Quantitative analysis of Pigment Epithelium Detachment response to different anti-VEGF agents in Wet Age-Related Macular Degeneration

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Short title: Quantification of response to anti-VEGF treatment of PEDs

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Key Words

Pigement Epithelium Detachment, Image Analysis, Differential Pharmacodynamics, Optical Coherence Tomography

Summary Statement

In this consecutive case series, reduction in PED height and volume and increase in PED reflectivity were more pronounced on treatment with aflibercept and were influenced by severity of disease at baseline. Aflibercept treatment achieves greater and faster improvement in quantifiable PED metrics at 3 months and 1 year from treatment onset.

Abstract

Purpose: To assess if Best Corrected Visual Acuity(BCVA) and Pigment Epithelium Detachment(PED) Height, Volume and Reflectivity in patients with Wet-AMD are influenced by baseline anatomical and functional parameters, including quantifiable metrics of PED morphology and choice of treatment.

Methods: 102 consecutive, treatment-naïve Wet-AMD patients with PED(>50 μm) treated with aflibercept(52) or ranibizumab(50) were retrospectively included. PED height, horizontal and vertical dimensions and volume were recorded at baseline, 3 months and 1 year. Bespoke image analysis software provided a quantifiable measure of reflectivity.

Results: BCVA at 3 months was influenced by baseline BCVA (p=0.006)

PED height was influenced by baseline height (p=0.009), SRF (p=0.008), CMT (p=0.006) and use of aflibercept (p=0.003) at 3 months and by baseline height (p=0.018), volume (p=0.017), vertical dimension (p=0.0004) and aflibercept (p=0.015) at 1 year.

PED reflectivity increased from 43.59 to 55.86 (3 months) and 57.35 (1 year) ($p<0.001$) and was influenced by its baseline values and, interestingly, use of aflibercept at 3 months (p=0.013).

Conclusions: Quantifiable metrics of PED morphology improve with treatment and PED content becomes hyper-reflective, more so on aflibercept. PEDs respond better in the context of more active disease. More hypo-reflective PED content may predispose to better treatment response, especially with aflibercept.

Introduction

Pigment epithelial detachments (PEDs) develop in more than 80% of eyes with exudative age-related macular degeneration (AMD).[1-3] However, the precise role of PEDs in the pathogenesis and management of wet AMD remains controversial.

Specifically the precise anatomical and functional benefits of PED treatment with anti-VEGF agents are still under investigation. Recent studies have shown variable outcomes associated with anti-VEGF therapy for PEDs in Wet AMD patients, perhaps partly due to differing diagnostic criteria, imaging standards and definitions of endpoints.[1-9,12-16] These studies have predominately focused on treatment-resistant cases of PED (non-responders to ranibizumab or bevacizumab) subsequently commenced on treatment with aflibercept.[13-15] There are limited studies placing emphasis on treatment-naïve patients and few extending beyond 3 months of follow up.[16] There are also conflicting conclusions with respect to the independent impact of PED flattening on visual function as this cannot be easily isolated from the broader impact of anti-VEGF treatment on retinal anatomy.[4-9]

The aim of the present work is to study response of PEDs to two different anti-VEGF treatments. The study is distinct in that it involves treatment-naïve patients with a follow-up extending to one year from commencing treatment. Furthermore both conventional and novel image analysis tools were used to provide more objective and detailed morphological assessments of pigment epithelial detachment changes.

The aim of this work is to assess if changes in visual acuity and PED height, volume and reflectivity at 3 months and 1 year from treatment onset are influenced by any of the morphological and functional metrics at baseline.

Methods

Patients under the care of the Macular Treatment Centre of the Manchester Royal Eye Hospital were recruited retrospectively into the study. A review of imaging databases was conducted and consecutive patients fulfilling the inclusion criteria were included into the study. Inclusion criteria were that patients had been newly diagnosed with wet AMD associated with a PED over the fovea. In addition patients should have been treatment-naïve previous to starting treatment with either aflibercept or ranibizumab. A minimum PED height of 50 microns was also required for the study. Imaging data was obtained from these patients as part of their usual care, including Optical Coherence Tomography scans extracted from MREH electronic databases and anonymized. The Eye Unit of the West Hertfordshire Hospital, Watford also contributed cases into this study. All data was extracted from the same imaging device model (TopCon RD-OCT 2000). Recruited patients first attended the relevant Eye Departments between March 2014 and January 2015. As per local research governance, ethics approval is not required for retrospective review of non-identifiable patient data.

