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Relative Retinal Blood Flow: A Novel and Informative Measure of Unilateral Retinal Vein Occlusion Severity

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Purpose: This study quantifies retinal vascular blood flow affected by unilateral central or branch retinal vein occlusion (CRVO or BRVO). We created a new, unitless metric for the severity of these diseases—relative blood flow (RBF)—and contextualized it with subject demographics, ocular presentation, and systemic conditions. Finally, we explored its efficacy as a predictor of future outcomes.

Methods: Data were collected from 20 control subjects and 32 clinically diagnosed CRVO (n = 15) or BRVO (n = 17) patients. We used laser speckle flowgraphy to quantify blood flow as mean blur rate and present RBF as the ratio between the blood flow in a subject's diseased and undiseased eyes. Because of our demonstration that blood flow has high intrapatient (between eyes and over time) but low interpatient correlation in eyes of healthy subjects, any differences between eyes can be attributed to the disease. These data were correlated with subject demographics and disease characteristics.

Results: In CRVO and BRVO eyes, average blood flow decreased by 26% and 7%, respectively. In CRVO, occlusion duration, central macular thickness, intraocular pressure, diabetes, previous laser and injection treatments, and injection within three months after measurement were significantly associated with RBF. In BRVO, no significant associations with RBF were found.

Conclusions: Blood flow in CRVO and BRVO was reduced compared to the unaffected fellow eye in most patients. RBF was useful in determining the severity of RVOs and predicting future treatment needs.

Translational Relevance: RBF is a promising new and informative metric for quantifying the severity of unilateral RVOs.

Introduction

Retinal vein occlusion (RVO) is second in prevalence to diabetic retinopathy among retinal diseases. In 2015, an estimated 28 million people were affected by RVO globally.[1] RVOs are divided into two subtypes based on the location of the occlusion and severity of symptoms. Central retinal vein occlusion (CRVO) affect the central retinal vein at or near the lamina cribrosa, whereas branch retinal vein occlusion (BRVO) generally affects smaller venules at arteriovenous crossings.[2] The symptoms of CRVO are more severe than BRVO, but both initially manifest as a painless loss of vision usually caused by occlusion-related macular edema.[3]

Diagnosis of RVO is primarily accomplished by a convergence of clinical evidence from fundoscopy, fluorescein angiography, and optical coherence tomography (OCT).[2] These methods focus primarily on the identification of signs such as tortuosity of retinal vessels, presence of macular edema, and areas of retinal nonperfusion. Recently, imaging techniques such as laser speckle flowgraphy (LSFG) and OCT angiography (OCTA) have emerged as more-precise and less-invasive ways of determining changes in retinal blood flow.[4,5]

LSFG, approved by the Food and Drug Administration in 2016, is a relatively new, noninvasive technology for measuring retinal blood flow.[6–9] Although other imaging techniques exist, such as

blue-field simulation, spectral-domain OCT, laser Doppler flowmetry, laser Doppler velocimetry, Doppler Fourier-domain OCT, and swept source OCT, LSFG is unique in that it is a noninvasive technique that allows for quantitative, dynamic imaging of blood flow through retinal vessels and the choroid in the optic nerve head (ONH) and macula.[10–16] OCTA is another new technology that quantitatively characterizes all layers of retinal vasculature.[5,17–22] When compared to LSFG, however, OCTA appears to have issues with limited field of view and lower internal consistency.[5]

LSFG metrics correspond well with our current understanding of retinal blood flow, both in patients with RVO and healthy subjects.[4,23] Mean blur rate (MBR), a quantitative measure of erythrocyte movement based on laser speckle patterns, is the main output of LSFG, and most studies use MBR or a ratio of MBR as a direct correlate of blood flow. In RVO eyes, there is a significant association between vascular MBR and early- and late-phase fluorescein angiography circulation times.[4] Similarly, two studies found a significantly lower blood flow in the ONH in ischemic RVO compared to nonischemic RVO, matching our understanding of severe perfusion deficits in eyes with ischemic RVOs.[24,25] LSFG findings also match our understanding of retinal autoregulation, because pure oxygen inhalation significantly decreases ONH MBR in healthy eyes.[26] Finally, two studies have examined the precision and reliability of relative flow volume (RFV) in retinal vessels, another metric generated by the LSFG software, by comparing it with absolute blood flow measurements from laser Doppler velocimetry and Doppler Fourier-domain OCT .[9,13] In healthy subjects, there is a significant correlation between venous RFV and absolute blood flow.[8,13]

