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Ultra-Widefield Fluorescein Angiography as a Biomarker for Response to Switch in Therapy in Persistent DME

Bahrami, B., Hong, T., Peto, T., & Chang, A. (2019). Ultra-Widefield Fluorescein Angiography as a Biomarker for Response to Switch in Therapy in Persistent DME. *OSLI Retina*, 50(12), 771-778.
<https://doi.org/10.3928/23258160-20191119-04>

Published in:
OSLI Retina

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

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TITLE: Ultrawidefield fluorescein angiography as a biomarker for response to switch in therapy in persistent diabetic macular edema

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Dr Andrew Chang has acted as a consultant for Allergan, Bayer and Novartis. Dr Tunde Peto has acted for a consultant for Novartis, Optos and Bayer.

Financial support was provided by Bayer Corporation Global. The sponsor had no role in the design, conduct or analysis of this research. None of the authors have any proprietary interest in any material or method presented.

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ABSTRACT

Purpose

To explore the effect of peripheral ischemia identified on ultrawidefield fluorescein angiography (UWFA) as a biomarker of response to switch in therapy from bevacizumab to aflibercept in persistent diabetic macular edema (DME).

Methods

Prospective clinical trial of 38 eyes from 38 patients previously treated with bevacizumab and persistent DME. Patients subsequently received aflibercept per protocol and were followed up for 48 weeks. UWFA was obtained on all patients at baseline and 48 weeks. Images were graded and used to calculate an overall ischemic index (II) and macular ischemic index (MII). II was compared with visual and central macular thickness (CMT) outcomes. Paired and independent samples t-tests and Fisher's exact tests were used to assess change and associations.

Results

Patients with an II greater than or equal to 50% at baseline had a poorer baseline visual acuity (60.1 ± 10.2 vs. 70.7 ± 9.0 letters, $p=0.005$) and a worse MII (6.9 ± 25 vs. $56 \pm 52\%$, $p<0.001$). These patients gained significantly more vision at 48 weeks (8.3 ± 9.3 vs. 2.6 ± 5.9 letters, $p=0.03$). At 48 weeks, there was no significant difference in visual acuity of patients with an II greater or less than 50% (68.4 ± 6.0 vs. 73.3 ± 9.6 letters, $p=0.16$).

54 **Conclusion**

55 Patients with persistent DME treated with bevacizumab and worse II had poorer
56 baseline visual acuity, potentially due to worse macular ischemia. These patients
57 had greater visual gain with similar final visual outcomes of those without
58 marked peripheral ischemia subsequent to switching to aflibercept.

59 **BACKGROUND**

60 Ocular imaging is a cornerstone of diagnosis and management of diabetic
61 macular edema (DME). Historically, fluorescein angiography (FA) has played a
62 pivotal role in qualifying vascular leakage and guiding therapy for DME. [1] In
63 the past two decades, optical coherence tomography (OCT) has revolutionized
64 diagnosis of DME, as well as led to the identification of prognostic biomarkers
65 such as distribution of edema, disruption to the inner segment ellipsoid (ISe)
66 band and external limiting membrane (ELM), and disorganization of the inner
67 retinal layers (DRIL). [2-5]

68
69 More recently, ultrawidefield (UW) imaging enabled documentation of
70 peripheral lesions, leading to more detailed description of disease severity and
71 prognosis of progression. [6, 7] When combined with FA, ultrawidefield
72 fluorescein angiography (UWFA) has been shown to identify 3.9 times more
73 nonperfusion, 1.9 times more neovascularization, and 1.1 times more retinal
74 pathology as compared with standard 7-fields photography. [8]

75
76 Peripheral ischemia may be quantified through the calculation of an ischemic
77 index (II). [9] Such an index gives a ratio of non-perfused to perfused retina and
78 has been shown to be associated with the prevalence of DME in a retrospective
79 study of 122 eyes (OR 3.75, 95%CI 1.26-11.13, $p<0.02$). [10] Furthermore, DME
80 that is recalcitrant to macular photocoagulation was found to be associated with
81 a worse II in a retrospective study of 148 eyes with persistent DME. [11] These
82 patients also required a greater number of treatments with macular laser
83 photocoagulation and had a lesser reduction in the CMT.

84

85 It is unclear what prognostic information can be gained from UWFA for patients
86 with DME being treated with anti-VEGF drugs. Herein we report the prognostic
87 value of UWFA from a prospective clinical trial where patients with persistent
88 DME were switched from bevacizumab to aflibercept.

89 **METHODS**

90 *Participants*

91 All patients gave informed consent to participate in this prospective, non-
92 randomized, open-label clinical trial. The study adhered to the Declaration of
93 Helsinki and was registered on the Australian and New Zealand Clinical Trials
94 Registry (ACTRN12614001307695).