Patients on aflibercept were treated as per drug label during the first year of treatment (three monthly injections, followed by 4 bi-monthly injections). Patients on ranibizumab were treated with a fixed dose of three monthly injections, followed by monthly PRN re-treatment. Patients that developed an RPE tear in the course of follow-up were excluded from statistical analysis.

Explanatory variables recorded for the purposes of this work included for every patient visit: Best-corrected visual acuity (BCVA), Central Macular Thickness (CMT), presence of Subretinal/ Intraretinal fluid (SRF/IRF), maximum PED height (defined as

the distance between the inner surface of Bruch's membrane and the outer surface of the Retinal Pigment Epithelium), PED horizontal and vertical dimensions and whether the patient had been treated with ranibizumab or aflibercept. Precise measurements for horizontal and vertical dimension of PED were measured for each case using the calibre function on the TopCon RD-OCT 2000 in-built software at baseline, 3 months and one year of follow-up. A mathematical formula was then used to calculate PED volume with great accuracy ($v = 2 \times \pi \times h \times a \times b / 3$, where h is the height, a and b the horizontal and vertical dimensions and v the volume).

In addition to the above standard metrics, we adapted image analysis software previously validated for retinal image analysis research projects.[17-18] The software was written in MatLab© and produces morphological descriptors of PEDs including pixel intensity of PED content ('reflectivity').

The final outcome dependent variables to be studied were; BCVA, PED height and volume and finally PED reflectivity, all at 3 months and one year.

Multiple linear regression analysis was used to determine the extent to which each of the explanatory variables was related to the dependent variables.

A sample size calculation for a linear regression model with 10 predictors (anticipated effect size of 0.15, desired statistical power level of 0.7 and probability level of 0.05) for the primary outcomes specified a minimum number of 98 cases. We were able to recruit 102 for the study. Linear regressions were used to assess the effect of each variable on change in outcome of interest; once uni-variable and once adjusted for baseline of the outcome. In the regression models for those variables for which the change did not follow a normal distribution, ratio of the two time points was used (instead of difference) to normalize the distribution. Logarithm of PED height and PED volume at baseline were used for the same reason.

Statistical analysis was performed on the SPSS platform (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp)

Results

102 consecutive patients were included into the study, of which 50 patients were on treatment with ranibizumab and 52 patients on treatment with aflibercept. There were no statistically significant differences in baseline characteristics between the two groups (including BCVA, CMT, presence of SRF/IRF and PED metrics such as height, volume and reflectivity). Patients received 6.7 injections of aflibercept and 6.5 injections of ranibizumab on average at the end of first year. 110 cases were considered for inclusion in the study, of which 7 were excluded due to development of an RPE rip in the course of follow-up. Of those, 4 patients were on treatment with ranibizumab and 3 on treatment with aflibercept.

Change in Baseline Variables (all patients)

There was a statistically significant improvement in BCVA at 3 months and 1 year from 60 to 64 ETDRS letters ($p=0.048$) and 65 letters ($p=0.01$), respectively. Corresponding changes in CMT at 3 months and 1 year were from 305 to 253 ($p<0.001$) and 242 ($p<0.001$) respectively. PED maximum height decreased on average from 297 microns to 236 microns at 3 months ($p<0.001$) and 225.5 microns at 1 year ($p<0.001$). Similarly, PED volume decreased from 12.1 mm^3 to 9.05 mm^3 ($p<0.001$) at 3 months and 8.877 mm^3 at 1 year ($p<0.001$). Interestingly, although

horizontal and vertical dimensions of PEDs decreased on average, these changes did not reach statistical significance. (Table 1)

