

# Treatment of Retinal Vein Occlusion with Ranibizumab in Clinical Practice: Longer-Term Results and Predictive Factors of Functional Outcome

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## Key Words

Branch retinal vein occlusion · Central retinal vein occlusion · Fluorescein angiography · Macular edema · Optical coherence tomography · Retinal vein occlusion · Vascular endothelial growth factor · Visual acuity

## Abstract

**Purpose:** To evaluate long-term results and predictors of efficacy in patients with macular edema due to retinal vein occlusion (RVO) treated with intravitreal ranibizumab in a clinical practice setting. **Methods:** The clinical records of patients with a minimum follow-up of 3 years were retrospectively analyzed. Sixteen eyes with branch RVO (BRVO) and 16 with central RVO (CRVO) were included. All patients performed cross-sectional evaluation with best-corrected visual acuity (BCVA), spectral domain optical coherence tomography and fluorescein angiography. The foveal avascular zone (FAZ) was assessed and microstructural morphology of the retina was characterized. **Results:** Follow-up was  $42.9 \pm 9.0$  and  $44.8 \pm 8.0$  months in the CRVO and BRVO groups, respectively. Patients with CRVO received on

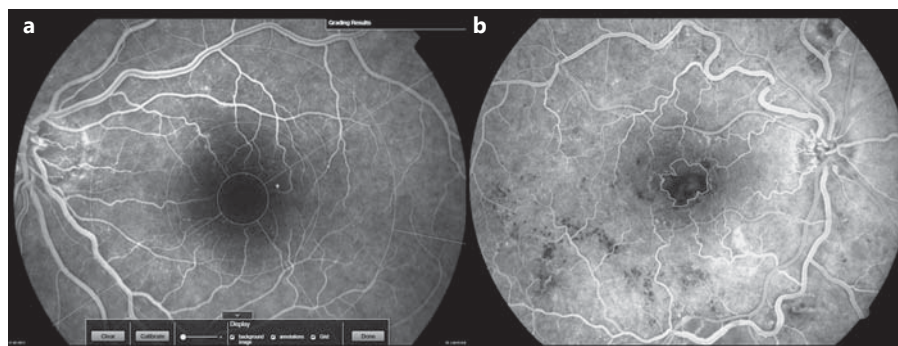
average 6.9 injections, with a final VA gain of  $8.3 \pm 15.0$  letters ( $p = 0.05$ ). BRVO eyes had on average 5.9 injections, with a final VA gain of  $1.6 \pm 21.0$  letters ( $p > 0.05$ ). The FAZ area remained stable in both groups ( $p > 0.05$ ). Baseline BCVA and disruption of the retinal pigment epithelium (RPE) were predictors of final BCVA ( $p = 0.001$  and  $0.011$ , respectively). **Conclusion:** Although functional outcomes were inferior to those reported in clinical trials, ranibizumab was satisfactory in the long-term treatment of macular edema secondary to RVO and was not associated with increased macular ischemia. Final BCVA depends on baseline BCVA and RPE integrity.

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## Introduction

Retinal vein occlusion (RVO) has different manifestations and may present as central RVO (CRVO), or hemi-central or branch RVO (BRVO). It is the second most common cause of reduced vision due to retinal vascular disease after diabetic retinopathy [1–5].

**Fig. 1.** Print screens of real-time grading using RetmarkerAMD®. After manually marking the optic disk diameter and the center of the fovea, the software generates a reference grid (a). The FAZ area is then manually delineated (b), and the respective area is automatically calculated.



Increased production of vascular endothelial growth factor (VEGF) occurs early in the disease process and is a major contributor to macular edema [6–8]. Thus, blockade of VEGF with anti-VEGF agents, such as ranibizumab (Lucentis®; Novartis, Basel, Switzerland), raised hopes to reduce macular edema and improve vision. Two pivotal trials finally established the role of anti-VEGF therapy in RVO: the Treatment of Macular Edema following Branch Retinal Vein Occlusion: Evaluation of Efficacy and Safety (BRAVO) [9] and the Treatment of Macular Edema following Central Retinal Vein Occlusion: Evaluation of Efficacy and Safety (CRUISE) trials [10]. Overall, the 12-month results showed that the visual gain was good and could be maintained. Moreover, early treatment with anti-VEGF led to a greater functional improvement than delayed therapy [11, 12]. The long-term outcomes in BRVO patients were excellent, although half still required occasional injections after 4 years. Most CRVO patients, however, still require frequent injections within a 4-year follow-up and had a more guarded prognosis [13]. These good results have been confirmed in many smaller studies [14–16]. An important aspect of anti-VEGF treatments, however, is the drug delivery protocol used in real-life clinical practice, which can be significantly different from that used in well-controlled clinical trials. Few reports exist on this matter in RVO, and none of them evaluated the associated long-term outcomes [17, 18]. Also, some debate has been raised about the role of anti-VEGF agents in promoting retinal nonperfusion (RNP) or, on the contrary, in reducing retinal ischemia by promoting retinal reperfusion [1, 19–21]. The impact of anti-VEGF agents in the choroid is also still poorly understood [22, 23].

The purpose of this study is to evaluate the long-term results of intravitreal ranibizumab (IVR) in the treatment of macular edema due to RVO in a clinical practice setting and to identify morphological factors related to functional outcome with multimodal retinal imaging.