We hypothesize that LSFG can improve RVO diagnosis and provide useful information to help assess the efficacy of RVO treatment. However, because MBR values are manually collected at the researcher's discretion from the ONH, choroid, or specific retinal vessels, it is difficult to compare findings between studies. Analyses are additionally complicated by significant intersubject heterogeneity that goes beyond simple demographic differences.[27,28] Therefore the first aim of this study is to define a novel metric—relative blood flow (RBF)—and demonstrate how it effectively describes the impediment of retinal blood flow in patients with unilateral RVO. The second aim of this study is to compare this metric to known characteristics of RVOs and use it to help predict future outcomes.

Methods

Study Design

This study was a prospective, exploratory analysis of retinal blood flow in patients with retinal vein occlusions.

Participants

The study cohort included 20 control subjects with no ocular conditions or systemic diseases. The experimental cohort consisted of 15 subjects with unilateral CRVO and 17 subjects with unilateral BRVO diagnosed by Beth Israel Deaconess Medical Center's retina services. Patients who had any additional macula-involving disease in either eye, media opacities, or recent (<3 month) history of injections, lasers, or surgery were excluded.

Data Collection

We used LSFG-NAVI (Softcare Co., Ltd., Fukuoka, Japan) to measure dynamics retinal circulation. This imaging technology has been used in numerous studies as a robust method of measuring and quantifying ocular blood flow.[8,9,13,26,29] The system consists of a fundus camera and laser. The laser, which is scattered by erythrocytes traveling through retinal vessels, creates an interference pattern that is subsequently captured by a Charge-coupled Device (CCD) video camera. Softcare software can then be used to mark an area of interest, typically an ellipse around the ONH. By comparing frame-by-frame measurements over the course of a subject's heartbeat, an arbitrary MBR unit can be calculated as a measure of blood flow. More detailed descriptions of this protocol have been described elsewhere.[29] Measurements for both eyes were taken in triplicate for each subject. For each eye, an ellipse rubber band was created to cover the entire ONH. For each measurement of a single eye, the rubber band was automatically placed in the same location to ensure consistency. For each rubber band, the MV – MT MBR measurement was used, which removes the mean MBR of the tissue area from the mean MBR of the vascular area.

Although Softcare Co has codified terminology in their manual, we chose to create our own for clarity and specificity (Fig. 1). "Frame MBR" was the instantaneous MBR value obtained in each frame of the measure. These frame MBRs were averaged over