Dependent Variables

Visual Acuity

Change in BCVA at 3 months was found to be significantly influenced only by baseline BCVA (p=0.0026), yet not with any other baseline variable, such as CMT, PED metrics, presence of IRF or SRF or choice of drug. After adjusting for baseline VA only baseline vertical dimension seems to be influential on change in VA at 3 months. (p=0.035) No basline variables influence visual acuity at 1 year. (Table 2)

PED height and volume

In the un-adjusted model, change in PED height at 3 months and 1 year were influenced strongly by baseline PED height values (p=0.009 and p=0.018 respectively).

After adjusting for baseline PED height, PED height at 3 months was found to be influenced by presence of SRF at baseline, (p=0.008), baseline CMT (p=0.006) and use of aflibercept (p=0.003). At 1 year, PED height reduction was significantly influenced by baseline PED volume (in the form of log(Baseline PED volume), p=0.017) and use of aflibercept (p=0.015) (Table 3)

As expected, change in PED volume was influenced by baseline values at 3 months (p<0.001) and 1 year (p<0.001) but also by use of aflibercept (p=0.028 at 3 months, p=0.018 at one year). At 3 months PED volume change was also influenced by baseline CMT (p=0.02) (Table 4)

PED Reflectivity

There was a significant increase in average PED reflectivity from 43.59 pixels at baseline to 55.86 pixels (p<0.001) at 3 months and 57.35 pixels at 1 year (p<0.001) Increase in PED reflectivity was influenced by its baseline values at 3 months (p=0.015) and one year (p=0.03) and, interestingly, use of aflibercept at 3 months (p=0.013). There was a borderline signficant influence of baseline BCVA (p=0.049) on PED reflectivity at 3 months. (Table 5)

Discussion

This study provides insight into the response of PEDs to treatment with anti-VEGF using quantifiable metrics, including height, volume and reflectivity. The differential pharmacodynamics of PED response to aflibercept and ranibizumab in treatment-naïve patients are also studied with a follow-up extending to 1 year. PEDs were assessed by means of a novel bespoke quantifiable metric (mean pixel intensity, or 'reflectivity') derived by previously validated image analysis software.[17-18]
We found all PED metrics and vision to improve with treatment including PED content becoming more hyper-reflective. We also found there was a significant benefit in terms of reduction in PED height and volume at both 3 months and one year from aflibercept treatment compared to ranibizumab. A number of recent reports have

highlighted the efficacy of aflibercept in achieving reduction in PED height, especially in cases refractory to prior treatment with anti-VEGF agents.[13-15] Similarly we found a clear benefit in terms of reduction in PED height and volume at both 3 months and one year from aflibercpet treatment, yet focusing on treatment-naïve patients with longer follow-up than previously reported.[16]

In terms of the regression analyses for visual acuity, all patients presented statistically significant improvement in BCVA at 3 months and one year, yet this was only influenced by baseline BCVA but no other baseline parameters, including CMT, presence of IRF/SRF or any of the PED metrics. Worse visual acuity at baseline was associated with a greater visual improvement both at 3 months and 1 year. After adjusting for baseline VA only baseline vertical dimension seems to be influential on change in BCVA at 3 months with longer PEDs presenting a greater visual improvement. A number of other reports have similarly demonstrated an anatomical response[4-12] to treatment of PEDs with anti-VEGF but a lack of correlation to functional outcomes.[4,5,9-11] It would be interesting to explore whether different clinically meaningful endpoints of visual function such as distortion and microperimetry might give alternative results. The significance of PED presence as a prognostic indicator for poor visual outcome in patients with Wet AMD on anti-VEGF treatment was recently highlighted.[19,20]