95
96 Full inclusion and exclusion criteria for the patients enrolled in this trial have
97 previously been reported. [12] Briefly, patients were aged 18 years or older and
98 had DME with a central macular thickness greater than 300µm in the spectral
99 domain optical coherence tomography (OCT; Spectralis, Heidelberg Engineering,
100 Heidelberg, Germany), best corrected visual acuity (BCVA) between 34 and 85
101 Early Treatment of Diabetic Retinopathy Study (ETDRS) letters and at least 4
102 previous intravitreal injections of bevacizumab (2.5 mg/0.1 mL) in the 6 months
103 prior to enrolment. Patients then received intravitreal aflibercept
104 (2.0mg/0.1mL) in one eye only every four weeks for a total of five injections at
105 which point the treatment interval was extended to injections every eight weeks
106 for a total follow up of 48 weeks. BCVA and CMT assessments occurred at every
107 four weeks with UWFA occurring at baseline and at 48 weeks.

108

109 *Image Acquisition*

110 UWFA images were acquired using the Optos 200TX (Optos Plc, Dunfermline,
111 Scotland). An intravenous bolus of 5mL of 10%w/v fluorescein was given and
112 images were obtained in the transit phase (up to 45 seconds) arteriovenous

phase (1 to 2 minutes) and during recirculation (up to 10 minutes). A single best image from the arteriovenous phase of the study eye was selected for grading.

Calculation of ischemic index

The ischemic index was calculated using the concentric rings method previously described. [13] Briefly, UWFA images for each patient at baseline and 48 weeks were overlaid with the template of seven concentric rings (Figure 1) as supplied in the supplement to the publication by Nicholson et al. [13] Using ImageJ software (NIH, Bethesda, Maryland), the template was resized and repositioned for each image such that the innermost ring was equal in size to the optic disc and the central point of the template was placed at the fovea. Each of these seven rings was divided into 12 equal segments subtending an angle of 30 degrees at the fovea. Each segment was graded as perfused, non-perfused or non-gradable if more than half of the segment consisted of one of the three. Grading was validated by an independent trained external grader (TP). Areas with scatter laser were deemed not gradable and were excluded from the calculation of the index.

The II was calculated from the grading by multiplying each segment by the total disc area represented and then dividing non-perfused retina by the total gradable area. An ischemic index of 50% or above was considered to be high as previously described and further analyses were based on this definition. [14]

The macular ischemic index (MII) was calculated from the 12 sectors comprising the innermost ring of the template.

Optical Coherence Tomography Grading

Images were graded for morphology of DME (intraretinal fluid and/or subretinal fluid), presence or absence of disorganization of the inner retinal layers (DRIL) >50%, inner segment ellipsoid (ISe) band disruption, external limiting membrane (ELM) disruption in a 1mm area centered around the fovea as previously described and reported. [2, 12, 15, 16]

Statistical Analysis

All statistical tests were performed and figures produced using IBM SPSS software (version 22; SPSS Inc, Chicago, Illinois, USA). Patients were grouped by baseline II greater than or less than or equal to 50%. Data was confirmed to be distributed normally using Shapiro-Wilk tests. Homogeneity of data was confirmed using Levene's test for all independent samples' t-tests. Adjustment was made to the analysis using the Welch-Satterthwaite method for data that was not homogenous. Fisher's exact test was used to analyze categorical data when sample sizes in groups were small. Pearson's correlation coefficient was calculated for correlation analyses. For all analyses, a p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Of the 43 patients recruited, one withdrew consent after the baseline visit, one was withdrawn due to a retinal detachment in the study eye after the second injection, three did not have UWFA performed at baseline and six did not have UWFA performed at 48-weeks. The characteristics of the 38 patients with complete UWFA data at baseline included for analysis are summarized in Table 1.

BCVA improved by a mean $\pm$ standard deviation of 4.0 ± 7.2 letters ($p=0.002$) to 72.2 ± 9.1 letters ($p=0.002$) and CMT reduced by $60 \pm 111\mu\text{m}$ ($p=0.002$) to $356 \pm 109\mu\text{m}$ ($p=0.002$) in the 38 patients with UWFA data at baseline over the 48-week study period.

There was no significant change in mean II ($24.9 \pm 32.5\%$ to $22.7 \pm 29.7\%$, $p=0.36$) or MII ($8.1 \pm 23.5\%$ to $7.9 \pm 19.7\%$, $p=0.88$) from baseline to 48-weeks. There was correlation between II and MII at baseline ($r=0.66$, $p<0.001$) and at 48-weeks ($r=0.57$, $p<0.001$).

