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The Diagnostic Accuracy of Double-Layer Sign in Detection of Macular Neovascularization Secondary to Central Serous Chorioretinopathy

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Running Head: Double-Layer Sign in CSCR-MNV

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Abstract

Purpose: To investigate the diagnostic value of elevated retinal pigment epithelium (RPE) and double-layer sign (DLS) in identifying macular neovascularization (MNV) secondary to central serous chorioretinopathy (CSCR).

Design: Retrospective, cross-sectional study.

Methods: Patients with CSCR underwent optical coherence tomography (OCT) and OCT angiography (OCT-A) scanning at Moorfields Eye Hospital. OCT scans were reviewed to identify the presence/absence of an RPE elevation. The maximum length and maximum height of the elevated RPE were measured. A minimum length of 1000 µm and a maximum height of 150 µm were used to define the "double-layer sign". Other qualitative anatomical features were also graded from OCT scans. OCT-A was examined to confirm the presence/absence of MNV. Binary logistic regression analyses were used to assess the association between OCT features and the detection of MNV on OCT-A. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to assess the diagnostic accuracy.

Results: One hundred and sixty-three eyes from 132 patients were included. Elevated RPE was detected in 148 eyes (91%). OCT-A-confirmed MNV was detected in 54 eyes (33%). The sensitivity and specificity of RPE elevation were 100% and 13.8%, respectively. DLS was identified in 95 eyes (58%). The sensitivity and specificity of DLS for detecting MNV were 87% and 56%, respectively. Hyper-reflectivity and non-homogeneity of the sub-RPE space were independently associated with MNV within the DLS (odds ratio, 17.7 and 14.8, $p<0.001$ and $p=0.02$, respectively). None of the other demographic or anatomical features associated with MNV. The presence of non-homogeneous hyper-reflective RPE elevation had a sensitivity and specificity of 98% and 67%, with PPV and NPV of 60% and 99%, respectively.

Conclusions: Non-homogeneous and hyper-reflective space under an elevated RPE of any length or height indicates an eye with higher risk of MNV than DLS. OCT-A should at least be performed for these eyes to confirm the presence of MNV and treat accordingly.

Introduction

Central serous chorioretinopathy (CSCR) is a chorioretinal disease typically characterized by serous subretinal detachment associated with choroidal and retinal pigment epithelium (RPE) abnormalities.[1, 2] Optical coherence tomography (OCT) is the most common device used to diagnose and monitor CSCR. In CSCR, subretinal fluid with or without serous pigment epithelial detachment (PED) can be detected.[3] On enhanced depth imaging, most eyes with CSCR also have a thick choroid.[4] The disease is self-resolving in most patients, requiring no treatment.[5]

However, recurrent or chronic CSCR may result in RPE irregularities best visualised on multimodal imaging especially the combination of OCT and fundus autofluorescence. With time, these changes may result in RPE atrophy and/or macular neovascularisation (MNV) and visual impairment.[6, 7] Up to a quarter of patients with chronic CSCR can develop MNV.[8]

Most MNV in CSCR are type 1 MNV as they are located under the RPE causing a shallow elevation of the RPE.[9] If such a shallow pigment epithelium has a length of at least 1000 µm and a maximum height of 150 µm, it is termed a “double-layer sign” (DLS).[10] The DLS sign may also occur in CSCR without MNV due to persistent accumulation of sub-RPE fluid or debris so the sub-RPE space may appear hypo-reflective due to fluid or hyper-reflective due to the subretinal accumulation of debris

or MNV.[11] Therefore, DLS often triggers an optical coherence tomography-angiography (OCT-A), fluorescein angiography (FA), or indocyanine green angiography (ICGA) to investigate the presence of MNV.

Although FA/ICGA are the gold standard diagnostic test for MNV,[12] angiographic diagnosis of MNV in chronic CSCR is often challenging due to the associated window defect, staining, and leaks associated with changes seen in RPE due to CSCR.[13] Additionally, the type 1 MNV complicating CSCR is often characterized by a late wash-out of the neovascular network determining the absence of a well-demarcated hyperfluorescent area in the late frames of ICGA images. The advent of OCT-A has overcome some of these diagnostic challenges. It is also a non-invasive test and so can be repeated regularly. It is capable of providing three-dimensional images of blood flow in retinal and choroidal microvasculature.[14] The use of OCT-A in CSCR allowed for a higher MNV detection rate than FA and ICGA.[15]

In a recent study, we showed that CSCR eyes with an OCT-A-confirmed MNV showed anatomical and functional improvement after anti-vascular endothelial growth factor (VEGF) treatment. This response was not seen in eyes without OCT-A evidence of MNV.[16]

As SRF may be seen in eyes with and without MNV, we rely on DLS as a sign of MNV. Other signs of MNV include fibrovascular PED, intraretinal fluid, and subretinal hyper-reflective material (SHRM). However, the diagnostic accuracy of DLS in identifying CSCR related MNV is unclear. It is also unknown whether any characteristics of DLS with or without other OCT features are more predictive of MNV. The aim of this study was to investigate the association of DLS with CSCR-

related MNV and the diagnostic accuracy of DLS in identifying MNV in CSCR patients that has been confirmed by OCT-A.

Methods

This retrospective observational study was approved by the Moorfields Clinical Effectiveness Department (CA18/MR/22-197). The “Standards for Reporting Diagnostic Accuracy Studies” (STARD) 2015 checklist was used to prepare this paper.[17]

Study Participants

Consecutive patients with a clinical diagnosis of CSCR, for whom spectral-domain OCT and OCT-A scans were acquired between November 2016 and March 2020, were retrospectively identified from Moorfields Eye Hospital electronic medical records. Both eyes were included if they met inclusion criteria. Eyes with other retinal or choroidal disorders that can affect retinal morphology were not included. History of treatment was not considered for excluding eyes from this analysis. In cases with more than one visit suitable for inclusion, the visit with the first OCT-A evidence of MNV was included.

