ABSTRACT

Objective To compare clinical characteristics of patients suffering from neovascular age-related macular degeneration (nAMD) with mature and immature choroidal neovascularisation (CNV) as assessed by optical coherence tomography angiography (OCTA). To explore the effect of total anti-vascular endothelial growth factor exposure on the occurrence of mature CNV when correcting for potential confounders.

Methods and analysis In this retrospective case series, we included 40 eyes of 36 patients with nAMD with CNV assessed by OCTA at the Manchester Eye Hospital between June 2016 and June 2017. A retinal specialist masked to patient information graded CNV depicted on OCTA scans. For statistical comparisons, we used t-tests, Fisher’s exact tests and a mixed-effects logistic regression model.

Results 18 patients (20 eyes) were treatment naïve, and the mean number of intravitreal injections (IVI) in the remaining eyes was 18.4 (range 2–71). The mean duration of nAMD was 19.3 months (range 0–87.4). 25 eyes (62.5%) exhibited mature CNV. Eyes with mature CNV did not differ from those with immature CNV regarding age (+2.8 years; p=0.288) or duration of disease (+9.4 months; p=0.061). However, they had a higher number of IVIs (+3.1; p=0.035). Among eyes with best corrected visual acuity over 25 letters, there was a strong association between the number of IVIs (0 vs 1–20: OR 68.01 [95% CI 1.30 to 3546.99; p=0.036], 0 vs >20 IVI: OR 380.01 [95% CI 2.60 to 55464.89; p=0.019]) and maturity status when correcting for potential confounders.

Conclusion Maturity status of CNV as assessed by OCTA may indicate treatment exposure of CNV in nAMD.

INTRODUCTION

Optical coherence tomography (OCT) is a non-invasive imaging technique that measures backscattered light to generate cross-sectional scans of biological tissues. In ophthalmology, OCT has contributed novel insights into choroidal and retinal anatomy significantly changing how we manage patients suffering from retinal disease, that is, neovascular age-related macular degeneration (nAMD). The most recent advance in OCT, so-called OCT angiography (OCTA), leverages improved acquisition speed and motion contrast techniques in order to detect choroidal and retinal blood flow. In contrast to fluorescein angiography, which masks the microvascular anatomy of choroidal neovascularisation (CNV) by leakage of fluorescent dye, OCTA allows investigating their phenotypical evolution in detail and in a non-invasive manner.

In CNV secondary to nAMD, OCTA has revealed two major microvascular patterns,
each associated with distinct disease activity and the requirement for antiangiogenic treatment. Immature CNV lesions exhibit high vessel density and branching index, capillary sprouting, and anastomoses, and have been reported to be more active and accordingly require a higher number of anti-vascular endothelial growth factor (VEGF) intravitreal injections (IVI). In contrast, mature CNV lesions show an increase in vessel diameter with a paucity of capillaries and branching points. CNV has been described to undergo cyclic regression (ie, reduction of flow area and pruning of anastomoses) in response to IVI and subsequent reproliferation. With continued anti-VEGF treatment, reproliferating CNV undergoes phenotypical maturation with distinctive vascular remodeling (so-called ‘vascular abnormally’) and concomitant decrease in leakage activity.

This preliminary evidence for an association between microvascular phenotype and disease activity has prompted a discussion about whether OCTA could inform anti-VEGF management decisions, such as selection of appropriate treatment intervals in a treat-and-extend anti-VEGF regimen or identifying when IVI ought to be stopped.

However, it is still unclear whether the phenotypical maturation of CNV truly reflects their response to anti-VEGF treatment or it is an effect of the natural disease course and inherent features of the membrane type. The association of anti-VEGF treatment exposure and disease duration has impeded the investigation of this problem.

In this exploratory study, we therefore sought to compare the clinical aspects of patients showing mature CNV with those with immature CNV and to examine the association of anti-VEGF treatment exposure with the maturity status of the CNV when correcting for potential confounders including AMD disease duration.

MATERIALS AND METHODS

Study design and setting, patient recruitment and enrolment
This is a cross-sectional case series of consecutive patients with nAMD with an OCTA scan presenting to the injection clinic of the Manchester Royal Eye Hospital between June 2016 and June 2017. As this study involved a retrospective analysis of deidentified retinal images obtained during routine clinical care, informed consent was not required.

