)

Spectral domain OCT (Heidelberg Engineering, Inc., Heidelberg, Germany), which became available for clinical use in 2005, is an extremely versatile, noninvasive imaging technique. It provides a cross-sectional image of the posterior aspect of the vitreous, the retina and its various optical layers, the choroid, and the sclera (Figure 7). The optical layers of the retina correspond to its histological layers. Moreover, OCT allows for the fast identification of macular edema, macular hole, epiretinal membrane, retinal detachment, and morphological changes in the layers of the retina (Theelen & Teussink, 2018).

In 2012, swept source OCT improved upon spectral domain OCT by allowing for greater depth of tissue penetration and faster data acquisition (Kishi, 2016). Doppler Fourier-domain OCT further extended this technology by allowing for retinal blood flow measurement using Doppler shift (Doblhoff-Dier et al., 2014). OCT angiography (OCTA) is another recent enhancement of OCT that reveals ocular blood flow and provides a high-resolution map the retinal vasculature. OCTA may be favored over FA because it does not require the injection of dye and provides information on all layers of retinal blood vessels. There are some drawbacks to OCTA. When compared to FA, OCTA does not allow for visualization of fluid leakage and has a limited field of view (Tsai et al., 2018).

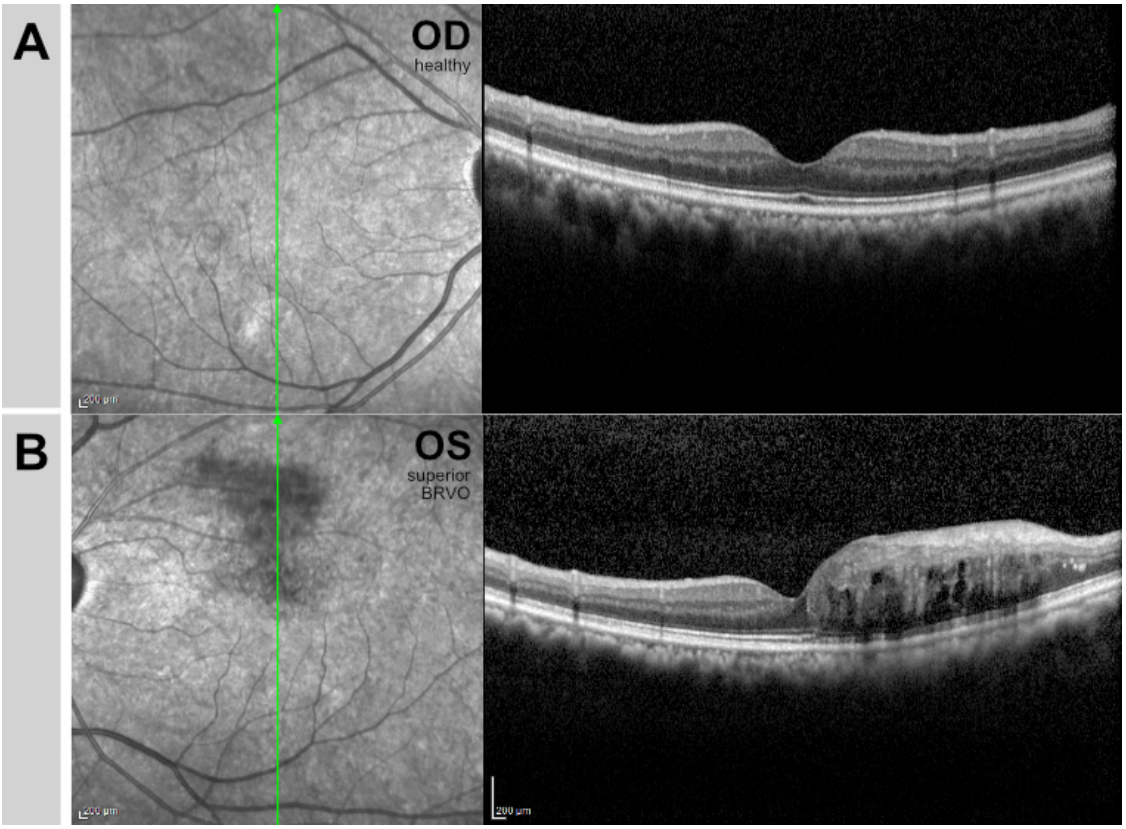


Figure 7. Spectral domain optical coherence tomography of the fovea. Panel A: A 90-degree line scan of the healthy, right eye (OD) of a patient. The fovea and various retinal layers are visible, and the retina is attached. There is no macular edema or epiretinal membrane. Panel B: A 90-degree line scan of the left eye (OS), diagnosed with superior BRVO, of the same patient. On the left side of the panel, cystoid macular edema is visible as a diffuse dark area in the upper half of the image. On the right side of the panel, in the cross-sectional scan, the thickening of the retina and black spots within the retina indicate CME. There is no epiretinal membrane. All images were obtained with an SD-OCT (Heidelberg Engineering, Inc., Heidelberg, Germany).

(3) Laser Speckle Flowgraphy (LSFG)

Laser Speckle Flowgraphy-NAVI (Softcare Co., Ltd., Fukuoka, Japan), approved by the FDA in 2016, is a relatively new, noninvasive technology for characterizing blood flow in the macula and optic nerve head. This method was first proposed in 1981 and, in the late 1990s, developed into a clinical apparatus, consisting of a laser and fundus camera for quantitative blood flow measurement (Fercher & Briers, 1981; Tamaki et al., 1997). The diode laser (Power: Laser Class 1) emits light at 830 nanometers to illuminate a six millimeter by four millimeter area on the retina. Erythrocytes moving through retinal vessels scatter this light, and the constructive and destructive interference creates a laser speckle pattern that is captured by a camera (Figure 8). The primary output of the system is mean blur rate (MBR), a numerical measure of blood flow, in arbitrary units. The unitless nature of MBR makes it difficult to compare with other techniques. For high quality data, there must be little to no opacity in the cornea, lens, and vitreous, and the subject must be able to fixate on a target easily (Sugiyama et al., 2010).

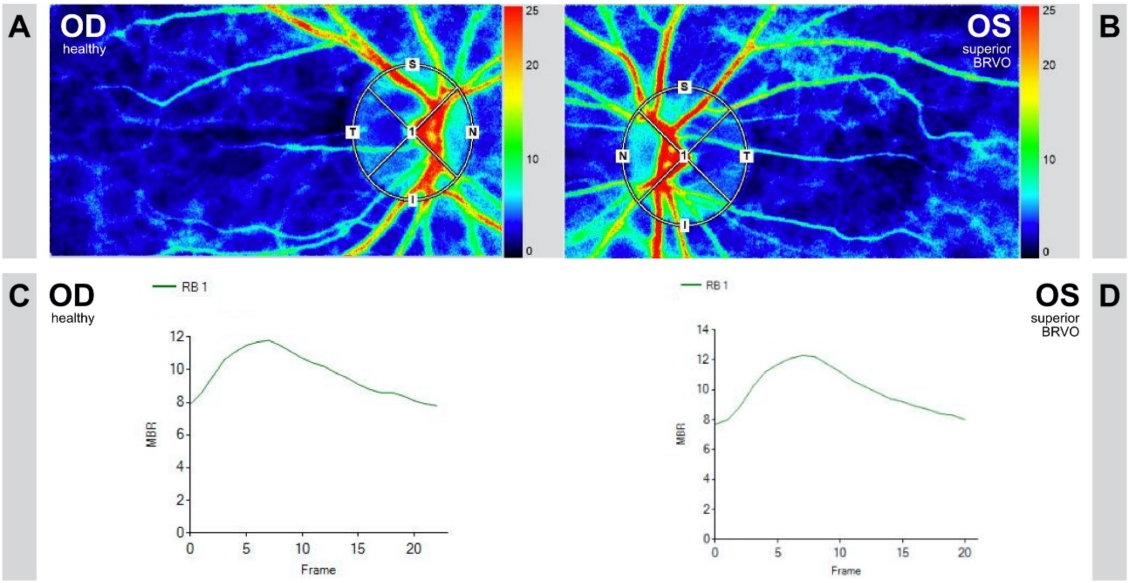


Figure 8. Laser speckle flowgraphy of the macula. The areas of high blood flow are red, while areas of low blood flow are blue. Panel A: A composite map of the healthy, right eye (OD) of a patient. Panel B: A composite map of the same patient's left eye (OS), which was diagnosed with BRVO in the superior quadrant. Panel C: The average mean blur rate (MBR) in the rubber band (RB) encircling the optic nerve head in the composite map in Panel A. The composite map is comprised 25 heartbeat maps, taken sequentially and overlaid on one another. These heartbeat maps are represented as 25 frames on the x-axis of the graph. Change in MBR over time is caused by cycling between systole and diastole; moreover, each graph represents one heartbeat. Panel D: The average mean blur rate (MBR) in the rubber band (RB) encircling the optic nerve head in the composite map in Panel B. All images were obtained with the LSFG-NAVI system and accompanying software (Softcare Co., Ltd., Fukuoka, Japan).

(4) Other Technologies

Many other technologies have been used to analyze retinal vasculature and blood flow (Wei et al., 2018). Some older noninvasive techniques include blue-field entoptic simulation and laser doppler velocimetry. Blue-field entoptic simulation enlists the patient in tracking the movement of their own leukocytes when looking into a blue background, but this technique is limited by bias introduced by patients (Riva & Petrig, 1980). Laser doppler velocimetry measures absolute erythrocyte velocity, but it makes multiple idealistic assumptions about retinal vessels and blood flow dynamics (Riva et al., 1979). Laser doppler flowmetry is a slightly newer technique that is similar in principle to LSFG, but the extent to which different layers of microcirculation contribute to the signal must be clarified (Riva et al., 2010). The adaptive optics scanning laser ophthalmoscope allows for measurement of absolute blood velocity in individual blood vessels (Zhong et al., 2011).

Ophthalmodynamometry is a diagnostic technique that is not based on visualization of the retina. Rather, it is a way of assessing the pressure within the central retinal vein and can potentially identify CRVO. However, in order to distinguish between the effect of an occlusion and IOP on retinal venous pressure, the patient's fellow eye must be healthy and have a similar IOP (Jonas, 2004; Robert, 2004).