Optical Coherence Tomography Imaging

Spectral-domain OCT (Spectralis, Heidelberg Engineering, Heidelberg, Germany) imaging was performed on all participants as part of standard care. Macular scans were reviewed by expert graders to extract structural features. Since OCT scans were acquired as standard of care, no specific acquisition protocol was followed.

Scanning covered a 6x6 mm area with a density ranged from 22 to 49 B-scans per volume.

Grading Definitions for OCT

All B-scans in the OCT volume were reviewed. OCT graders were blinded to the outcome of OCT-A grading. The presence of RPE elevation was identified as a separation of the RPE from the Bruch's membrane. If an RPE elevation was detected, graders identified the B-scan with the greatest horizontal extension. Maximum length and maximum height in that frame were measured, and the length-to-height ratio was calculated. A shallow RPE elevation with a minimum length of 1000 μm and a maximum height of 150 μm was considered as a "double-layer sign".

The RPE elevation was then further graded for reflectivity in the sub-RPE space. Reflectivity of the sub-RPE space was graded as predominantly hyper-reflective or hypo-reflective relative to the reflectivity of the outer nuclear layer (ONL). The sub-RPE space was also graded as homogeneous or heterogeneous.

Other qualitative characteristic features were also recorded, including RPE layer irregularity, photoreceptor layer disruption, and presence of SHRM. RPE layer was considered irregular when it loses its smooth contour. Photoreceptor disruption was defined as disruption of the ellipsoid zone, external limiting membrane or thinning ONL. SHRM was defined as "a hyper-reflective material located external to the retina, and internal to the RPE".[18]

A subset of OCT scans (20 scans) was re-graded by another grader to assess agreement.

OCT Angiography Imaging and Grading

Spectral-domain OCT-A scans were acquired using the AngioPlex instrument (Carl Zeiss Meditec, Inc., Dublin, CA, USA). Macular 3x3 mm or 6x6 mm images were examined by an expert grader to detect MNV from *en face* and B-scan images. A minimum OCT-A quality index of 6 was required to be included in the analysis. The *en face* outer retina to choriocapillaris (ORCC) slab along with the flow signal on the OCT B-scans were used to confirm the presence/absence of MNV on OCTA. Projection artifacts from retinal vasculature was considered when reviewing images. Dynamic adjustments to the slab boundaries were carried out whenever needed to achieve the best visibility of outer retinal flow signal. Since OCT-A has proved to be more reliable than dye-based angiography for detecting neovascularization secondary to CSCR,[15] detecting an evidence of MNV on OCT-A was considered as the reference standard for the diagnostic accuracy analyses in this study. The default OCT-A review software provides both structural and angiography data. Thus, blinding the OCT-A grader to the structural OCT scans is not feasible.

Statistical Analysis

All statistical analyses were performed on Excel 2013 (Microsoft Corporation, Redmond, WA, USA), SPSS v 27 (IBM Corporation, Armonk, NY, USA) and RStudio IDE (RStudio PBC, Boston, MA, USA). Data were presented as either mean ± standard deviation (SD) or frequency and percentage. Inter-rater reliability for OCT parameters were assessed by intraclass correlation coefficient or percentage of agreement between two graders. Contingency tables with chi-squared test were used to assess the association between characteristic OCT features and MNV. Univariable binary logistic regression was used to explore the structural DLS features that are associated with the presence of MNV on OCT-A. Generalised estimating equation (GEE) with robust standard error estimation was used to adjust

for the intrasubject correlation between both eyes. Odds ratio (OR) with 95% confidence interval (CI) and *p* value were calculated. Penalised maximum likelihood estimation method was used to deal with quasi-complete separated data (R package logistf).[19, 20] However, this method does not account for potential correlation between eyes of the same participant. Parameters with statistically significant associations were then included in a multivariable logistic regression model to detect independent predictive features. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to assess the diagnostic accuracy. No indeterminate or missing OCT or OCT-A data were present in this analysis.

Results

One hundred and sixty-three eyes from 132 patients were included in the analysis (Figure 1). At time of eye scanning, the mean age was 55.2 ± 12.2 years, with 46 eyes (28.2%) were from female patients. An elevation of the RPE was detected in more than 90% of the included eyes (148 out of 163 CSCR). OCT-A-confirmed MNV was detected in 54 eyes (33.1%). Graders subjectively reported that the smaller 3x3 mm OCT-A scans provided better visualization of neovascularization, and thus, they were more useful than the 6x6 scans for confirming the presence/absence of MNV in challenging cases.

The analysis of inter-grader agreement in a subset of OCT scans revealed acceptable reliability of graded parameters. The measurement of the length and height of elevated RPE had a good agreement (0.83 and 0.98, respectively, intraclass correlation coefficient). The detection of qualitative features also showed strong agreement between graders (range, 83% - 100%).

Table 1 shows the 2x2 contingency table representing the relationship between MNV and RPE elevation. A significant association was detected between elevated RPE and MNV (p = 0.002, chi-squared test). The presence of any RPE elevation had a 100% sensitivity for the detection of MNV on OCT-A (Table 2). However, the specificity was very low (13.8%).