Inclusion and exclusion criteria
We included all patients with fluorescein angiography confirmed diagnosis of nAMD that presented to the injection clinic of the Manchester Royal Eye Hospital, either newly diagnosed (treatment naïve) or treated with anti-VEGF agents (ranibizumab [Lucentis] or aflibercept [Eylea]) and had an OCTA scan, either acquired at or after the date of first nAMD diagnosis. Ranibizumab was given on a fixed monthly dosing for the first 3 months followed by pro re nata. In line with the licensed posology, aflibercept was given monthly for the first 3 months followed by two monthly until 1 year from commencement. This was then followed by either pro re nata or treat-and-extend regimes.

We excluded patients with nAMD who had OCTA scans without visible CNV.

Swept-source OCTA
In each case, we used a swept-source OCTA system (Topcon Triton, Topcon, Tokyo, Japan) for image acquisition. Manual segmentation of the OCTA image fine-tuned the depth of imaging to ensure optimal visualisation of the CNV in each case. An experienced and board-certified Medical Retina specialist (KB) graded and classified morphological features of CNV. Following the observations of Al-Sheikh and colleagues, he classified the CNV as immature if they harboured a dense vascular network with a high branching index, high number of anastomoses and a smooth outline (figure 1). We classified the CNV as mature if the vascular network involved loosely packed and larger (trunk) vessels, with a small branching index and loss of the capillary fringe (figure 2).

The retinal specialist graded the CNV without the knowledge of the corresponding treatment exposure or disease course. We extracted possible confounders including patients’ age, disease duration, number of injections at the time of OCTA imaging, best corrected visual acuity, number of injections at the first visit of the treatment clinic and knowledge of the corresponding treatment exposure or disease course. We extracted possible confounders including patients’ age, disease duration, number of injections at the time of OCTA imaging, best corrected visual acuity, number of injections at the first visit of the treatment clinic and knowledge of the corresponding treatment exposure or disease course.
visual acuity (BCVA), presence of subretinal fluid (SRF) and presence of intraretinal fluid (IRF). We operationalised disease duration by the time difference between the date of first nAMD diagnosis and OCTA imaging. It was standard practice for OCTA to be done prior to IVI if both were on the same day.

**Statistical analysis**

**Descriptive statistics**

We summarised continuous variables with means and SDs and dichotomous variables as percentages. Differences in population characteristics between patients with immature and mature CNV were tested using parametric or non-parametric tests as appropriate. A p value of less than 5% was considered statistically significant.

To assess the association between the number of IVIs and the occurrence of mature CNV, we categorised the number of injections into three groups (no IVI, ≤20 IVI, >20 IVI) using two indicator variates. To correct for possible confounding we fitted a mixed-effects logistic regression model using an indicator variate for mature state of CNV (yes/no) as the dependent variate, and categories of IVI along with the possible confounders stated above into the model. A mixed-effects model with an indicator variate for patient ID as a random factor was chosen to account for the fact that four subjects provided data on both eyes. For this analysis, we excluded seven eyes with very low BCVA (≤25 letters) as these represented extreme cases who did not receive standard care according to the UK National Institute for Health and Care Excellence (NICE) guidelines on nAMD and therefore the number of injections in these cases did not reflect any standardised treatment regimen. 13

Analyses were done using the Stata V.14.2 statistical software package (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp).

**RESULTS**

**Patients’ descriptive statistics**

A total of 40 eyes (25 eyes with mature and 15 eyes with immature CNV) were studied. Four patients provided both eyes to the analysis. In those four patients, we classified CNV of one patient contributing two eyes, as immature in one and as mature in the other eye. Mean age was 80.1 years (SD ±5.82), and 19 patients (51.4%) were female. Twenty eyes were treatment naïve, the mean age was 80.1 years (SD ±5.82), and 19 patients (51.4%) were female. Twenty eyes were treatment naïve, the mean age was 80.1 years (SD ±5.82), and 19 patients (51.4%) were female.

