

Association of retinal vessel calibre and visual outcome in eyes with diabetic macular oedema treated with ranibizumab

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Abstract

Purpose The study aims to identify the association between the baseline retinal vascular calibre and visual outcome of patients with diabetic macular oedema (DMO) treated with intravitreal ranibizumab.

Methods The 1-M field (as defined in the ETDRS study) of the digital colour fundus photographs of DMO patients who had been treated primarily with ranibizumab in a clinical trial was assessed. Of the 84 patients, 25 had gradable retinal photographs that could be subjected to analyses by the Interactive Vessel Analysis (IVAN) software at baseline. The average retinal vascular calibre of the six largest venules (CRVE) and the six largest arterioles (CRAE) in the peripapillary area (0.5 and 1 disc diameter from the optic disc margin) was measured. The relationship between CRVE and CRAE at baseline and the change in visual acuity at month 12 was assessed using the Mann–Whitney *U* test.

Results Ten eyes from 10 patients who had shown an improvement of ≥ 2 lines of best corrected visual acuity (BCVA) at month 12 had a wider baseline CRVE ($248.3 \pm 24.5 \mu\text{m}$) compared with the 15 eyes from 15 patients who did not show the improvement of ≥ 2 lines ($226.6 \pm 44.8 \mu\text{m}$, $P < 0.05$). The baseline CRAE did not differ significantly in these patients (156.1 ± 22.7 vs $142 \pm 17.5 \mu\text{m}$, $P = 0.17$).

Conclusions A wider baseline retinal venular calibre may be a predictor of better visual outcome in DMO eyes treated with ranibizumab. Further prospective studies with a larger sample size and a broader range of disease severity and visual acuity are needed to confirm this finding.

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Introduction

Changes in retinal vascular calibre (broadening or narrowing) have been considered a risk factor for the development of various systemic diseases in several large population-based studies including the Beaver Dam Eye Study, the Blue Mountain Eye study, and the Wisconsin Epidemiologic Study of Diabetic Retinopathy.^{1–4} Furthermore, wider retinal venular calibre has been regarded as an independent risk factor for the subsequent incidence and progression of diabetic retinopathy (DR).⁵

The discovery of anti-vascular endothelial growth factor (anti-VEGF) therapy has considerably transformed the management and treatment of diabetic macular oedema (DMO). Ranibizumab (Lucentis, Genentech, San Francisco, CA, USA) (RBZ), an anti-VEGF agent, is a recombinant, humanised monoclonal antibody fragment that binds to all isoforms of VEGF-A, which is a critical stimulus in the pathogenesis of DMO.⁶ The RISE and RIDE studies demonstrated that ranibizumab is an effective treatment for DMO. The RISE study showed that 44.8% of patients receiving 0.3 mg RBZ and 39.2% of patients receiving 0.5 mg RBZ gained ≥ 15 letters, while the RIDE study demonstrated that the matching proportions were 33.6% and 45.7%, respectively.⁷ The factors that predict the treatment response to RBZ in DMO patients are currently not apparent to clinicians and scientists. Bressler *et al* recently evaluated 361 eyes that were randomly assigned to intravitreal RBZ with prompt or

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deferred laser treatment within a trial of RBZ, triamcinolone acetonide, and laser treatment for centre-involved DMO. The study was done to identify factors that predict the success or failure of treatment with intravitreal RBZ. The authors have demonstrated that younger age, milder DR on clinical examination, the absence of surface-wrinkling retinopathy, and the reduction in central subfield thickness during the first treatment year better predicted visual acuity outcomes.⁸

Furthermore, studies have demonstrated that diabetic patients have a wider retinal arteriolar calibre⁴ and these patients have a greater chance of developing incident retinopathy. Therefore, changes in retinal vascular calibre are considered to be a potential subclinical marker of DR.⁹ The Blue Mountains Eye Study also revealed that the severity of DR is associated with widening of the retinal venular calibre.⁴ Klein *et al*⁵ have confirmed this finding in a recently published study. They concluded that increase in the retinal venular calibre, but not the arteriolar calibre, was independently associated with the occurrence and advancement of DR.