We explored the diagnostic accuracy of different height cut-offs of the RPE elevation. Using a 100 and 120 µm cut-off allowed for detecting 78% and 87% of the eyes with MNV, respectively, leading to missing a significant proportion of MNV cases in our cohort. Meanwhile, the elevated RPE in 93% of MNV cases had a height of less than 150 µm. Thus, a 150 µm maximum height, along with a minimum length of 1000 µm were chosen to define the double-layer sign in this study.

The DLS was identified in 95 eyes (58.3%) in our cohort (Figure 1). The presence of DLS was significantly associated with MNV detection in CSCR patients (p < 0.001) (Table 1). The sensitivity of DLS for detecting MNV was 87.0% with a specificity of 56.0%. The PPV and NPV were 49.5% and 89.7%, respectively (Table 2). Using this definition of DLS missed 13% of the eyes with MNV in our cohort. Seven eyes with OCT-A-evidence of MNV had an RPE elevation which did not meet the dimensional criteria for DLS (Figure 1). These cases will be discussed in more detail later.

Eyes with Double-Layer Sign

Characteristic demographic and OCT features in eyes with DLS were summarized in Table 3. On unilateral logistic regression, older age associated significantly with MNV (p = 0.002). None of the dimensional parameters of the DLS were significantly associated with MNV. However, hyper-reflectivity and non-homogeneity of the sub-RPE space were strong predictors of neovascular activity within the DLS (OR, 50.0

and 39.9, respectively, $p < 0.001$) (Figure 2). Similarly, the presence of SHRM was significantly correlated with the presence of MNV (OR = 2.7, $p = 0.02$). Although RPE irregularity and photoreceptor disruption were commonly present in eyes with CSCR, they were not associated with MNV (Table 3).

On multivariable regression, hyper-reflectivity and non-homogeneity in the sub-RPE were independently associated with MNV (Table 3). However, age, gender, and the presence of SHRM did not show significant correlation with MNV after adjusting for other confounders (Table 3).

In eyes with DLS, the combined significant features (hyper-reflective and non-homogeneous) had a 97.9% sensitivity for predicting MNV with 60.4% specificity. Positive and negative predictive values of the DLS features combined were 70.8% and 96.7%, respectively (Table 2).

In all CSCR eyes, incorporating the significant features with DLS caused a slight decrease in the sensitivity of MNV detection to 85.2% while improving the specificity to 82.6%, as compared to 87.0% and 56%, respectively, for DLS alone (Table 2). The combined features also improved the PPV and NPV (Table 2).

Eyes without Double-Layer Sign

Sixty-eight eyes from 61 CSCR patients did not meet the criteria for the presence of DLS. Fifty-three (76.8%) of them had an RPE elevation with a length of less than 1000 µm and/or a height of more than 150 µm. The remaining 15 eyes did not show any signs of elevated RPE (Figure 1).

Seven eyes without DLS but with RPE elevation showed an OCT-A evidence of MNV (2 females and 5 males, mean age, 54.3 ± 13.6 years, range, 37 – 69 years).

OCT and OCT-A scans were reviewed again and the absence/presence of DLS/MNV was confirmed. All 7 eyes had an RPE elevation that did not follow the definition of DLS. However, all of them displayed hyper-reflectivity and non-homogeneity in the sub-RPE space. Two eyes had an RPE elevation longer than 1000 µm, but slightly higher than the 150 µm cut-off (183 and 195 µm). One of them is demonstrated in Figure 3 (panels A1 and A2). Three other eyes had an RPE elevation of less than 150 µm in height, but with a maximum length of less than 1000 µm (ranged 622 – 749 µm). These lesions showed a relatively small MNV on OCT-A (Figure 3, B1 and B2). The last 2 eyes showed relatively short and high RPE elevations (length, 866 and 851 µm, height, 412 and 293 µm, respectively) (Figure 3, C1 and C2).

Our cohort included a total of 5 eyes with a relatively highly elevated (> 150 µm) RPE with non-homogeneous and hyperreflective sub-RPE space. Interestingly, 4 of them showed evidence of MNV on OCT-A. All 15 eyes with no RPE elevation did not reveal MNV on OCT-A.

Discussion

This study aimed at investigating the diagnostic value of the elevated RPE and double-layer sign in identifying eyes with MNV secondary to CSCR. All MNV cases in our cohort had an elevated RPE, which is believed to accommodate the neovascular membrane.[21] However, the majority of the included CSCR eyes had some form of RPE elevation, which limits its specificity in identifying MNV. Thus, we sought to further characterise these lesions to define their MNV-specific features. The DLS has been previously described as a shallow and long RPE elevation.[22, 23]

DLS is associated with the presence of neovascularisation in several chorioretinal diseases including CSCR,[24] age-related macular degeneration (AMD),[22] and polypoidal choroidal vasculopathy.[25] In this analysis, we used a minimum length of 1000 µm and a maximum height of 150 µm to describe the DLS. Although, there was a significant association between the presence of DLS on OCT and the detection of an OCT-A-evidence of MNV, several cases with neovascularisation did not manifest as a DLS. We were able to detect MNV in eyes with as an elevated RPE that was as short as 622 µm and height of 412 µm. Additionally, the height and length of the RPE elevation were not significantly associated with MNV. Thus, we believe that the size of the RPE elevation in CSCR should not be used to exclude the possibility of the presence of neovascularisation.

