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Case Series

Case series of retinal vein occlusions showing early recovery using oral L-methylfolate

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Abstract: This case series describes the aggregate rate of recovery in five consecutive subjects (six eyes) with retinal vein occlusion (RVO) who received L-methylfolate and other vitamins *via* Ocufolin®, a medical food. Subjects were followed for 10–33 months by a single ophthalmologist. Ocufolin® was prescribed at the time of diagnosis and subjects remained on the regimen throughout the time of observation. Examinations were performed in an unmasked fashion at 3-month intervals with recording of best corrected visual acuity (BCVA), average retinal nerve fiber layer (ARNFL) and central macular thickness (CMT), and fundus (examination of the retina, macula, optic nerve, and vessels) photography. Testing was done for vitamin deficiencies, vascular and coagulable risk factors, and methylenetetrahydrofolate reductase (MTHFR) polymorphisms. Vitamin deficiencies and vascular risk factors were found in all subjects, and all four tested subjects carried at least one MTHFR polymorphism. By the end of the study period BCVA in all subjects was 20/25 or better. Cystoid macular edema was identified and measured by optical coherence tomography (OCT). The percent change was calculated and plotted at 3-month intervals using the percent change in thickness from the time of diagnosis and percent change toward normative values for ARNFL and CMT. The total reduction in thickness of ARNFL and CMT from time of diagnosis was 44.19% and 30.27%, respectively. The comparison to normative data shows a reduction of ARNFL from 164.2% to 94% and CMT from 154.4% to 112.7% of normal thickness (100%). Plots showed the aggregate recovery was most rapid over the first 3 months and slowed over the next 3 months with most of the recovery taking place within 6 months of treatment. The rate of improvement in BCVA and resolution of retinal thickening was found to be better than predicted on historical grounds. No subjects progressed from nonischemic to ischemic RVO. Vitamin deficiencies, vascular risk factors, and genetic predisposition to oxidative stress were common in this RVO series. It appears that addressing these factors with Ocufolin® had a salutary effect on recovery.

Keywords: L-methylfolate, MTHFR polymorphism, nutritional deficiency, retinal ischemia, retinal vein occlusion

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Introduction

Retinal vein occlusion (RVO) is a relatively common retinal vascular disease. Pooling large RVO population studies from the USA, Europe, and Australia, RVO prevalence was found to be 5.2 per 1000 patients.[1] Many first RVOs are silent, one study of 49 cases of RVO revealed that 18% had a previously undiagnosed RVO.[2] RVO presents a continuum of mild to severe ischemia which is the final common pathway to vision loss. The division between 'nonischemic RVO' and 'ischemic RVO' is somewhat arbitrary and was made to anticipate prognosis. Classical 'nonischemic RVO' shows mild findings of ischemia, such as mild capillary dropout and microscopic non-perfusion, while classical 'ischemic RVO' has more generalized capillary distention, drop out, and non-perfusion with more pronounced venous dilation, exudation, lower blood flow, and by implication increased oxidative stress.[3]

Hayreh *et al.* reviewed over 1000 cases of RVO and found that the cumulative probability of developing a recurrence within 4 years was 2.5%

in the same eye, and up to 11.9% in the fellow eye. The cumulative probability of progression to ischemic RVO increased with age and longer follow-up. In people aged 45–64, the incidence at 6 and 18 months was 6.7% and 8.1%, respectively. In persons 65 and older, the rate of ischemic progression was 13.2% and 18.6%.[3] A review of 24 RVO studies involving subjects who lost vision due to RVO confirmed that the degree of ischemia and resultant structural loss were major factors in the prognosis for vision loss or recovery and that improvement of acuity to better than 20/40 was uncommon.[4] McIntosh *et al.* reviewed 53 central retinal vein occlusion (CRVO) studies, finding that ultimate best corrected visual acuity (BCVA) outcomes were poor, with none better than 20/40, most declining further over time, and with 25% becoming ischemic.[5]

There are parallels between the issues we describe with RVO and diabetic retinopathy (DR). Emily Chew of the National Institute of Health Department of Epidemiology has reviewed the changing landscape in the treatment of DR from 1979 with the Early Treatment of Diabetic Retinopathy Study (ETDRS) through 1989. The ETDRS study showed that focal and grid photocoagulation was superior to observation in subjects with diabetic retinopathy and macular edema (ME), making photocoagulation the standard therapy for decades.[6,7] Dr Chew then points to the 2010 studies published demonstrating that intravitreal injections of anti-vascular endothelial growth factor (anti-VEGF) drugs were superior to grid photocoagulation in preventing further vision loss in a patient population similar to the ETDRS, once again revolutionizing the treatment of DR.[7] Further, Chew comments on a 2019 study by the Diabetic Retinopathy Clinical Research Retina Network showing that subjects with DR and ME who had not yet lost vision did as well with observation and anti-VEGF only when vision loss was identified, as those who were treated early with anti-VEGF injections.[6,8] The goal for the immediate future is not to replace intravitreal injections but rather to reduce the number of patients requiring them to an optimal minimum since, although they have revolutionized the treatment of retinal ischemic disease, they are painful, expensive, and not always effective.[6,10–12]

Several studies have now established injectable anti-VEGF drugs as the mainstay of management for cystoid macular edema (CME) and neovascularization in patients with RVO.[13–15] However, two major RVO trials, BRAVO and CRUISE, found sham injections were nearly as effective at achieving 20/40 or better vision, with neither treatment nor sham achieving 20/40 BCVA 70% of the time. Steroid injections had modest benefits in less than 30% of subjects with significant glaucomatous side effects.[16] Injectable therapy's lifetime quality-adjusted life year costs were recently estimated to range between $17,000 and $114,000.[16] The incidence of endophthalmitis is low, but it is devastating when it occurs.[10] We conjecture, on the basis of these and other studies, that while anti-VEGF therapy is efficacious for vision loss with ME and RVO, the response may be better in populations with DR than with RVO.[17,18]

There is also concern that long-term repetitive anti-VEGF injections may themselves cause or allow macular atrophy due to temporarily increased oxidative stress. In animal models this side effect may be prevented with concurrent administration of the antioxidant *N*-acetyl cysteine (one of the components of Ocufolin®).[19] A complete list of Ocufolin® ingredients is shown in Table 1.

