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CLINICAL STUDY

Influence of baseline diabetic retinopathy status on initial anatomical response of intravitreal ranibizumab therapy for diabetic macular oedema

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Abstract

Purpose **Intraocular vascular endothelial growth factor (VEGF) levels increases with the severity of diabetic retinopathy. Response of diabetic macular oedema (DMO) to ranibizumab is driven by VEGF suppression. We hypothesised that the initial reduction of central macular thickness by ranibizumab should be maximum in severe diabetic retinopathy until the levels of VEGF decreases to the levels observed in eyes with mild retinopathy.**

Methods **Consecutive patients with centre-involving DMO (central subfield thickness (CSFT) > 300 μm) who had three consecutive monthly ranibizumab injections followed by as needed therapy were included. Retinopathy status was graded as mild non-proliferative diabetic retinopathy (NPDR) (G1), moderate to severe NPDR with no prior panretinal photocoagulation (G2), and treated PDR (G3).**

Results **Two hundred and thirty-nine eyes from 204 patients with a mean age of 64.9 years were included. The distribution was 31.4 G1, 32.2 G2, and 36.4% G3. Mean baseline CSFT for all eyes was 458.5 ± 110.8 μm. Baseline CSFT for G1, G2, and G3, respectively, were 437.6 ± 90.9, 472.3 ± 109.8, and 464.7 ± 124.9 μm ($P = 0.2155$). Mean change in CSFT after three consecutive injections was 128.5 ± 116.6 μm. The mean changes were 95.8 ± 101.4 μm for G1, 137.2 ± 112.9 μm for G2, and 148.9 ± 126.9 μm for G3. The changes in CSFT between groups adjusted for baseline CSFT were statistically significant ($P = 0.0473$). At 6 and 12 months after a mean of 4.5 and 7.7 injections, the changes between groups were no longer significant, $P = 0.4783$ and $P = 0.8271$, respectively.**

Conclusions **The initial anatomical response of DMO with intravitreal ranibizumab injections was maximum in eyes with treated PDR, suggesting that the higher the VEGF levels, the better the response with ranibizumab.**

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Introduction

Diabetic macular oedema (DMO) is caused by breakdown of the blood–retina barrier and an increase in vascular permeability.[1] Several cytokines contribute to the pathogenesis of DMO, but vascular endothelial growth factor (VEGF) is thought to be the prime stimulus.[2] There has been a paradigm shift of treatment of DMO from conventional macular laser therapy for clinically significant macular oedema (CSMO) to intravitreal agents that inhibit VEGF (anti-VEGF) for centre-involving DMO. Ranibizumab, a recombinant, humanised monoclonal antibody fragment was the first anti-VEGF licensed for this indication. It decreases vascular permeability in DMO and results in reduction in retinal thickness.

The efficacy and anatomical outcomes of intravitreal ranibizumab are well proven. Several studies including Diabetic Retinopathy Clinical Research (DRCR) Protocol I, RESTORE, and READ-2 have investigated the efficacy of intravitreal ranibizumab monotherapy in DMO with a reported reduction in central retinal

thickness by 102 μm in 6 months and 119–194 μm in 12 months.[3–6] Intraocular VEGF is raised in DMO and eyes with any diabetic retinopathy.[2,7,8] Increasing severity of DR is related to increased intraocular VEGF levels.[9] As the response of DMO to ranibizumab is by suppression of VEGF alone with no effect on other cytokines such as ICAM-1, MCP-1, and IL-6, the response of DMO should in theory vary with severity of DR.[10] The DRCR network have investigated factors affecting anatomical outcomes at 12 months and found severity of diabetic retinopathy and previous panretinal photocoagulation (PRP) treatment not to affect significantly the change in central subfield thickness (CSFT) on optical coherence tomography.[11] However, there was a larger change in CSFT for severe non-proliferative diabetic retinopathy compared with milder forms, although not statistically significant.[11] The reduction of CSFT is at a maximum after the first three loading doses of ranibizumab, suggesting that the maximum decline in VEGF levels may be during the loading phase. Therefore, we hypothesised that the initial response to ranibizumab in terms of reduction of CSFT may vary with severity of diabetic retinopathy. The aim of this study was to test this hypothesis by a retrospective analysis of the initial outcome data on consecutive patients initiated on ranibizumab therapy for DMO in a real-life setting.

Materials and methods

This retrospective study was performed in the NIHR Biomedical Research Centre, Moorfields Eye Hospital and University College London, United Kingdom. Local institutional review board approval was obtained for this retrospective analysis of anonymised data and the local registration number was 14/047. The study was conducted in accordance to the tenets of the Declaration of Helsinki.