We also explored the demographic and anatomical features that can predict the presence of MNV on OCT-A. Although older age was significantly associated with higher risk for detecting MNV, the association fell slightly short of significance on multivariable regression (p = 0.07). In contrast, Jingli et al. observed that older age was an independent risk factor for the presence of neovascularization beneath the elevated RPE.[26] On structural OCT, non-homogeneity and hyper-reflectivity of the sub-RPE space under any-sized RPE elevation were the only predictors of the detection of MNV. Only one eye with MNV in our cohort was graded to have a hypo-reflective sub-RPE (Figure 4). However, both graders agreed that although it was predominantly hypo-reflective, some hyperreflective signal was still detected in the sub-RPE space. A similar finding was reported by Bousquet et al. where they described it as being “partially hyperreflective”.[27] Fibrovascular tissue is believed to be the cause of the non-homogeneous hyperreflective elevation of the RPE.[28] These combined features had a very high sensitivity for detecting MNV in our cohort. The

NPV was almost 99%, implying that it is very unlikely to detect a MNV in a CSCR eye with the absence of a non-homogeneous hyperreflective elevation of the RPE. However, a PPV of about 60% indicates that approximately 1 in every 3 eyes with these features might still not have MNV.

Although univariable analysis in this cohort showed a significant association between the presence of SHRM on OCT and the detection of MNV on OCT-A, the association was not found on multivariable regression. This finding disagrees with our recent report where SHRM was still significantly linked to MNV on multivariable analysis.[16] This can be attributed to the different patient cohorts as well as the different variables that were included in each analysis. In the previous study, we had already investigated whether subretinal fluid, intraretinal hyperreflective foci, subretinal hyperreflective foci, intraretinal cysts, and pachychoroid were associated with MNV. We found that none of these were associated with MNV and so we did not include them in the analysis in this paper.[16]

Disruption of photoreceptor layers and irregular RPE are common features in cases with chronic CSCR, irrespective of the presence or absence of MNV.[29] Thus, these features were not helpful for predicting the detection of neovascular activity on OCT-A. Meanwhile in AMD, the presence of RPE irregularity was significantly more common in eyes with MNV as compared to non-neovascular AMD. Additionally, large drusenoid PEDs can be commonly detected in AMD. In contrast, this feature is not typically expected in eyes with CSCR. Thus, the presence of a relatively highly elevated RPE with hyper-reflectivity in the sub-RPE space in CSCR should be suspected of neovascularization. In our cohort, 4 out of the 5 eyes with this feature had an OCT-A-evidence of MNV.

Our findings are consistent with the results of previous reports studying the double-layer sign in CSCR eyes with and without secondary MNV. Sheth and colleagues observed significantly higher prevalence of DLS with hyperreflective core in eyes with MNV as compared to CSCR eyes with no evidence of MNV.[11] More recently, Lee et al. also observed a significant association between DLS and MNV.[30] However, both reports had a relatively small sample size for eyes with MNV, and they did not have a specific dimensional definition for the DLS. They also confirmed the presence of MNV by FFA and/or ICGA, but not OCT-A. Lee and colleagues also reported a significant association between the presence of MNV and the ellipsoid zone disruption and RPE changes,[30] which we did not observe in our analysis. This discrepancy can be attributed to differences in the included cohorts and analyses between both studies. In our study, we used OCT-A to diagnose MNV, rather than dye-based angiography. Additionally, Lee et al. analysed a wide spectrum of angiography- and OCT-based variables using univariable regression analyses.[30] Multivariable regression analysis can adjust for confounders and potentially alter the significance of some parameters.

Elevated RPE and DLS have been also described in neovascular AMD. A recent report by Narita et al. identified the characteristics of double-layer sign associated with non-exudative MNV in eyes with AMD.[10] Similar to our findings, nonhomogeneous reflectivity was a significant predictor of the presence of MNV in eyes with large drusen. However, unlike CSCR, the dimensions of the elevated RPE had a significant role in identifying eyes with MNV. Defining a minimum length of 1000 μm for the DLS was able to detect all cases of MNV in their cohort. Additionally, smaller height and log height-to-area ratio were significantly associated with the presence of MNV in eyes with large drusen. RPE irregularity as well as

hyper-reflectivity and non-homogeneity of the sub-RPE space were also correlated with the detection of MNV. Thus, they described these shallow irregular RPE elevation (SIRE) lesions to be able to detect all cases of non-exudative MNV in eyes with large drusen.[10] However, the findings of our study suggest that this finding cannot be readily extended to eyes with CSCR. As we presented earlier, the proposed size limit of SIRE can miss a significant proportion of MNV in CSCR eyes. This size discrepancy between the two studies may be also due to the variation in activity and/or chronicity level of the neovascular lesion in both studies.

In our study, a consecutive cohort of CSCR patients were analysed to investigate structural OCT features that can predict the detection of neovascularisation on OCT-A. Since OCT is more widely available than OCT-A, identifying these features can be valuable in defining high risk eyes. Diagnosing MNV in eyes with CSCR can significantly alter the management of such cases as they benefit from intravitreal anti-VEGF treatment to improve/stabilise vision.[16] The findings of our study imply that OCT might be able to exclude the presence MNV in eyes without elevated RPE with non-homogeneous hyperreflective core. However, when these structural OCT features are present, OCT-A would still be needed to provide a definitive diagnosis of MNV in these eyes.