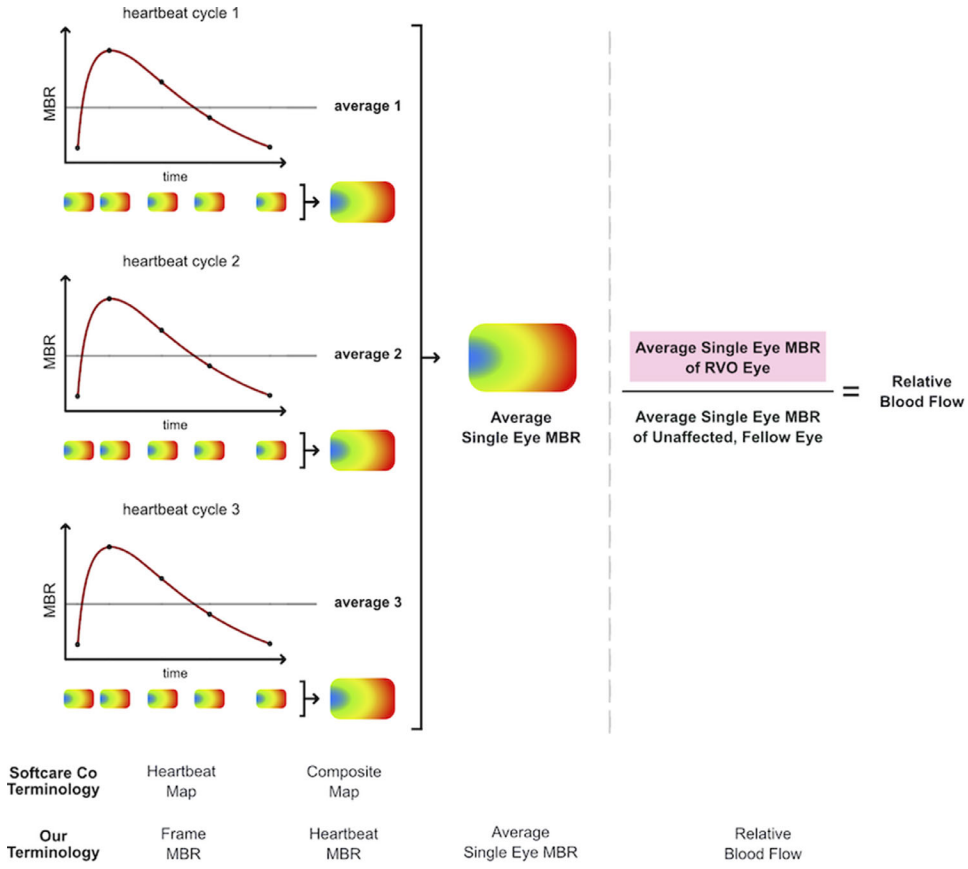

Figure 1. LSFG output, data analysis workflow, and terminology. Each heartbeat cycle, measured over the course of a few seconds, generates a finite number of heartbeat maps (usually around 20–30). Here we have depicted five frames per heartbeat cycle, with the corresponding heartbeat maps below the x-axis. 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 unaffected, fellow eye of the same patient to give the relative blood flow.

the course of a subject's heartbeat to calculate the "heartbeat MBR," the most basic unit of measure and the output of a single LSFG-NAVI video image. The three measurements in each eye were then averaged to obtain a single eye MBR that could be used for comparisons between eyes and to estimate measurement error. Finally, a subject's RBF was calculated by dividing their diseased eye's average MBR by their fellow unaffected eye's average MBR or, in the case of healthy controls, their average left eye MBR by their average right eye MBR. This ratio is our primary metric and is unitless. Therefore an RBF of 0.70 in a CRVO patient indicates that their CRVO eye has 70% of the blood flow in their unaffected fellow eye.

Demographics Variables and Disease Characteristics

Through medical chart review, we identified sex, race, diabetes, hypertension, and high cholesterol status as general demographic variables that put our new metric in context. We also evaluated whether patients had glaucoma, cataract, a posterior chamber intraocular lens, and, in the diseased eye only, epiretinal membrane or neovascularization of the optic disk. We subdivided BRVO patients on the basis of the location of their occlusion—superior or inferior—and calculated duration of RVO as the time between first diagnosis and measurement of blood flow. Before the blood flow measurement, we determined whether

patients had received surgery, injection, or laser treatment on either eye. After the measurement, we identified patients who were prescribed or received injection within three months of that date. Total number of injections takes into account all injections the patient received for RVO, both before and after the measurement. Finally, we examined central macular thickness (CMT), intraocular pressure (IOP), and best-corrected visual acuity at the time of blood flow measurement (month zero) and repeated these measurements one and three months later on a subset of patients. CMT was measured on an OCT Spectralis (Heidelberg Engineering, Heidelberg, Germany) and IOP was measured using a Tono-Pen (Reichert Inc., Depew, New York, USA).

Statistical Analyses

Initial analysis consisted of analysis of variance tests to explore the consistency of MBR between control eyes and the reliability of our consistency assumption. The primary analysis consisted of a Welch's *t*-test between the RBF of CRVO and BRVO eyes to healthy controls. We then performed a series of univariate analyses between demographic variables and disease characteristics to confirm that our metric matches the current understanding of RVOs. Finally, to determine whether RBF was useful in predicting the need for an injection within the next three months, we created a logistic regression model using RVO duration, CMT at time of imaging, and RBF as predictors. The significance of model coefficients and predictive power in the form of both qualitative descriptions and optimism-adjusted bootstrapped C-statistics from the logistic regression model were analyzed.[30] All descriptive statistics were created using SPSS (IBM Co., Armonk, NY, 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). 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.