For PED height, reduction was influenced by its baseline values but also baseline CMT and presence of SRF, linking PED response to treatment with other markers of disease activity in Wet AMD. (Figure 1) Thus PEDs in the context of more aggressive disease, as suggested by SRF presence and higher CMT, might be more responsive to anti-VEGF treatment. Similarly, for PED volume, reduction was influenced by its

baseline value and also baseline CMT, suggesting again a greater response in more active disease. PED flattening occurs rapidly within the first 3 months of treatment and reaches a plateau with small further reduction in height thereafter up to 1 year of treatment. Higher and more voluminous PEDs present a greater reduction in all morphological metrics on average. The algorithm for volume calculation in this work used precise calibre measurement of all three PED dimensions (height, horizontal and vertical), thus potentially offering a more accurate measurement of actual PED volume. In contrast, relevant volume measurements in previous reports have been drawn from in-built OCT software, using a standard, common base diameter for all PEDs.[19,21]

With regard to reflectivity analysis, the pattern of response to treatment in terms of reflective properties of PED content seems to be one of increasing reflectivity. Interestingly, the difference in pixel intensity, albeit significant as measured by the image analysis software, was in most cases not discernable by the naked eye. (Figure 3) We found that reduction in the hypo-reflective content of PEDs was associated with use of aflibercept at 3 months. (Figure 2) Broadhead et al[13] found that solid PEDs treated with aflibercept were less likely to experience reductions in PED dimensions than either hollow or mixed PEDs. The authors concluded that aflibercept is effective in reducing PED dimensions in treatment-resistant patients and is most effective in PEDs demonstrating some hyporeflective optical coherence tomography characteristics. Similar conclusions are drawn from our present cohort using more objective measures and applying to treatment-naïve rather than treatment-resistant Wet AMD. Our work takes a step further as reflective properties of PEDs were quantified by means of a bespoke image analysis platform and were

shown to be responsive to treatment with anti-VEGF, with the effect being more pronounced in patients on aflibercept treatment. It could thus be assumed that more hypo-reflective PED content at baseline may be associated with a better response to treatment, especially with aflibercept. We hypothesise that increasing reflectivity of the PED content is due to loss of hypo-reflective, serous elements rather than increasing hyper-reflective elements, such as fibrosis. Alternatively, increasing reflectivity may be due to loss of serous content, leaving behind the more hyper-reflective CNV complex harboured within the PED.

This work was retrospective, yet great care was taken to provide matched and comparable groups in terms of follow-up. At baseline, the groups did not differ in any of the PED metrics or disease activity features. Worthy of note, both patient groups received on average a similar number of injections over the course of 1 year. We also found a similar number of RPE tears in both groups, yet the numbers are too small to draw meaningful conclusions on RPE tear incidence. The study was powered to assess its endpoints, however a larger sample size would have provided a more robust data set and increased the reliability of the results.

Quantifiable metrics of PED morphology were used offering an objective portrayal of PED evolution in response to treatment. We found that the impact of PED morphology and response to treatment on visual outcome is limited. All PED metrics improve with treatment and PED content becomes less hypo-reflective, yet in a more pronounced way on aflibercept. The difference in rate of response is more prominent in the first three months of treatment and less so at one year. PEDs respond better in the context of more active disease, as suggested by higher CMT and presence of IRF/SRF. More hypo-reflective PED content may pre-dispose to better response to

anti-VEGF treatment, especially with aflibercept. These results might be useful in terms of prognostic value and for the purposes of patient stratification to one of different anti-VEGF treatments. Further prospective studies are required to test these findings further.

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Figure Captions

Figure 1:

Top: Case of Wet AMD with high PED accompanied by IRF and SRF, prior to treatment commencement

Bottom: Same patient after 6 injections of ranibizumab at the end of year 1 of treatment. Significant reduction in PED height, yet persistent features of active disease (IRF/SRF)

Figure 2:

Top: Case of Wet AMD with PED. The PED outline is produced in a semi-automated fashion by the image analysis software.