## Materials and Methods

A retrospective and cross-sectional study was conducted at the Ophthalmology Department of the Centro Hospitalar e Universitário de Coimbra and the Association for Innovation and Biomedical Research on Light and Image (AIBILI). Signed informed consent was obtained from all subjects, and the study was conducted in accordance with the tenets of the Declaration of Helsinki and after institutional review board approval.

The medical records of consecutive patients with RVO followed in our Department were examined. Eyes with a history of RVO treated with IVR and a minimum follow-up of 3 years were included if there was no history of other vitreoretinal disease, uveitis, dense cataract or other pathologies that could compromise visual acuity (VA). Patients with a history of previous vitrectomy and/or a scleral buckling procedure were also excluded. All patients started treatment with 0.5 mg ranibizumab (Lucentis), using a pro re nata (PRN) regimen without a loading dose, if best-corrected VA (BCVA) was inferior to 20/32 due to macular edema, which was confirmed with optical coherence tomography (OCT). During follow-up, retreatment was performed if there was BCVA loss superior to 5 Early Treatment of Diabetic Retinopathy Study (ETDRS) letters (L), and/or in the presence of intraretinal or sub-retinal fluid in OCT.

Patients' medical records were reviewed for data, including demographics, follow-up time, BCVA evolution (with ETDRS charts), central macular thickness (CMT) evolution acquired by automated fast macular thickness map in spectral domain OCT (SD-OCT), number of IVR injections, other treatments performed previously for macular edema, development of neovascular disease and treatment with panretinal photocoagulation, other ocular comorbidities and intraocular surgery, and systemic or adverse effects related with IVR treatment.

A final cross-sectional evaluation was also performed in all patients including: BCVA (ETDRS charts), slit lamp examination and dilated fundus stereoscopic examination with +90- and +60-diopter lenses, color fundus photography, fluorescein angiography (FA) and SD-OCT with Spectralis® OCT (Heidelberg Engineering, Heidelberg, Germany) in both retinal and choroidal mode [enhanced depth imaging OCT (EDI-OCT)].

The foveal avascular zone (FAZ) area was measured in the first available angiogram and in the angiogram performed in the final cross-sectional visit. For this purpose, 2 independent observers (C.F. and J.P.M.) used RetmarkerAMD® software (Critical Health

SA, Coimbra, Portugal) to manually delineate the FAZ and the area was automatically calculated by the software. The software divides the posterior pole in 10 subfields with a circular grid centered in the macula and calibrated through the edges of the optic disk, identical to the ETDRS-style macular grid (which comprises 1-, 3- and 6-mm concentric circles). The central subfield overlaps with the central macula (fig. 1) [24]. Other areas of RNP were also qualitatively analyzed for classification into ischemic/nonischemic subtypes in the final visit. It was considered as ischemic if the RNP area was greater than 10 disk areas in eyes with CRVO and 5 disk areas in eyes with BRVO.

Microstructural qualitative analysis of the retina using SD-OCT was performed before baseline treatment and at the final cross-sectional evaluation by the same 2 authors (C.F. and J.P.M.). The hyper-reflective lines corresponding to the external limiting membrane (ELM), inner (IS) and outer segment (OS) junction and retinal pigment epithelium (RPE) were also classified as being disrupted or intact. Any disagreement was resolved by the senior author (R.S.).

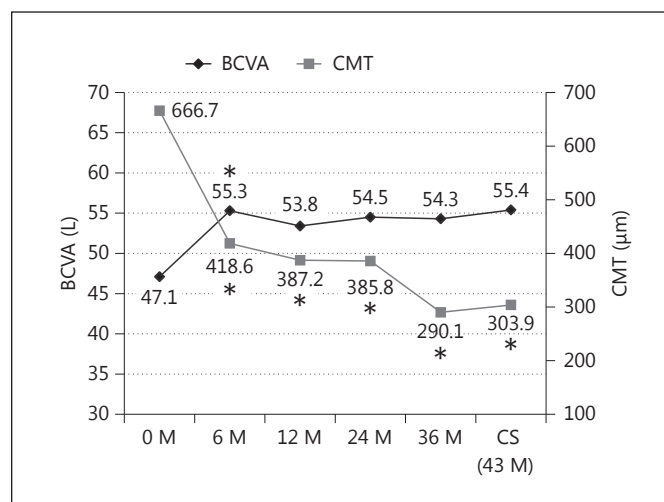
EDI-OCT with Spectralis OCT (Heidelberg Engineering) was performed to measure the choroidal thickness (CT). The method of obtaining EDI-OCT images and CT measurements has been reported previously [25, 26]. Both the horizontal and vertical sections passing through the center of the fovea were used for the CT measurements, which were obtained manually under the fovea using the scale supplied with the software, and at 1,000- and 3,000- $\mu$ m distance to the fovea in the nasal, temporal, superior and inferior directions. Each image was measured by 2 independent observers (C.F. and J.P.M.) and the average of the 2 was considered for analysis. Data with discrepancies of 15% were reanalyzed by the senior author (R.S.). The average of 10 readings taken in each eye was considered the macular CT. The average of the 2 subfoveal readings (vertical and horizontal) was considered the subfoveal CT.