Risk Factors for Retinal Vein Occlusions

In general, advanced age and vascular or hematological diseases are risk factors for retinal vein occlusion. Studies on RVO, therefore, tend to evaluate the traditional risk factors for atherosclerosis: hypertension, hyperlipidemia, and diabetes (O'Mahoney, 2008). A recent meta-analysis examining epidemiological studies of RVO identified age and hypertension as the strongest risk factor for the disease. In 2015, the global prevalence of RVO was less than 1% for people in their 30s, 40s, and 50s, and greater than 1% for those in their 60s, 70s, and

80s. Heart attack and stroke history, high cholesterol, and high creatinine were also significantly correlated with RVO. Diabetes mellitus was not a significant risk factor (Song et al., 2019). In a 12-year longitudinal study conducted in South Korea, researchers found an increased risk of RVO in patients with end-stage renal disease, which is associated with cardiovascular and thrombotic disorders (K. S. Lee et al., 2018).

Some sources suggest that there are etiological differences between young and old people who present with CRVO. So-called nontraditional risk factors—disorders causing aberrant inflammatory responses, connective tissue abnormalities, or hypercoagulability states—are associated with CRVO in people under the age of 50 (Rothman et al., 2019). Hypercoagulability and endothelial changes may also be a result of oral contraception usage (Stowe et al., 1978). Additionally, in patients with CRVO, the sclera and lamina cribrosa of their affected eye was significantly thicker than that of sex- and age-matched control subjects (Adiyeke et al., 2020).

The anatomical distribution of retinal vessels seems to play a role in the genesis of BRVO. When the superior and inferior temporal retinal veins were closer to the foveal center than both paired temporal retinal arteries, patients were less likely to develop BRVO (Oztas et al., 2017).

Complications of Retinal Vein Occlusions

Decreased vision and cystoid macular edema (CME) are the most common complications of RVO. CME is swelling of the retina caused by interstitial fluid entering into the tissue due to a breakdown of the outer or inner blood-retinal barrier. Using OCT cross-sectional scans of the retina, it is possible to measure the central macular thickness (CMT) in micrometers. CMT reflects the height of the fovea and is increased where there is fluid buildup (Rotsos & Moschos, 2008). Studies examining the correlation of vision loss and increased CMT due to macular edema in patients with RVO found no or poor correlations between CMT and visual acuity (VA) (Ou et al., 2017; Scott et al., 2009). These findings suggest that other factors beyond the amount of macular edema at the fovea are driving vision loss.

The exact mechanism of vision impairment in people with RVO is unclear, though CME is thought to play a major role. Theories for the causes of vision loss fall into three main categories: damage of photoreceptor cells, damage of cells in the inner retinal layers, and retinal swelling (Iijima, 2018). Blood and lipoproteins infiltrating the subretinal space have been shown to cause photoreceptor cell death in rabbit and mouse models (Bhisitkul et al., 2008; Notomi et al., 2013; Tsujikawa et al., 2010). Apoptosis of cells in the inner nuclear and ganglion cell layers was hypothesized to be detrimental to vision because the cells in these layers convey signals from the photoreceptors to the optic nerve. However, research in this area has been inconclusive (Finkelstein, 1992; Tadayoni et al., 2016). Finally, macular edema does not affect the transmission of light through the outer layers of the retina to the photoreceptor layer. Rather, CME may affect neural transduction between bipolar cells and photoreceptors by changing the extracellular matrix composition (Yasuda et al., 2015). While macular edema is reversible, deterioration of photoreceptor cells and inner retinal layers may be irreversible and responsible for poor visual outcomes even after resolution of macular edema.

With RVO the formation of an epiretinal membrane and consequent visual distortion is sometimes observed. An epiretinal membrane is a clear, contractile tissue that forms between the vitreous and the retina and exerts a mechanical force, or traction, on the retina. Retinal traction manifests as visual distortion. The reason for growth of an epiretinal membrane secondary to BRVO is unknown (Kang et al., 2015).

Prompt treatment of RVO is necessary to prevent or minimize and delay more severe complications. Left untreated, RVO may lead to neovascularization of the iris, angle, or the retina, retinal detachment, and, in rare cases, macular infarction (Kim et al., 2015; Tsujikawa et al., 2010). Patients with ischemic CRVO tend to develop neovascularization of the iris, or rubeosis iridis, and neovascularization of the angle which can lead to secondary neovascular glaucoma (Boyd et al., 2002; Rong et al., 2019).

CRVO often gives rise to dilation of small vessels on the optic veins called optociliary shunt vessels connecting the retinal vasculature to the choriocapillaris at the optic nerve head (Masuyama et al., 1990). In contrast, neovascularization of the retina mainly occurs in patients with severe BRVO (Sakimoto et al., 2020). These complications can be debilitating, painful, and require urgent treatment.

Treatments for Retinal Vein Occlusions

The current standard of care for RVO is generally targeted at relieving symptoms rather than addressing an underlying, systemic cause. Treatment can differ based on how long a patient has had an occlusion. When patients present with acute RVO, they receive a series of intravitreal anti-VEGF injections and laser treatments to prevent decreases in visual acuity (VA) and increases in macular edema. Additional treatment options include intravitreal corticosteroid injections, gas inhalation therapies, surgery, and close observation (Wong, 2010).

(1) Injection

Two main types of intravitreal injections exist for use in RVO patients: anti-VEGF and corticosteroids. Retinal tissue hypoxia due to RVO leads to the upregulation of vascular endothelial growth factor (VEGF), which promotes vascular leakage. Fluid escape from leaking vessels causes cystoid macular edema (Aiello et al., 1994; Pe’er & Folberg, 1998). Anti-VEGF injections work by preventing breakdown of the blood-retinal barrier. Several studies have demonstrated the efficacy of three different anti-VEGF agents: ranibizumab (Lucentis), aflibercept (Eylea), and bevacizumab (Avastin).

In clinical trials with end points at 6 months, ranibizumab reduced macular edema in BRVO and CRVO patients (Brown et al., 2010; Campochiaro et al., 2010). In two year-long clinical trials, aflibercept reduced macular edema in

BRVO and CRVO patients (Clark et al., 2016; Garcia-Arumi et al., 2018). A systematic review found that bevacizumab improves VA and reduces CME over three months, and statistically significantly improves VA within the first month (Yilmaz & Cordero-Coma, 2012). Bevacizumab reduces CME in patients with ischemic CRVO but does not improve visual outcomes (Hall et al., 2019). One recent clinical trial compared all three intravitreal anti-VEGF agents for reducing macular edema in CRVO. At 100 weeks, ranibizumab and aflibercept were functionally interchangeable, though the same could not be claimed for bevacizumab (Hykin et al., 2019). The optimal dosage schedule for these anti-VEGF agents for both types of RVO has yet to be established (Lip et al., 2018).

Studies aiming to elucidate the molecular mechanisms by which these anti-VEGF agents ameliorate retinal vein occlusion are ongoing. One study found that bevacizumab increased levels of transthyretin, a tetrameric protein normally synthesized by RPE cells in the retina. Transthyretin may somehow prevent vascular leakage and development of neovascularization, but more evidence is needed to support this hypothesis (Cehofski et al., 2018).

Corticosteroids have an inhibitory effect on VEGF and the inflammatory response. Triamcinolone acetonide is an injectable corticosteroid that seems to improve macular edema over 10-12 months in patients with nonischemic CRVO (Bashshur, 2004; Ip et al., 2009). In BRVO, however, triamcinolone is less effective than bevacizumab (Higashiyama et al., 2013). Dexamethasone (Ozurdex), an intraocular steroid implant, improves VA outcomes in patients with

BRVO and CRVO, but has side effects of cataract and increased IOP (Haller et al., 2011). Between corticosteroid and anti-VEGF injections, the latter is more often used to treat RVO due to is greater efficacy and better risk profile.

(2) Laser

Panretinal laser photocoagulation is thought to prevent development of neovascularization, vitreous hemorrhage, and improve visual acuity. According to one study, macular edema in patients with CRVO and BRVO did not improve with scatter, panretinal laser photocoagulation (Campochiaro et al., 2015). However, a recent systematic review found that panretinal laser therapy for ischemic CRVO prevents neovascularization of the retina and iris and improves macular edema (Li et al., 2018). Scatter photocoagulation was found to prevent neovascularization in BRVO (The Branch Vein Occlusion Study Group, 1984). Macular grid laser therapy does not, however, appear to improve macular edema in patients with BRVO (Callizo et al., 2019).

(3) Gas Inhalation

Blockage of a retinal vein deprives an area of tissue of blood flow and the delivery of oxygen. Lower oxygen saturation in the occluded retinal vein was found to be associated with the level of ischemia in CRVO, but not BRVO (Šínová et al., 2018). Some studies have tried to examine the effect of hyperoxia on resolution of macular edema. The reasoning behind this is two-fold: first, oxygen causes vessels to constrict; second, oxygen will ameliorate tissue ischemia. Constriction of vessels may decrease leakage, while increased oxygen saturation in the blood may prevent cell death. Oxygen was also found to downregulate the VEGF pathway in a mouse model of ischemic retinopathy, which may help improve macular edema (Liu et al., 2013).

Carbogen, a mixture of 95% oxygen and 5% carbon dioxide, has also been tested as an alternative to pure, 100% oxygen. Carbon dioxide may prevent oxygen-induced vasoconstriction and thereby allow for greater oxygen delivery to the retina. When carbogen inhalation was coupled with acetazolamide injection in a porcine model of ischemic retinopathy, it decreased ischemia due to acute BRVO (Pournaras et al., 2004). With more testing, hyperoxia may represent a possible noninvasive therapy for the reduction of ischemia and better visual outcomes in people with RVO.

(4) Other Therapies

Since RVOs may be caused by thrombosis, some studies have examined the efficacy of systemic anticoagulant and antiplatelet therapies with drugs such as heparin, ticlopidine, and streptokinase (Arroyo, 2001; Kohner et al., 1976; Lazo-Langner et al., 2010). Researchers have also attempted surgical intervention and intravitreal injection of tissue plasminogen activator (tPA) on animal models and humans, respectively (Ameri et al., 2008; Chhablani et al., 2010; Ghazi et al., 2003; Hattenbach et al., 2009; Lahey et al., 1999; Tameesh et al., 2004; Weizer & Fekrat, 2003).