Patients with an II greater than 50% ($n=9$) at baseline had a poorer baseline visual acuity (60.1 ± 10.2 vs. 70.7 ± 9.0 letters, $p=0.005$; Figure 2A) and a worse MII ($6.9 \pm 25\%$ vs. $56 \pm 52\%$, $p<0.001$) compared to patients with a lower II ($\leq 50\%$). These patients gained significantly more letters of vision at 48 weeks (8.3 ± 9.3 vs. 2.6 ± 5.9 letters, $p=0.03$). At 48 weeks, there was no significant difference in absolute visual acuity in patients with an II greater than 50% compared to those with an index less than or equal to 50% (68.4 ± 6.0 vs. $73.3 \pm$

9.6 letters, $p=0.16$; Figure 2B). There was no significant difference in baseline
(69.4 ± 9.3 vs. 63.0 ± 13 letters, $p=0.14$) or final (72.6 ± 9.4 vs. 70.3 ± 7.9 letters,
 $p=0.55$) BCVA in patients with the absence or presence of macular ischemia, nor
were the BCVA gains in these two groups different (3.2 ± 6.9 vs. 7.3 ± 8.1 letters,
 $p=0.18$).

There was no difference in CMT at baseline or 48-weeks for patients with an II greater than 50% at baseline compared with those less than or equal to 50% ($429 \pm 61\mu\text{m}$ vs. $412 \pm 101\mu\text{m}$, $p=0.63$ and $395 \pm 112\mu\text{m}$ vs. $343 \pm 107\mu\text{m}$ $p=0.22$, respectively; Figure 3). There was no difference in change in CMT for patients with an II greater than 50% ($-34 \pm 73\mu\text{m}$ vs. $-68 \pm 120\mu\text{m}$, $p=0.42$).

There was no significant correlation between CMT at baseline ($r=0.18$, $p=0.29$) or change in CMT at 48-weeks ($r=0.16$, $p=0.33$) with the MII.

There was no significant correlation between baseline II and HbA1c ($r=0.16$, $p=0.40$), duration of diabetes ($r=0.25$, $p=0.13$), or number of previous anti-VEGF injections for DME ($r=-0.31$, $p=0.06$). There was no significant correlation between baseline MII and HbA1c ($r=0.01$, $p=0.96$), duration of diabetes ($r=0.21$, $p=0.20$), or number of previous anti-VEGF injections for DME ($r=-0.23$, $p=0.17$).

There was no correlation between II or MII and presence of subretinal fluid, disorganization of the inner retinal layers, external limiting membrane or inner segment ellipsoid band disruption (Table 2 data not shown).

DISCUSSION

Among patients with persistent DME and significant prior treatment with bevacizumab, a high baseline II greater than 50% was associated with a poorer baseline visual acuity. However, these patients had a similar final visual acuity to those with a low baseline II when therapy was switched to aflibercept.

Visual acuity is dependent on the health of the macula, both in the available blood supply as well as the integrity of the various cells involved in phototransduction. In this study, visual acuity was not associated with a thicker CMT but with worse macular ischemia. Recent studies utilizing OCT angiography have identified a negative correlation between macular capillary density and visual acuity. [17] OCT biomarkers such as ISe band and ELM disruption, DRIL and presence of subretinal fluid are all associated with a poorer visual acuity. [2-5] Whilst ischemia may be postulated to explain these structural abnormalities, presence of these factors did not correlate with macular ischemia in this study.

There are several potential explanations for a greater gain in vision for the patients with a higher baseline II. Firstly, the starting visual acuity was significantly lower, meaning that there was more potential for vision gain. Secondly, there may be a “ceiling effect”, to the amount of vision that can be gained in this cohort of patients with persistent DME. Finally, ischemia and hypoxia are strongly implicated in the pathogenesis of DME. Areas of untreated retinal non-perfusion may stimulate the production of mediators such as VEGF-A and placental growth factor (PlGF) that contribute to the formation and persistence of DME. Whilst there is no control group to compare to in this study,

these factors may be more effectively inhibited by aflibercept leading to improved outcomes in these patients. [18]

It has been hypothesized that scatter photocoagulation to areas of peripheral ischemia may help in the management of DME. Complete resolution of macular edema following panretinal photocoagulation was demonstrated in a case series of 17 eyes with florid proliferative DR and DME. [19] Worsening of DME was reduced over a period of six months in a clinical trial of 52 patients randomized to a single dose of bevacizumab either with or without targeted photocoagulation. [20] Reduced levels of VEGF in the eye following panretinal photocoagulation (PRP) may be responsible for this effect. [21] However, PRP is also known to exacerbate macular edema likely through transient increases in inflammatory cytokines and VEGF. [22, 23]