Inclusion and exclusion criteria

Consecutive patients with DMO with CSFT $>300\,\mu$m who were initiated on ranibizumab therapy from 1 June 2013 to 31 October 2014 in Moorfields Eye Hospital (London, UK) were included. Inclusion criteria included patients who successfully completed three consecutive monthly intravitreal ranibizumab. Bilateral cases, if eligible, were included and each eye studied separately. Spectral domain Optical Coherence Tomography measurements obtained with Topcon 3D-OCT-2000 (Topcon, Tokyo, Japan) using the macula cube protocol must be available for the visits within the baseline and three further reviews. Patients were excluded if any of the three injections or the follow-up appointments following the third injection was outside the designated interval of 30 ± 11 days. Patients without Topcon 3D-OCT-2000 measurements for the visits in the study period were excluded. Patients receiving previous anti-VEGF, intravitreal, or periorbital steroid treatment to the study eye within 90 days or macular laser within 120 days of the start of the three consecutive treatments were excluded. Cataract surgery in the study eye within 60 days of the baseline visit was an exclusion criterion. Previously vitrectomised eyes and eyes with other coexisting retinal disease were excluded. Eyes that underwent cataract surgery, macular laser or intravitreal, or periorbital steroid therapy during the study period were also excluded.

Data collection

Patient demographics, type of diabetes, and date of ranibizumab injections were collected from the local electronic database (Open Eyes). All patients had baseline colour fundus photograph and fundus fluorescein angiography as per local ranibizumab treatment pathway for DMO. The retinopathy status and evidence of previous PRP were confirmed from the colour fundus photographs and clinical examination findings obtained before initiation of treatment. The study period was defined as from commencement of the first ranibizumab injection to date of 12 months follow-up. Data on CSFT at each visit up were obtained from SD-OCT imaging of the macula using Topcon 3D-OCT-2000. The central circle in the ETDRS grid from the retinal thickness report was used as the CSFT. The grid was repositioned to ensure centration. The CSFT measurements were recorded at baseline, month 1, 2, 3, 6, and 12 months.

Data analysis

Groups studied are G1, G2, and G3. In this study, we classified mild non-proliferative diabetic retinopathy, ETDRS level 10 and 35, with no prior PRP as G1, moderate to severe non-proliferative diabetic retinopathy, ETDRS levels 43–47 and 53, with no prior PRP as G2, and stable treated proliferative diabetic retinopathy with evidence of PRP as G3. Change in CSFT was calculated from baseline. The change in CSFT was also analysed by performing a logarithmic transformation as previously described by the DRCR network.[12] In brief, the CSFT values were transformed to a log base 10 of the ratio of the CSFT divided by 200 and rounded to the nearest 100th. This was performed for change in CSFT from baseline to month 1, 2, 3, 6, and 12.

Subanalysis of month-by-month change in CSFT was analysed using mean change in CSFT and change in logarithmic transformation as described. The change

described is compared with the previous visits measurements, that is, month 2 change describes the change from month 1 to month 2 and month 6 change describes the change from months 3 to 6 (Figure 1).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 22 and MedCalc for Windows, version 15.0 (MedCalc Software, Ostend, Belgium). Statistical analyses comparing mean CSFT values were performed using the Kruskal–Wallis test and for change in CSFT of the three retinopathy grades, an ANCOVA adjusting for baseline CSFT measurements as a concomitant variable was used. A Levene's test for equality of error variances and homogeneity of regression slopes were assessed. A χ^2 test was used to analyse the differences between percentage of eyes achieving a CSFT of 300μm or less at month 3 between the three groups. The sample size required for a Type 1 error of 0.05 and a Type 2 error of 0.80 or 80% power to assess change in CSFT between groups was determined to be 75 eyes per group for the primary outcome at month 3. The criterion for statistical significance was set at $P \leq 0.05$.