Although in theory these therapies provide a way to address an important cause of RVO, the risks of hematological disorders with ticlopidine and vitreous hemorrhage with streptokinase seem to outweigh the benefits of these drugs (Ageno et al., 2016). The relative dearth of clinical trials evaluating the efficacy of and optimal dosage schedules for heparin and tPA has likely hindered their implementation in the clinic (Shahid, 2006).

One proof-of-concept study in a rabbit model lays the foundation for a photochemical method of targeted drug delivery to a blood vessel in the retina (Arroyo et al., 1997). Although the aim of the study was to create a blood clot using a form of thrombin, their technique could theoretically be used to dissolve a blood clot with tPA. The researchers created a caged version of thrombin by covalently bonding a photosensitive inhibitor to a key active site serine. Exposing caged thrombin to violet light through the eyes released the inhibitor from the active site serine, regenerating active thrombin enzyme at the site of light exposure (Arroyo et al., 1997). Given that tPA is also a serine protease, it may be amenable to caging and photolysis in the same fashion as thrombin. At present, no studies have attempted to adapt this photoactivation approach to tPA (Lee et al., 2009).

Studies have also examined the benefits of combining injection with laser therapies (Chew, 2009; McAllister et al., 2018; Moon et al., 2016; Tadayoni et al., 2017). Regardless of the treatment plan, patients with RVO should be kept under close observation. Recently, improvements on mass-spectrometry have enabled scientists to uncover changes in protein dynamics associated with RVO, which may lead to the development of new treatments. Proteomics have been performed on retina, vitreous humor, and aqueous humor samples from humans and animals with RVO. In general, the proteins that change with RVO seem to be associated with cell-adhesion processes and extracellular matrix remodeling. Proteomic profiling may be a promising avenue for the development of innovative therapies for RVO (Cehofski et al., 2017).

Outcomes and Prognosis for Retinal Vein Occlusions

The main outcomes of interest in RVO are best-corrected visual acuity (BCVA), macular edema, and neovascularization. While visual outcomes are usually moderate in both CRVO and BRVO, the average visual acuity tends to be lower in CRVO than in BRVO (McIntosh et al., 2010; Rogers et al., 2010).

According to one study, patients who present with a VA of 20/200 or worse due to CRVO were more likely to develop neovascularization and less likely to experience improved vision (The Central Vein Occlusion Study Group, 1997). More specifically, ischemic CRVO is correlated with development of secondary neovascular glaucoma within 15 months while nonischemic CRVO is not (McIntosh et al., 2010). CRVO patients younger than 50 years old have better final VA than those aged 50 and older (Rothman et al., 2019).

In BRVO, outcome and prognosis for BCVA are worse with advanced age, greater central macular thickness, and a poor baseline VA. When the BRVO occurs where the vein crosses over the arteriole, the nonperfusion area was significantly higher and the crossing pattern may be associated with risk for neovascularization (Iida-Miwa et al., 2019). Intraretinal hemorrhage after acute

BRVO can exacerbate the treatment burden and worsen clinical outcomes (Powers et al., 2019).

Although some RVOs resolve spontaneously or after a small number of anti-VEGF injections, the majority of patients with RVOs require numerous injections to keep macular edema under control. One prospective case series examined outcomes for patients with CRVO and BRVO after 78 months and 58 months, respectively. Within 6 months of follow-up, 75% of CRVO and 58% of BRVO patients required anti-VEGF injection. This suggests that more sustained anti-VEGF therapy is necessary to stabilize macular edema and improve outcomes (Iftikhar et al., 2019). With our current standard of care, RVO becomes a chronic disease for most patients.

Differential Diagnoses

RVO should be distinguished from proliferative diabetic retinopathy and from ocular ischemic syndrome. All three diseases are associated with hemorrhage, macular edema, dilated retinal vessels, and neovascularization (Batterbury & Murphy, 2019; Yanoff & Sassani, 2014). If a patient does not have diabetes, it is more likely to be RVO. If a patient has diabetes, ophthalmodynamometry can help distinguish RVO from diabetic retinopathy (Robinson & Halpern, 1992). Measuring the transit time of dye when performing an FA can also elucidate the disease, since the flow of dye out of the eye or a region of the retina would be slower than expected in an RVO eye than in a PDR eye. Since ocular ischemic syndrome is caused by atherosclerosis of the carotid artery, measuring the transit time of fluorescein dye is once again useful. In the case of ocular ischemic syndrome, the flow of dye from the time of injection in the area to the eye would be delayed (Mizener et al., 1997).

Other chief causes of sudden, painless monocular vision loss include anterior ischemic optic neuropathy, optic neuritis, papillophlebitis, and Terson’s syndrome. Retinal detachment and vitreous hemorrhage also cause sudden vision loss, though these may be secondary to RVO (Batterbury & Murphy, 2019; Yanoff & Sassani, 2014).

SPECIFIC AIMS

The goal of this exploratory, nonrandomized study was to quantitatively describe retinal vascular blood flow in patients with RVO and to create and explore a new measurement of RVO severity: relative blood flow (a unitless value). Blood flow data was collected using a fairly new technology called Laser Speckle Flowgraphy. The first objective was to characterize LSFG output in healthy eyes by establishing intra-patient consistency and stability of blood flow measurements over time. The second objective was to compare the blood flow in eyes with CRVO and BRVO with the blood flow in healthy eyes. The third objective was to identify any statistically significant correlations between demographic factors and RVO. This investigation will supplement current knowledge on retinal hemodynamics and may provide retina specialists and their patients with a more nuanced understanding of retinal vein occlusions.

METHODS

Study Design

This study was an exploratory, preliminary analysis of retinal vascular blood flow in patients with retinal vein occlusions.

Participants

We studied 20 control subjects with no ocular conditions or systemic diseases, and 32 patients diagnosed with unilateral retinal vein occlusion. Patients who presented to the clinic with sudden, painless vision loss in the entire visual field or in one quadrant of one eye were evaluated by a retina specialist. Diagnosis was primarily accomplished through a convergence of evidence from medical history, ophthalmoscopy, fluorescein angiogram (Optos California, Dunfermline, UK), and optical coherence tomography (Spectralis, Heidelberg Engineering, Heidelbergy, Germany). We identified 15 eyes with CRVO, and 17 eyes with BRVO.

Patients who had any additional ocular disease in either eye, such as diabetic retinopathy or age-related macular degeneration, were excluded. Patients with dense cataracts were excluded from the study since cataracts interfere with data collection by LSFG. Patients with epiretinal membranes or secondary neovascular glaucoma associated with RVO were included in this study.

The institutional review board at Beth Israel Deaconess Medical Center authorized this study, all patients provided informed consent, and research was conducted in accordance with the Declaration of Helsinki.

Data Collection

A comprehensive ophthalmological exam was conducted on the patients at each clinic visit. A recent medical history was collected to document changes in visual acuity, including flashing lights and floaters, and any reports of ocular pain. Risk factors such as diabetes and hypertension, major systemic health conditions, and any other recent changes in health were noted. Current medications and eye drops were confirmed.

The patients' visual acuity was evaluated using a Snellen or an Early Treatment Diabetic Retinopathy Study (ETDRS) chart and a handheld occluder. Patients performed the visual exam wearing distance glasses or bifocals, if applicable, and an occluder. In some cases, the pinhole attachment on the occluder was used to obtain the best corrected visual acuity (BCVA). All BCVA values were converted in the Logarithm of Minimum Angle of Resolution (LogMAR) scale using the LogMAR equation (Table 2) (Holladay, 1997).

Table 2. Conversion between Snellen visual acuity and LogMAR values

Snellen Visual Acuity (feet)	LogMAR Equivalent	Snellen Visual Acuity (feet)	LogMAR Equivalent
20/16	-0.10	20/100	+0.70
20/20	0.00	20/125	+0.80
20/25	+0.10	20/160	+0.90
20/32	+0.20	20/200	+1.00
20/40	+0.30	20/250	+1.10
20/50	+0.40	20/320	+1.20
20/63	+0.50	20/400	+1.30
20/80	+0.60	CF 2 feet*	+2.00
LogMAR = -Log(20 / distance in feet of best line read) *CF 2 feet = eye can count fingers at a distance of 2 feet			

IOP was measured in millimeters of mercury (mmHg) using a Tonopen (Reichert Inc., Buffalo, NY, USA) after applying topical anesthetic to each eye (proparacaine hydrochloride 0.5%). Eyes were not dilated unless required for subsequent blood flow measurements.

As a supplement to fundoscopy, various ocular imaging techniques were used to diagnose RVO and monitor the macula for complications. Upon first encounter, a wide-field fluorescein angiogram was acquired to identify the area of venous occlusion. At each visit, three types of OCT scans of the macula were taken: two line-scans at 0 and 90 degrees, and a fast-scan for a 25-scan composite image of the macula. The CMT measurements were obtained from the foveolar area of the fast scan output.

We used LSFG-NAVI to measure the dynamics of retinal circulation. This imaging technology (Softcare Co., Ltd., Fukuoka, Japan) has been used in numerous studies as a robust method of measuring and quantifying ocular blood flow (Iwase et al., 2015; Luft et al., 2016a; Shiga et al., 2014, 2015; Sugiyama et al., 2010). The system is comprised of a laser and fundus camera. Erythrocytes traveling through retinal vessels scatter the laser, and the constructive and destructive interference creates a laser speckle pattern that is captured by a CCD camera.

Softcare Co., Ltd. has devised a terminology to describe the output of LSFG (Figure 9). Over the course of about four seconds, the LSFG software generates “heartbeat maps” for each frame based on the laser speckle pattern. These heartbeat maps are then overlaid on one another to form a “composite map.” Markers called rubber bands allow for targeted analysis of various regions such as the macula or individual blood vessels. The elliptical rubber band, which is partitioned into four quadrants (superior, temporal, inferior, and nasal) can be used to analyze the optic nerve head (ONH) area. The light intensity map allows for accurate, manual placement of the elliptical rubber band onto the heartbeat map. The software can then analyze the area within the rubber band to generate quantitative data.