Several studies have evaluated the association between intravitreal treatments and retinal vascular calibres. Wickremasinghe *et al*,¹⁰ using Interactive Vessel Analysis (IVAN) software, demonstrated that intravitreal triamcinolone acetate has a significant narrowing effect on both retinal arteriolar and venular calibres after a single injection over a 3-month time period. This study also demonstrated that there was no change in retinal arteriolar or venular calibres in those who were treated with sham injections. Additionally, Tatlipinar *et al*¹¹ assessed eight DMO patients treated with intravitreal injections of 1.25 mg bevacizumab over a period of 1 month. They had reported that there would be a trend towards vasoconstriction in arterioles and venules at day 7, but this difference did not reach statistical significance ($P > 0.05$). Further, Wickremasinghe *et al*'s¹² findings demonstrated a potential association between wider baseline venular calibre and a worst response in eyes with neovascular age-related macular degeneration (AMD) treated with RBZ.

Therefore, the measurement of retinal vascular calibre may potentially prove to be an anatomical indicator of underlying disease severity in patients with DMO and may also provide the scientific community with a better understanding of the factors that determine visual outcome in patients needing anti-VEGF treatment.

Materials and methods

Study population

This study was based on data from the READ-2 study, which was a phase II randomised multicentre clinical

trial designed to evaluate the safety and efficacy of intravitreal RBZ in patients with DMO. The study protocol and primary outcomes have been reported.^{13,14} The READ-2 study subjects whose data were included in this vessel calibre analysis included those who had been treated primarily with RBZ alone or in combination with laser and had gradable retinal photographs at baseline.

Measurement of retinal vascular calibre

Optic disc-centred digital colour fundus photographs (field 1) of DMO patients were assessed at baseline.¹⁵ All photographs were taken prior to the first treatment with RBZ or laser plus RBZ in the READ-2 Study. The retinal vascular calibre was measured using the computer program IVAN version 1.30 (University of Wisconsin, Madison, WI, USA) according to a detailed protocol that has been described by Hubbard and Wong.^{16,17} All vessels coursing through an area one-half to one-disc diameter from the optic disc margin (double arrow in Figure 1) were measured. The mean vessel calibre of the largest six arteries was termed central retinal artery equivalent (CRAE) while that of the largest six venules was coined central retinal vein equivalent (CRVE) using formulas developed by Hubbard *et al*¹⁶ and later modified by Knudtson *et al*.¹⁸

Main outcome measures

In the READ-2 Study, best-corrected visual acuity (BCVA) was measured using the ETDRS charts. Optical coherence tomography (OCT) was obtained by experienced technicians who were using the fast macular scan protocol (Stratus OCT Carl Zeiss Meditec, Dublin, CA, USA) at baseline; no spectral domain OCT was available at the time the READ-2 was conducted. Primary outcome measures included changes in visual acuity and central macular thickness (CMT) at month 12 compared to baseline. In addition, demographics (age, gender, race, and ethnicity), HbA1c level, and mean arterial blood pressure (MAP) were collected and correlated to outcome measurements.

Statistical analysis

In this vessel calibre analysis, improvement in visual acuity at month 12 was defined as a ≥ 10 letter gain in vision after the initiation of RBZ or RBZ plus laser treatment for DMO. The association between baseline CRVE and CRAE and the change in BCVA and CMT at month 12 was assessed using the Mann-Whitney *U* test.