Despite the relatively large sample size and the detailed qualitative and quantitative analyses, this study was limited by its retrospective and cross-sectional design. Our OCT data are based on real-life clinical data, leading to variations in the used scanning protocols. Additionally, OCT-A imaging is not routinely performed for all CSCR patients in our practice. Thus, including patients for whom OCT-A was performed might have induced a selection bias towards patients with a clinically-suspected MNV. This can explain the relatively high prevalence of MNV in our

cohort. The majority of the detected neovascular lesions in these eyes are likely to be long-standing and some eyes might have been previously treated. Chronicity and treatment might influence the morphology of MNV and/or DLS. Early signs of developing MNV in eyes with CSCR may be different. Our study was also limited by the subjective nature of many of the OCT/OCT-A gradings. However, our inter-grader reliability analyses indicated acceptable degree of variability between graders. Another potential limitation of our study is that OCT-A may fail to detect the presence of MNV in cases of slow flow. Future larger prospective and longitudinal studies are needed to confirm the findings of this study.

In conclusion, the widely-available structural OCT can be a valuable tool to identify suspected MNV in eyes with CSCR. Detecting a non-homogeneous and hyperreflective space under an elevated RPE indicates an eye with higher risk of neovascularisation. OCT-A should be performed for these eyes to confirm the presence of MNV and treat accordingly.

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Table of contents statement

In this retrospective study of 163 eyes with central serous chorioretinopathy, characteristic features of double-layers sign were analyzed to investigate its

association and diagnostic accuracy for identifying macular neovascularization. Detecting a non-homogeneous and hyperreflective space under an elevated pigment epithelium, irrespective of its size, indicates an eye with higher risk of neovascularization. Optical coherence tomographic angiography should be performed for these eyes to confirm the presence of macular neovascularization and treat accordingly.

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Figure Legends

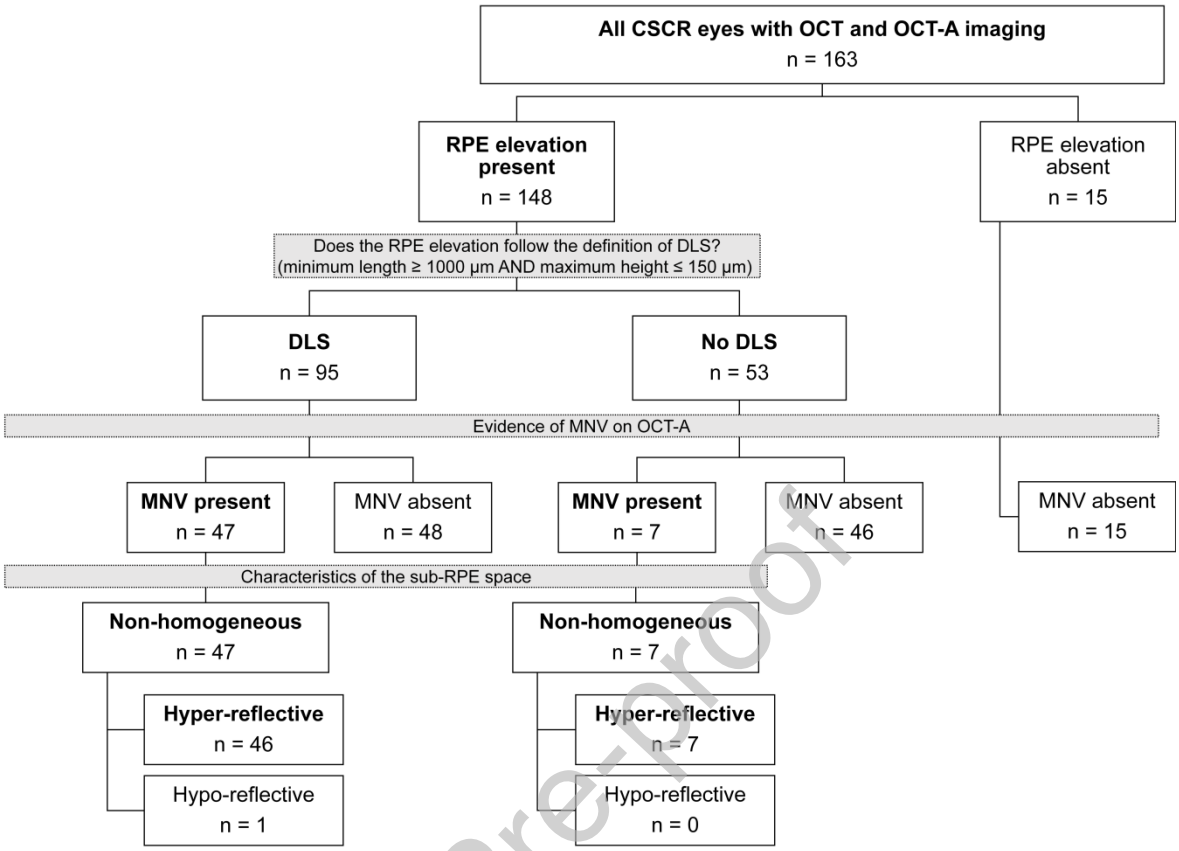

FIGURE 1. Flow diagram of study participants. CSCR: central serous chorioretinopathy, OCT: optical coherence tomography, OCT-A: OCT angiography, RPE: retinal pigment epithelium, DLS: double-layer sign, MNV: macular neovascularization.

