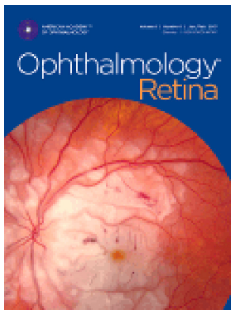
Accepted Manuscript



Predictive Value of the OCT Double-Layer Sign for Identifying Subclinical Neovascularization in Age-Related Macular Degeneration

Yingying Shi, MD, Elie H. Motulsky, MD, PhD, Raquel Goldhardt, MD, FACS, Yehoshua Zohar, MD, MHA, Marie Thulliez, MD, William Feuer, MS, Giovanni Gregori, PhD, Philip J. Rosenfeld, MD, PhD

PII: S2468-6530(18)30495-0

DOI: https://doi.org/10.1016/j.oret.2018.10.012

Reference: ORET 419

To appear in: *Ophthalmology Retina*

Received Date: 8 August 2018

Revised Date: 15 October 2018

Accepted Date: 19 October 2018

Please cite this article as: Shi Y., Motulsky E.H., Goldhardt R., Zohar Y., Thulliez M., Feuer W., Gregori G. & Rosenfeld P.J., Predictive Value of the OCT Double-Layer Sign for Identifying Subclinical Neovascularization in Age-Related Macular Degeneration, *Ophthalmology Retina* (2018), doi: https://doi.org/10.1016/j.oret.2018.10.012.

Predictive Value of the OCT Double-Layer Sign for Identifying Subclinical Neovascularization in Age-Related Macular Degeneration

Yingying Shi, MD, Elie H. Motulsky, MD, PhD, Raquel Goldhardt, MD, FACS, Yehoshua Zohar, MD, MHA, Marie Thulliez, MD, William Feuer, MS, Giovanni Gregori, PhD, Philip J. Rosenfeld, MD, PhD

Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, Florida, USA

Corresponding Author:
Philip J. Rosenfeld MD, PhD
Bascom Palmer Eye Institute
900 NW 17th street
Miami, FL, 33136
Voice: 305-326-6148
Fax: 305-326-6538
E-mail: prosenfeld@miami.edu

Meeting Presentation: This research will be presented during Retina Subspecialty Day at the American Academy of Ophthalmology Meeting, October 2018.

Financial Support: Research supported by grants from Carl Zeiss Meditec, Inc. (Dublin, CA), the Salah Foundation, an unrestricted grant from the Research to Prevent Blindness, Inc., New York, NY, and the National Eye Institute Center Core Grant (P30EY014801) to the Department of Ophthalmology, University of Miami Miller School of Medicine. The funding organization had no role in the design or conduct of this research.

Conflicts of Interest:

Dr. Gregori and Dr. Rosenfeld received research support from Carl Zeiss Meditec, Inc. Dr. Gregori and the University of Miami co-own a patent that is licensed to Carl Zeiss Meditec, Inc. Dr. Rosenfeld also received additional research support from Genentech and Tyrogenex; consultancy for Boehringer-Ingelheim, Carl Zeiss Meditec, Chengdu Kanghong Biotech, Genentech, Healios K.K, F. Hoffmann-La Roche Ltd., Isarna Pharmaceuticals, MacRegen Inc., Ocudyne, Ocunexus Therapeutics, Tyrogenex, and Unity Biotechnology; equity interests in Apellis, Digisight, and Ocudyne.
The other authors have no disclosures.

Running head: Identification of subclinical MNV using double layer sign

Address for reprints:
Philip J. Rosenfeld, MD, PhD
Bascom Palmer Eye Institute,
900 NW 17th street,
Miami, FL, 33136

Abstract

Purpose: Structural optical coherence tomography (OCT) images from eyes with non-exudative age-related macular degeneration (AMD) were graded for the presence of a double layer sign to determine if the double-layer sign predicted subclinical macular neovascularization (MNV).

Design: Prospective, observational study.

Participants: Non-exudative AMD patients with and without subclinical MNV identified by swept source OCT angiography (SS-OCTA).