Statistical analysis was performed using Stata software (version 12.1; StataCorp LP). Univariate analysis was performed on a basis of variable selection for the multivariate analysis, using Mann-Whitney U and paired Wilcoxon tests, and Spearman's correlation coefficient. A multiple regression model was used to identify independent prognostic factors for final BCVA, including the variables selected in the univariate analysis.

## Results

### Patient Demographic Data

Thirty-two eyes of 32 patients (15 females and 17 males) were included. Sixteen eyes were included in the CRVO group (15 eyes with CRVO diagnosis and 1 eye with hemi-central vein occlusion) and 16 eyes in BRVO group. In the latter, 14 eyes had major BRVO and 2 eyes had macular BRVO. Age, gender, follow-up time and systemic risk factors for RVO are presented by group in table 1. Five eyes (31%) in the CRVO group and 2 eyes (13%) in the BRVO group were pseudophakic. Four eyes (25%) in the CRVO group and 2 (13%) eyes in the BRVO group had a previous diagnosis of glaucoma, all of them controlled with topical medication. Considering the previous treatments for mac-



**Fig. 2.** Evolution of BCVA and CMT in the CRVO group during follow-up and in the cross-sectional (CS) evaluation. M = Months. \*  $p < 0.05$ .

**Table 1.** Patient demographics by group

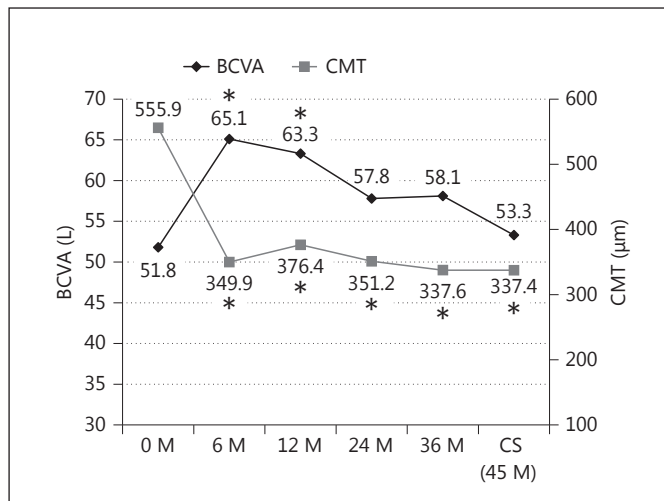
	CRVO	BRVO
Age at treatment initiation, years	63.9 $\pm$ 14.3	68.8 $\pm$ 10.5
Follow-up since IVR treatment, months	42.9 $\pm$ 9.0	44.8 $\pm$ 8.0
Time between diagnosis and 1st IVR treatment, months	5.4 $\pm$ 5.4	8.0 $\pm$ 10.7
Gender, males/females	12/4	5/11
Systemic risk factors, n (%)		
Diabetes mellitus type 2	1 (6)	3 (19)
High blood pressure	8 (50)	9 (56)
Dyslipidemia	6 (38)	7 (44)

Means  $\pm$  SD. n = Number of eyes.

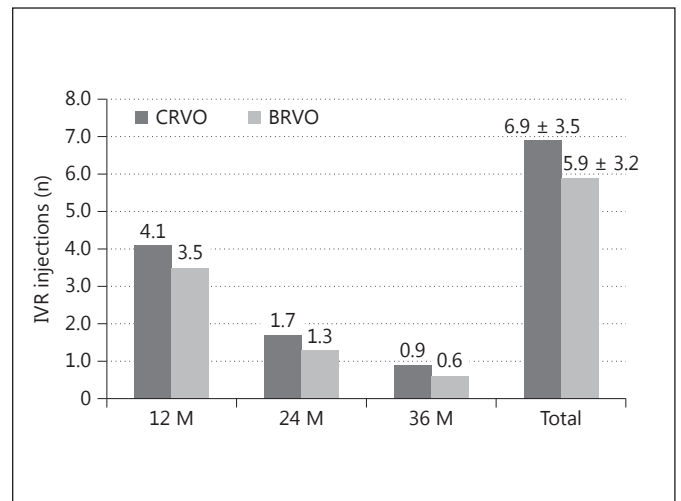
ular edema: 3 eyes in the CRVO group had already received intravitreal triamcinolone and/or bevacizumab (2 injections maximum), and 4 eyes in the BRVO group had been treated previously with macular grid laser and/or intravitreal triamcinolone (1 injection maximum).

### Evolution and Cross-Sectional Evaluation of BCVA and CMT

**CRVO Group.** Gain in BCVA was significant only at the 6-month evaluation ( $p = 0.018$ ). The final mean BCVA in the cross-sectional evaluation was  $55.4 \pm 23.6$  L, which represents a final mean gain of  $8.3 \pm 15.0$  L ( $p = 0.05$ , borderline significant; fig. 2; table 2).



**Fig. 3.** Evolution of BCVA and CMT in the BRVO group during follow-up and in the cross-sectional (CS) evaluation. M = Months. \*  $p < 0.05$ .