Results

Blood Flow in Healthy Eyes

While heartbeat MBR varied between subjects ($F_{19} = 14.60$, $P < 0.001$, Fig. 2), there was no statistically significant difference between the eyes of a single control subject ($F_1 = 0.83$, $P = 0.37$) or in repeated imaging sessions ($F_1 = 0.012$, $P = 0.91$). Thus, the heartbeat MBR measurement is consistent between images and eyes of a single patient but not between patients. We then calculated the RBF of healthy eyes for use as a comparison group (mean ± SD = 1 ± 0.01, Fig. 3, blue box).

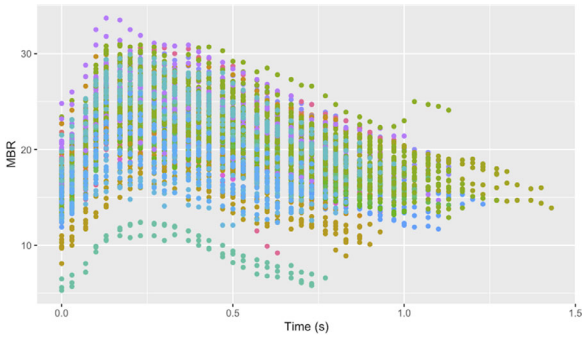

Figure 2. Frame mean blur rate measurements of the healthy eyes of all control subjects (n = 20). Each color represents a different patient. Time is measured in seconds (s) and MBR in arbitrary units. The overall shape of the graph represents the heartbeat cycles of multiple subjects. There is a statistically significant difference in heartbeat MBR values between patients ($F_{19} = 14.60$, $P < .001$).

Blood Flow in Eyes With Unilateral RVO

We examined the blood flow in patients with unilateral CRVO or BRVO. Patients with both CRVO (n = 15, mean ± SD = 0.74 ± 0.05, $P < .001$) and BRVO (n = 17, mean ± SD = 0.93 ± 0.05, $P = 0.015$) had lower RBF than our healthy patients (Fig. 3, red and green boxes). Thus there was an average reduction in blood flow of 30% in eyes with CRVOs and

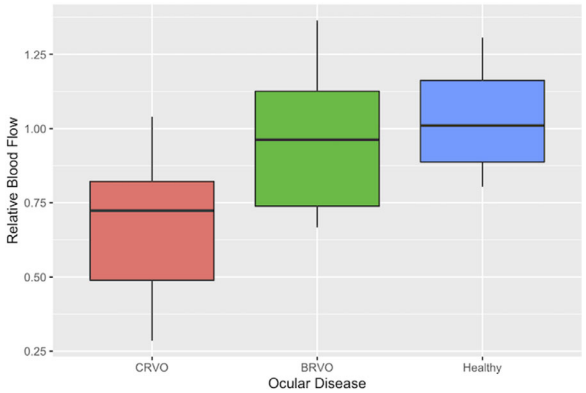

Figure 3. Average relative blood flow in eyes with central and branch retinal vein occlusion compared to healthy eyes. Compared to the average relative blood flow of healthy eyes (*blue box*, n = 32, mean ± SD = 1 ± 0.01), the average relative blood flow of eyes with CRVO (*red box*, n = 15, mean ± SD = 0.74 ± 0.05, $P < 0.001$) and BRVO (*green box*, n = 17, mean ± SD = 0.93 ± 0.05, $P = .015$) were significantly lower. The average relative blood flow of healthy eyes took the patient's left eye as the "diseased" eye during calculations.

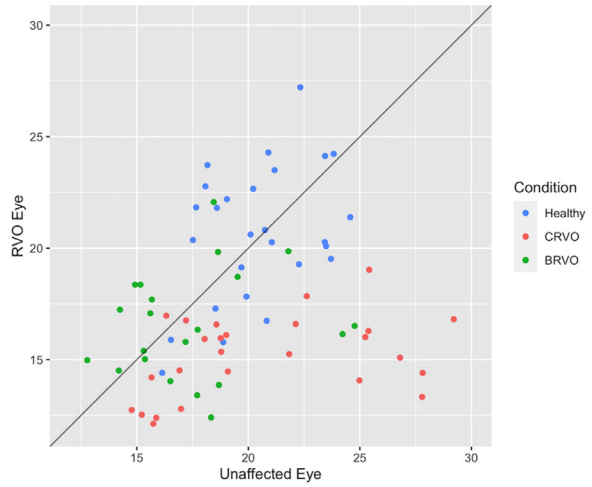

Figure 4. Comparison of MBR between eyes. In healthy control subjects, the average single eye MBRs are consistent between both eyes, clustering around the line of equality. In RVO patients, reduction of MBR in the diseased eye places these patients below the line of equality, with CRVO patients showing a more consistent clustering than BRVO patients. For controls, the left eye was taken to be the "RVO Eye."