Identifying the metabolic risk factors for ischemic progression offers an opportunity to intervene with less risk and possibly greater effectiveness in cases that have not yet suffered severe visual loss and the possibility to augment the effectiveness of anti-VEGF therapy once it is required. A recent meta-analysis found that folate insufficiency was a risk factor for RVO.[20] The methylenetetrahydrofolate reductase (*MTHFR*) *C677T* polymorphism is a known cause of hyperhomocysteinemia.[21,22] Elevated homocysteine has given a clearer indication of association with RVO, hypertension (HTN), stroke, and pulmonary embolism.[21–24] The common *MTHFR A1298C* polymorphism is less associated with elevated homocysteine, but some studies suggest an association with venous occlusive disease.[25,26]

We contend that investigating and managing such risk factors are as important for RVO as for heart disease and stroke. Nutritional and lifestyle approaches have been shown to be effective in reducing, slowing, and even reversing many of these risk factors.[23,28–36] Atherosclerosis, autoimmune disease, diabetes, dyslipidemia, glaucoma, HTN, hyperviscosity, obesity, smoking, and thrombotic syndromes (antiphospholipid syndrome, antithrombin deficiency, factor 5 Leiden mutations, hyperhomocysteinemia, protein C and

Table 1. Shows the active ingredients and corresponding quantities contained within each capsule of Ocufolin®

Ocufolin® ingredients	
L-Methylfolate	900 µg
Vitamin C (ascorbic acid)	45 mg
Vitamin D (as cholecalciferol)	1500 IU
Vitamin E natural complex (as alpha, beta, gamma, and delta tocopherols)	7.5 IU
Vitamin B1 (as thiamine hydrochloride)	1.5 mg
Vitamin B2 (riboflavin)	10 mg
Vitamin B6 (as pyridoxal 5′-phosphate)	3 mg
Vitamin B12 (as methylcobalamin)	500 µg
Pantothenic acid (as calcium-D-pantothenate)	5 mg
Zinc (as zinc oxide)	26.6 mg
Selenium (as l-selenomethionine)	20 µg
Copper (as cupric oxide)	0.667 µg
N-acetyl cysteine	180 mg
Lutein	3.35 mg
Zeaxanthin	700 µg

S deficiency, and prothrombin gene mutations) are familiar modifiable risk factors for RVO/CRVO.[19,2122,24,37–40] Overall, a best practices protocol should be developed and become the standard of care for ophthalmologists as well as other practitioners managing vascular disease.

Addressing the detrimental consequences of MTHFR-reduced function variants, vitamin insufficiency, and homocysteine elevation with strategic combinations of vitamins and related compounds (particularly with folate, B12, and *N*-acetyl cysteine) has been successful for increasing retinal blood flow and visual acuity. Schmidl demonstrated that one Ocufolin® daily reduced homocysteine by 30% in diabetic subjects with modestly improved retinal blood flow.[32] Pilot studies of Ocufolin® found that using three daily capsules better demonstrated improved retinal perfusion, macular superficial capillary density, conjunctival perfusion, and visual acuity in subjects with early DR.[33,34] In a study of subjects with glaucoma, one Ocufolin® daily decreased both retinal venous pressure (RVP) and homocysteine, suggesting an appropriate mechanism of action to address RVO/CRVO ischemia.[35]

Rapid and favorable response without recourse to intravitreal anti-VEGF therapy was observed in five patients (six eyes) seen consecutively and treated with three Ocufolin® capsules for acute RVO or CRVO prompted us to evaluate the clinical course retrospectively. These subjects are discussed and considered in the context of what we know of homocysteine, *MTHFR* polymorphisms, RVO, RVP, and progression to ischemic RVO. We offer a brief discussion on how improved perfusion with reduced RVP may speed the resolution of RVO, yielding improved visual outcomes over the published benchmarks.

Case presentation

Five consecutive subjects (six affected eyes) with acute symptoms consistent with RVO and treated with Ocufolin® were assessed during the study. The patients presented to The Eye Center in Fayetteville, Arkansas, from 2015 to 2021. They were provided an explanation of treatment options available, including but not limited to treatment with Ocufolin®. The patients declined anti-VEGF therapy and chose close observation

Table 2. The demographic and diagnostic information for each of the five subjects.

Clinical characteristics of study cases										
Case	**Age**	**Eye**	**Diagnosis**	**BCVA at start of Ocufolin**	**BCVA after resolution**	**Time to resolution**	**Duration of remission**	**Recurrence**	**MTHFR polymorphism**	**Predisposing conditions noted on workup**
1	71	OS	CRVO	20/40	20/25	10 months	66+ months	None	A1298C heterozygous	HTN, hypercholesterolemia, hyperhomocysteinemia, deep calf pain, former smoker, maternal retinal
2	58	OS	BRVO	20/60	20/20	10 months	61+ months	None	A1298C heterozygous	DM2, HTN, hypercholesterolemia, hypertriglyceridemia, hyperhomocysteinemia, former smoker, Vitamin D and B12 deficient
3	70	OD	CRVO	20/60	20/20	4 months	33+ months	None	A1298C homozygous	HTN, hypercholesterolemia, hypertriglyceridemia, hyperhomocysteinemia
4	34	OD	CRVO	20/20	20/20	2 months	30+ months	None	Declined testing	Hypothyroidism, hyperhomocysteinemia, ANA+
5	68	OU	CRVO	20/30 OU	20/20 OU	15 weeks	10+ months	None	A1298C and C677T	ERM with cystoid change (OS), MGUS paraproteinemia, AFib, CABG

The BCVA at the time of diagnosis and resolution, time to resolution, duration of remission, and non-recurrence are noted. Additionally, the MTHFR polymorphism test results and other comorbidities are listed for each of the subjects. Subject 5 had bilateral CRVO corresponding to the fifth and sixth eyes; these data were independent with respect to statistical analysis.
AFib, atrial fibrillation; ANA+, anti-nuclear antibody positive; BCVA, best corrected visual acuity; BRVO, branch retinal vein occlusion; CABG, coronary artery bypass graft; CRVO, central retinal vein occlusion; DM2, diabetes mellitus type 2; ERM, epiretinal membrane, HTN, hypertension; MGUS, monoclonal gammopathy of unknown significance; MTHFR, methylenetetrahydrofolate reductase; OD, oculus dexter (right eye); OS, oculus sinister (left eye); OU, oculus uterque (both eyes).

with oral Ocufolin®. They were followed with serial clinical examination, fundus photography, and OCT imaging. Fluorescein angiography was unavailable. None had an afferent pupillary defect (APD), and all had an initial BCVA of 20/80 or better, suggesting these were all nonischemic RVOs. All had elevated homocysteine, a marker for methylation impairment. Four of the five consented to genetic testing and were found to carry the *MTHFR* reduced function polymorphism *A1298C*. One patient also carried the *C677T* polymorphism. Following the observed trend of rapid recovery without recurrence, the five consecutive patients were presented with and explained a thorough written informed consent, including the potential publication of results which they understood, agreed to and signed.