**Fig. 4.** Total number of injections given and by year of follow-up, on average, in both groups. M = Months.

**Table 2.** Evolution of BCVA and CMT in the CRVO and BRVO groups during follow-up and in the cross-sectional (CS) evaluation

	CRVO				BRVO			
	BCVA, L	p value	CMT, μm	p value	BCVA, L	p value	CMT, μm	p value
Initial visit	47.1±26.0	–	666.7±221.4	–	51.8±24.1	–	555.9±225.9	–
6 months	55.3±19.2	0.018	418.6±183.5	0.001	65.1±20.6	0.023	349.9±150.0	0.005
12 months	53.8±21.4	0.087	387.2±175.5	0.002	63.3±19.5	0.044	376.4±136.2	0.013
24 months	54.5±21.8	0.163	385.8±195.5	0.006	57.8±25.0	0.201	351.2±188.3	0.019
36 months	54.3±24.1	0.057	290.1±151.5	0.003	58.1±26.0	0.344	337.6±100.4	0.013
CS visit	55.4±23.6	0.052	303.9±160.3	0.001	53.3±24.8	0.815	337.4±153.5	0.009

Means ± SD. Differences were evaluated versus the initial visit.

**Table 3.** BCVA in the final cross-sectional evaluation

Group	Stabilization (≤5 L)	Gain >5 L	Gain ≥15 L	Gain ≥30 L	Loss >5 L	Loss ≥15 L	Loss ≥30 L
CRVO	38% (n = 6)	6% (n = 1)	31% (n = 5)	13% (n = 2)	6% (n = 1)	6% (n = 1)	0% (n = 0)
BRVO	63% (n = 10)	0% (n = 0)	13% (n = 2)	6% (n = 1)	0% (n = 0)	13% (n = 2)	6% (n = 1)

n = Number of eyes; % = rate of eyes.

**BRVO Group.** Gain in BCVA was significant at the 6- and 12-month follow-up visits ( $p = 0.023$  and  $0.044$ , respectively). The final mean BCVA in the cross-sectional evaluation was  $53.3 \pm 24.8$  L, corresponding to a final mean gain of  $1.6 \pm 21.0$  L ( $p > 0.05$ ; fig. 3; table 2). The

percentage of eyes that maintained, gained or lost VA is presented in table 3 for both groups. The CMT was significantly inferior at all evaluation points compared to baseline in both the CRVO and the BRVO group ( $p < 0.05$ ; fig. 2, 3; table 2).



**Table 4.** Microstructural changes in SD-OCT, CT and FAZ area at initial and final visits in both groups

	CRVO		BRVO	
	initial	final	initial	final
Macular fluid (cystoid macular edema/subretinal fluid), %	100 (n = 15)	31 (n = 5)	100 (n = 14)	69 (n = 11)
Disruption of the ELM, %	93 (n = 14)	88 (n = 14)	43 (n = 6)	69 (n = 11)
Disruption of the IS/OS line, %	100 (n = 15)	88 (n = 14)	86 (n = 12)	69 (n = 11)
Disruption of the RPE, %	60 (n = 9)	25 (n = 4)	43 (n = 6)	38 (n = 6)
Vitreomacular traction, %	7 (n = 1)	6 (n = 1)	21 (n = 3)	6 (n = 1)
Epiretinal membrane, %	20 (n = 3)	75 (n = 12)	43 (n = 6)	63 (n = 10)
Loss of foveal contour due to macular atrophy, %	0 (n = 0)	31 (n = 5)	0 (n = 0)	6 (n = 1)
Macular CT, $\mu\text{m}$	–	254.0 $\pm$ 76.3	–	192.6 $\pm$ 55.7
Subfoveal CT, $\mu\text{m}$	–	284.2 $\pm$ 93.7	–	205.6 $\pm$ 66.2
FAZ area, $\text{mm}^2$	0.43 $\pm$ 0.34 (n = 9)	0.39 $\pm$ 0.21 (n = 12)	0.40 $\pm$ 0.31 (n = 12)	0.43 $\pm$ 0.27 (n = 13)

% = Rate of eyes; n = number of eyes. One eye in the CRVO group and 2 eyes in the BRVO group had poor baseline OCT quality preventing morphological analysis.

**Table 5.** Final FAZ area and BCVA in ischemic and nonischemic eyes in both groups (means  $\pm$  SD)

	CRVO			BRVO		
	ischemic	nonischemic	p value	ischemic	nonischemic	p value
Final FAZ area, $\text{mm}^2$	0.48 $\pm$ 0.25	0.33 $\pm$ 0.11	>0.05	0.47 $\pm$ 0.37	0.42 $\pm$ 0.23	>0.05
Final BCVA, L	38.6 $\pm$ 17.6	68.4 $\pm$ 17.2	0.013	37.7 $\pm$ 18.4	62.7 $\pm$ 22.0	0.020
Baseline BCVA, L	30.0 $\pm$ 18.3	60.3 $\pm$ 21.6	0.022	45.0 $\pm$ 10.0	55.8 $\pm$ 29.4	>0.05

### Number of Injections and Complications

The average number of injections performed during follow-up is presented in figure 4. In the CRVO group, 75% (n = 12) of the eyes required IVR injections after the 1st year of treatment, and 44% (n = 7) required treatment after 2 years of follow-up. In the BRVO group, 50% (n = 8) of the eyes required IVR injections after the 1st year, and 31% (n = 5) after 2 years of follow-up. The mean time without treatment since the last injection was 21.6  $\pm$  14.5 months in the CRVO group and 24.5  $\pm$  17.6 months in the BRVO group.