9% in eyes with BRVOs. There was, however, substantial heterogeneity within these two groups. In comparing the MBR of the healthy control group eyes to the unaffected eyes in the RVO groups, there was no difference between the control ($n = 40$, mean $= 19.58 \pm 1.13$) and CRVO groups ($n = 15$, mean $= 18.79 \pm 2.09$, $P = 0.52$), but there were lower values in the BRVO group compared to the control group ($n = 17$, mean $= 16.03 \pm 1.93$, $P = .004$) (Fig. 4).

RBF and Other Factors

We examined the association of a variety of demographic factors, systemic and ocular conditions, treatments, and visual acuity with the relative blood flow (Tables 1, 2, and 3A, 3B). Of the descriptive variables, RVO duration ($r = 0.59$, $P < 0.001$), CMT ($r = 0.44$, $P = 0.003$), diabetic status ($t_{19} = 3.44$, $P = 0.003$), history of previous laser ($t_{23} = 3.30$, $P = 0.003$), and history of injection treatments ($t_{27} = 2.96$, $P = 0.006$) were positively associated with RBF in CRVO patients (Fig. 5). Need for injection within three months ($r = -2.61$, $P = 0.013$) and IOP ($r = -0.34$, $P = 0.029$) were negatively associated with RBF in CRVO patients (Fig. 5). No descriptive variables were significantly associated with BRVO patients. Excluding four subjects with glaucoma did not significantly change RBF findings for the CRVO (mean $= 0.75$, $t_{102} = 0.48$, $P = 0.63$) or BRVO (mean $= 0.92$, $t_{66} = 0.12$, $P = 0.90$) groups.

Table 1. Patient Demographics

	CRVO	BRVO	RVO Total	Control
Eye				
Left	8 (53.3)	7 (41.2)	15 (46.9)	—
Right	7 (46.7)	10 (58.8)	17 (53.1)	—
Total	15	17	32 (100)	—
Sex				
Female	6 (40)	12 (70.6)	18 (56.3)	13 (65)
Male	9 (60)	5 (29.4)	14 (43.8)	7 (35)
Age, Mean (yrs)	62	74	68	37
PCIOL				
Disease eye	3 (20)	8 (47.1)	11 (34.4)	—
Fellow eye	3 (20)	5 (29)	8 (25)	—
RVO eye—Other Disease				
Epiretinal membrane	2 (13.3)	2 (11.8)	4 (12.5)	—
Neovascularization of the disk	0 (0)	0 (0)	0 (0)	—
Hypertension	8 (53.3)	12 (70.6)	20 (62.5)	—
Diabetes	4 (26.7)	6 (35.3)	10 (31.3)	—
Cataract	6 (40)	8 (47.1)	26 (59.4)	—
Glaucoma	2 (13.3)	2 (11.8)	4 (12.5)	—
High cholesterol				
Yes	5 (33.3)	8 (47.1)	13 (40.6)	0 (0)
No	5 (33.3)	5 (29.4)	10 (31.3)	20 (100)
Not specified	5 (33.3)	4 (23.5)	9 (28.1)	0 (0)
Race				
Asian	2 (13.3)	1 (5.9)	3 (9.4)	7 (35)
Black/African American	3 (20)	5 (29.4)	8 (25)	1 (5)
Hispanic	1 (6.7)	3 (17.7)	4 (12.5)	4 (20)
White	7 (46.7)	8 (47.1)	15 (46.9)	8 (40)
Not specified	2 (13.3)	0 (0)	2 (6.3)	0 (0)
BRVO type				
Superior	—	5 (29.4)	—	
Inferior	—	8 (47.1)	—	
Not specified	—	4 (23.5)	—	
Total	—	17 (100)	—	
Prior surgery				
Disease eye	3 (20)	8 (47.1)	11 (34.4)	
Fellow eye	3 (20)	4 (23.5)	7 (21.9)	
Prior laser				
Disease eye	12 (80)	16 (94.1)	28 (87.5)	
Fellow eye	1 (6.7)	—	1 (3.1)	
Prior injection				
Disease eye	11 (73.3)	14 (82.4)	25 (78.1)	
Fellow eye	0 (0)	0 (0)	0 (0)	
Injection within 3 months	6 (40)	5 (29.4)	11 (34.4)	
Duration of RVO (mos)				
Mean	35.50 (3.0)	44.82 (3.7)	40.61 (3.4)	
Median	25.50 (2.1)	36	31	
SD	35.90 (3.0)	32.28 (2.7)	33.70 (2.8)	
Range	120	118	121	
Min	0	3	0	
Max	120	121	121	
Total number of injections				
Mean	8.13	5.41	6.69	
Median	9	4	5	
SD	7.1	5.17	6.2	
Range	20	20	20	
Min	0	0	0	
Max	20	20	20	