Mean blur rate (MBR) is a numerical measure of the blood flow within the rubber band area. MBR is calculated by comparing the mean velocity of all cells in the measurement area (the static cells of the tissue and the erythrocytes moving through vessels) with the mean velocity of the dynamic cells (the erythrocytes) in the measurement area. MBR is therefore a unitless value that represents relative blood flow velocity in an area and can vary based on tissue characteristics. In particular, increasing retinal pigmentation leads to decreasing MBR values, so laser intensity must be adjusted using the Pigmagic software to account for differences between subjects.

Using the elliptical rubber band and the composite map, we can obtain various MBR-based readouts of relative blood flow in the four quadrants of the ONH area. The primary values of interest to us in this study are MV, MT, MA, and MV-MT (Table 1). MT is a measure of the movement of erythrocytes within the choriocapillaris. MV-MT isolates the relative blood flow within the retinal vessels by excluding the mean blur rate of the underlying choroidal tissue.

Table 3. Definition of blood flow measures using mean blur rate (MBR) obtained from a composite map

MV	mean MBR of vascular area (higher MBR area) within the rubber band
MT	mean MBR of tissue area (lower MBR area) within the rubber band
MA	mean MBR of the entire area within rubber band, usually equivalent to average
MV – MT	the difference of the mean vascular MBR and the mean tissue MBR within the rubber band

For all subjects of this study, we performed LSFG in both eyes. Patients were dilated only if necessary with 1% tropicamide, which does not affect retinal blood flow (Luft et al., 2016b). We have two sets of controls. First, we used control subjects' eyes, which have no previous history of ocular disease or procedures, no diabetes, no hypertension, and are otherwise healthy. These were primarily used to determine the inter- and intra-patient MBR correlation and to establish the feasibility of using patients' healthy fellow eye as an expected value. Second, we used patients' healthy fellow eye, having been established as a satisfactory expectation. This will be our primary point of comparison for analytical purposes to mitigate the effects of the observed inter-patient variability.

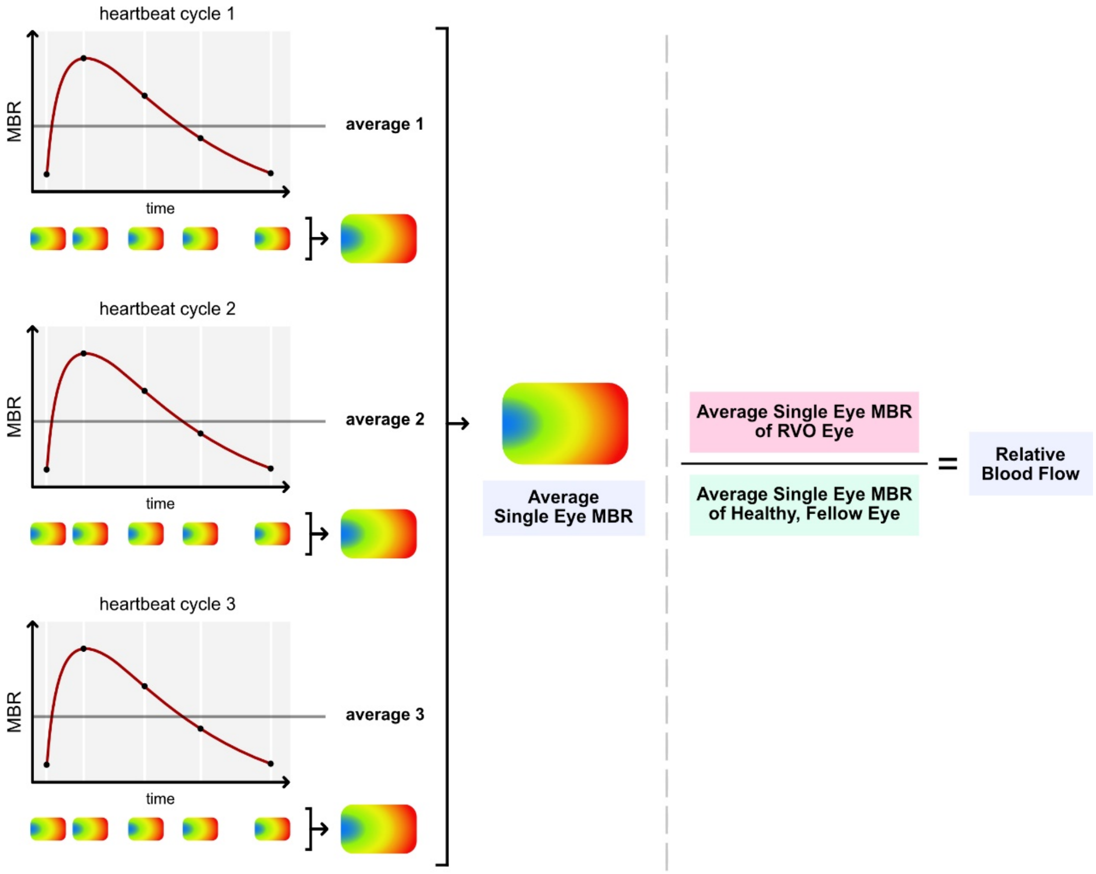
We will now introduce our own terminology, which more specifically describes the data we collected and analyzed from LSFG (Figure 9). We replace the term heartbeat map with "frame MBR" to reflect the instantaneous MBR values we obtain with each frame via an elliptical rubber band on the ONH area. We used MV-MT values to isolate the blood flow in the retinal vessels, and from now on, any reference to MBR values will imply the MV-MT values. When heartbeat maps are combined to form a composite map, these MBR values form a graphical representation of a heartbeat cycle. For ease of analysis, we average the frame MBRs over a single heartbeat cycle to get a "heartbeat MBR," disregarding frame-by-frame variation. For both eyes of each patient, we acquired three heartbeat MBRs from three different heartbeat cycles to minimize any variation that may be due to differences in blood flow between a patient's heartbeats. We average three heartbeat MBR values to get an "average single eye MBR" with standard deviation. Finally, we divide a patient's average single eye MBR from their RVO eye by the average single eye MBR in their healthy fellow eye to get a "relative blood flow." We use relative blood flow (RBF) as our primary metric of blood flow. Since LSFG represents a way to quantify retinal blood flow, we believe it will provide a deeper understanding of blood flow changes in patients with retinal vein occlusions and optimize treatment schedules.

Demographics Variables and Disease Characteristics

Through medical chart review, we identified whether patients had received surgery or laser treatment on either eye prior to the blood flow measurement. Duration of RVO is the amount of time between first diagnosis and measurement of blood flow. Total number of injections takes into account all injections the patient has received for RVO, both before and after the measurement (Table 7). We examined BCVA for 31 patients at the time of blood flow measurement, month zero, and repeated measurements one month and three months later.

Statistical Analyses

The primary analysis consisted of a Welch's t-test between the relative blood flow of CRVO and BRVO eyes to healthy controls. The relative blood flow of healthy controls considered patients' left eye to be the "diseased" eye and numerator of the RBF ratio. We then performed a series of univariate analyses between demographic variables and disease characteristics. These analyses consisted of either Welch t-tests or Pearson's Correlation Coefficients, depending on the nature of the grouping variable. All descriptive statistics and graphs in the Appendix were created using SPSS (IBM Co., Armonk, New York, USA). Calculations of RBF and all related analyses and graphs were generated using R statistical software (version 3.5.2, R Core Team, Vienna, Austria, 2018).



Softcare Co Terminology	Heartbeat Map	Composite Map		
Our Terminology	Frame MBR	Heartbeat MBR	Average Single Eye MBR	Relative Blood Flow

Figure 9. LSFG output, data analysis workflow, and terminology. Each heartbeat cycle, measured over the course of a few seconds, generates a finite number of heartbeat maps (usually around 20-30). Here we have depicted five frames per heartbeat cycle, with the corresponding heartbeat maps below the x-axis. These heartbeat maps are combined to form one composite map for one heartbeat cycle. Although the manufacturers of LSFG, Softcare Co, have devised a terminology for LSFG outputs, we have formulated our own terminology to clarify our data analysis. We imaged three heartbeat cycles per eye per patient, generating a total of six composite maps per visit. The heartbeat MBR values are the average MBRs of each heartbeat cycle. The three heartbeat MBRs for each eye are averaged to give two average single eye MBRs. The average single eye MBR for the RVO eye of a patient is divided by the average single eye MBR of the healthy, fellow eye of the same patient to give the relative blood flow.

RESULTS

Blood Flow in Healthy Eyes

In order to characterize the expected retinal blood flow in the optic nerve head area of healthy eyes, we obtained the LSFG output of the eyes of 20 control patients with no ocular conditions. Figure 10 is a graphical representation of all frame MBR values collected from one subject with no ocular conditions at two imaging sessions, and Figure 11 displays data from all 20 healthy subjects. For ease of analyses, we will compare the average MBR values over the course of a heartbeat in the upcoming sections (heartbeat MBR).

We found no statistically significant difference between the eyes of each control subject ($F_1 = 0.83$, $P = .37$). We also found no statistically significant difference between repeat imaging sessions of each control subject ($F_1 = 0.012$, $P = .91$). We found statistically significant differences when we compared the heartbeat MBR values obtained from the healthy eyes of multiple control subjects ($F_{19} = 14.60$, $P < .001$) (Figure 11). Thus, the heartbeat MBR measurement is consistent between images and eyes but not between patients.