This study was conducted with the approval of Johns Hopkins University School of Medicine Institutional

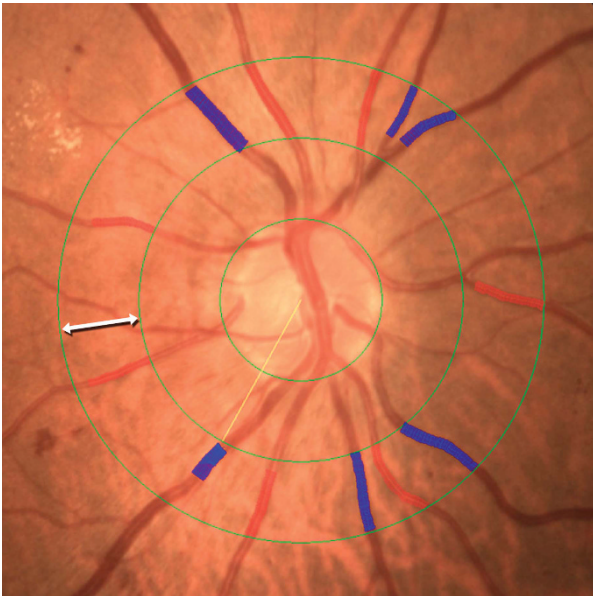


Figure 1 An optic disc-centred digital colour fundus photograph of the right optic nerve head of a DMO patient. All vessels coursing through an area one-half to one-disc diameter from the optic disc margin (double arrow) were measured and the largest six were termed central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE).

Review Board and in accordance with the principles of the Declaration of Helsinki.

Results

In this analysis, 25 patients (25 eyes; male: 15, female: 10, mean age: 61.9 years (SD 9.1)) had baseline photographs that were gradable by IVAN software. The visual acuity information was collected for these patients at baseline and month 12. Table 1 shows the demographics, mean HbA1c level, mean MAP, and the treatment group assignment at baseline, as well as the association between the characteristics and visual outcome of the eyes at month 12. Group 1 (G1) includes 10 eyes of 10 patients who showed visual improvement that was ≥ 2 lines over the study period. These patients have been classified as demonstrating improved visual acuity. Group 2 (G2) includes 15 eyes of 15 patients with < 2 -line vision gain or a decrease in visual acuity when compared to the baseline.

Anatomical (CRVE, CRAE) and functional (BCVA) characteristics of the eyes in this study and their correlations with visual outcome after 12 months have been summarised in Table 2. Race, sex, and baseline CRVE were significantly different in both groups ($P < 0.05$). Marginal correlations were demonstrated when baseline mean MAP and mean HbA1c were compared ($P < 0.10$).

The eyes that showed improvement in this study also had a wider baseline venular calibre (CRVE = $248.3 \pm 24.5 \mu\text{m}$) when compared with the eyes that did not show significant improvement (CRVE = $226.6 \pm 44.8 \mu\text{m}$) ($P < 0.05$). On the other hand, the baseline arteriolar calibre did not demonstrate a significant correlation with visual outcome, since there was no significant difference between the two groups (CRAE: 156.1 ± 22.7 vs $142 \pm 17.5 \mu\text{m}$, $P = 0.17$). The mean BCVA at baseline has no significant difference in G1 and G2 (26.5 vs 25.6 letters, $P = 0.7$), and thus it did not correlate with visual outcomes at month 12 (The mean number (SD) of RBZ injections was 5.12 ± 1.91 and no significant difference in treatment frequency between the two groups was appreciated (4.88 ± 2.02 vs 5.26 ± 1.90 , $P > 0.5$)). There was no significant difference between the mean number of injections of RBZ in the group that gained ≥ 10 letters and the group with < 10 letters (4.88 ± 2.02 vs 5.26 ± 1.90 , $P > 0.5$).

Discussion

The index study is among the first to analyse and find a correlation between retinal vessel calibre and visual outcome in patients with DMO. Our study has demonstrated that eyes that showed moderate improvement (≥ 10 ETDRS letters) had a wider baseline venular calibre when compared to eyes that did not show moderate improvement. A wider baseline CRVE was significantly correlated with vision improvement, while a wider baseline CRAE was not. As Klein *et al*⁵ have suggested that CRVE can be used to predict DR progression, it is possible that retina specialists may use CRVE to predict visual improvement in patients with DMO treated with RBZ.