Max, maximum; Min, minimum; PICOL, posterior chamber intraocular lens.

Table 2. Best-Corrected Visual Acuity Measurements (LogMAR)

	CRVO	BRVO	No Injections Within 3 Months	Injections Within 3 Months
Month 0				
n	15	17	14	11
Mean	0.3	0.4	0.29	0.49
Median	0.3	0.3	0.3	0.35
SD	0.29	0.29	0.16	0.42
Range	1.15	1.24	0.53	1.28
Min	0	0.06	0	0.02
Max	1.15	1.3	0.53	1.3
Month 1				
n	5	6	8	2
Mean	0.12	0.27	0.2	0.23
Median	0.13	0.24	0.21	0.23
SD	0.15	0.11	0.16	0.14
Range	0.43	0.27	0.5	0.2
Min	−0.1	0.13	−0.1	0.13
Max	0.33	0.4	0.4	0.33
Month 3				
n	2	7	4	4
Mean	0.17	0.5	0.24	0.65
Median	0.17	0.48	0.19	0.65
SD	0.09	0.3	0.17	0.29
Range	0.13	0.86	0.38	0.7
Min	0.1	0.14	0.1	0.3
Max	0.23	1	0.48	1

Max, maximum; Min, minimum.

In subjects with CRVO, RBF had significant predictive power of need for injection within three months, independent of that visit's CMT and RVO duration (Table 4). Among six subjects who had CMT in normal ranges, three subjects had RBF < 0.60 (0.30, 0.57, 0.57), whereas the other three had RBF > 0.75 (0.86, 0.80, 0.76). Those with RBF < 0.60 needed an injection in three months, whereas those with RBF > 0.75 did not. We also observed the predictive power of RBF quantitatively in our optimism-adjusted bootstrapped c-statistics in models: RVO duration and CMT without RBF (0.80) differed from RVO duration and CMT with RBF (0.85) (Fig. 6). This means the model can correctly predict the need for injections within three months 80% of the time using RVO duration and CMT alone and 85% of the time if RBF is used as well.

Discussion

Relative blood flow, the extent to which the blood flow in an eye is compromised by a retinal vein occlusion compared to the blood flow in the unaffected fellow eye, represents a promising new and informative metric for quantifying the severity of unilateral retinal vein occlusions at a single time point. In this study, we first demonstrated the consistency of blood flow between a healthy subject's eyes and observed decreased blood flow in eyes with retinal diseases before using this knowledge to consider a patient's fellow eye as a healthy expectation of their diseased eye. By finding the unitless ratio between a single patient's eyes, we then measured the extent to which the RVO impedes blood flow in the form of relative blood flow. Finally, we found associations between RBF and established descriptive variables and used it as an independent predictor of a patient's need for injection in the next three months.

Our novel RBF measure has substantial advantages over previously used metrics. Although previous studies use LSFG to evaluate changes to retinal blood flow in eyes with RVO, there is a lack of consensus on how best to analyze and interpret various MBR values within and between patients. Many studies are unable to interpret MBR values collected from one session and rely on MBR ratios generated over

Table 3A. Test Statistics, Correlations, and *P* Values Comparing RBF and Demographic Variables in RVOs

	CRVO RBF			BRVO RBF		
	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

Statistically significant values ($P < .05$) are bold.