### Table 1 Characteristics and comparisons of patients with immature versus mature membranes

| Subgroup of treated eyes | Immature (15 eyes) | Mature (25 eyes) | P value |
|--------------------------|--------------------|-----------------|--------|
| Mean age in years (SD)   | 81.80 (5.82)       | 79.04 (8.81)    | 0.288  |
| Female (proportion)      | 7/15 (46.7%)       | 14/25 (56.0%)   | 0.745  |
| Treatment-naïve eyes (proportion) | 11/15 (73.3%) | 9/25 (36.0%)    | 0.048† |
| Median number of IVI (IQR) | 0 (0–8)            | 6 (0–18)        | 0.042† |
| IVI groups (injections, n) |                  |                 |        |
| 0                        | 10                 | 5               |        |
| ≤20                      | 3                  | 9               |        |
| >20                      | 1                  | 5               |        |
| Overall                  | 0.035†             |                 |        |
| Mean IVI per month (SD)  | 0.18 (0.36)        | 0.33 (0.33)     | 0.045† |
| Subgroup of treated eyes | 0.69 (0.39)        | 0.52 (0.28)     | 0.329  |
| Anti-VEGF type            |                    |                 |        |
| Ranibizumab               | 3 (20.0%)          | 6 (24.0%)       |        |
| Afibercept                | 1 (6.7%)           | 6 (24.0%)       |        |
| Both                      | 1 (6.7%)           | 4 (16.0%)       |        |
| Overall                   | 0.875              |                 |        |
| Mean disease duration (months) | 13.39 (28.14)      | 22.77 (26.41)   | 0.061* |
| Mean BCVA in letters (SD) | 58.27 (17.19)      | 44.48 (24.09)   | 0.081* |
| Presence of IRF (proportion) | 3/15 (20.0%)       | 4/25 (16.0%)    | 0.747  |
| Presence of SRF (proportion) | 7/15 (46.7%)       | 3/25 (12.0%)    | 0.014† |

*Wilcoxon rank-sum (Mann-Whitney) test.†Statistically significant

BCVA, best corrected visual acuity; IRF, intraretinal fluid; IVI, intravitreal injection; SRF, subretinal fluid; VEGF, vascular endothelial growth factor.

Eyes with mature CNV did not significantly differ from those with immature CNV regarding age (+2.8 years; p=0.288) or duration of disease (+9.4 months; p=0.061). However, eyes with mature CNV were more often treated with anti-VEGF (14/22 vs 4/15; p=0.045). They also had a significantly higher median number of total IVIs (+6, IQR 0–18, p=0.042) and included a smaller proportion of treatment-naïve eyes (36.0% vs 73.3%, p=0.048). However, within the subgroup of treated eyes, treatment frequency and the median number of total IVIs were comparable. The mean BCVA in eyes with immature CNV was higher (58.27 letters, SD ±17.19) than in patients with mature membranes (44.48 letters, SD ±24.09, p=0.081). Eyes with immature CNV also showed SRF in more cases than eyes with mature membranes (46.7% vs 12.0%, p=0.014). However, the occurrence of IRF was comparable in both groups (20.0% vs 16.0%, p=0.747).

Table 1 shows the characteristics and comparisons of patients with immature versus mature CNV. When excluding seven eyes with very low BCVA (≤25 letters), there was a strong association between the number of IVIs (0 vs 1–20 IVI: OR 68.01 [95% CI 1.30 to 3546.99; p=0.036], 0 vs >20 IVI: OR 380.01 [95% CI 2.60 to 55

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Figure 3  (A) A colour fundus image, (B) a cross-sectional foveal optical coherence tomography (OCT) image, (C) and the corresponding en face OCT angiography (OCTA) image showing the choriocapillaris slab of a patient with neovascular age-related macular degeneration (nAMD) with mature choroidal neovascularisation (CNV) treated by 41 anti-vascular endothelial growth factor (VEGF) intravitreal injection (IVI).
As in every study using real-world clinical data, we faced the problem of potential patient selection due to various reasons including, that is, variability in patient referral or availability of clinical resources for upstream evaluations. This might have introduced selection bias to an unknown extent. In view that these data should stimulate further evaluation of the biological phenomenon of CNV maturation in treated nAMD, we believe that our data will inform this discussion. It is also noted that the CNV membrane may progress between morphological phases following anti-VEGF injection. Future work visualising the evolution of CNV structural change following anti-VEGF would be helpful. Finally, while grading was performed twice for the majority of cases with no change in CNV maturity status resulting, we did not formally assess intragrader variability.

In this study, we demonstrate an association between anti-VEGF treatment exposure and CNV morphology as visualised on OCTA, after accounting for confounding factors including disease duration. We show a strong association between mature CNV phenotype with an increasing number of anti-VEGF injections.

Implications for research
Given the results presented in this case series, evaluations in a prospective cohort study of reasonable size enrolling patients who are presenting with suspected nAMD should be performed. The analysis should consider assessing several clinical subgroups including the different membrane types, the type of anti-VEGF drug and clinical activity.

Implications for practice and conclusions
This study further highlights the potential of OCTA to have a substantial impact on patient care. For instance, the response to anti-VEGF therapy in nAMD may be assessed by phenotypical maturation, thereby influencing clinical decision-making. In conclusion, the assessment of the maturity status of CNV in nAMD by OCTA may be a complementary way to follow the response to anti-VEGF therapy, providing additional information for treatment decisions.

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Patient consent for publication Not required.

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