Klein *et al* evaluated the correlation of change in retinal vascular calibre to the subsequent 6-year incidence and progression of DR and incidence of proliferative diabetic retinopathy (PDR) and macular oedema in patients with diabetes mellitus. Their results showed that widening of the retinal venular, but not arteriolar, calibre was associated with subsequent incidence and progression of DR, independent of the DR severity level, glycemic control, and other factors. They believe the CRVE may provide additional information about the risk of incidence and progression of DR beyond traditional risk factors.⁵

In a recently published study, Tatlipinar *et al*¹¹ evaluated the short-term effects of a single intravitreal bevacizumab injection on the retinal vascular calibres in patients with DMO. There appeared to be a trend towards vasoconstriction, but did not reach statistical significance. The results suggested that intravitreal injection of bevacizumab might induce retinal vasoconstriction.

Table 1 Baseline characteristics of eyes treated with intravitreal ranibizumab injections for diabetic macular oedema

Characteristics	Total (n = 25)	Improvement in BCVA of ≥ 10 letters (n = 10)	No improvement in BCVA (n = 15)	P-value
<i>Age, years</i>				
Mean \pm SD	61.92 \pm 9.11	59.1 \pm 7.5	63.8 \pm 9.82	0.2
<i>Sex, n (%)</i>				
Female	10 (40%)	1 (10%)	9 (60%)	
Male	15 (60%)	9 (90%)	6 (40%)	
<i>Race, n (%)</i>				
Asian	1 (4%)	1 (10%)	0	
African-American	1 (4%)	1 (10%)	0	
Caucasian	21 (84%)	6 (60%)	15 (100%)	
Others	2 (8%)	2 (20%)	0	
<i>Ethnicity, n (%)</i>				
Hispanic	1 (4%)	1 (10%)	0	
Non-Hispanic	24 (96%)	9 (90%)	15 (100%)	
<i>Mean HbA1c, %</i>				
Mean \pm SD	7.26 \pm 1.37	7.01 \pm 1.7	7.42 \pm 1.14	0.09
<i>MAP</i>				
Mean \pm SD	97.7 \pm 13.4	103.4 \pm 15.84	93.91 \pm 10.55	0.08
<i>Treatment group</i>				
0.5 mg RBZ	11 (44%)	4 (40%)	7 (46.47%)	
Laser + RBZ	14 (56%)	6 (60%)	8 (53.33%)	

Abbreviations: BCVA, best corrected visual acuity; Hb, haemoglobin; MAP, mean arterial blood pressure; n, number; RBZ, ranibizumab. Patients were divided into two groups based on the visual outcomes at month 12.

However, the small number of subjects might have prevented the difference from reaching statistical significance. In our study of patients receiving treatments with RBZ the results have demonstrated that patients with a wider CRVE at baseline exhibited greater improvement in visual acuity at 12 months. CRVE may thus be considered as an indirect marker and indicator of how an eye with DMO may respond to anti-VEGF therapies such as RBZ. Perhaps, the intraocular level of VEGF may correlate with CRVE.

Furthermore, Sacu *et al*¹⁹ investigated the effect of intravitreal ranibizumab (0.5 mg) on retinal vascular calibres and retrobulbar blood velocities in patients with acute branch retinal vein occlusion (BRVO). Their study using retinal vessel analyser (RVA) showed significant vasoconstriction in retinal veins and arteries of the affected eye as well as a reduction in flow velocities in the eyes with BRVO. However, it appeared that the reduction in retinal vascular calibres and flow velocities did not correlate with change in visual acuity or number

Table 2 Anatomic and functional characteristics of eyes treated with intravitreal ranibizumab injections for diabetic macular oedema