Table 3B. Test Statistics, Correlations, and *P* Values Comparing RBF and Demographic Variables in RVOs

	Correlation Coefficient	*P* Value	Correlation Coefficient	*P* Value
Age	0.16	0.31	−0.28	0.15
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 ($P < 0.05$) are bold.
BCVA, best-corrected visual acuity.

Table 4. Predictive Power of RBF on Need for Injection Within the Next Three Months

	Coefficient	SE	*P* Value
Central macular thickness (month 0)	0.017	0.007	0.013
RVO duration	−0.037	0.017	0.032
Relative blood flow	−7.49	2.99	0.018
Optimism-adjusted bootstrapped C-statistic			
Duration + CMT	0.80		
Duration + CMT + RBF	0.85		

Analysis of coefficients and c-statistic of logistic regression model.

multiple visits.[6,24,26,31,32] Furthermore, RFV is reliable only when examining venous flow, but heterogeneity between the vasculature of a patient's eyes, as well as between the eyes of many patients, limit the practicality of this measure. RBF is an intuitive, unitless metric that can be collected in one session and, in theory, be applied to any empirical measure of retinal blood flow, not just the LSFG.

RBF appears to be more useful in describing CRVOs than BRVOs. This is primarily because CRVOs impede blood flow around the entire optic nerve, whereas BRVOs only affect blood flow around part of the nerve (30% reduction as opposed to 9% reduction). CRVO RBF also correlates with multiple descriptive factors and predicts need for future injections, whereas BRVO RBF does not. As such, we will primarily be discussing the implications of CRVO RBF here.

RBF correlates with a variety of descriptive characteristics of CRVOs. Older RVOs and those with previous treatments had less obstructed blood flow,

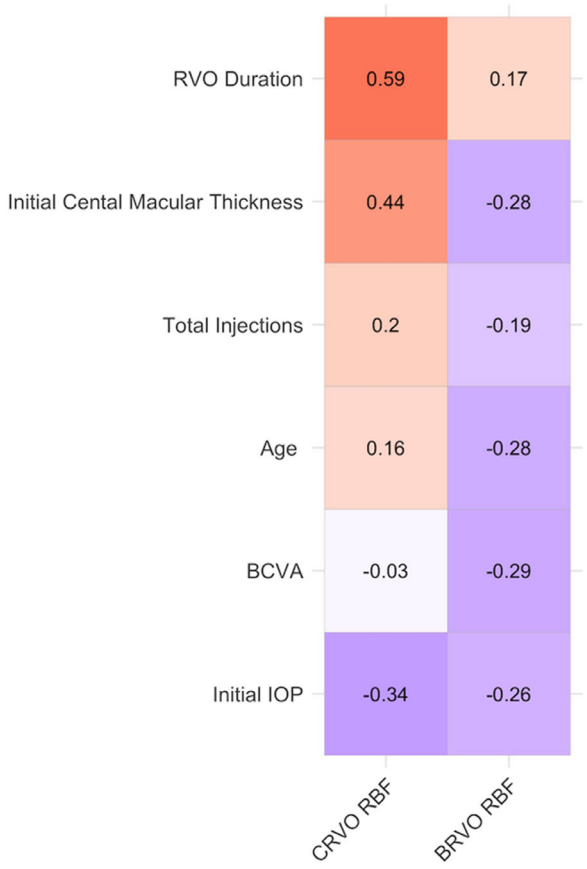

Figure 5. Correlations between relative blood flow and covariates in eyes with CRVO and BRVO. A heat map of the correlations between the continuous covariates and outcomes. *Red cells* indicate positive correlations, whereas *blue* indicate negative correlations. The intensity of the color indicates the strength of the correlation.

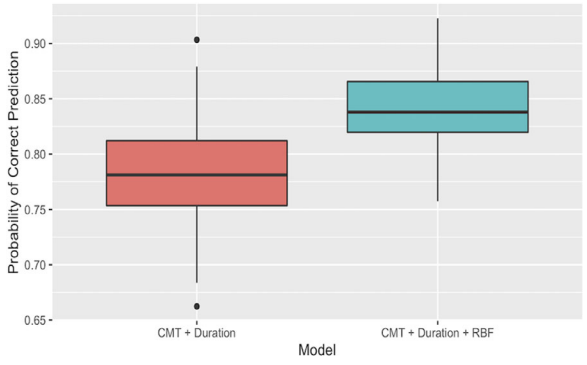

Figure 6. Predictive power of logistic regression model with and without the novel RBF metric. Relative blood flow added significant predictive power to a logistic regression model predicting need for injection within three months of measurement in patients with CRVO. This power was in addition to (and thus independent of) central macular thickness and the duration of time since the onset of patients' symptoms. Thus RBF adds new and important information that a clinician may wish to use while evaluating patients with this condition.