Most recently, monotherapy with ranibizumab was shown to have similar outcomes to combination therapy with ranibizumab and targeted laser photocoagulation in a three year, randomized trial of 40 eyes from 29 patients with DME and significant peripheral ischemia. [24] There were no differences in treatment burden or visual or anatomical outcomes. The authors suggested areas of non-perfusion may represent dead rather than stressed tissue and thus do not contribute to increased production of factors driving DME. This is similar to the outcomes presented in the RELATE study, where patients with branch and central retinal vein occlusion who were randomized to intravitreal ranibizumab therapy with targeted scatter laser had similar vision and anatomical outcomes to those treated with ranibizumab monotherapy. [25]

256

257 Contrary to Wessel et al, we did not find any correlation between diabetes
258 control and II, nor did we find a relationship between duration of diabetes and II.
259 [10] Furthermore, we did not confirm findings of other studies presenting data
260 suggesting a reversal of areas of non-perfusion with intravitreal injection of anti-
261 VEGF drugs or dexamethasone implant. [26, 27]

262

263 The strengths of this study are in the prospective and standardized nature of
264 data collection in a clinical trial setting. The trial participants received the per
265 protocol treatment during the study period and had retinal imaging performed
266 at standardized time points. The methodology for grading ischemia has been
267 previously validated.

268

269 There are inherent limitations in the data and analyses performed due to lack of
270 a control arm as well as a relatively small sample size from a single center.

271 Patients included had heterogeneous prior treatments including macular and
272 panretinal photocoagulation. Furthermore, correction for peripheral distortion
273 and introducing validated, reliable computer based segmentation for the
274 calculation of peripheral ischemia may yield more accurate results in the future.
275 [28, 29]

276

277 The exploratory analyses from this study demonstrate that significant ~~peripheral~~
278 retinal ischemia may correlate with poorer visual acuity and a greater capacity
279 for vision improvement in patients with persistent DME switched to aflibercept.
280 Additionally, there appears to be no clear association between degree of

281 peripheral ischemia and severity of macular edema, suggesting that other factors
282 may be involved in macular thickening in DME. Future directions for these
283 findings are to assess the effect of peripheral ischemia as a biomarker of
284 treatment response to anti-VEGF drugs in treatment naïve eyes as well as those
285 treated with other modalities such as corticosteroids that target different
286 pathological pathways.

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419 **TITLES AND LEGENDS TO FIGURES**

420

421 Figure 1: Ultra-widefield fluorescein angiography with concentric rings overlay

422

423 Figure 2. Boxplot showing baseline (A) and 48-week (B) visual acuities grouped

424 by baseline ischemic index

425

426 Figure 3. Boxplot showing baseline (A) and 48-week (B) central macular

427 thickness grouped by baseline ischemic index

Table 1. Baseline characteristics of patients included in analysis

Characteristic	Data
Number of patients	38
Age (years), mean $\pm$ SD	62.6 $\pm$ 9.7
Male, n (%)	26 (68)
Right eyes, n (%)	18 (47)
Duration of diabetes (years), mean $\pm$ SD	17.5 $\pm$ 11.1
HbA1c (%), mean $\pm$ SD	7.9 $\pm$ 1.7
Duration of anti-VEGF treatment (months), mean $\pm$ SD	25.0 $\pm$ 22.2
Total number of anti-VEGF injections, mean $\pm$ SD	16.4 $\pm$ 11.2
Prior treatments in study eye	
Focal/grid macular photocoagulation, n (%)	15 (39.5)
Panretinal photocoagulation, n (%)	15 (39.5)
Vitrectomy, n (%)	5 (13.1)
Baseline BCVA (letter score), mean $\pm$ SD	68.2 $\pm$ 10.2
Baseline CMT (microns), mean $\pm$ SD	416 $\pm$ 93

SD = standard deviation, VEGF=vascular endothelial growth factor, BCVA=best corrected visual acuity, CMT=central macular thickness

Table 2. Correlation of optical coherence tomography parameters with baseline ischemic index and macular ischemic index

Characteristic	External limiting membrane disruption	Presence of subretinal fluid	Presence of inner segment ellipsoid band disruption	Disorganization of the retinal inner layers >50%
Baseline Ischemic Index	r= 0.24, p=0.14	r=-0.07 , p=0.66	r= 0.14, p=0.42	r=0.08 , p=0.65
Baseline Macular Ischemic Index	r=0.35 , p=0.06	r=-0.14 , p=0.42	r= 0.17, p=0.30	r=0.18 , p=0.28

A**Baseline Visual Acuity (letters)**90
80
70
60
50
40 **Baseline Ischaemic Index** $\leq 50\%$ $> 50\%$ **B****48-Week Visual Acuity (letters)**90
80
70
60
50
40 **Baseline Ischaemic Index** $\leq 50\%$ $> 50\%$