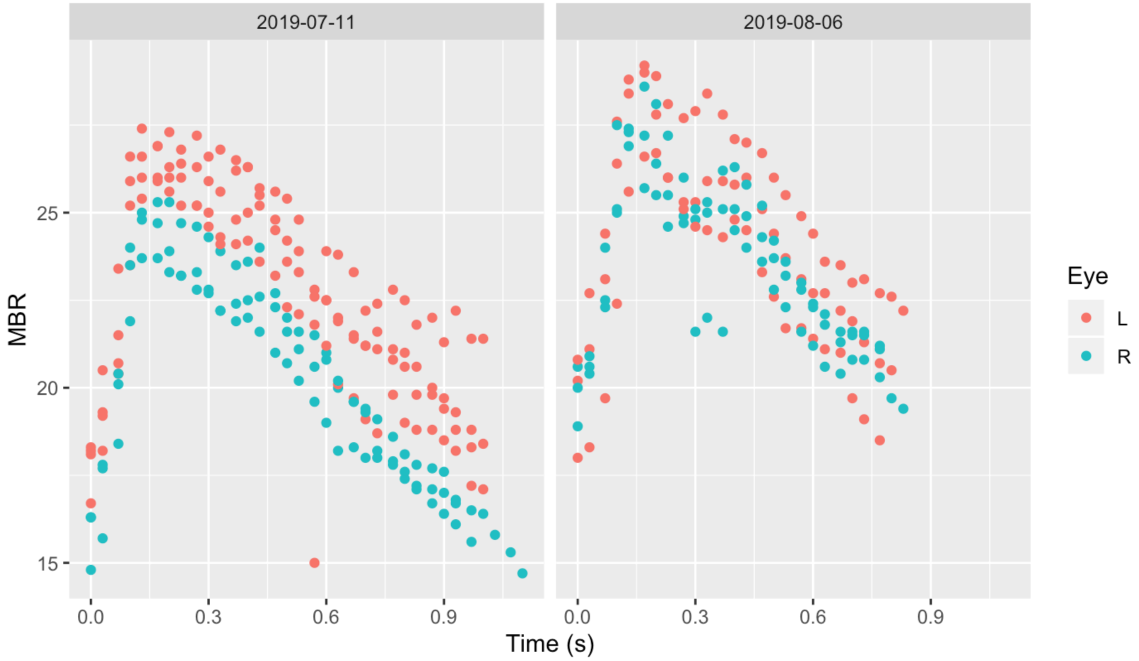


Figure 10. Frame mean blur rate measurements of the healthy eyes of one control subject with no ocular conditions at two separate sessions. Aqua dots represent frame MBR values collected from the ONH are of the right eye (R). Red dots represent frame MBR values collected from the ONH area of the left eye (L). The imaging sessions occurred on two dates: 11 July 2019 (left panel) and 06 August 2019 (right panel). Time is measured in seconds (s) and MBR in arbitrary units. At each imaging session, three composite maps were collected. Images for the composite map were taken automatically over a heartbeat cycle, which is reflected in the MBR values and shape of the graphs. There are no significant differences between the left and right eyes of this patient ($F_1 = 0.83$, $P = 0.37$). Additionally, there are no significant differences between measurements collected at the two sessions ($F_1 = 0.013$, $P = .91$).

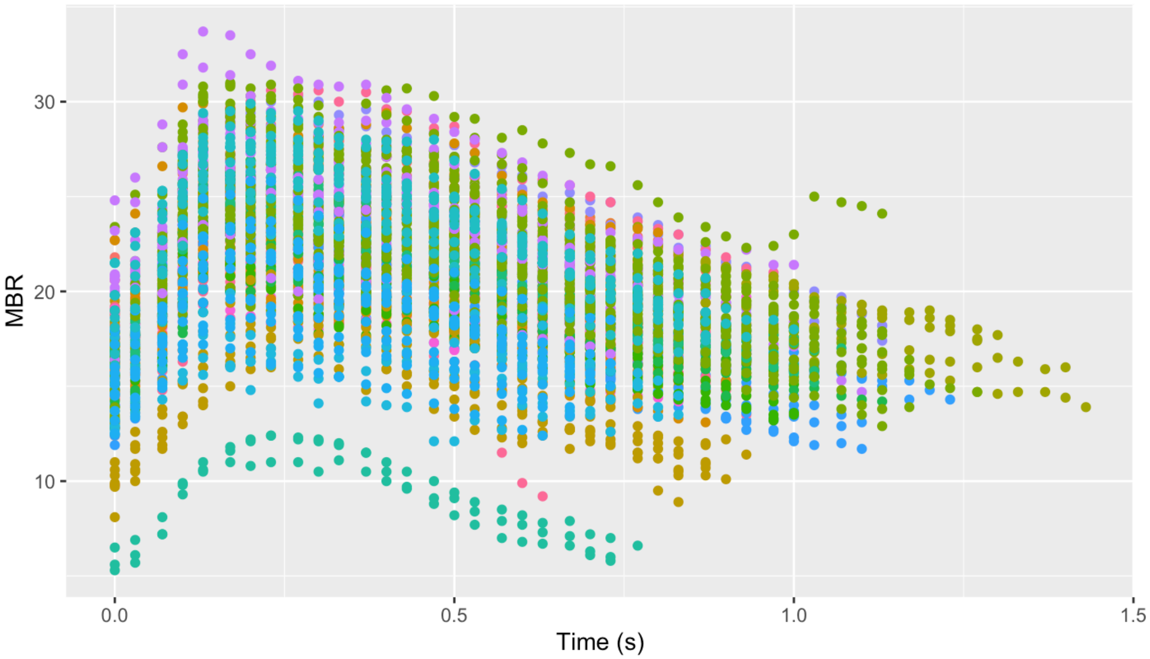


Figure 11. Frame mean blur rate measurements of the healthy eyes of all control subjects (n = 20). Each color represents a different patient. Time is measured in seconds (s) and MBR in arbitrary units. The overall shape of the graph represents the heartbeat cycles of multiple subjects. There is a statistically significant difference in heartbeat MBR values between patients ($F_{19} = 14.60$, $P < .001$).

Patient's Healthy Fellow Eye as an Expectation

The goal of analyzing healthy control patients was to determine if patients with monocular diseases could use the blood flow in their healthy, fellow eye as an expectation for blood flow in their diseased eye. Specifically, we wanted to know if the mean blur rate of a patient's healthy, fellow eye could serve as an expected value for their diseased eye. If their healthy, fellow eye has a fairly consistent mean blur rate, then we can divide the average single eye MBR of their diseased eye by the average single eye MBR of their healthy eye to get a relative blood flow measurement. The importance of the relative blood flow measurement is that it quantifies, as a percentage, the extent to which their disease has affected the retinal vascular blood flow in that eye.

Figure 12 shows the distribution of the differences in heartbeat MBR between the two eyes of each control subject. It is a nearly normal distribution with 95% of patients' eyes differing by 7 units or less. One way to measure the potential error in using patients' fellow eyes as reference is to calculate the root-mean-squared-error (RMSE) of a linear regression model predicting a patient's right eye MBR from their left eye's value. RMSE is particularly useful as it is a measurement of the average difference between values that gives greater weight to larger deviations. We wanted to avoid the possibility of large discrepancies producing false relative blood flow measurements and skewed results. The RMSE of a linear regression model predicting right eye MBR using the same patient's left eye MBR and an intercept of 0 is 2.79, a difference of 13.8% from the average single eye MBR value.

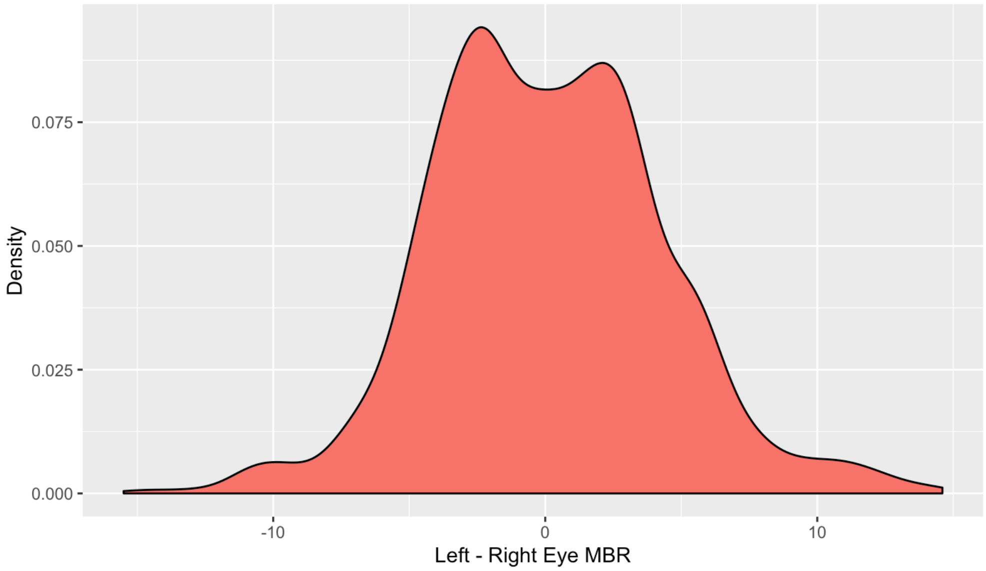


Figure 12. Difference in heartbeat mean blur rate between the eyes of all control subjects (n = 20). The goal of this initial analysis was to determine if patients' healthy, fellow eyes could be used as healthy expectations for their diseased eyes. If so, their relative blood flow (MBR of disease eye divided by their MBR of healthy eye) would be a useful metric that describes the effect of their disease on retinal vascular blood flow. The first step in this process was to explore the difference in heartbeat MBR between the eyes of control subjects. For each control subject, heartbeat MBR values from the right eye were subtracted by the heartbeat MBR values from the left eye and plotted. 95% of patients had MBR within 7 units of their other eye, with an RMSE 2.79.

Blood Flow in Eyes with Retinal Vein Occlusions

The average single eye MBR of the CRVO eye of patients was lower than that of their healthy, fellow eye. We found that, on average, the blood flow of a CRVO eye was 74±5% that of the blood flow of the healthy eye, indicating a 26% average reduction in blood flow due to the occlusion (Figure 13). The average single eye MBR of the BRVO eye of patients was also lower than that of their healthy, fellow eye. We found that, on average, the blood flow of a BRVO eye was 93±5% that of the blood flow of the healthy eye, indicating a 7% average reduction in blood flow due to the occlusion (Figure 13). To incorporate the terminology that we defined earlier, the relative blood flow of CRVO eyes was 74±5% on average, while the relative blood flow of BRVO eyes was 93±5% on average.