Long-term follow-up was available in all five of the patients and revealed stable BCVA 20/25 or better with no recurrence, illustrating a rapid trajectory of recovery (compared to historical data) when homocysteine elevation and other risk factors are identified and addressed. Additionally, there were no adverse or unanticipated events, and none of the subjects had another RVO event during the study period. The clinical characteristics of each of the study cases are outlined in Table 2.

OCT analysis

One case showed trace CME, while two others showed significant CME. One case had mild preexisting CME due to a unilateral preexisting epiretinal membrane that remained stable. The rapid administration of Ocufolin® was done to

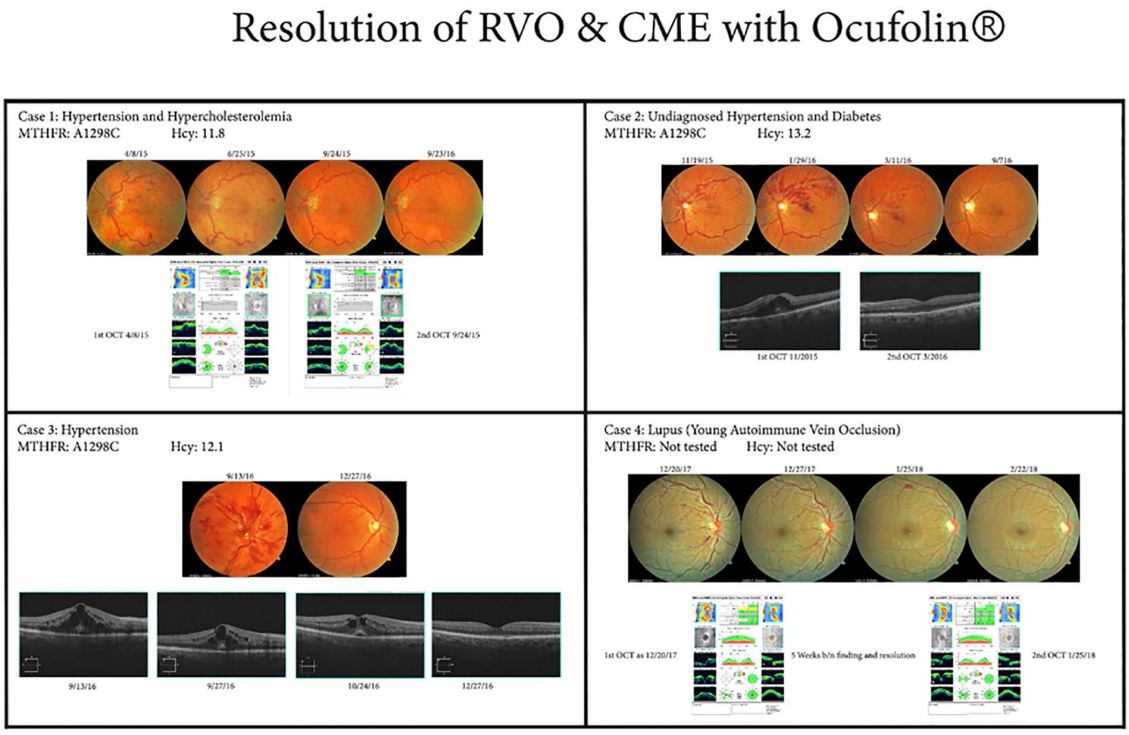

Figure 1. Clinical course of retinal venous occlusion.
Serial imaging of cases 1–4 documenting rapid resolution of edema, hemorrhage, and venous stasis. Cases 1 and 4: Serial fundus photographs show resolution of venous stasis and hemorrhage. OCT's document resolution of RNFL swelling. Cases 2 and 3: Fundus photographs show resolution of venous stasis and hemorrhage. OCT's documents rapid resolution of significant macular edema.
RNFL, retinal nerve fiber layer.

address methylation impairment manifesting as homocysteine elevation, suboptimal vitamin levels, oxidative stress, and ischemia. In retrospect, we realize that it also served to improve perfusion and lower RVP. This may have prevented the progression to ischemic RVO with attendant permanent vision loss, as might have been predicted.[41,42] Figure 1 shows a photomontage of fundus photography and OCT at the time of diagnosis juxtaposed with imaging at the time of clinical resolution in four of the five subjects (OCT was unavailable for the fifth case).

The retinal recovery index (RRI) was plotted as shown in Figures 2 and 3 by calculating the percent change toward normal values for average retinal nerve fiber layer (ARNFL) and central macular thickness (CMT) by 3-month intervals. The resulting plot shows a slope of 9.1% improvement per 3-month interval over the course of the first 12 months for ARNFL and 6.5% improvement in CMT over the same segments and time period representing a total reduction in thickness of 44.19% and 30.27%, respectively. When the ARNFL and CMT are compared to normative data, the slope measurements are 14.3% and 6.4% representing a reduction from 164.2% to 94% and 154.4% to 112.7% of normal thickness, respectively. These figures demonstrate the triphasic form of the RRI. We note that the cases had a slight lag period after starting medication before demonstrating a more positive prognosis (usually in the first 30–60 days). We were then able to see a considerable improvement of prognostic indicators in 3–6 months, followed by a slow return to normal over the 6–12 month period. That is to say, sometimes the edema did not crest immediately, but typically in the fourth-month rapid improvement ensued on only Ocufolin®, without anti-VEGF injection (although this was offered).

Figure 4(a) and (b) were made to show the slope of recovery over the first 0–8 months of treatment and the subsequent 8–16 months of treatment, with the outcome variables of interest being ARNFL as a percent of normative ARNFL data and CMT as a percentage of normative CMT data, respectively. Outside of the baseline measurements, observations up to 16 months post-treatment were