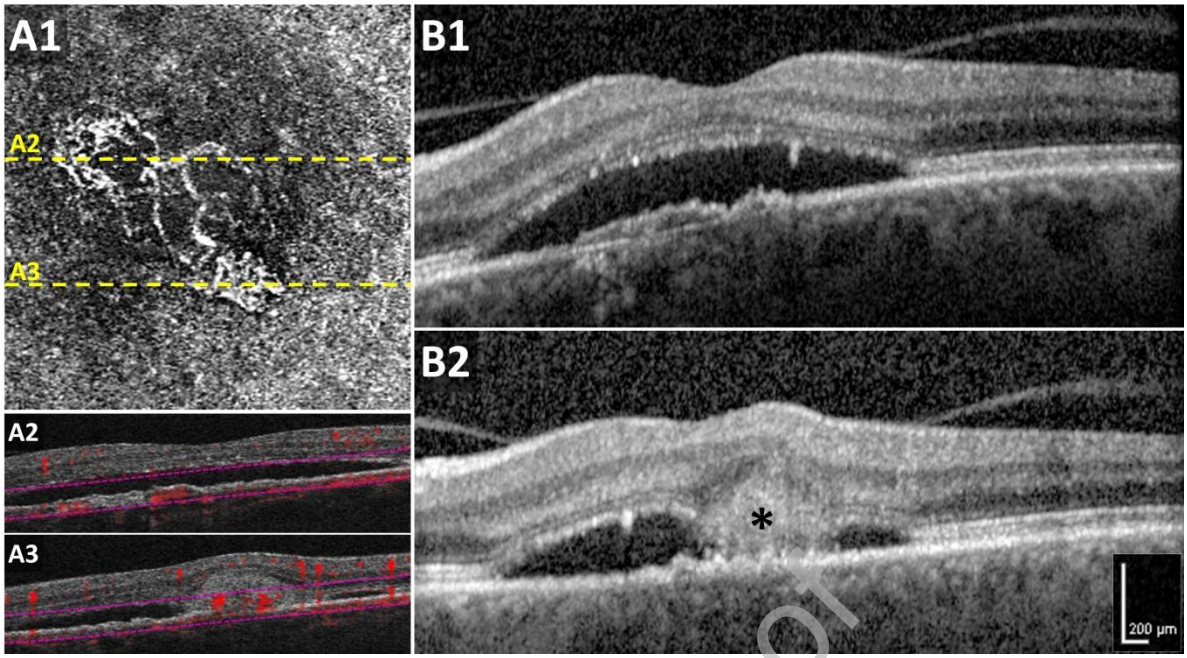

FIGURE 2. Macular 3x3 mm optical coherence tomography angiography (OCT-A, A1-A3) and 6x6 mm structural OCT (B1 and B2) scans of the left eye of a 63-year-old patient with chronic central serous chorioretinopathy. (A1) *En face* OCT angiogram of the outer retina to choriocapillaris (ORCC) slab showing a macular neovascularization (MNV). The ORCC slab boundaries are demonstrated by the purple dashed lines in A2 and A3. Yellow dashed lines in A1 correspond to B-scans in A2 and A3. (A2 and A3) Cross-sectional structural OCT B-scans with overlaid red-coded OCT-A flow signal. OCT/OCT-A B-scans revealed a mixed type 1 and type 2 MNV. The superior part of the neovascular membrane (A2) was detected in the sub-retinal pigment epithelium (RPE) compartment, while the inferior portion of the membrane can be detected above the RPE, in the subretinal compartment. (B1) Structural OCT B-scan showing the largest horizontal extension of the elevated RPE (length, 1493 µm, maximum height, 46 µm). Non-homogeneous hyper-reflectivity is evident in the sub-RPE space. Subretinal fluid, outer retinal disruption, and RPE irregularity can be also observed in the structural OCT. Subretinal hyperreflective

material (SHRM, black asterisk) colocalized with type 2 part of the MNV. The scale bar in the lower right corner belongs to the structural OCT scans in B1 and B2.