In the CRVO group, one eye presented with rubeosis and vitreous hemorrhage and another presented with retinal neovascularization during follow-up. Both were successfully treated with IVR and panretinal photocoagulation, and had a final VA loss of 5 L and a gain of 31 L, respectively. In the BRVO group, 4 eyes developed retinal neovascularization and were treated with scatter photocoagulation. The final VA of these eyes ranged from 5 to 60 L (median = 30.5 L), and VA changed between a loss of 45 L and a gain of 25 L (median = –12 L).

No cases of retinal tears, detachment or infections were observed in both groups, and there were no reported systemic events.

### OCT and FA

Table 4 presents the most common retinal morphological changes found in OCT when starting treatment and in the final visit, and subfoveal and macular CT as well as FAZ area measurements performed in both groups. There was a statistically significant difference between both groups for subfoveal CT (p = 0.024) but not for age (p > 0.05). The first angiogram was performed on average 8.6  $\pm$  10.4 months after the diagnosis of RVO. The change between mean baseline and mean final FAZ areas was nonsignificant in both groups (p > 0.05).

Seven eyes (44%) in the CRVO group and 6 eyes (38%) in the BRVO group were classified as ischemic in the final visit. Differences between ischemic and nonischemic eyes for the final FAZ area and BCVA are presented in table 5. In the CRVO group, and in spite of the worse final VA, ischemic eyes gained 8.6  $\pm$  16.4 L on average (p > 0.05). CRVO eyes without ischemia gained on average 8.1  $\pm$

12.8 L ( $p > 0.05$ ). In the BRVO group, ischemic eyes had a final mean loss of  $7.3 \pm 21.5$  L ( $p > 0.05$ ) compared to a final mean gain of  $6.9 \pm 17.4$  L in nonischemic eyes ( $p > 0.05$ ). Also, as shown in table 3, two eyes lost 15 L or more and 1 eye lost 30 L or more in the BRVO group. A detailed analysis of these patients with severe or very severe VA loss showed that the first one who lost 45 L was considered ischemic in the final visit and had permanent changes in the external layers of the retina in OCT; the second lost 15 L because of poor response to treatment with recurrent edema through follow-up in spite of the 11 injections performed; the third patient lost 19 L and also showed poor response to treatment after 5 injections, with permanent fluid in OCT until the final visit, and he was also considered ischemic in the final visit.

#### *Univariate Analysis*

**CRVO Group.** Final BCVA correlated with initial BCVA ( $p < 0.001$ ,  $r = 0.82$ ). Of the baseline and final morphological changes found in OCT, only a disrupted RPE status in the final OCT was associated with worse final VA ( $p = 0.018$ ).

**BRVO Group.** Final BCVA correlated with initial BCVA ( $p = 0.020$ ,  $r = 0.61$ ). Of the morphological changes, baseline disruption of RPE and the final status of ELM and IS/OS line negatively affected the final BCVA ( $p = 0.008$ ,  $0.036$  and  $0.036$ , respectively). Also, a negative correlation was found between final macular and subfoveal CT, and final BCVA ( $p = 0.026$ ,  $r = -0.57$ , and  $p = 0.030$ ,  $r = -0.56$ ).

**All Eyes Together.** RPE disruption at baseline ( $p = 0.003$ ) and disruption of RPE, ELM and IS/OS line at the final visit ( $p = 0.006$ ,  $0.006$  and  $0.015$ , respectively) negatively affected the final VA. Final BCVA was positively correlated with initial BCVA ( $p < 0.001$ ,  $r = 0.74$ ).

As reported, 5 eyes were previously treated with bevacizumab and/or triamcinolone, which could be a confounding factor. For this reason, we repeated the analysis excluding these 5 eyes. Again, only the initial RPE disruption ( $p = 0.012$ ), and the final ELM, IS/OS line and RPE disruption in OCT were associated with worse final BCVA ( $p = 0.021$ ,  $0.021$  and  $0.041$ , respectively). The final BCVA was again correlated with initial BCVA ( $p < 0.001$ ,  $r = 0.76$ ). The final CMT was also positively correlated with the total number of IVR injections performed during follow-up ( $p = 0.012$ ,  $r = 0.48$ ).

#### *Multiple Regression Analysis*

Regression analysis was performed to identify the predictive variables for final BCVA considering all eyes to-

gether. For this purpose, we included the OCT morphological changes found to be significant in univariate analysis and adjusted for initial BCVA.

Being strongly correlated with the final BCVA, initial BCVA was found as a positive predictor of the final BCVA ( $p = 0.001$ ), as expected. Among the morphological changes in OCT, only the disruption of the RPE before starting treatment was predictive of worse final BCVA ( $p = 0.011$ ). The remaining morphological variables had no predictive value once initial BCVA was considered in multivariate analysis.

The analysis was repeated excluding the 5 eyes previously treated with bevacizumab and/or triamcinolone. The variables with predictive value remained the same: initial BCVA ( $p < 0.001$ ) and baseline status of RPE in OCT ( $p = 0.010$ ).

## **Discussion**

We analyzed the long-term results of intravitreal ranibizumab in the treatment of macular edema due to RVO in clinical practice, and we identified predictors of functional outcome using multimodal retinal imaging. Our results show that baseline disruption of macular RPE and initial BCVA are good predictors of functional outcome, that intravitreal ranibizumab did not prevent rubeosis or retinal neovascularization, and that no increase in macular ischemia was observed after 3 years or more of treatment.