consistent with the idea that laser or injection treatments may help improve perfusion and that the retinal vascular occlusion recannulates or develops collateral vessels, reducing resistance over time. Our findings appear to confirm two recent studies that find a correlation between intravitreal injection and subsequent increase in blood flow.[6,33] Subjects with diabetes had greater (more normal) RBF than those without, although it is notable that these patients had no history of diabetic retinopathy. This finding is consistent with previous reports that patients with diabetes but no retinopathy have increased retinal blood flow compared to healthy controls.[34] Finally, CMT was positively correlated with RBF, indicating that subjects with a significant amount of edema actually have fairly normal blood flow. We do not believe that macular edema interfered with data quality, because our images and data were collected from the ONH area, not the macular area. For a similar reason, we do not believe that the presence of an epiretinal membrane should interfere with RBF measurement at the ONH, consistent with previous studies comparing LSFG measurements before and after vitrectomies.[35,36]

It has long been known that RVOs result in reduced retinal perfusion, but they are generally described in terms of the related symptoms (such as macular edema and CMT, tortuosity of blood vessels, and presence of nonperfusion)—rather than as a direct, quantitative measurement of the blood flow. In general, qualitative, symptom-based characterization is adequate, and outcomes can often be quite good after treatment. However, there are opportunities for additional data to be clinically useful. Current treatment protocols rely heavily on clinician intuition, and it is not always clear when to transition patients from monthly schedules and how frequently injections should be administered after symptoms have diminished.[37–39] Our RBF measure, which aids in predicting need for anti-Vascular Endothelial Growth Factor (VEGF) injections independently of from RVO duration and CMT, could help to inform treatment strategies and perhaps decrease the frequency of injections required for effective RVO management.

In our patients, we found that there was no difference in the MBR of the unaffected CRVO eyes when compared to the control group, but that the MBR of unaffected BRVO eyes was significantly lower. This difference is most likely explained by the higher age of BRVO patients when compared to the other groups and the well-documented finding that MBR decreases with age.[13,40] Notably, there was no correlation between RBF and age in either group, indicating that the metric is associated with disease-related differences rather than demographic

changes. Age-related changes to MBR also highlight why it is useful to use the intrapatient control of patient's fellow eye when making comparisons of blood flow.

This study has several limitations. First, the sample size was relatively small and may be ungeneralizable and underpowered to detect small differences or to perform subgroup analyses. Our recruitment base was fairly small, and labor-intensive data collection exacerbated patient recruitment, which may be an issue for this device's practical utility. Second, the RBF metric cannot be used on patients with bilateral retinal disease, because it relies on a patient's unaffected fellow eye as an internal control. Third, like all imaging devices, LSFG was less effective on patients with media opacities or who had difficulty fixating their vision. Fourth, 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 but may also be informative, especially in BRVO. Because our metric is unitless, other imaging techniques that capture blood flow in different areas should be able to use RBF to expand our findings. Fifth, arterial blood pressure and ocular perfusion pressure were not obtained, because RBF values were calculated within each patient at one time point. It is possible that significant differences in IOP between the eyes of a patient may affect RBF; however, a previous study only noted significant changes in MBR at the ONH when IOP was increased by 20 mm Hg.[41] The average IOP difference between eyes for our patients was 1.6 mm Hg, with the highest difference being 6 mm Hg. Previous studies show no significant correlation between LSFG indices and mean arterial pressure or ocular perfusion pressure.[8,9,26] Finally, the limited timeframe of this study impeded our ability to collect the full range of longitudinal data.

In this exploratory study, we were able to validate LSFG as a reliable technology for the measure of intrapatient 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 may further elucidate these findings—in particular, studies should assess whether relative blood flow can be used as a predictive factor for whether a patient will need an injection in the next three months and explore this metric in other imaging devices, such as OCTA. 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.

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