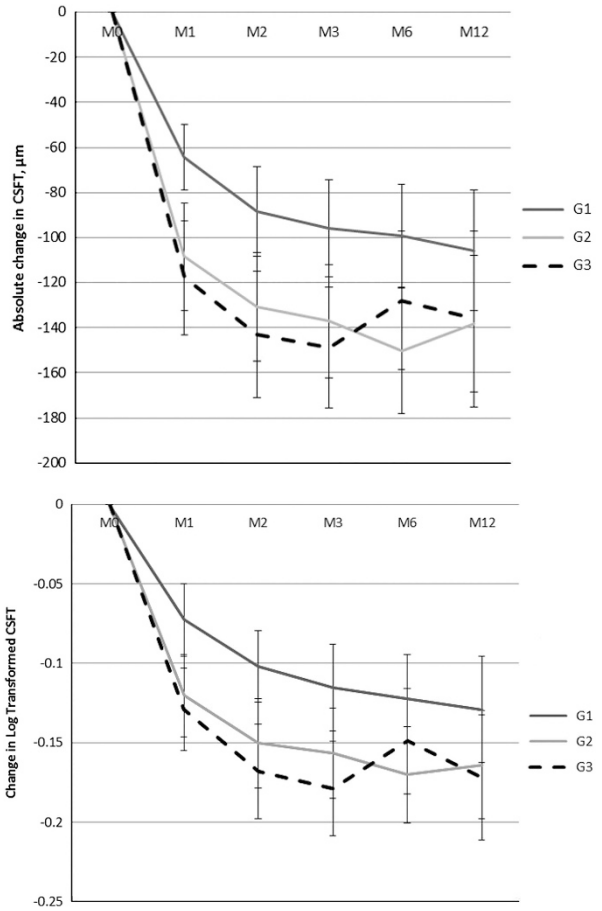

Figure 1 Mean absolute change with 95% confidence interval error bars in CSFT at month 0, 1, 2, 3, 6, and 12 for mild non-proliferative diabetic retinopathy (G1), moderate to severe non-proliferative diabetic retinopathy with no prior panretinal photocoagulation (G2) and treated proliferative diabetic retinopathy (G3) for the three categories of retinopathy (top graph) and mean absolute change with 95% confidence interval error bars in log-transformed CSFT at 0, 1, 2, 3, 6, and 12 for the three categories of retinopathy (bottom graph).

Results

A total of 239 eyes from 204 patients were eligible and included in the study for analysis. The mean age of patients was 64.9 years with a male to female ratio of 1.6 : 1. Four patients (2.0%) were type 1 diabetes mellitus and the rest, type 2 diabetes mellitus. The distribution between groups were fairly even with 75 eyes (31.4%) classified as G1, 77 eyes (32.2%) as G2, and 87 eyes (36.4%) as G3. The availability of OCT data for months 0–3, 6, and 12 are detailed in Table 1.

Mean baseline CSFT for all eyes was 458.5 ± 110.8 μm. The mean baseline CSFT for G1 was 437.6 ± 90.9 μm, 472.3 ± 109.8 μm for G2, and 464.3 ± 124.9 μm for G3 ($P = 0.2155$). Table 2 describes the baseline characteristics of each group. All eyes received three injections from baseline to month 3. Mean number of injections at month 6 were 4.5 and 6.8 at month 12. Mean number of injections by groups are described in Supplementary Information File 1.

Change in CSFT between groups

Mean change in CSFT after three consecutive ranibizumab injections at month 3 was 128.5 ± 116.6 μm. For individual groups, the mean changes were 95.8 ± 101.4 μm for G1, 137.2 ± 119.9 μm for G2, and 148.9 ± 126.9 μm for G3. The difference between the three groups after adjusting for baseline CSFT as a concomitant variable were statistically significant ($P = 0.0474$). Change in CSFT at month 1, 2, 3, 6, and 12 are detailed in Table 3. The variances between the three groups were not significantly different ($P = 0.683$) and there is homogeneity of the regression slopes ($P = 0.856$).

Table 1 The number of eyes with available central subfield thickness data for months 0–3, 6, and 12

	Months 0–3	*Month 6*	*Month 12*
G1	75 (100%)	72 (96%)	51 (68%)
G2	77 (100%)	68 (88%)	69 (90%)
G3	85 (100%)	79 (93%)	74 (87%)

Percentage or eyes achieving CSFT of 300μm or less

At month 3 following three consecutive intravitreal ranibizumab injections, 40.0% of eyes in G1 and 38.9% of eyes in G2 achieved a CSFT of 300μm or less, whereas 56.3% of eyes achieved this result in G3. This difference was found to be statistically significant ($P=0.0418$).

Subanalysis of month-by-month change in CSFT

The mean change in CSFT in month 1 compared with baseline levels and adjusting for baseline CSFT were significantly different between G1, G2, and G3 ($P=0.0216$). The month-by-month change in subsequent months, month 2 and month 3, did not show a significant difference between the three groups (Table 4).

Table 2 Baseline characteristics including number of patients, age, CSFT, and log-transformed CSFT[a]

	G1	*G2*	*G3*	P-*value*
Number of patients	75	77	87	
Age	68.0	64.8	61.6	0.008
CSFT (μm)	437.6	472.3	464.3	0.2155
Log-transformed CSFT	0.33	0.37	0.35	0.2155

Abbreviation: CSFT, central subfield thickness. [a] For mild non-proliferative diabetic retinopathy (G1), moderate to severe non-proliferative diabetic retinopathy with no prior panretinal photocoagulation (G2), and treated proliferative diabetic retinopathy (G3).