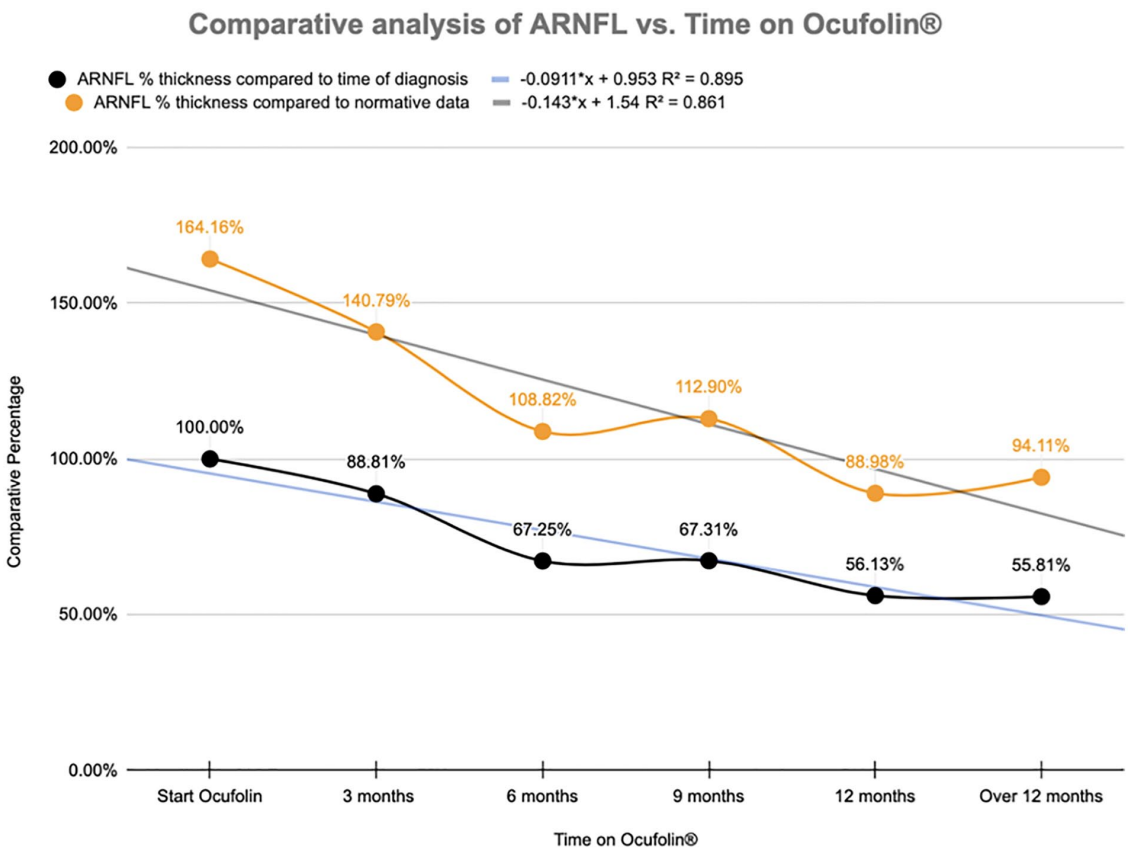

Figure 2. The orange curve in the figure represents the aggregate ARNFL as a percentage return to normative data* for all subjects over the course of treatment. The corresponding light orange line is the linear regression of this curve. The black curve in Figure 2 represents the reduction in the aggregate ARNFL for all subjects as a percentage thickness from the time of diagnosis over the course of treatment. The corresponding blue line is the linear regression of this curve.
Source: Normative data was taken from the Zeiss 510k summary.[43]
ARNEL, average retinal nerve fiber layer.

binned in 4-month intervals (0–4, 4–8, etc.) up to the highest month value in their interval, and all observations collected after 16 months were binned at the 16-month mark. The ARNFL RRI over the first 8 months was −6.228% per month, while the ARNFL RRI for the second period was −2.529% per month. The CMT RRI over the first 8 months was −2.165% per month, while the CMT RRI for the second period was −0.989% per month.

This retrospective case series aims to evaluate the efficacy of treatment with an L-methyl folate antioxidant cocktail in five subjects with mild to moderate RVO. The intervention for all subjects was incrementally increased each week of treatment to the optimal dosage of 3 Ocufolin® qD (1 Ocufolin® qD the first week, 2 Ocufolin® qD the second week, and 3 Ocufolin® qD the third week) and remained on the regimen throughout the time of observation.

All subjects were tested for vitamin deficiencies and vascular and coagulable risk factors. Four of the five subjects were tested for *MTHFR* polymorphisms. Subjects were examined in an un-masked fashion at clinically relevant intervals (approximately every 3 months) throughout the recovery period. During these examinations, BCVA, OCT ARNFL, CMT, and fundus photography were recorded. Tables of clinical improvement were created, and statistical plots were drawn of the rate of retinal recovery as assessed by improvement of ARNFL and CMT. We chose to express this recovery curve as a calculated RRI as has been done in previous treatment trials in DR. The index is based on these six eyes and the numbers themselves may or may not prove useful in the future, but we believe the concept of an index may stand the test of time.

These data were analyzed in several ways: (1) on the basis of percent change from baseline, (2)