Methods: Subjects were enrolled prospectively into a SS-OCTA imaging study. A set of test scans with and without subclinical MNV was compiled to assess the ability of trained graders to identify non-exudative type 1 MNV. The graders only evaluated the structural OCT B-scans of those eyes. The presence of a double-layer sign was used as a predictive sign for subclinical type 1 MNV. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) from two separate gradings were calculated and compared.

Main Outcome Measures: The association between the presence of a double-layer sign and subclinical type 1 MNV.

Results: One hundred eyes with non-exudative AMD from 94 patients were used for this study. The test set contained 64 eyes with intermediate AMD (iAMD), which included 20 eyes with subclinical MNV, and 36 eyes with late AMD, which included 13 eyes with subclinical MNV. Two junior graders read the scans separately then reached a consensus grading. They detected a double-layer sign in 24 out of 33 eyes with subclinical MNV and did not detect a double-layer sign in 56 out of 67 eyes without MNV. Their sensitivity, specificity, PPV, and NPV were 73%, 84%, 69%, and 86%, respectively. The senior grader detected a double-layer sign in 29 out of 33 eyes with subclinical MNV and did not detect a double-layer sign in 58 out of 67 eyes without MNV, achieving a sensitivity, specificity, PPV, and NPV as 88%, 87%, 76%, and 94%, respectively. For all graders, there were statistically significant associations between the type 1 MNV and the double-layer sign ($P < 0.001$).

Conclusions: The double-layer sign on structural OCT B-scans was associated with subclinical type 1 MNV and can be used to identify these lesions with good predictive values in eyes with non-exudative AMD.

Introduction

Sato et al. were the first to describe the double-layer sign on optical coherence tomography (OCT) B-scans.[1] Using a time domain OCT system (Stratus OCT; Carl Zeiss Meditec, Dublin, CA), they scanned 44 eyes with polypoidal choroidal vasculopathy (PCV) and found two highly reflective layers in 59% of these eyes and these layers corresponded to the regions with branching vascular networks identified by indocyanine green angiography (ICGA). The double-layer sign was comprised of two highly reflective layers that corresponded to a separation between the retinal pigment epithelium (RPE) and another highly reflective layer beneath the RPE, which was presumed to be Bruch's membrane (BM). This double-layer sign represented a low lying, irregular pigment epithelial detachment.

In subsequent OCT imaging studies, the double-layer sign was found to correspond to type 1 macular neovascularization (MNV) in eyes with non-exudative age-related macular degeneration (AMD).[2-6] In 2013, Querques et al. observed 11 eyes with non-exudative AMD using multimodal imaging that included fluorescein angiography (FA), ICGA, and spectral-domain OCT (SD-OCT).[2] The type 1 MNV was visualized using FA and ICGA. Meanwhile, at the site of subclinical MNV, SD-OCT imaging revealed an irregularly, slightly elevated RPE with moderately reflective material in the sub-RPE space that was distinct from the underlying BM layer.

The presence of a double-layer sign appears to correlate with the presence of non-exudative type 1 MNV. However, direct visualization of these non-exudative neovascular lesions requires the use of ICGA or OCT angiography (OCTA).[6-17] It is important to identify these lesions in routine clinical practice since AMD eyes with asymptomatic, subclinical type 1 MNV have a higher annual risk of exudation compared with eyes without these lesions.[3] OCTA is a simple, safe, non-invasive strategy for the detection of type 1 MNV, which is much more practical than ICGA for routine screening. Moreover, SS-OCTA provides better visualization of the full extent of the type 1 MNV compared with SD-OCTA imaging.[3, 4, 10, 18] However, these OCTA instruments are more expensive and not as widely available in clinical practices as the routine structural OCT instruments.

In order to determine whether structural OCT images can be used to detect subclinical MNV reliably, this study tests the predictive value of a double-layer sign seen on structural OCT B-scans for the detection of subclinical MNV compared with SS-OCTA imaging in eyes with intermediate and late non-exudative AMD.

Material and Methods

Patients with non-exudative AMD were enrolled in a prospective OCT study at Bascom Palmer Eye Institute from April 2016 through January 2018. The institutional review board of the University of Miami Miller School of Medicine approved this study. Informed consent was obtained from all patients. The study was performed in accordance with the tenets of the Declaration of Helsinki and complied with Health Insurance Portability and Accountability Act of 1996.