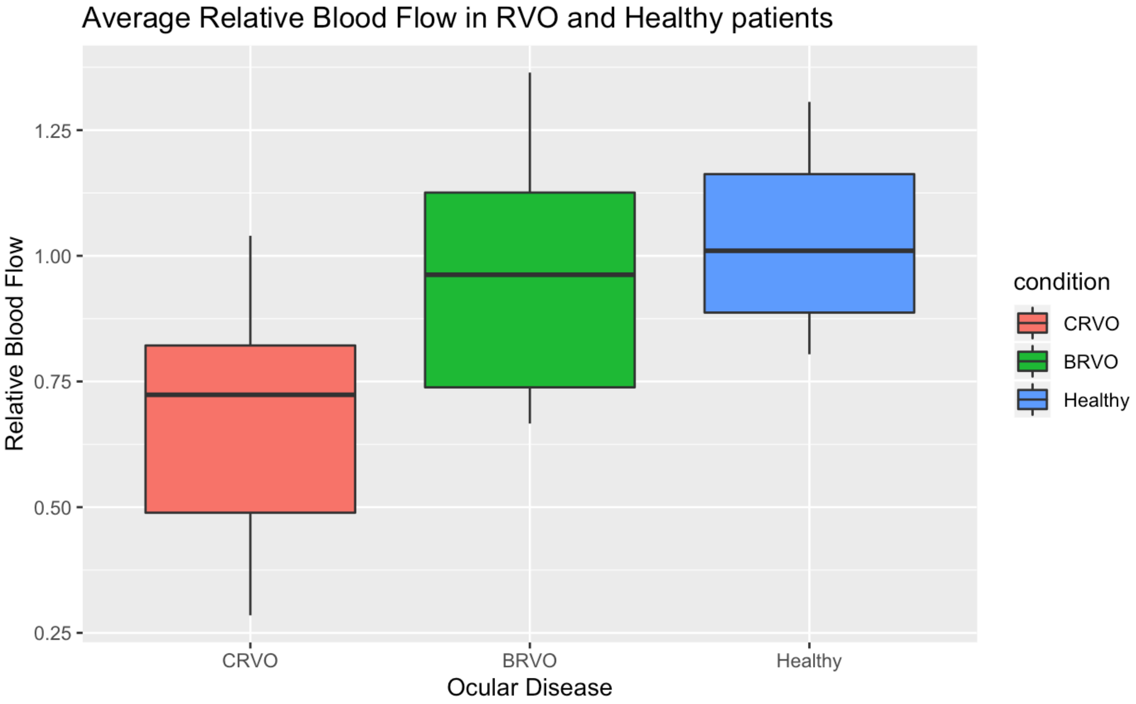


Figure 13. Average relative blood flow in healthy eyes and eyes with central and branch retinal vein occlusion. The red box plot shows the average relative blood flow of eyes with CRVO (*n* = 15, mean ± SD = 0.74 ± .05, *P* < .001). The green box plot shows the average relative blood flow of eyes with BRVO (*n* = 17, mean ± SD = 0.93 ± .05, *P* = .15). The blue box plot shows the average relative blood flow of healthy eyes (*n* = 32, mean ± SD = 1 ± .01). The average relative blood flow of healthy eyes took the patient's left eye as the "diseased" eye during calculations.

Demographics of Retinal Vein Occlusions

We examined the association of a variety of demographic factors, systemic and ocular conditions, treatments, and visual acuity (Tables 4-6) with the relative blood flow. The average age of patients was 67.53 years, the median age was 69.5 years, the standard deviation (SD) was 12.3 years, the minimum was 33 years, and the maximum was 93 years of age (Appendix, Figure A).

For the duration of RVO data set, even though an outlier at 240 months was excluded, the data is left skewed. Likewise, the data set for total number of injections has left skew. For these two variables, median is a better metric for analysis (Appendix, Figures B-C). The statistics of these two variables are summarized in Table 7.

BCVA was recorded for 31 patients at the time of blood flow measurement (month zero), for 11 patients one month after the blood flow measurement, and 9 patients three months after the blood flow measurement (Table 8). BCVA at month zero, one, and three are shown in Table 9 and in boxplot form, stratified by need for injection within 3 months after blood flow measurement (Appendix, Figure D). BCVA was recorded at all three time points for only five patients (Appendix, Figure E). Only one of those patients, signified by the orange line, received an injection within three months after blood flow measurement. There was no correlation between RBF and BCVA at month zero in either CRVO eyes (r = -0.03, P = .84) or BRVO eyes (-0.29, P = .14) eyes.

In CRVO patients, we found no significant association between RBF and phakic status, cataract, glaucoma, high cholesterol, hypertension, and total number of injections. We found there was a significant association between RBF and diabetes (t_{19} = -3.44, P = .0028), previous laser (t_{23} = -3.30, P = .0032), previous injection (t_{27} = -2.96, *P* = .0064), need for an injection within three months after blood flow measurement (t_{33} = 2.61, *P* = .013), the duration of CRVO (t_{39} = 4.56 , *P* < .001), CMT (t_{40} = 3.12, *P* = .0034), and IOP (t_{40} = -2.27, *P* = .029). In BRVO patients, we found no significant association between RBF and any demographic variable. All P-values for RBF and RVO are summarized in Table 10.

Table 11 lists the RBFs of patients with or without the dichotomous demographic factors significantly correlated with CRVO. Patients with diabetes, previous laser, or previous injection displayed increased RBFs at the time of measurement in relation to those who did not (Table 11). Patients who needed an injection within three months after the time of measurement had decreased RBF at the time of measurement in relation to those who did not need an injection (Table 11).

Table 4. Frequencies and percentages of dichotomous and categorical demographic characteristics

Characteristic	Frequency	Percent	Characteristic	Frequency	Percent
Eye			**Phakic Status**		
Left	32	50	PCIOL	19	29.7
Right	32	50	Phakic	45	70.3
Total	64	100	Total	64	100
Sex			**Cataract**		
Female	18	56.3	Yes	26	59.4
Male	14	43.8	No	38	40.6
Total	32	100	Total	64	100
Hypertension			**Glaucoma**		
Yes	40	62.5	Yes	8	12.5
No	24	37.5	No	56	87.5
Total	64	100	Total	64	100
Diabetes			**High Cholesterol**		
Yes	20	31.3	Yes	26	40.6
No	44	68.8	No	20	31.3
Total	64	100	Not specified	18	28.1
			Total	64	100
Race			**Hispanic**		
Asian	3	9.4	Yes	5	16.1
African American	8	25.0	No	26	83.9
Hispanic	4	12.5	Not specified	1	--
White	15	46.9	Total	32	100
Not specified	2	6.3			
Total	32	100			
Disease			**BRVO Type**		
BRVO	14	43.8	Inferior	5	29.4
BRVO; ERM	2	6.3	Superior	8	47.1
BRVO; NVD*	1	3.1	Not specified	4	23.5
BRVO Total	17	53.1	Total	17	100
CRVO	13	40.6			
CRVO; ERM	2	6.3			
CRVO Total	15	46.9			
Total	32	100			

*NVD = neovascularization of optic disk

Table 5. Frequencies and percentages of treatments received before blood flow measurement

	Frequency	Percent		Frequency	Percent
Surgery prior to blood flow			**Laser prior to blood flow**		
BRVO			**BRVO**		
Yes	8	47.1	Yes	16	94.1
No	9	52.9	No	1	5.9
Total	17	100	Total	17	100
CRVO			**CRVO**		
Yes	3	20	Yes	12	80
No	12	80	No	3	20
Total	15	100	Total	15	100
Disease Eye			**Disease Eye**		
Yes	11	34.4	Yes	28	87.5
No	21	65.6	No	4	12.5
Total	32	100	Total	32	100.0
Fellow Eye			**Fellow Eye**		
Yes	7	21.9	Yes	1	3.1
No	25	78.1	No	31	96.9
Total	32	100	Total	32	100.0

Table 6. Frequencies and percentages of injection before and after blood flow measurement

Injection prior to blood flow			**Injection within 3 months after blood flow**		
BRVO			**BRVO**		
Yes	14	82.4	Yes	5	29.4
No	3	17.6	No	9	52.9
Total	17	100	Not specified	3	17.6
			Total	17	100
CRVO			**CRVO**		
Yes	11	73.3	Yes	6	40.0
No	4	26.7	No	6	40.0
Total	15	100	Not specified	3	20.0
			Total	15	100
Disease Eye			**Disease Eye**		
Yes	25	78.1	Yes	11	34.4
No	7	21.9	No	15	46.9
Total	32	100.0	Not specified	6	18.8
			Total	32	100.0

Table 7. Statistics on the duration of retinal vein occlusion and total number of injections received

	n	Mean	Median	SD	Range	Min	Max
Duration of RVO in months (in years)	31	40.61 (3.38)	31 (2.58)	33.71 (2.81)	121	0 (0)	121 (10.1)
Total Number of Injections	32	6.69	5	6.20	20	0	20

Table 8. BCVA values of RVO patients 0, 1, and 3 months after blood flow measurement

Disease			BCVA Month 0* (LogMAR)	BCVA Month 1 (LogMAR)	BCVA Month 3 (LogMAR)
BRVO	*n*	Valid	17	6	7
		Missing	0	11	10
	Mean		0.40	0.27	0.50
	Median		0.30	0.24	0.48
	SD		0.29	0.11	0.30
	Range		1.24	0.27	0.86
	Minimum		0.06	0.13	0.14
	Maximum		1.30	0.40	1.00
CRVO	*n*	Valid	14	5	2
		Missing	1	10	13
	Mean		0.30	0.12	0.17
	Median		0.30	0.13	0.17
	SD		0.29	0.15	0.09
	Range		1.15	0.43	0.13
	Minimum		0.00	-0.10	0.10
	Maximum		1.15	0.33	0.23

*BCVA Month 0 is the best corrected visual acuity of patients at the time of the blood flow measurement

Table 9. BCVA values of RVO patients 0, 1, and 3 months after blood flow measurement stratified by need for injection within 3 months after blood flow measurement