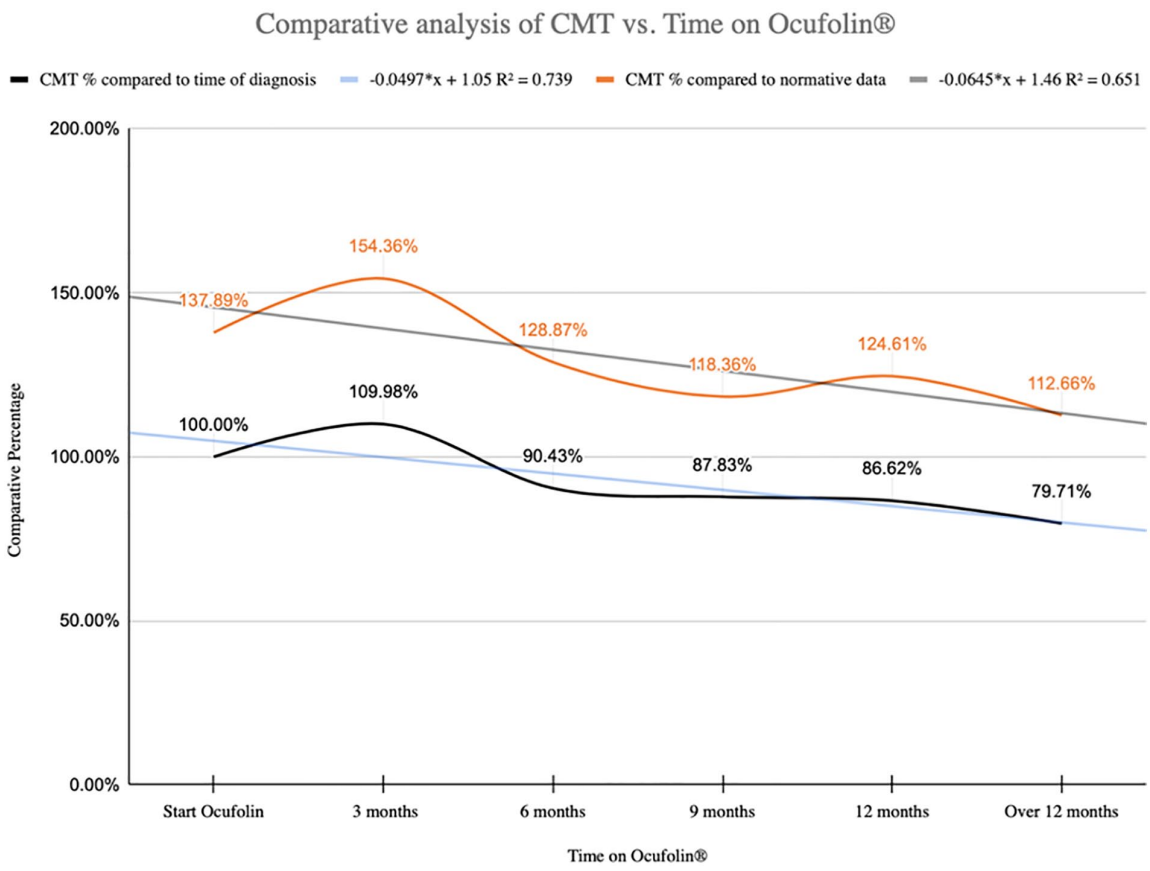

Figure 3. The orange curve in the figure represents the aggregate CMT as a percentage return to normative data* for all subjects over the course of treatment. The corresponding light orange line is the linear regression of this curve. The black curve in Figure 3 represents the reduction in the aggregate CMT for all subjects as a percentage thickness from the time of diagnosis over the course of treatment. The corresponding blue line is the linear regression of this curve.
Source: Normative data was taken from the Zeiss 510k summary.[43]
CMT, central macular thickness.

percent thickness compared to normative data, and (3) raw data average for ARNFL and CMT. Additionally, the data sets were grouped to show the RRI by observation period (see statistical plots in Supplemental Appendices). This illustrates that the majority of the recovery takes place in the period of observation from diagnosis to 6 months of treatment with Ocufolin®. After 6 months, the RRI levels off as it approaches the retinal thickness of a non-diseased eye. Linear regression analysis and paired *t*-tests were performed for statistical significance.

Given that subjects were not measured with completely equal intervals of time between observations and that one subject had no observations beyond 10 months, we conducted our analysis on the raw dataset, as well as an imputed version of the data designed to create a balanced dataset. Specifically, the dataset is set up such that each eye has five observations at 0, 3, 6, 9, and 12 months. The ARNFL value associated with a given eye-month is the ARNFL and CMT value for the given eye from the nearest observation that occurred prior to the given month. This approach is in keeping with standard statistical practice for treatment studies.

Regression analysis

We utilize a linear regression model in which the outcome variable is the subject's ARNFL and CMT, respectively, and the explanatory variable of interest is the number of months since starting treatment with Ocufolin®. Eye-fixed effects are included, and standard errors are clustered at the eye level. For this model, we subset the data to only include observations

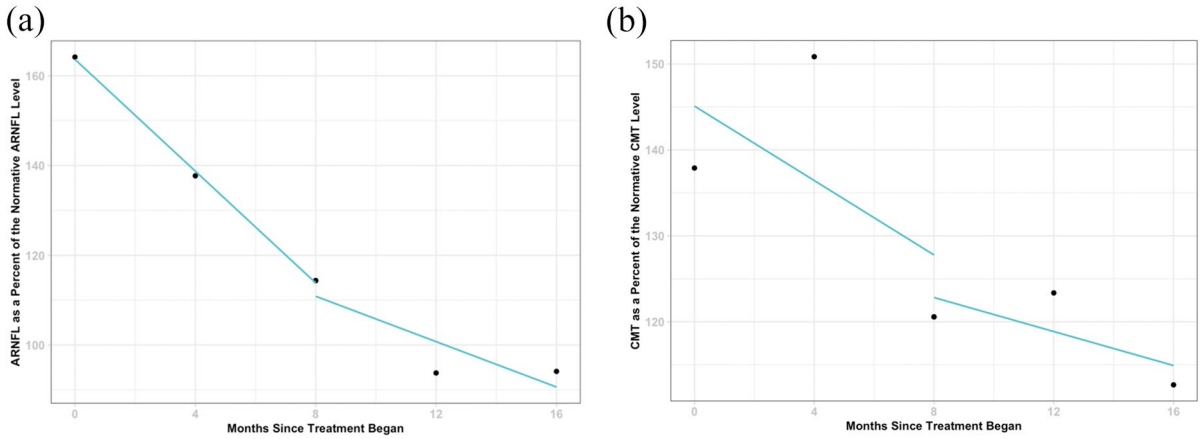

Figure 4. Figures (a) and (b) report the average ARNFL and CMT for the observed eyes as a percent of the normative levels. Values are reported in 4-month intervals, where, for 4, 8, and 12 months, any value recorded within the interval is included in the average value reported at the end of the interval. The values for 0 months are set as the average of the baseline ARNFL and CMT values, respectively, and the values for 16 months reflect observations post-16 months. If a given eye appeared more than once in a given interval, then the observed values of that eye were averaged within the interval in question. Thus, all eyes included in calculating the average for a given interval are weighted equally. If an eye did not have an observation within a given interval, then that eye was not included in calculating the average ARNFL value for that interval. Thus, each interval does not necessarily include the same eyes in calculating its average ARNFL. Two lines of best fit are included for the intervals from 0 to 8 and 8 to 16+ months. The lines are reported separately to demonstrate the steeper initial decline in the ARNFL value, followed by a more gradual decline, representing biphasic edema resolution. The slope of the first and second ARNFL lines reflect a 6.2 and 2.5 percentage points decline per month, respectively. The slope of the first and second CMT lines reflect a 2.165 and 0.988 percentage point decline per month, respectively. In each case, the figures reveal a steep initial decline in ARNFL and CMT, followed by a more gradual resolution of edema over time.

within 12 months of beginning treatment. In columns (1) and (2), we include every observation for every patient that occurred within 12 months of beginning treatment and examine months since starting treatment with Ocufolin® as a continuous variable.

Table 3 shows the ARNFL model. In column (1), we find that, holding all else constant, an additional month of treatment is associated with a statistically significant 7.455 point reduction in ARNFL level, $p = 0.003$, within-$R^2 = 0.582$. From column (2), we find that holding all else constant, an additional month of treatment is associated with a statistically significant 8.016 percentage point reduction in ARNFL level as a percent of the normative ARNFL level.[43] In columns (3) and (4), we use the imputed version of the dataset. In this rendition of the data, utilizing the same model, we find that, holding all else constant, an additional month of treatment is associated with a statistically significant 4.983 point reduction in ARNFL level, $p = 0.003$, within-$R^2 = 0.673$, and a 5.358 percentage point reduction in ARNFL level as a percent of the normative ARNFL level,[43] $p = 0.003$, within-$R^2 = 0.673$.