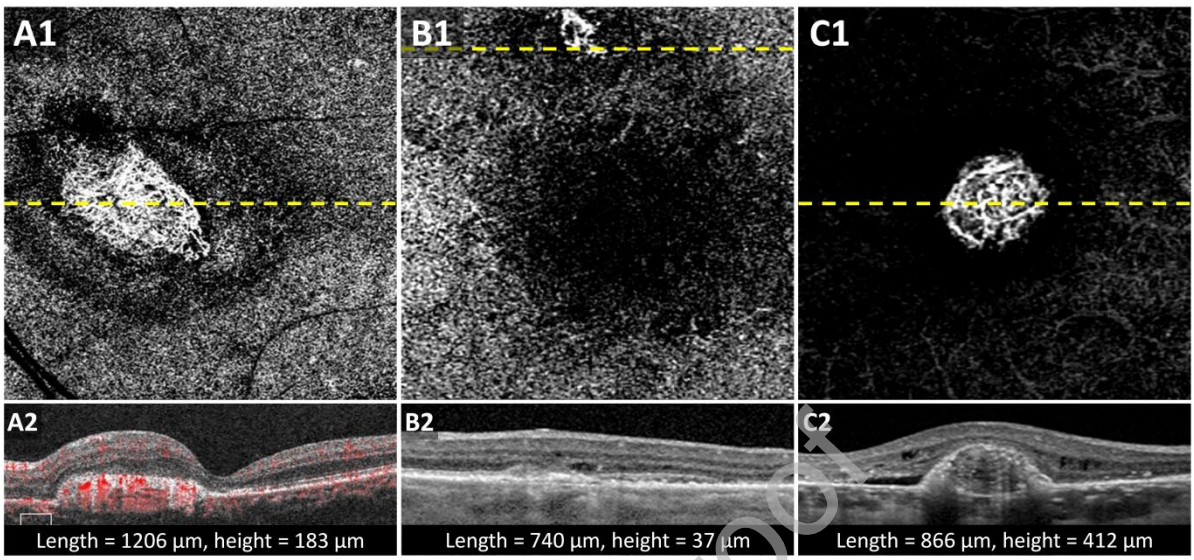

FIGURE 3. Macular neovascularization (MNV) secondary to central serous chorioretinopathy in eyes without double-layer sign (DLS). (A1 – C1) *En face* optical coherence tomography angiograms (OCT-A) of the outer retinal slab. Yellow dashed lined correspond to the cross-sectional images in A2 – C2. B-scans demonstrate the greatest horizontal extension of a retinal pigment epithelium (RPE) elevation with non-homogeneous hyper-reflectivity in the sub-RPE space. (A) Macular 6x6 mm OCT/OCT-A scan of the left eye of a 37-year-old male patient. The cross-sectional image demonstrates a type 2 MNV lying within a fibrovascular membrane. The length and height of the RPE elevation are 1206 µm and 183 µm, respectively. (B) Macular 3x3 mm OCT/OCT-A scans of the left eye of a 58-year-old male patient. Images display a small type 1 MNV with an elevated PED that did not meet the 1000 µm minimum length threshold of a DLS (740 µm). (C) Macular 3x3 mm OCT/OCT-A scans of the right eye of a 37-year-old female patient. A type 1 MNV can be seen in the *en face* OCT angiogram of the outer retina (C1). Structural

OCT B-scan (C2) shows a RPE elevation with a maximum length and height of 866 µm and 412 µm, respectively.

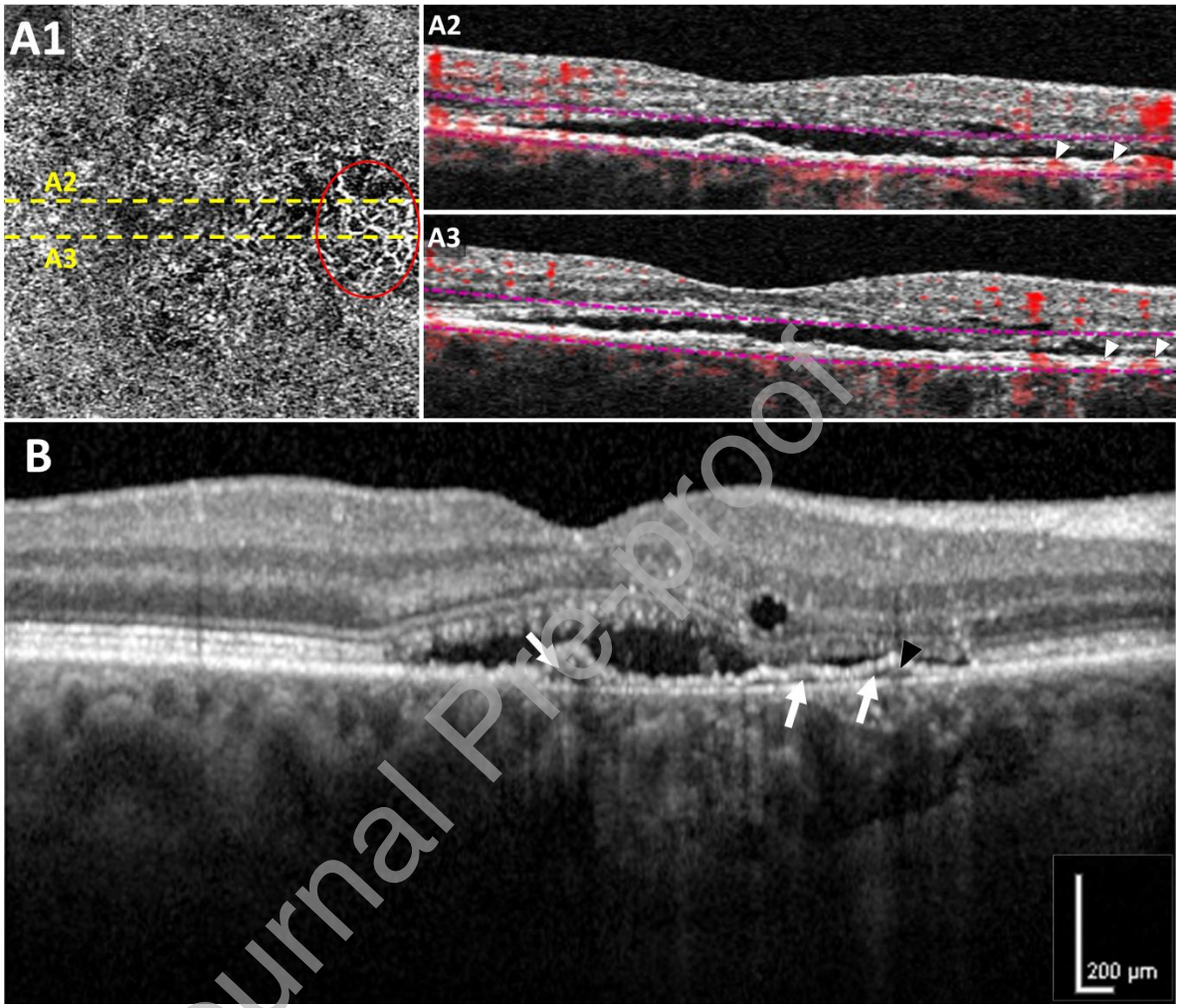

FIGURE 4. Macular 3x3 mm optical coherence tomography angiography (OCT-A, A1-A3) and 6x6 mm structural OCT (B) scans of the right eye of a 72-year-old female patient with chronic central serous chorioretinopathy. (A1) *En face* OCT angiogram of the outer retina to choriocapillaris (ORCC) slab showing flow signal that was suspected of macular neovascularization (MNV) nasal to the fovea (red circle). The ORCC slab boundaries are demonstrated by the purple dashed lines in A2 and A3. Yellow dashed lines in A1 correspond to B-scans in A2 and A3. (A2 and A3) Cross-sectional structural OCT B-scans with overlaid red-coded OCT-A

flow signal. OCT/OCT-A B-scans revealed a type 1 MNV, with the flow signal located under the retinal pigment epithelium (RPE) (white arrow heads). (B) Structural OCT B-scan showing the largest horizontal extension of the elevated RPE (length, 2472 µm, maximum height, 53 µm). This was the only MNV case in our cohort that was graded to have a predominantly hypo-reflective sub-RPE space (white arrows). However, some hyper-reflectivity can still be observed within the elevated RPE (black arrowhead). The scale bar in the lower right corner belongs to the structural OCT scan in B.