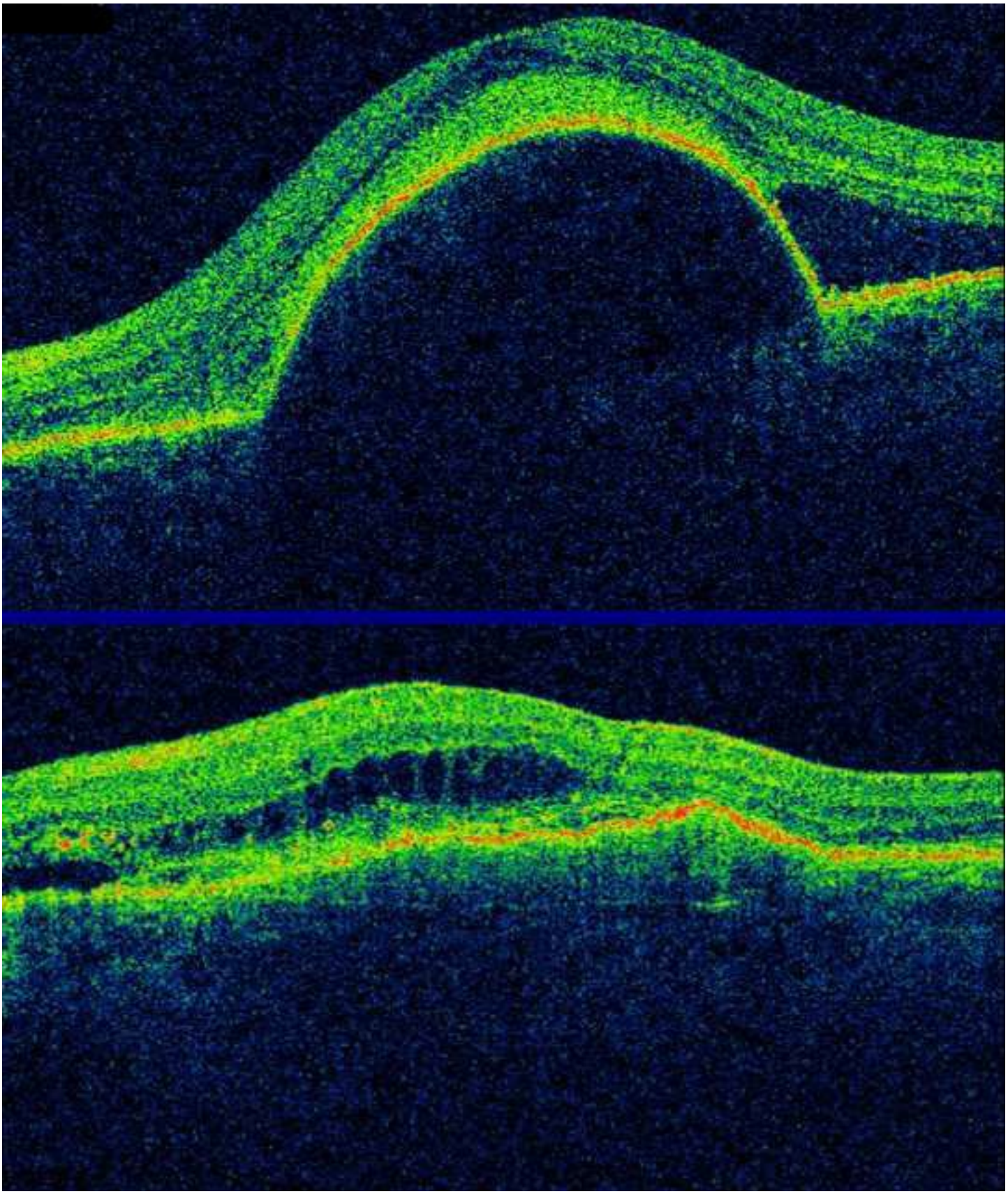
Bottom: Same patient at the end of year 1 of treatment with aflibercept. Pixel intensity has increased from 18 to 50.

Figure 3:

Top: Case of Wet AMD with PED. The PED outline is produced by the image analysis software.

Bottom: Same patient after 6 injections of ranibizumab at the end of year 1 of treatment. PED outline remains fairly unchanged, yet pixel intensity has increased from 32 to 49.

In the Description Box, Fig 1, Fig 2, etc.) Figure 1



In the Description Box, Fig 1, Fig 2, etc.) Figure 2