Table 4 shows the CMT model. In column (1), we find that holding all else constant, an additional month of treatment is associated with a 10.74-point reduction in CMT (not statistically significant) within-$R^2 = 0.201$. From column (2), we find that holding all else constant, an additional month of treatment is associated with a 4.197 percentage point reduction in CMT (not statistically significant) as a percent of the normative CMT level.[43] In columns (3) and (4), we use the imputed version of the dataset. In this rendition of the data, utilizing the same model, we find that, holding all else constant, an additional month of treatment is associated with a 5.211-point reduction in CMT (not statistically significant) and a 2.036 percentage point reduction in CMT (not statistically significant) as a percent of the normative CMT.[43] This statistical treatment, once again, demonstrates that most of the reduction in retinal thickness has occurred by the 12th month of treatment and further treatment shows little change. These data do not support the cessation of treatment at the 12th month as we have no knowledge of the tendency for recurrence, but if the anti-VEGF studies are any indication, then continued treatment would be the prudent decision.

Table 3. ARNFL regression analysis.

Continuous month regression (3 month interval imputed)				
	(1) β (SE)	**(2) β (SE)**	**(3) β (SE)**	**(4) β (SE)**
Months since Start	−7.455**	−8.016**		
	(1.366)	(1.469)		
Months since Start			−4.983**	−5.358**
			(0.937)	(1.008)
Constant	147.3***	158.4***	143.9***	154.8***
	(4.344)	(4.671)	(5.622)	(6.046)
N R^2	28	28	30	30
Within R^2	0.713	0.713	0.748	0.748
	0.582	0.582	0.673	0.673
Eye fixed effects	✓	✓	✓	✓
Cluster	Age	Age	Age	Age
Raw ARNFL	✓		✓	
% Normative ARNFL		✓		✓
Data imputed			✓	✓

*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.
ARNFL, retinal nerve fiber layer; SE, standard error.

Table 4. CMT regression analysis.

Continuous month regression (3-month interval imputed)				
	(1) β (SE)	**(2) β (SE)**	**(3) β (SE)**	**(4) β (SE)**
Months since Start	−10.74	−4.197	−6.609	−2.582
	(5.789)	(2.261)	(3.585)	(1.400)
Constant	410.6*** (19.20)	160.4*** (7.499)	355.0*** (14.30)	138.7*** (5.585)
N	27	27	33	33
Within R^2	0.201	0.201	0.231	0.231
Eye fixed effects	✓	✓	✓	✓
Cluster	Age	Age	Age	Age
Raw cmt	✓		✓	
% Normative cmt		✓		✓
Data imputed			✓	✓

*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.
CMT, central macular thickness; SE, standard error.

Discussion

RVO natural history benchmark studies

The natural history of CRVO in an era before there was any effective treatment has been studied. A series of 667 consecutive subjects between 1973 and 2000 were reviewed in the literature in 2011.[44] CRVO was divided into nonischemic and ischemic forms, observing that frequent loss of central vision was especially common in ischemic CRVO.[45]

The Central Vein Occlusion Study prospective study of 725 subjects found that conversion rates for nonischemic CRVO to ischemic-CRVO were 15% at 4 months and 34% at 3 years[46] and even higher likelihood in subjects with a previous history of stroke.[44]

Nonischemic CRVO

In Hayreh *et al.*'s series, nonischemic CRVO eyes seen within 3 months of onset achieved a final BCVA of 20/100 or better in only 78% of cases. Of nonischemic eyes presenting with a BCVA of better than 20/60, 17% of those deteriorated by 3 months, and 20% deteriorated over the 2.5 year follow-up. Of those subjects with an initial BCVA of 20/70 or better; after resolution of macular edema, 59% had improved BCVA. Of those with moderate to severe visual field loss initially, 86% improved. Long term visual loss correlated with the development of an epiretinal membrane or foveal pigmentary degeneration. Untreated, even the less affected subjects failed to show clinical improvement 41% of the time.[44] These statistics show that even nonischemic CRVO has a poor visual prognosis if left untreated.

In our six eyes with nonischemic RVO/branch retinal vein occlusion treated with Ocufolin®, all achieved and maintained BCVA of 20/25 or better with no recurrences over their follow-up periods of 10, 30, 33, 61, and 81 months, respectively, as depicted in Table 2. This exceeds what might be expected from reported benchmarks. Further, the graphs in Figures 1 and 3 show the ARNFL in these cases at diagnosis had an average thickness of 164.16% of normal, which decreased to normal (94.11% of the normative value) after 1 year on Ocufolin® with rate of retinal recovery averaging 6.2% per month for the first 8 months and 2.5% per month for the second 8-month period. Figures 1 and 3 show the CMT in these cases began at 137.89% at diagnosis before decreasing to levels much closer to the normal value after 1 year on Ocufolin® (112.66%), with a rate of retinal recovery averaging 2.165% per month for the first 8 months and 0.988% per month for the second 8 months.

Ischemic CRVO

Although untreated nonischemic CRVO has a relatively poor prognosis, the prognosis for ischemic CRVO is much worse. Ischemic CRVO subjects tended to be older with HTN, diabetes, and previous stroke. They recovered BCVA 20/100 or better in only 1% of the cases. Visual field defects were significant in 18% of subjects. Resolution of macular edema brought no improvement of BCVA or visual fields.[44] Ischemic CRVO usually begins as nonischemic CRVO.[44] Today, the standard of care treatment is periodic intravitreal injection of anti-VEGF monoclonal antibodies. Even that is often not sufficient to prevent progression of ischemia.[47] A key associated factor for conversion from a nonischemic state to an ischemic state appears to be central retinal venous blood flow velocity. Greater venous blood flow velocity in nonischemic CRVO has been shown to be associated with stability of the nonischemic state.[48]

Progression to neovascular glaucoma (NVG) is a serious concern for ischemic CRVO, occurring in 22–50% of patients. Some NVG prognostic markers include ischemic retinopathy, poor initial BCVA, HTN, and a positive APD. NVG is commonly termed '100-Day Glaucoma' acknowledging it to be a late complication of the initial RVO.[41] The standard of care for preventing NVG is lifelong repeated intravitreal injections to bind VEGF. This is a temporizing measure, not a cure, with the mean time to development of NVG being 212 days after the last injection.[41] Anti-VEGF antibody injections do nothing to reduce the underlying ischemia. Clearly, preventing the progression from nonischemic to ischemic CRVO or to NVG should be a major goal for preventing vision loss and disability.