SS-OCTA (PLEX® Elite 9000; Carl Zeiss Meditec, Inc, Dublin, CA) images were acquired using a 6x6 mm scan pattern centered on the fovea. The SS-OCT laser operates at a central wavelength of 1060 nm (1000-1100 nm full width) and a speed of 100,000 A-scans per second. At the level of the retina, the full width at half-maximum axial resolution is approximately 5 microns with an estimated lateral resolution at the retinal surface of appropriately 14 microns. In the 6x6 mm scan pattern, a single B scan consisted of 500 A-scans and two B-scans are repeated at each of 500 B-scan positions. *En face* flow images were generated by the instrument using the OCT microangiography (OMAG) algorithm, as previously described.[17, 19, 20] For the visualization of the MNV, a custom segmentation strategy was used to create slabs extending from the RPE to BM. This feature is available on the commercial instrument and allows the reviewer to select pre-specified boundary layers, and in this study, the boundary layers selected for segmentation were the RPE and the RPE-fit, also known as BM. Obvious artifacts in the boundaries generated by the automated segmentations were corrected using the editing tool.

In addition to SS-OCTA, all the patients underwent routine clinical examination. All AMD eyes included in this study had no evidence of exudation based on the absence of macular fluid after review of the retina thickness maps and B-scans from the SSOCT images. Non-exudative AMD eyes were classified by clinical examinations as

either intermediate AMD (iAMD) or late AMD.[21] Eyes with drusen of at least 125 microns in diameter or pigmentary changes, but without evidence of geographic atrophy (GA) or exudation, were defined as iAMD. Late AMD was defined by the presence of GA, defined as complete RPE and outer retinal atrophy (cRORA), in the absence of exudation.[22] The diagnosis of subclinical MNV was based on SS-OCTA imaging as previously described.[3, 4] Eyes with subclinical MNV, which included eyes with both iAMD and late AMD, were chosen based on the knowledge that the graders had not previously evaluated these cases. The eyes without MNV were chosen based on the presence of intermediate or late non-exudative AMD and their clinical characteristics were similar to the eyes with MNV. Eyes with GA, which not fully contained within the 6X6 mm scan area were excluded.

Three graders (PJR, RG and ZY) graded the study cases without any prior knowledge or review of the cases and whether subclinical MNV was present. Prior to the official grading, the senior grader (PJR) with experience in visualizing MNV on SS-OCTA imaging trained two junior graders (RG and ZY) to recognize double-layer sign using a small training set of cases that were separate from the grading set of cases. The graders only graded whether the B-scans contained a double-layer sign. The double-layer sign term used in this study was characterized by a low-lying irregular RPE detachment with reflective material in sub-RPE space, which was above the highly reflective layer identified as BM. Each grader reviewed the eyes in the validation set, in a random order, using only the *en face* total retina structure image and structural B-scans, but without any flow information. The eyes in the validation set were graded for the presence or absence of a double-layer sign, furthermore the numerical positions of the B-scans corresponding to the double-layer sign was recorded. When there was disagreement between the two junior graders, they were asked to reach a consensus grading.

Two sets of gradings, the consensus results from the two junior graders and the one from the senior grader, were analyzed separately and then compared. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the double-layer sign for identifying subclinical MNV were calculated. Proportions were compared with the Fisher exact test and agreement between ratings was quantified with

percent agreement and Cohen's Kappa statistic. Kappa adjusts percent agreement for marginal prevalence and a guide to its interpretation is: < 0.40, poor agreement; > 0.75, excellent agreement; 0.40 to 0.75, fair to good agreement.[23]

Results

One hundred eyes with non-exudative AMD from 94 patients were graded for the presence of subclinical MNV based on structural OCT information alone (Table 1). Of the 100 eyes enrolled in this study, 64 eyes were in the iAMD group and 36 eyes were in the late AMD group. Of the 64 eyes with iAMD, 20 eyes were diagnosed with subclinical MNV and 44 eyes did not contain subclinical MNV. Of the 36 eyes with late AMD, 13 eyes were diagnosed with subclinical MNV and 23 eyes did not contain subclinical MNV (Table 1).