VA increased steadily in the first 6 months as expected in both groups; however, the observed gain was not as good as those reported in the BRAVO and CRUISE trials [9, 10]. This is probably related to the PRN regimen used from the beginning instead of fixed monthly injections in the first 6 months performed in these trials. In fact, the mean number of injections in the 1st year in our study was 4.1 in the CRVO group and 3.5 in the BRVO group. The protocols implemented in clinical trials are often difficult to apply in regular clinical settings, and the frequent restrictions found in clinical practice may be associated with the limited number of injections performed. Recent studies analyzed the protocols and frequency of anti-VEGF treatments for macular edema secondary to RVO in clinical practice in the United States. One reported average bevacizumab injections of 2.5, 3.1 and 3.3 in BRVO, and 3.1, 3.1 and 3.5 in CRVO across the years 2008, 2009 and 2010, respectively. Another reported mean numbers of injections of  $4.4 \pm 2.8$  and  $4.7 \pm 2.9$  in patients treated with ranibizumab or aflibercept, respectively, for CRVO

in the 1st year [17, 18]. This picture is much different from the above-mentioned clinical trials and very similar to our own experience.

In the long term, after 43 months of treatment, we found a mean gain of 8.3 L in CRVO patients, and 31% gained 15 L or more. This was inferior compared to CRVO patients from the RETAIN study [13], where patients gained on average 14.0 L from the CRUISE study baseline, and 53.1% gained 15 L or more after a mean follow-up of 51.4 months. The mean number of injections per year in the RETAIN study was, however, also superior: 4.5 in year 2 and 3.6 in year 3 (3.3 in year 4) compared to 1.7 in year 2 and 0.9 in year 3 in our study. Although the initial VA gain for CRVO patients was not as high, VA remained stable in the long term, with fewer injections.

In BRVO patients, the RETAIN study [13] reported an improvement of 20.1 L at the end of follow-up, with 61.8% of the patients showing an improvement of 15 L or more from the BRAVO trial baseline. Our results are different, as the gain in VA did not remain constant, but declined, with a final mean gain of 1.6 L after 45 months of treatment, and only 13% gained 15 L or more. A more profound analysis revealed, however, that 38% were ischemic in the final visit. In fact, 2 eyes lost more than 15 L and 1 lost more than 30 L, which, considering the small sample size of the group, helps to explain the lower final VA. Also, excluding ischemic eyes from the analysis, a VA gain of 6.9 L would be achieved. More ischemia is associated with higher levels of VEGF, and in the long term this may have a negative impact on VA despite the treatment with anti-VEGF. Another important factor is again the number of injections performed, which was superior in the RETAIN study: 2.6, 2.1 and 2.0 in years 2, 3 and 4, respectively, for BRVO eyes [13]. Instead, we performed on average 1.3 injections in year 2 and 0.6 in year 3. This also may have contributed to the progressive loss in VA.

CMT decreased steadily and significantly in the first 6 months, paralleling the most expressive VA gain, in both groups. In the CRVO group, CMT continued to decrease during follow-up, but VA remained stable. Also, 31% of these eyes still had fluid in the final OCT. This is not unexpected, and Campochiaro et al. [13] also reported resolution of macular edema in only 43.8% of CRVO cases in the long term. In the BRVO group, CMT remained relatively stable, but VA still declined. Sixty-nine percent of our BRVO patients still had fluid in the final OCT compared to 50% in RETAIN study. Recurrent and chronic edema is thought to lead to loss in VA due to photoreceptor damage in the long term [13]. Probably the morpho-

logical changes at microstructural level from chronic and recurrent edema are the reason for this divergence between CMT and VA. A more aggressive strategy preventing fluid recurrence could, therefore, lead to improved results.

CT was also assessed in both groups in the final visit, and we found that it was superior in the CRVO group compared to the BRVO group. Tsuiiki et al. [22] have already reported increased subfoveal CT in CRVO eyes, and they also suggested that this is related to vessel dilation and increased permeability by VEGF. Considering this assumption, and as CRVO is a global event associated with higher levels of VEGF, the choroid of these eyes could be expected to be thicker compared to BRVO eyes. The age difference between the 2 groups, although without significance, may also have contributed to this outcome, as CT decreases with age [26]. Tsuiiki et al. [22] also found that the subfoveal CT of CRVO eyes decreased significantly after intravitreal bevacizumab treatment. Therefore, inhibition of VEGF by bevacizumab could be the cause of this reduction. This can also aid to explain the relatively normal values for CT found in the CRVO group and the apparently decreased CT readings found in the BRVO eyes.

Acutely after BRVO or CRVO, there may be mild or no RNP (nonischemic RVO), or it may be severe (ischemic RVO). Measurements of the area of RNP on FA have shown that enlargement of this area is common in both BRVO and CRVO [27, 28]. The mechanism is unknown, but concern has been expressed that it may be exacerbated by VEGF itself [1, 19], or on the contrary from its inhibition [20, 21]. For this purpose, we also analyzed the changes in the FAZ area in eyes with RVO treated with IVR in the long term. These changes were, however, small and nonsignificant in both groups. The initial concerns of worsening of macular ischemia after prolonged treatment with ranibizumab are, therefore, not supported by the findings of our study.