Need for Injection Within 3 Months After Blood Flow Measurement			BCVA Month 0 (LogMAR)	BCVA Month 1 (LogMAR)	BCVA Month 3 (LogMAR)
No	*n*	Valid	14	8	4
		Missing	26	32	36
	Mean		0.29	0.20	0.24
	Median		0.30	0.21	0.19
	SD		0.16	0.16	0.17
	Range		0.53	0.50	0.38
	Minimum		0.00	-0.10	0.10
	Maximum		0.53	0.40	0.48
Yes	*n*	Valid	11	2	4
		Missing	0	9	7
	Mean		0.49	0.23	0.65
	Median		0.35	0.23	0.65
	SD		0.42	0.14	0.29
	Range		1.28	0.20	0.70
	Minimum		0.02	0.13	0.30
	Maximum		1.30	0.33	1.00
NA	*n*	Valid	6	1	1
		Missing	7	12	12
	Mean		0.26	0.08	0.27
	Median		0.25	0.08	0.27
	SD		0.15	--	--
	Range		0.40	0.00	0.00
	Minimum		0.10	0.08	0.27
	Maximum		0.50	0.08	0.27

Table 10. Test statistics, correlations, and P values comparing relative blood flow and demographic variables in retinal vein occlusions

	CRVO			**BRVO**		
	t statistic	**df**	**P value**	**t statistic**	**df**	**P value**
Phakic Status	1.28	10	0.23	0.219	24	0.83
Cataract	-1.52	13	0.15	-0.37	22	0.72
Glaucoma	1.56	5	0.18	1.39	7	0.21
High Cholesterol	0.41	22	0.69	0.57	14	0.58
Hypertension	-0.71	38	0.48	-0.47	8	0.65
Diabetes	-3.44	19	**0.0028**	-0.96	20	0.35
Previous Laser	-3.30	23	**0.0032**	0.96	3	0.42
Previous Injection	-2.96	27	**0.0064**	0.34	9	0.74
Injection within 3 months after blood flow measurement	2.61	33	**0.013**	0.56	14	0.58
	correlation coefficient		**P value**	**correlation coefficient**		**P value**
RVO Duration	0.59		**<0.001**	0.17		0.40
CMT (Month 0)	0.44		**0.0034**	-0.28		0.15
IOP (Month 0)	-0.34		**0.029**	-0.26		0.29
BCVA (Month 0)	-0.03		0.84	-0.29		0.14
Total Injection	0.20		0.21	-0.19		0.33

Statistically significant values, with a $P < 0.05$ have been bolded.

Table 11. Average relative blood flow for statistically significant dichotomous demographic variables

Average Relative Blood Flow* in CRVO		
	Yes	**No**
Diabetes	0.82	0.60
Previous Laser	0.70	0.52
Previous Injection	0.70	0.53
Need for injection within 3 months of blood flow measurement	0.59	0.76

*Relative blood flow = average single eye MBR of CRVO eye / average single eye MBR of healthy, fellow eye. In this chart, we averaged the relative blood flows for patients with CRVO (*n* = 15). Numbers closer to 1 indicate blood flow closer to the healthy expectation.

DISCUSSION

In this study, we used LSFG to quantify and compare the blood flow in the eyes between and within control subjects. We used this data to validate the blood flow in patients' healthy eyes as an expectation for the blood flow in their RVO eye. We created a metric called relative blood flow to compare the blood flow in diseased eyes to healthy eyes. Finally, we identified the demographic factors that were significantly associated with differences in relative blood flow.

We found that blood flow (average single eye MBR) in each eye of the same control subject were not different, but that the blood flow in the eyes of different control subjects varied significantly. In patients with unilateral RVO, this finding allowed us to use the blood flow of their healthy, fellow eye as a healthy expectation for blood flow in their diseased eye. This allowed us to create a novel metric: relative blood flow, which is the blood flow in the RVO eye divided by the blood flow in the healthy eye. Relative blood flow is a descriptor of the extent to which a retinal vein occlusion impedes blood flow--the severity of an RVO.

We found a 25% average reduction in the relative blood flow of CRVO eyes, and a 7% average reduction in the relative blood flow of BRVO eyes. Since central retinal vein occlusions occur in larger veins than branch retinal vein occlusions, it makes sense that we would see a greater reduction in blood flow in CRVO eyes.

When we looked at the effects of dichotomous demographical variables on relative blood flow in CRVO eyes, patients with diabetes had significantly higher relative blood flow on average than those without. The precise mechanism of this unclear. It is possible that our sample size was too small, the patients with diabetes were controlling it well, or that the diabetic patients had little macular edema. A recent meta-analysis showed that diabetes is not a significant risk factor for RVO (Song et al., 2019). More research must be done to elucidate why patients with diabetes had less severe CRVO.

Having laser treatment or injection prior to blood flow measurement were also significantly associated with greater relative blood flow. Laser photocoagulation and anti-VEGF injections both work to prevent neovascularization. This suggests that decreasing VEGF levels leads to improvement in blood flow. A more detailed follow-up study, taking into account the number and timing of previous laser and injection treatments, may further elucidate the details of this finding.

There was a significant association between relative blood flow and need for an injection within three months after blood flow measurement in patients with CRVO. CRVO eyes with, on average, a 41% reduction in blood flow (59% relative blood flow) did need an injection. CRVO eyes with, on average, a 24% reduction in blood flow (76% relative blood flow) did not need injection. This indicates patients with severe CRVOs, ones resulting in lower relative blood flow, are more likely to receive an injection within three months. Therefore, LSFG may assist clinicians in assessing risk of additional injection in their patients with CRVO.

In CRVO, we found that relative blood flow was significantly positively correlated with the duration of the occlusion, suggesting that the retinal blood flow in the occluded eye recovers over time. Interestingly, in BRVO, this was not the case. Blood flow did not recover significantly over time, but the average reduction is only 7% so the size of the effect may be too small to detect with our current sample size. It is also possible that restoration of blood flow is accomplished in a unique way following CRVO.

There was also a positive correlation between relative blood flow and CMT, suggesting that greater blood flow in CRVO eyes may contribute to increased macular edema. We found a negative correlation between relative blood flow and IOP, perhaps because high IOP may cause venous compression and thereby additionally inhibit blood flow.

We did not uncover any significant associations or correlations with relative blood flow and BRVO. This may be because the reduction in blood flow is so small that detecting significant changes with our sample size is not possible. The location of a BRVO may also play a larger role in impeding blood flow, which may not have been captured by our LSFG analysis methods.

Limitations

This study has several limitations. First, the sample size was relatively small and may be ungeneralizable and underpowered to detect small differences. This was caused primarily by our small recruitment base (patients in BIDMC's Eye Clinic), but was exacerbated by the labor-intensive data collection, which may be an issue for this device’s practical utility. Second, like all imaging devices, LSFG was less effective on patients with media opacities or who had difficulty fixating their vision. Third, we only collected and analyzed blood flow data from the entire ONH area of the retina. Changes to relative blood flow in certain quadrants of the ONH area, or other regions of the retina, were not examined. Finally, the limited timeframe of this study impeded our ability to collect the full range of longitudinal data.

CONCLUSION

In this exploratory study, we were able to validate LSFG as a reliable technology for the measure of intra-patient retinal blood flow and created a new metric of retinal vein occlusion severity, relative blood flow. We used this measure to quantify the decreased blood flow in eyes with retinal vein occlusion. We found significant associations between relative blood flow in CRVO and diabetes, previous laser, previous injection, need for injection within three months of blood flow measurement, duration of occlusion, central macular thickness, and intraocular pressure. Future studies can be done to elucidate these findings—in particular, to assess if relative blood flow can be used as a predictive factor for whether or not a patient will need an injection in the next three months. As a severity metric for retinal vein occlusion, relative blood flow may add depth to diagnoses and streamline treatment plans for patients with retinal vein occlusion.

APPENDIX

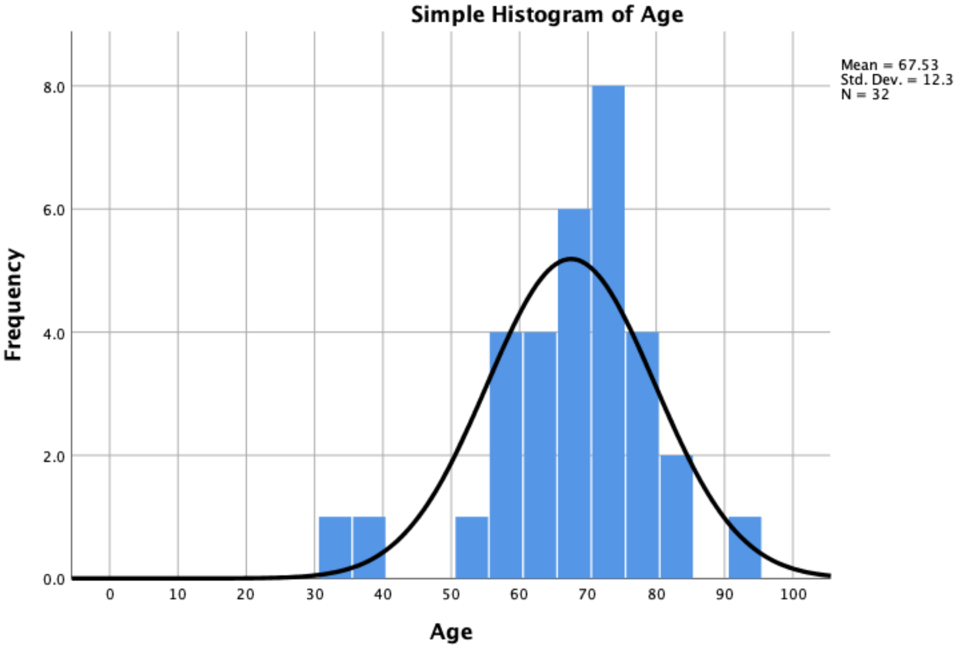


Figure A. A histogram of age (in years). The data follows a fairly normal distribution. Most patients with RVO around 70 years of age.