The senior and junior graders agreed on the presence of a double-layer sign in 24 eyes out of the 33 eyes with subclinical MNV. In 4 eyes with subclinical MNV, both gradings agreed on the absence of a double-layer sign. There was disagreement between the junior and senior graders on 5 eyes with subclinical MNV (Table 2).

The two gradings agreed on the absence of a double-layer sign in 52 out of the 67 eyes without subclinical MNV. In 5 eyes without subclinical MNV, both gradings agreed on the presence of a double-layer sign. There was disagreement between the junior and senior graders on 10 eyes without subclinical MNV (Table 2). Agreement between the senior grader and junior graders was excellent for iAMD (% agreement = 89%, Kappa=0.761), moderate for advanced AMD (% agreement = 78%, kappa = 0.546), and intermediate for all AMD (% agreement = 85%, Kappa=0.677).

The sensitivity, specificity, PPV, and NPV of the junior graders were 73%, 84%, 69% and 86% respectively. The sensitivity, specificity, PPV and NPV of the senior grader were 88%, 87%, 76% and 94%, respectively. Overall, the association between the double-layer sign and subclinical neovascularization for both gradings was found to be highly statistically significant ($P < 0.001$). However, the two gradings showed that the double-layer sign was a better predictor of subclinical MNV in eyes with drusen ($P < 0.001$) than eyes with GA ($P = 0.056$ for the junior grading, $P = 0.001$ for the senior grading) as shown in Table 3.

Figure 1 shows two eyes with iAMD and subclinical MNV in which both gradings identified a double-layer sign. SS-OCTA *en face* images show the MNV using a custom slab that extended from the RPE to BM. A color-coded B scan also demonstrates the flow information under the RPE that correlated with the MNV. Structural B scans through the lesion show a typical double-layer sign as a low-lying, irregular elevation of the RPE above BM. Within the sub-RPE space, moderately reflective material is detected. Figure 2 shows examples of two eyes with subclinical MNV in which both gradings did not identify a double-layer sign. SS-OCTA *en face* imaging showed small foci of MNV that corresponded to drusen-like elevations of the RPE seen on B-scans. Figure 3 shows examples in which there was a disagreement between graders as to whether a double-layer sign was present. This disagreement existed even though the B-scans through the MNV showed an apparent double-layer sign. Figure 4 shows two eyes with iAMD in which MNV was not diagnosed based on SS-OCTA *en face* and B-scan flow imaging, yet a double-layer sign was identified. Both gradings identified a double-layer sign in the eye shown in panels A through D. In panels E through H, there was disagreement between the gradings as to whether a double-layer sign was present. Figure 5 shows two eyes with late AMD and subclinical neovascular lesions in which both gradings identified a double-layer sign. SS-OCTA *en face* images show the MNV using a custom slab that extended from the RPE to BM. A color-coded B scan also demonstrates the flow information under the RPE that correlated with the MNV. Structural B scans through the lesion show the double-layer sign that is adjacent to the GA. Figure 6 shows two eyes with late AMD in which MNV was not present based on SS-OCTA *en face* and B-scan flow imaging, yet a double-layer sign was identified. Both gradings identified a double-layer sign in the eye shown in panels A through E. In panels F through J, there was disagreement among the gradings as to whether a double-layer sign was present.

Discussion

Previous OCTA studies have shown that subclinical type 1 MNV corresponded to a double-layer sign on structural OCT B-scans.[3, 4, 10, 11, 24, 25] However, it was not known if the presence of this double-layer sign on structural OCT images could be used to

predict the presence of subclinical neovascularization. In our current study, we used SS-OCTA imaging as the gold standard for the detection of MNV, we found that all the eyes with subclinical MNV did show a double-layer sign, and the graders correctly identified the MNV based on the presence of the double-layer sign seen on structural B-scans in most of these eyes. While the sensitivity and specificity were good, we feel the positive and negative predictive values, which take into account the prevalence of MNV, are more useful.[26] These reflect the real world scenarios in which the absolute truth of the presence of MNV is not known and the clinician wants to gauge how likely it is that the presence or absence of a double-layer sign finding predicts the presence or absence of MNV. In this study, the subclinical MNV prevalence was 33%. If in a different clinical setting the prevalence were lower, say 10%, then the senior grader's PPV for the presence of double-layer sign in iAMD would be reduced to 59%, but the NPV for the absence of double-layer sign would increase to 99%.