Another important finding was that ischemic eyes had larger final FAZ areas and worse functional outcome. This meets the concept of the influence of higher levels of VEGF in the progression of RNP, and the fact that, as stated by Sophie et al. [1], in patients with RVO infrequent ranibizumab injections to control edema may not be sufficient to prevent progression of RNP in all cases, which in turn may contribute to loss of visual gains in the long term. It is also important to notice that in our study, treatment with ranibizumab did not prevent the development of retinal neovascularization and rubeosis. This was also reported in a recent paper [29]. Therefore, the role of

ranibizumab in preventing progression of ischemia and subsequent development of neovascular disease must be further addressed.

In the present study, we found that only baseline BCVA and microstructural morphological changes in OCT were related to the final visual outcome in univariate analysis. Among the morphological changes, only disruption of the external layers of the retina (ELM, IS/OS junction and RPE) reached statistical significance. Regression analysis was also performed to identify the predictive variables for final BCVA. Only the baseline BCVA and the baseline disruption of the RPE were significant predictors of visual outcome. Similarly, Coscas et al. [5] also reported that final visual prognosis depends on the initial VA and on the presence and ELM integrity, and IS/OS photoreceptors (IS/OS interface). There was no other factor found to have predictive value for functional outcome, but this may only be due to the small sample size. This matter should be further addressed in future studies, as optimization of treatment strategies is necessary in clinical practice, as supported by our study.

There are important limitations to this study. Besides its retrospective design and small sample, the morphological measurements of the choroid and FAZ were obtained manually by 2 independent observers, because there is no automated software available at present. New

automated software will reduce the bias involved and the time required for measurements. Although the overall group was not small, the subgroups were; however, we provide further insight into the functional and morphological evolution of eyes with RVO treated with ranibizumab in the long term in a real-life clinical setting, and we explored different factors related to functional outcome.

In conclusion, treatment with ranibizumab as needed to control macular edema is safe and provides satisfactory long-term results in patients with CRVO and BRVO, although functional outcome was not as good as reported in clinical trials with higher retreatment rates. Ranibizumab does not seem to contribute to macular ischemia, but it did not prevent the development of retinal neovascularization and rubeosis. Final VA depends on the baseline VA and RPE integrity assessed by means of SD-OCT.

### Disclosure Statement

Rufino Silva is a member of the Advisory Board for Bayer, Allergan, Novartis, Alcon, THEA and Alimera. João Figueira is a member of the Advisory Board for Alcon, Novartis, Allergan, Bayer, Pfizer and Alimera. The other authors have no proprietary or financial interest.

### References

- Sophie R, Hafiz G, Scott AW, Zimmer-Galler I, Nguyen QD, Ying H, Do DV, Solomon S, Sodhi A, Gehlbach P, Duh E, Baranano D, Campochiaro PA: Long-term outcomes in ranibizumab-treated patients with retinal vein occlusion; the role of progression of retinal nonperfusion. *Am J Ophthalmol* 2013;156:693–705.
- Rehak M, Wiedemann P: Retinal vein thrombosis: pathogenesis and management. *J Thromb Haemost* 2010;8:1886–1894.
- Duker JS, Brown GC: Anterior location of the crossing artery in branch retinal vein obstruction. *Arch Ophthalmol* 1989;107:998–1000.
- Frangieh GT, Green WR, Barraquer-Somers E, Finkelstein D: Histopathologic study of nine branch retinal vein occlusions. *Arch Ophthalmol* 1982;100:1132–1140.
- Coscas G, Loewenstein A, Augustin A, Bandello F, Battaglia Parodi M, Lanzetta P, Monés J, de Smet M, Soubrane G, Staurenghi G: Management of retinal vein occlusion – consensus document. *Ophthalmologica* 2011;226:4–28.
- Campochiaro PA, Hafiz G, Shah SM, Nguyen QD, Ying H, Do DV, Quinlan E, Zimmer-Galler I, Haller JA, Solomon SD, Sung JU, Hadi Y, Janjua KA, Jawed N, Choy DF, Arron JR: Ranibizumab for macular edema due to retinal vein occlusions: implication of VEGF as a critical stimulator. *Mol Ther* 2008;16:791–799.
- Spaide RF, Chang LK, Klancnik JM, Yannuzzi LA, Sorenson J, Slakter JS, Freund KB, Klein R: Prospective study of intravitreal ranibizumab as a treatment for decreased visual acuity secondary to central retinal vein occlusion. *Am J Ophthalmol* 2009;147:298–306.
- Hahn P, Fekrat S: Best practices for treatment of retinal vein occlusion. *Curr Opin Ophthalmol* 2012;23:175–181.
- Campochiaro PA, Heier JS, Feiner L, Gray S, Saroj N, Rundle AC, Murahashi WY, Rubio RG, BRAVO Investigators: Ranibizumab for macular edema following branch retinal vein occlusion: six-month primary end point results of a phase III study. *Ophthalmology* 2010;117:1102–1112.
- Brown DM, Campochiaro PA, Singh RP, Li Z, Gray S, Saroj N, Rundle AC, Rubio RG, Murahashi WY, CRUISE Investigators: Ranibizumab for macular edema following central retinal vein occlusion: six-month primary end point results of a phase III study. *Ophthalmology* 2010;117:1124–1133.
- Brown DM, Campochiaro PA, Bhisitkul RB, Ho AC, Gray S, Saroj N, Adamis AP, Rubio RG, Murahashi WY: Sustained benefits from ranibizumab for macular edema following branch retinal vein occlusion: 12-month outcomes of a phase III study. *Ophthalmology* 2011;118:1594–1602.
- Campochiaro PA, Brown DM, Awh CC, Lee SY, Gray S, Saroj N, Murahashi WY, Rubio RG: Sustained benefits from ranibizumab for macular edema following central retinal vein occlusion: twelve-month outcomes of a phase III study. *Ophthalmology* 2011;118:2041–2049.
- Campochiaro PA, Sophie R, Pearlman J, Brown DM, Boyer DS, Heier JS, Marcus DM, Feiner L, Patel A; RETAIN Study Group: Long-term outcomes in patients with retinal vein occlusion treated with ranibizumab: the RETAIN study. *Ophthalmology* 2014;121:209–219.
- Kinge B, Stordahl PB, Forsaa V, Fossen K, Haugstad M, Helgesen OH, Seland J, Stene-Johansen I: Efficacy of ranibizumab in patients with macular edema secondary to central retinal vein occlusion: results from the sham-controlled ROCC study. *Am J Ophthalmol* 2010;150:310–314.