Discussion

This retrospective analysis of 239 eyes has revealed that the initial anatomical response to intravitreal ranibizumab varies with severity of diabetic retinopathy. The more severe forms of retinopathy, groups G2 and G3 in our study, presented a more substantial initial decrease in retinal thickness, 137.2 and 148.9 μm, respectively, after the first three injections compared with mild diabetic retinopathy. G1 produced a modest reduction in CSFT, 95.79 μm, almost a third less than that observed in the G2 and G3 groups. Interestingly, when month-by-month changes were studied, the change at month 1 revealed the biggest variation with subsequent months not manifesting a significant difference. Given the nature of ranibizumab as an inhibitor of VEGF and VEGF levels proven to reflect the severity of diabetic retinopathy,[7–9] the results obtained in our study corroborates the theory that the initial response in retinal thickness is dependent on retinopathy status.

Our study also revealed that by month 6 and including month 12, there was no significant difference in CSFT from baseline between retinopathy grades. This is in keeping with the observations noted in the analysis of the

Table 3 Absolute change in central subfield thickness and log-transformed absolute change in central subfield thickness from baseline at month 1, 2, 3, 6, and 12[a]

	Month 1		*Month 2*		*Month 3*		*Month 6*		*Month 12*	
	μ*m*	*Lg*	μ*m*	*Lg*	μ*m*	*Lg*	μ*m*	*Lg*	μ*m*	*Lg*
G1	64.2	0.07	88.5	0.10	95.8	0.12	99.2	0.12	105.7	0.13
G2	108.5	0.12	130.7	0.15	137.2	0.16	150.1	0.17	138.3	0.16
G3	117.8	0.13	142.9	0.17	148.9	0.18	127.9	0.15	136.2	0.17
P-value (ANCOVA)	0.0216	0.0187	0.0327	0.0087	0.0474	0.0209	0.478	0.5077	0.827	0.4508

Abbreviation: ANCOVA, analysis of covariance. [a] For mild non-proliferative diabetic retinopathy (G1), moderate to severe non-proliferative diabetic retinopathy with no prior panretinal photocoagulation (G2), and treated proliferative diabetic retinopathy (G3).

Table 4 Month-by-month central subfield thickness change

Category	*M1*		*M2*		*M3*		*M6*		*M12*	
	μ*m*	*Lg*	*μm*	*Lg*	μ*m*	*Lg*	μ*m*	*Lg*	μ*m*	*Lg*
G1	64.2	0.073	24.3	0.029	7.3	0.013	5.0	0.007	15.2	0.018
G2	108.5	0.120	22.2	0.030	6.5	0.006	9.8	0.013	− 2.3	0.004
G3	117.8	0.129	25.1	0.039	6.0	0.011	− 13.5	− 0.020	5.1	0.019
P-value	0.022		0.951		0.990		0.756		0.705	

For mild non-proliferative diabetic retinopathy (G1), moderate to severe non-proliferative diabetic retinopathy with no prior panretinal photocoagulation (G2), and treated proliferative diabetic retinopathy (G3) at month 1, 2, 3, 6, and 12.

Protocol I data on factors associated with change in OCT thickness after treatment with ranibizumab for DMO that also showed no statistically significant change in CSFT in relation to severity of diabetic retinopathy at 12 months. This does not imply that retinopathy severity has entirely no effect on the anatomical outcome following intravitreal ranibizumab therapy, but merely at month 6 onwards and with subsequent treatments, the delayed improvement in mild retinopathy results in a statistically insignificant difference between retinopathy severities. This is evident when the difference in the mean CSFT between groups at month 12 were compared with month 3. Eyes in G1 showed a 9.9 μm decrease in CSFT at month 12 compared with month 3, whereas eyes in G2 only had a 1.1 μm reduction and eyes in G3 increased by 12.7 μm. This suggests other inflammatory or angiogenic cytokines further down the cascade at a later stage may contribute to the pharmacodynamics of ranibizumab. However, the initial change does differ between retinopathy grades as early as month 1, which also corresponds to the maximum change in our cohort and resembles that of previous landmark studies.[5,13]

Changes in CSFT between groups is a challenging avenue due to the nature of differences in CSFT between different retinopathy severities, which is in itself a finding, and the fact that change in CSFT is possibly dependent on this varying baseline measurements. We attempted to minimise this by also adopting a log change in CSFT as proposed by the DRCR network and the significance of the difference adjusted for baseline CSFT measurements as a concomitant variable.[12] We acknowledge the retrospective nature of the study and therefore included consecutive cases that fulfilled the predetermined eligibility criteria and thus reducing ascertainment bias. Another limitation is the retinopathy status, which was graded based on images and clinical examination findings in the case notes. Although not assessed in a reading centre, investigators reviewed clinical images along with case notes of patients to ensure the retinopathy severity is as accurate as possible. The association between clinical grading and far peripheral ischaemia is also questionable.