Characteristics	Total (n = 25)	Improvement in BCVA of ≥ 10 letters (n = 10)	No improvement in BCVA (n = 15)
Mean BCVA, letters	25.96 \pm 8.3	26.5 \pm 9.89	25.6 \pm 7.57
Mean baseline CRAE	147.63 \pm 20.55	156.12 \pm 22.74	141.97 \pm 17.47
Mean baseline CRVE	235.31 \pm 38.89	248.33 \pm 24.50	226.63 \pm 170.35
Mean CMT at BL	447.16 \pm 160.79	443.9 \pm 124.19	449.33 \pm 185.45
<i>Number of injections</i>			
Mean \pm SD	5.12 \pm 1.91	4.88 \pm 2.02	5.26 \pm 1.90
Range	2-8	2-8	2-8
Mean CMT at month 12	349.52 \pm 117.47	326.8 \pm 109.84	364.66 \pm 123.65

Abbreviations: BCVA, best corrected visual acuity; BL, baseline; CMT, central macular thickness; CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; n, number; RBZ, ranibizumab; SD, standard deviation.

of re-treatments. Additionally, they found that there was no correlation between the degree of vasoconstriction and visual outcome when RBZ was used, while there was a correlation when laser treatment was used. Our study showed that there was a statistically significant relationship between baseline CRVE and visual acuity.

Wickremasinghe *et al*¹² using IVAN software evaluated patients with neovascular AMD treated with intravitreal RBZ and showed that wider baseline CRVE significantly correlated with poor visual outcome after 12 months. Our study in DMO demonstrated contradictory findings, suggesting the difference in pathophysiology and oxygenation effect of the two diseases, even though both neovascular AMD and DMO appear to be driven by VEGF. However, it does suggest that baseline CRVE may be considered as a predictor of visual acuity in patients with macular diseases treated with RBZ.

Several studies have employed the IVAN software to measure the retinal vascular calibres in different diseases and its association with the progression of ocular or systemic diseases. No study employing IVAN to evaluate the relationship between baseline retinal vascular diameters and response to RBZ in DMO patients has been reported. Although our pilot study employed the IVAN software to measure and analyse effectively the average retinal vascular calibres of the CRVE and the CRAE, and to show evidence that there is a potential correlation between baseline CRVE, but not CRAE, and visual outcomes, there are several limitations. First, the sample size is too small to generalise our results. Second, there may be limited generalisability of our findings to the general population, as our study population was derived from a multicentre clinical trial, where study subjects were carefully selected to meet the study criteria. Further, there may be inherent biases among the 25 fundus photographs of sufficient quality to be evaluated by IVAN software in

this analysis. Applying similar analyses to a larger number of DMO eyes may yield different outcomes. Additionally, other associated factors such as cigarette smoking, diet, and body mass index (BMI) were not considered in this study using the READ-2 data. Finally, there might be measurement errors in the retinal vascular diameters measurement, given the limitations of the IVAN computer software when applied to retinal images of insufficient quality. Further studies with a greater number of DMO eyes managed with RBZ or other VEGF antagonists and a broader range of disease severity and visual acuity are needed to confirm our initial findings.

Summary

What was known before

- Studies have evaluated the association between intravitreal treatments and retinal vascular calibres.
- Widening of the retinal venular, but not arteriolar, calibre was associated with subsequent incidence and progression of diabetic retinopathy.
- Intravitreal injection of bevacizumab might induce retinal vasoconstriction in eyes with DMO.

What this study adds

- The present study is among the first to analyse and demonstrate a correlation between retinal vessel calibre and visual outcome in patients with DMO.
- Our study has demonstrated that eyes that showed moderate improvement in visual acuity had a wider baseline venular calibre when compared to eyes that did not show moderate improvement.

Conflict of interest

Dr Nguyen serves on the Steering Committees for the studies of ranibizumab and aflibercept for retinal vascular diseases sponsored by Genentech and Regeneron. Dr Do serves on the Steering Committee for the studies of aflibercept in diabetic macular oedema sponsored by Regeneron. The READ-2 Study was supported by the Juvenile Diabetes Research Foundation.

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