TABLE 1. Contingency tables of OCT features and macular neovascularization

		Macular neovascularization			*P* value*
		Absent	**Present**	**Total**	
Retinal pigment epithelium (RPE) elevation	**Absent**	15	0	15	0.002
	Present	94	54	148	
	Total	109	54	163	
Nonhomogeneous and hyperreflective RPE elevation	**Absent**	73	1	74	< 0.001
	Present	36	53	89	
	Total	109	54	163	
Double-layer sign (DLS)	**Absent**	61	7	68	< 0.001
	Present	48	47	95	
	Total	109	54	163	
Nonhomogeneous and hyperreflective DLS	**Absent**	90	8	108	< 0.001
	Present	19	46	55	
	Total	109	54	163	
Nonhomogeneity and hyperreflectivity	**Absent**	29	1	30	< 0.001
	Present	19	46	64	

(in eyes with DLS)	**Total**	48	46	94	

**P* values were based on chi-squared test. Double-layer sign is defined as an elevated RPE with a minimum length of 1000 um and a maximum height of 150 um.

TABLE 2. Diagnostic accuracy of double-layer sign and other OCT features for detecting macular neovascularization

	All CSCR eyes				**CSCR eyes with double-layer sign**
	Retinal pigment epithelium (RPE) elevation	**Nonhomogeneous and hyperreflective RPE elevation**	**Double-layer sign (DLS)**	**DLS with combined significant features**	**Nonhomogeneity and hyperreflectivity**
Sensitivity	100%	98.1%	87.0%	85.2%	97.9%
Specificity	13.8%	67.0%	56.0%	82.6%	60.4%
Positive predictive value	36.5%	59.6%	49.5%	70.8%	70.8%
Negative predictive value	100%	98.6%	89.7%	91.8%	96.7%

CSCR: central serous chorioretinopathy. Double-layer sign (DLS) is defined as an elevated RPE with a minimum length of 1000 um and a maximum height of 150 um.

TABLE 3. Characteristic features of double-layer sign in eyes with CSCR

	All	macular neovascularization		Univariable logistic regression		Multivariable logistic regression	
		Present	Absent	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Number of eyes	95	47 (49.5%)	48 (50.5%)				
Gender							
Male	65 (68.4%)	28 (59.6%)	37 (77.1%)	2.3 (0.9 – 5.7)	0.08	1.2 (0.4 – 3.7)	0.8
Female	30 (31.6%)	19 (40.4%)	11 (22.9%)				
Age (years)	58.9 ± 11.6	63.1 ± 9.8	54.8 ± 11.9	1.074 (1.028 – 1.122)	0.002	1.0 (0.996– 1.1)	0.07
Range	27 – 88	43 – 78	27 – 88				
Length (100 µm increments)	21.80 ± 10.37	22.78 ± 7.81	20.84 ± 12.38	1.020 (0.972 – 1.071)	0.42		

Height (10 µm increments)	6.37 ± 3.19	6.73 ± 2.96	5.81 ± 3.37	1.022 (0.912 – 1.144)	0.71		
Length/height ratio	43.5 ± 36.2	43.5 ± 45.2	43.6 ± 24.8				
Log length/height ratio	1.56 ± 0.25	1.55 ± 0.24	1.57 ± 0.26	0.87 (0.17 – 4.4)	0.87		
Reflectivity of sub-RPE space							
Hyporeflective	26 (27.4%)	1 (2.1%)	25 (52.1%)		< 0.001		**< 0.001**
Hyperreflective	69 (72.6%)	46 (97.9%)	23 (47.9%)	50.0 (6.3 – 395.5)		17.7 (3.9 – 168.6)	
Homogeneity of sub-RPE							
Nonhomogeneous	81 (85.3%)	47 (100%)	34 (70.8%)	39.9 (5.0 – 5166.8)	< 0.001	14.8 (1.4 – 2079.3)	**0.02**
Homogeneous	14 (14.7%)	0	14 (29.2%)				
Disruption of photoreceptors							
Absent	5 (5.3%)	3 (6.4%)	2 (4.2%)		0.64		
Present	90 (94.7%)	44 (93.6%)	46 (95.8%)	0.6 (0.1 – 4.1)			

RPE irregularity							
Absent	5 (5.3%)	2 (4.3%)	3 (6.3%)		0.67		
Present	90 (94.7%)	45 (95.7%)	45 (93.7%)	0.7 (0.1 – 4.3)			
Subretinal hyperreflective material							
Absent	54 (56.8%)	21 (44.7%)	33 (68.7%)		0.02		0.47
Present	41 (43.2%)	26 (55.3%)	15 (31.3%)	2.7 (1.1 – 6.5)		1.5 (0.5 – 4.3)	

CSCR: central serous chorioretinopathy; OR: Odds ratio; CI: confidence interval; RPE: retinal pigment epithelium.

Biosketch - Ahmed Hagag

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