Review of causes and risk factors

Microvascular retinal diseases, including RVO, share common risk factors with atherosclerosis. Autoimmune states, diabetes, endothelin-1 secretion, hypercholesterolemia, hyperhomocysteinemia, HTN, inflammation, smoking, and VEGF secretion may all independently and collectively

contribute to atherosclerosis and microvascular damage.[42,49–54] The long-ranging Beaver Dam Eye Study confirms that diabetes, glaucoma, and migraine are associated with microvascular disease, including RVO.[55]

Many studies have shown that diabetes is associated with microvascular disease and RVO.[19,21,22,24,31,37,39,42,55] Vitamin D deficiency, although widespread in the general population, is more frequent and problematic for patients with any of the aforementioned risk factors. Severity of disease manifestation appears to be proportional to the degree of vitamin D deficiency.[56–58] Recently serum cobalamin (Vitamin B12) was shown to be lower in RVO subjects than matched controls. Interestingly, higher B12 levels favored better response to intravitreal anti-VEGF therapy, while lower B12 levels predicted worse clinical outcomes.[59]

Flammer syndrome is a primary vascular dysregulation affecting many vascular beds and end organs. Although often unrecognized in the United States, it is associated with low-tension glaucoma, disk hemorrhages, and increased oxidative stress.[60,61] There appears to be an overlapping relationship between Flammer Syndrome with low tension glaucoma, retinal venous dysregulation, and increased RVP with increased risk for retinal vascular disease, including RVO.[39,62,63] Devogelaere and others have specifically shown that subjects with glaucoma and ocular vascular diseases have increased RVP which responds to Ocufolin®.[35,64,65]

Elevation of homocysteine is inversely correlated with retinal vessel diameter and choroidal perfusion and positively correlated with retinal occlusive disease.[66–69] Elevation of homocysteine also causes apoptosis and atrophy of retinal neuronal tissue, particularly the photoreceptors and ganglion cells forming the optic nerve.[70,71] Homocysteine is converted to methionine by L-methylfolate and B12, key ingredients of Ocufolin® (Table 1). Folate type, availability, and folate receptor efficiency regulate intracellular homocysteine. Folate transport receptor pharmacokinetics are increasingly thought to be important for proper function of the brain and visual system and at the root of many central and retinal neuropathies.[72–77] Folate receptor 1 alpha transport protein is the principle gateway for folate into the cell, selecting for L-methylfolate while blocking transport of folic acid.[72–76] Folate has three major transporters: (i) reduced folate carrier 1 (RFC1; RFC; SLC19A1), (ii) proton-coupled folate transporter (PCFT; SLC46A1), and (iii) folate receptor alpha (FOLR1). RFC1 protein has been determined to be the particular transporter expressed in the inner blood–retinal barrier, hence retinal microvessels and mural cells.[77] Gurler and colleagues have recently shown that good folate carrier function is crucial for the integrity of the inner retinal blood barrier and resistance to ischemia.[77]

Impairment of this transport protein and the resulting impairment of folate metabolism may occur for many reasons, including excess folic acid. Excess folic acid (above 500 mcg/day) causes the buildup of unmetabolized folic acid on the blood–brain barrier (BBB) and blood–retinal barrier (BRB) with competitive inhibition of active L-methylfolate uptake.[73,74] This leads to relative folate deficiency (central folate deficiency) in the retina, optic nerve, and brain, often masked by high peripheral serum folate levels (as folic acid).[73,74] Intracellular transport of L-methylfolate lowers homocysteine.[72] It also reduces vasoconstriction by release of nitric oxide (NO). Impaired intracellular folate availability causes increased homocysteine, and vasoconstriction.[76] Stover and colleagues have suggested the use of reduced folates such as leucovorin or L-methylfolate to address this.[74] Recent observations that aspects of early DR may be reversed with the use of a high dose-methylfolate cocktails suggest a practical way to overcome central and retinal folate insufficiency due to deficient intake, methylation impairment, or transport.[23,78] This has important implications for RVO ischemia.

Review of RVO risk and severity

The current state of RVO treatment is to observe, inject, and photocoagulate as deemed necessary. Injection and photocoagulation are clearly effective but also expensive, inconvenient, invasive, and painful. Pan retinal photocoagulation also creates permanent visual field loss and loss of night vision, impairing driving, and loss of self-sufficiency. Anti-VEGF injections run a small risk of endophthalmitis and glaucoma crisis and must be repeated for life to prevent the development of NVG. Although anti-VEGF injections have revolutionized the management of DR, they are only partially effective in RVO patients.[13,15] Whether to treat every RVO with anti-VEGF is a serious question the clinician must face. One might question whether

addressing the risk factors of homocysteine and HTN earlier and folate metabolism polymorphisms at the nonischemic stage would improve RVO outcomes? While only a proper double-blind placebo-controlled trial can answer these questions, the cited literature and the results we report here are suggestive.

Recent evidence suggests that addressing similar risk factors slows or reverses cerebral and retinal small vessel disease. Currently, elevations of homocysteine can only be addressed nutritionally. Lowering serum homocysteine with riboflavin, pyridoxal 5′-phosphate (B6), folate, and B12 lowers blood pressure and improves ocular perfusion.[20,23,79] This has been shown to be neuroprotective and possibly reverse early DR.[20,33] Increasing NO and lowering homocysteine appear to be common mechanisms of microvascular benefit with these vitamins. Folate and B12 lower homocysteine by donating a methyl group to homocysteine, converting it to methionine. The L-methylfolate and methylcobalamin forms readily cross the BBB and BRB and lower homocysteine more effectively because they already are primed with a methyl group for ready transfer.[80–83]

MTHFR C677T and *A1298C* code for reduced function genetic variants of the MTHFR enzyme, which methylates folate, a critical step in many aspects of cellular metabolism.[40] These polymorphisms are present in as much as 70% of many world populations.[83] Common *MTHFR* variants are most burdensome when coupled with common deficiencies of riboflavin (B2), folate (B9), or cobalamin (B12).[84–86] Conversely, *MTHFR* impairments are less burdensome with optimal vitamin cofactor availability.[87–90] Worldwide, these are common vitamin deficiencies.[85] Such nutritional stressors increase in incidence and severity with age and age-related loss of vitamin absorption, paralleling the increase of homocysteine, HTN, microangiopathy, and thromboembolism.[21,87,89,91–94] It is not surprising that insufficiencies of these same vitamins increase the risk of diabetes, prediabetes, and RVO.[29,31,76,89,90,95] Additionally, vitamin D deficiency impairs folate transport across the BBB and also appears to impair homocysteine control.[96,97] As previously noted, serum B12 deficiency is a negative predictor for response to anti-VEGF therapy, which immediately suggests a simple, safe, and inexpensive B_{12} adjunctive therapy with intravitreal anti-VEGF.[59]

Using the natural active forms of these vitamins avoids the toxicity seen with synthetic forms. Pyridoxal-5-phosphate is the natural active form of B6 with no reported toxicity. L-Methylfolate is the natural active form of folate with no reported toxicity, readily crossing the blood–brain and retinal barriers while possessing seven times greater efficacy than folic acid.[74,80] High doses of L-methylfolate are safe and beneficial during long-term management of depression.[74]

Similarly, methylcobalamin is the natural active form of B12 and is very effective for lowering homocysteine.[98] Methylcobalamin is nontoxic even at high intravenous doses.[99] It is also safer than cyanocobalamin in the presence of renal failure, a condition frequently associated with elevated homocysteine and RVOs.[98,100]

Investigation for treatable etiologies

The five consecutive subjects were investigated for causes that increase risk and severity of RVO and several treatable risk factors that were identified and addressed or referred for management. Among them, we found diabetes, hyperlipidemia, HTN, hyperhomocysteinemia, lupus-like autoimmune retinal vasculitis, *MTHFR A1298C* and *C677T* polymorphisms, vitamin D deficiency, and paraproteinemia (monoclonal gammopathy of unknown significance).