- 15 Chang LK, Spaide RF, Klancnik JM, Sorenson J, Slakter JS, Freund KB, Yannuzzi LA, Tseng JJ, Klein R: Longer-term outcomes of a prospective study of intravitreal ranibizumab as a treatment for decreased visual acuity secondary to central retinal vein occlusion. *Retina* 2011;31:821–828.
- 16 Tan MH, McAllister IL, Gillies ME, Verma N, Banerjee G, Smithies LA, Wong WL, Wong TY: Randomized controlled trial of intravitreal ranibizumab versus standard grid laser for macular edema following branch retinal vein occlusion. *Am J Ophthalmol* 2014;157:237–247.
- 17 Lotery AJ, Regnier S: Patterns of ranibizumab and aflibercept treatment of central retinal vein occlusion in routine clinical practice in the USA. *Eye (Lond)* 2015;29:380–387.
- 18 Kiss S, Liu Y, Brown J, Holekamp NM, Almony A, Campbell J, Kowalski JW: Clinical utilization of anti-vascular endothelial growth-factor agents and patient monitoring in retinal vein occlusion and diabetic macular edema. *Clin Ophthalmol* 2014;8:1611–1621.
- 19 Campochiaro PA, Bhisitkul RB, Shapiro H, Rubio RG: Vascular endothelial growth factor promotes progressive retinal nonperfusion in patients with retinal vein occlusion. *Ophthalmology* 2013;120:795–802.
- 20 Manousaridis K, Talks J: Macular ischaemia: a contraindication for anti-VEGF treatment in retinal vascular disease? *Br J Ophthalmol* 2012;96:179–184.
- 21 Feucht N, Schönbach EM, Lanzl I, Kotliar K, Lohmann CP, Maier M: Changes in the foveal microstructure after intravitreal bevacizumab application in patients with retinal vascular disease. *Clin Ophthalmol* 2013;7:173–178.
- 22 Tsuike E, Suzuma K, Ueki R, Maekawa Y, Kitaoka T: Enhanced depth imaging optical coherence tomography of the choroid in central retinal vein occlusion. *Am J Ophthalmol* 2013;156:543–547.
- 23 Yamazaki T, Koizumi H, Yamagishi T, Kinoshita S: Subfoveal choroidal thickness after ranibizumab therapy for neovascular age-related macular degeneration: 12-month results. *Ophthalmology* 2012;119:1621–1627.
- 24 Marques JP, Costa M, Melo P, Oliveira CM, Pires I, Cachulo ML, Figueira J, Silva R: Ocular risk factors for exudative AMD: a novel semiautomated grading system. *ISRN Ophthalmol* 2013;2013:464218.
- 25 Spaide RF, Koizumi H, Pozzoni MC: Enhanced depth imaging spectral-domain optical coherence tomography. *Am J Ophthalmol* 2008;146:496–500.
- 26 Margolis R, Spaide RF: A pilot study of enhanced depth imaging optical coherence tomography of the choroid in normal eyes. *Am J Ophthalmol* 2009;147:811–815.
- 27 Ip MS, Scott IU, VanVeldhuisen PC, Oden NL, Blodi BA, Fisher M, Singerman LJ, Tolentino M, Chan CK, Gonzalez VH; SCORE Study Research Group: A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with observation to treat vision loss associated with macular edema secondary to central retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 5. *Arch Ophthalmol* 2009;127:1101–1114.
- 28 Scott IU, Ip MS, VanVeldhuisen PC, Oden NL, Blodi BA, Fisher M, Chan CK, Gonzalez VH, Singerman LJ, Tolentino M; SCORE Study Research Group: A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with standard care to treat vision loss associated with macular edema secondary to branch retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 6. *Arch Ophthalmol* 2009;127:1115–1128.
- 29 Brown DM, Wykoff CC, Wong TP, Mariani AF, Croft DE, Schuetzle KL; RAVE Study Group: Ranibizumab in preproliferative (ischemic) central retinal vein occlusion: the rubeosis anti-VEGF (RAVE) trial. *Retina* 2014;34:1728–1735.