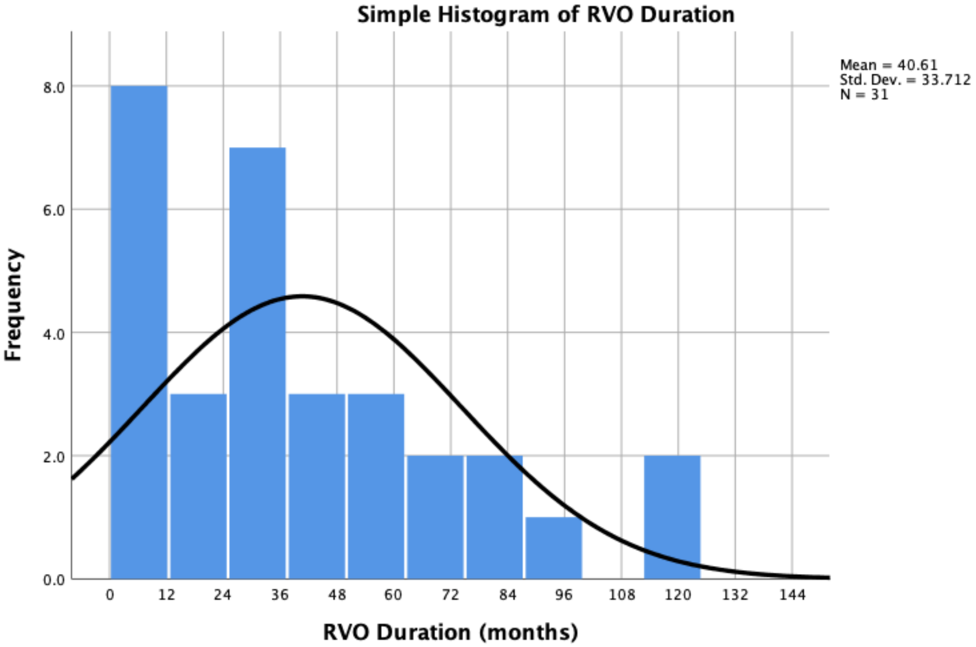


Figure B. A histogram of RVO duration. Note that an outlier at 240 months was excluded from this data set. The data is left skewed.

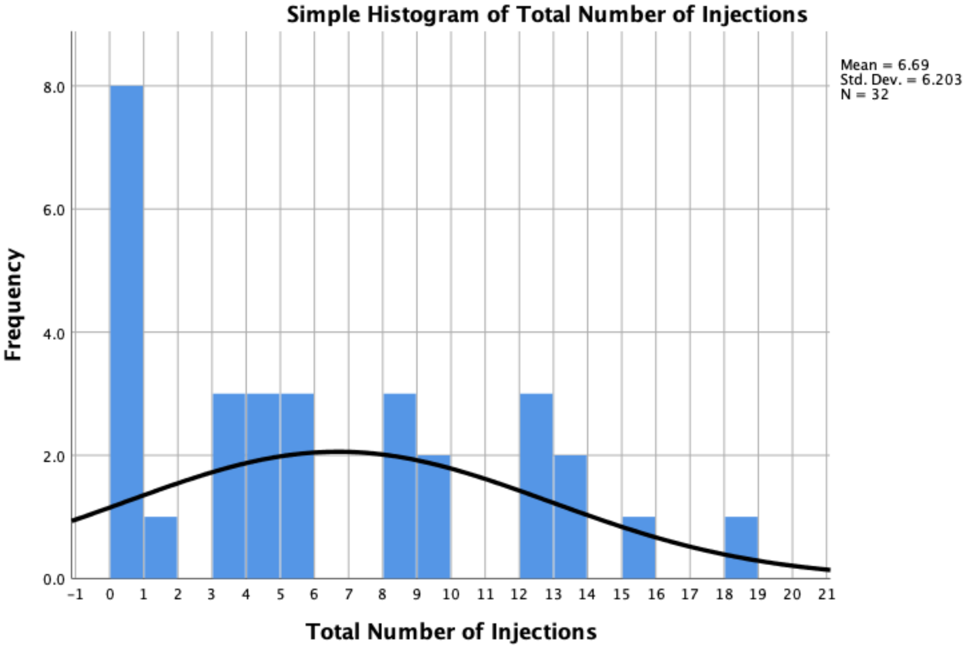


Figure C. A histogram of total number of injections. The data is left skewed, with eight patients receiving no injection.

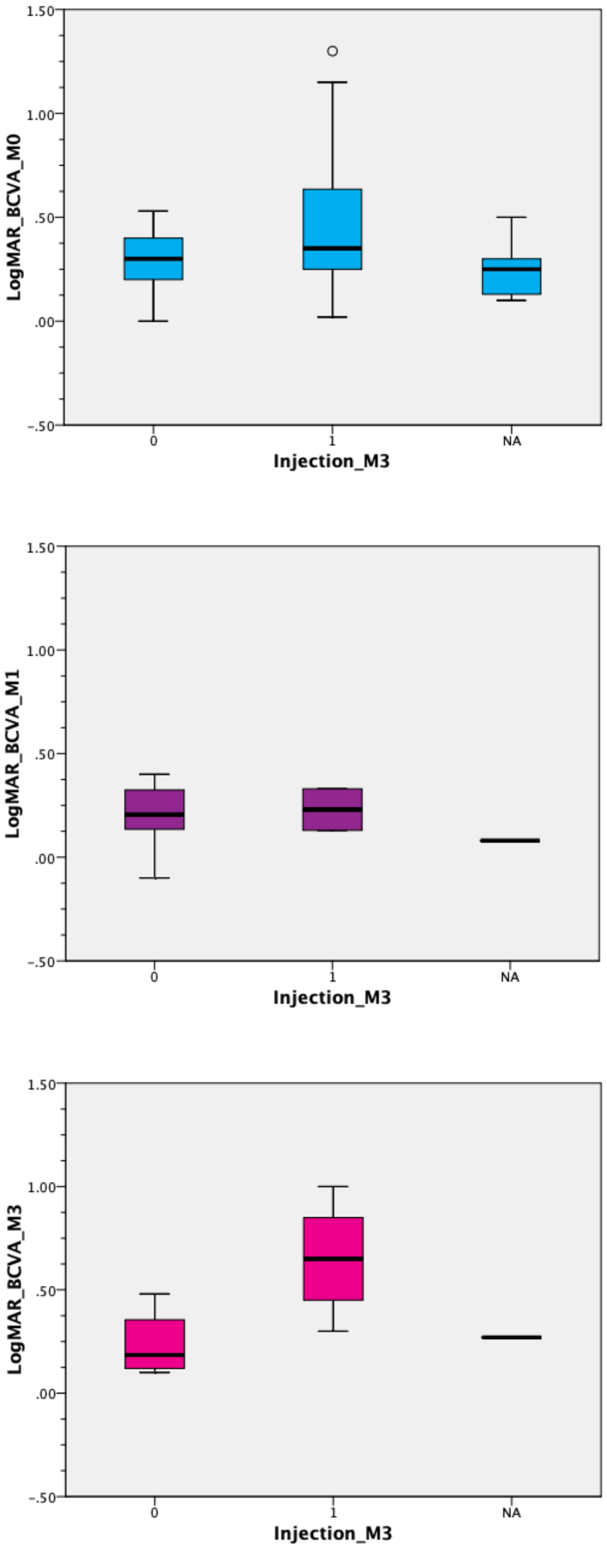


Figure D. Boxplots of best corrected visual acuity at month 0, 1, and 3 stratified by need for injection within 3 months after blood flow measurement. LogMAR_BCVA_M0, LogMAR_BCVA_M1, and LogMAR_BCVA_M3 on the y-axes, are the BCVAs converted to LogMAR units for Month 0, Month 1, and Month 3, respectively. Smaller numbers correspond to better vision, see Table 2 for information. Injection_M3, on the x-axes, is the need for injection within 3 months after blood flow measurement. If no, value = 0. If yes, value = 1. If data was not available, value = NA. Top panel: data from Month 0, *n* = 31. One patient was excluded from data set. Middle panel: data from Month 1, *n* = 11. Bottom panel: data from Month 3, *n* = 9. See Table 9 for statistical data.

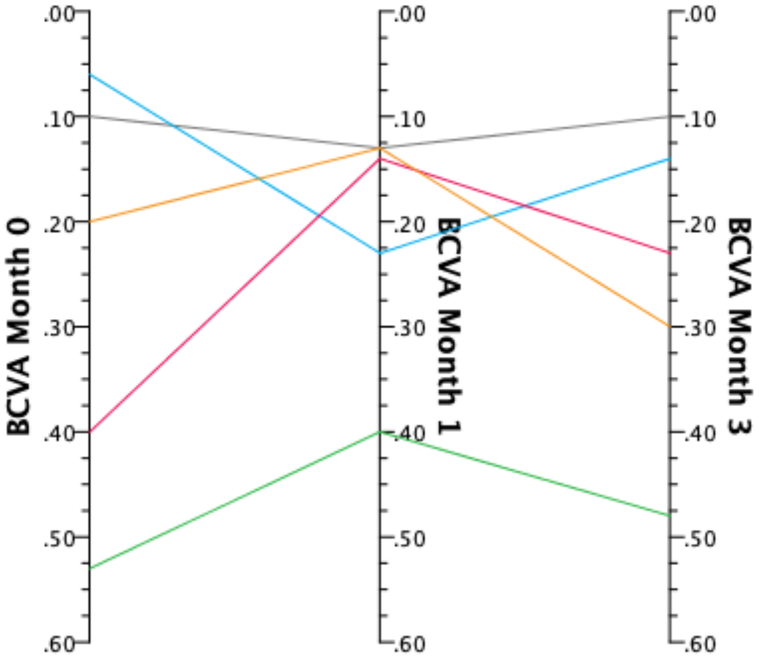


Figure E. Change in visual acuity of five patients over 3 months following blood flow measurement. The visual acuities of five patients were recorded over the course of one and three months following the visit at which their retinal blood flow was measured. The scale is in LogMAR units, where smaller numbers correspond to better vision. Only the patient represented as an orange line received an injection within 3 months after the blood flow measurement. It is worth noting that this patient is also one of two patients who has received the maximum number of total injections (20) within our patient sample group.

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