Marcucci *et al.*[101] have developed an excellent protocol for RVO work up that can be copied and shared. We have used a similar testing protocol that includes blood pressure, fasting blood sugar, hemoglobin A1c (HbA1c), MTHFR status, complete blood count, sed rate (sedimentation rate), C-reactive protein (CRP), anti-nuclear antibody, rheumatoid arthritis, serum protein electrophoresis (SPEP), lipids, homocysteine, and vitamins B2, B6, B12, and D. Serum folate may be added in countries without folate fortification.

The eye specialist should ensure these tests are done and that the patient's primary care medical team seriously address all underlying systemic diseases and nutritional insufficiencies. Due to their unfamiliarity with small vessel disease of the eye, primary care physicians appreciate and need written guidance if they are asked to evaluate for and manage these underlying etiologies. We recommend the Marcucci protocol, adding CRP, HbA1c, SPEP, and vitamins B12 and D.

Strengths

Addressing RVO metabolically is affordable, convenient, safe, and self-administrable, and if begun early, may reduce the risk of progression to ischemia, glaucoma, NVG, vision loss, and the need for intravitreal injections.

With normalization of retinal blood flow and capillary density, reversal of microangiopathy may be possible and provide the clinician with an opportunity to bend the arc of RVO disease progression away from ischemia and irrecoverable vision loss. Addressing RVO drivers metabolically may be initiated prior to, during, or after intravitreal anti-VEGF therapy and panretinal photocoagulation laser. This supports rather than competes with the current therapies for RVO.

Ocufolin® shows promise for conveniently addressing the nutrigenomic drivers of RVO based on the ingredient mechanisms of action. Ocufolin® has been shown to lower homocysteine, lower RVP, lower systolic blood pressure, increase conjunctival and retinal perfusion, increase retinal venous blood flow, and increase capillary density in the macula.[32–35,78] The ingredients of Ocufolin® (Table 1) have previously been published.[32] Additionally, coupled with anti-VEGF therapy, Ocufolin® has sufficient B12 to maintain optimal anti-VEGF effectiveness.[59]

Limitations

The six eye cohort is not appropriately powered, and the numbers are too few for the sophisticated statistical analysis we would like to see. We are aware that using percentage change from baseline to demonstrate clinical recovery has been thought to be less effective a statistical methodology than using analysis of covariance in large clinical trials; however, it allowed us to show quantitative return to clinical normalcy and correlated well with the regression line using percentage change compared to published norms. An additional limitation of data availability was an incidence where the baseline CMT data was not recorded until 7 days after starting treatment Ocufolin®, this measurement was used as the baseline measurement for CMT. We do not believe this created substantive errors, but it is worth mentioning. Biases of selection are difficult to detect in a clinical practice; however, these were five sequential subjects seen by the same ophthalmologist. Compared to benchmark RVO data, the results suggest a trend toward early and more complete non-ischemic resolution. Coupled with the newly discovered mechanism of Ocufolin® induced reduction of RVP, they suggest a new clinical opportunity that should be verified through properly controlled trials.

Future directions

Considering the strengths and weaknesses implicit in these observations, we recommend a controlled trial that follows anatomical outcomes, BCVA, retinal perfusion, RVP, oxidative stress, and biochemical markers of ischemia and could potentially use a form of the RRI as a metric for evaluating therapeutic efficiency and comparison with future studies. We also recommend investigation of the MTHFR 1298C polymorphism as a potential risk factor for occlusive small vessel disease of the brain and eye.

Conclusion

We cannot yet cure the consequences of retinal vascular disease. However, addressing the risk factors for progressive vascular insult with nutritional supplementation appears to have prevented progression to ischemic RVO with vision loss in our cohort.

Currently ophthalmologists either observe RVO, waiting to see if ischemia and severe vision loss will require anti-VEGF treatment, or immediately treat all RVO patients with anti-VEGF treatment. We have frequently observed that little effort is made to identify and address the underlying risk factors of microangiopathy, which drive recurrence, severity (ischemia), and vision loss. Such efforts might improve the outcomes of RVO as documented above and substantially reduce the disease burden on our patients and society. Clearly, this is a worthy goal.

- Dates in text, such as 1/7/2020 are in the Month/Day/Year format.
- In bilateral measurements, if the eye is not specified, the first value is OD and the second value is OS, for example:
  - Goldmann Applanation Tonometry: 17/16 means that the intraocular pressure is 17 mm Hg OD and 16 mm Hg OS.
  - Cup-to-disk ratio: 0.3/0.4 means that the cup-to-disk ratio is 0.3 OD and 0.4 OS.

Declarations

Ethics approval and consent to participate

Recent studies linking Ocufolin® to improved retinal perfusion and decreased RVP led to a review of the outcomes of five consecutive subjects with RVO treated with Ocufolin®. Ocufolin® was initially chosen for its benefit in conditions characterized by ischemia and oxidative stress. All were treated by the same physician, CJB. This retrospective anonymized cohort was reviewed by the Washington Regional Medical Center Institutional Review Board.

Consent for publication

Written informed consent to participate was obtained for all subjects in this case series. All subjects gave permission for the use of their anonymized data.

Author contributions

Steven Baker: Conceptualization; Data curation; Formal analysis; Resources; Validation; Visualization; Writing – original draft; Writing – review & editing.

Dylan Baker: Data curation; Formal analysis; Software; Validation.

Robert Baker: Conceptualization; Resources; Validation; Visualization; Writing – original draft; Writing – review & editing.

Craig J. Brown: Conceptualization; Data curation; Investigation; Project administration; Resources; Supervision; Writing – original draft; Writing – review & editing.

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Competing interests

Steven Baker, Dylan Baker, and Robert Baker declare no conflicts of interest. Craig J. Brown holds patents for applications of folates for addressing ocular health conditions, including migraine, and an ownership interest in Global Healthcare Focus, the maker of Ocufolin®.

Availability of data and materials

Access to anonymized data and materials is available upon request.

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