The seminal ETDRS and DRS papers have showed that PRP treatment is successful in preventing severe visual loss in diabetic retinopathy. Despite being introduced more than 50 years ago and still a treatment of choice in diabetic retinopathy management, the mechanism of action attracts a number of theories. PRP destroys the outer retina that accounts for up to two-thirds of the total oxygen consumption.[14] It is widely believed that this in turn, improves inner retinal oxygenation by increasing the oxygen delivery from the choroidal circulation.[15–17] Hypoxia and ischaemia upregulates VEGF and by improving retinal oxygenation, VEGF levels may potentially decrease.[18,19] It is well understood that VEGF levels are higher in DMO and diabetic retinopathy, with higher levels observed with more severe forms.[7–9] Interestingly, VEGF levels following PRP treatment has not been as clear. Aiello *et al*[7] have shown that VEGF levels in quiescent proliferative diabetic retinopathy are reduced, whereas Shimura *et al*'s[20] work on the other hand revealed VEGF levels to be similar prior and following PRP treatment in patients. Although PRP does improve oxygenation and potentially reduce VEGF levels, there are still raised levels of VEGF present. This would explain our finding that despite PRP treatment, the anatomical change in CSFT following anti-VEGF treatment mimics that of naïve severe non-proliferative diabetic retinopathy eyes. This conforms to our understanding that VEGF levels may be reduced following PRP but still raised. Persistent active neovascularisation do occur despite complete PRP treatment. Treatment with intravitreal bevacizumab, an anti-VEGF agent, is successful in these circumstances with 70–100% showing complete regression of new vessels.[21–23] This augments the argument that VEGF levels and the effects of VEGF is still very much present in eyes despite significant PRP treatment.

The theory put forward in our hypothesis simplifies the pathogenesis of DMO and the pharmacodynamics of ranibizumab in assuming that retinopathy severity is a surrogate to VEGF levels and VEGF is the sole cytokine involved in DMO; thus, the anatomical response to an anti-VEGF agent represents *in vivo* VEGF levels. As VEGF is driven by ischaemia, retinopathy severity is likely to reflect VEGF levels but not necessarily other cytokines.[18,19] We acknowledge that there is a complex interplay with several other cytokines, such as insulin like growth factor-1, basic fibroblast growth factor, angiopoietin-1 and -2, stromal derived factor-1 and tumour necrosis factor in this orchestra that is diabetic retinopathy and macular oedema; however, VEGF is believed to be a major factor.[24,25] Comparing diabetic retinopathy and central retinal vein occlusion, macular oedema baseline values in landmark studies differs, 412–482 μm in diabetic retinopathy and 639–689 μm in central retinal vein occlusion.[5,13,26–28] This may be a reflection of prior treatment or duration of disease however appears to be consistent despite more recent DMO studies. As for the change in CSFT at 12 months with ranibizumab injections, it was 119 μm for DMO and 452–462 μm in central retinal vein occlusion, again a marked difference.[5,29] The manifestation of DMO in mild diabetic retinopathy with minimal observed areas of retinal non-perfusion also suggests that DMO in mild retinopathy is not primarily driven by ischaemia, but it does manifest a response to anti-VEGF. This may suggest that the treatment with anti-VEGF in DMO with mild retinopathy involves a cascade of events rather than just

primary VEGF inhibition like that seen in more severe forms of retinopathy. This may explain the delayed but eventually comparable response observed in our study.

Clinically, acknowledging a slower response in mild retinopathy encourages us to be patient with treatment of such cases as the response is not rapid and also invites further research into alternate and more focused therapies in managing DMO related to mild retinopathy. In summary, our findings support the hypothesis that the initial anatomical response in DMO treated with intravitreal ranibizumab depends on the severity of baseline diabetic retinopathy severity with more significant response in eyes with severe retinopathy.

Summary

What was known before

- VEGF levels are higher in more severe forms of diabetic retinopathy Intravitreal anti-VEGF is successful in treating DMO.

What this study adds

- The initial outcome of treatment of DMO is influenced by baseline diabetic retinopathy severity.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on Eye website (http://www.nature.com/eye)