At the beginning of the study, we had assumed that graders would have more difficulty in identifying a double-layer sign in eyes with drusen compared with eyes that had GA, since both drusen and type 1 MNV cause elevations of the RPE. Thus, we expected that the presence of drusen in the eyes with iAMD would be a confounding feature for the graders compared with the presence of GA in late AMD. However, this was not the case. For the most part, graders were able to distinguish typical drusen as focal elevations of the RPE and distinguish these small RPE detachments or focal double-layer signs from the more irregular double-layer sign associated with MNV. While the graders did miss small focal areas of MNV that had configurations similar to drusen, they were better at identifying true double-layer signs associated with MNV in iAMD than in eyes with GA. This came as a surprise. While most MNV in eyes with GA can be found along the margin of the GA, the graders also identified areas at the margins of GA that contained a presumed double-layer sign, but did not contain MNV based on SS-OCTA imaging. This feature of GA, which may be a consequence of the ongoing degeneration of the outer retina and RPE at the margins of the lesions, may be a confounder when assessing for a double-layer sign associated with MNV or may serve as a potential space for the subsequent growth of MNV. Another possibility is that MNV could have been present in these areas with a double-layer sign and just not

detected using SS-OCTA imaging. To determine which possibility is likely, ongoing natural history studies using SS-OCTA to follow eyes with late AMD should provide answers.

The ability of graders to distinguish typical drusen from a double-layer sign associated with MNV suggests that the differences between these two types of RPE detachments can be easily learned based on certain structural OCT characteristics. The double-layer sign term used in this study was characterized by two highly reflectively layers (RPE and BM) seen on OCT imaging, an irregular contour of the RPE, an elevation with a width usually greater than typical drusen as seen on OCT B-scans, and an internal reflectivity within RPE detachments harboring MNV that appeared different from the reflectivity seen with typical non-vascularized drusen (as showed in Figure 5, panel J). However, there may be other subtler features that are used to distinguish drusen from double-layer signs associated with MNV. The next obvious step would be to try to establish a machine-learning algorithm that might be able to better identify MNV based on the above-mentioned B-scan features and other subtler structural OCT features associated with subclinical type 1 MNV.

Previous studies have shown that eyes with chronic central serous chorioretinopathy (CSC) and PCV also contain double layer signs detectable on B-scans, which have been correlated with type 1 MNV.[27-30] While Bousquet et al.[29] found that only one third of these double-layer signs in CSC correlated with type 1 MNV (CNV), Dansingani et al.[28] found that in eyes with PCV, the double layer sign was a good diagnostic predictor of a branching vascular network or type 1 MNV. These results may be related to different features of these diseases and different imaging strategies, but the presence of drusen and GA in AMD present their own unique set of challenges.

In AMD, the ability to identify subclinical MNV is important when recruiting patients into clinical trials, especially when the outcome of the trial depends on preventing exudation or slowing disease progression.[31, 32] Whether to exclude such eyes from participation in trials will be at the discretion of the study organizers, but it stands to reason that these subclinical lesions are common and need to be studied. Consequently, we believe these eyes should be included and stratified between groups in a randomized trial. Moreover, subclinical MNV will arise during the course of any

AMD trial, so it will be important to detect them and determine if the treatment under study affected their formation or progression to exudation. Therefore, it is important to use OCTA in all AMD trials to identify and monitor these lesions. In the absence of OCTA, our study suggests that reviewing B-scans for the presence of a double-layer sign might suffice, but it is labor intensive, small lesions will be missed, and double-layer signs at the edge of GA might be misinterpreted as MNV. If the aforementioned machine-learning algorithm can be developed to identify subclinical MNV, then it will be a more objective, efficient, and cost-effective way of screening for subclinical MNV in clinical trials where OCTA is not performed. In addition, in clinical practices without OCTA capability, an algorithm capable of identifying subclinical type 1 MNV would be very useful to use in conjunction with routine structural OCT imaging to identify these lesions that are at risk for exudation.

A limitation of this study was the use of SS-OCTA as the gold standard for the diagnosis of type 1 MNV. While ICGA is the historical gold standard for the detection of these subclinical lesions, our experience is that SS-OCTA is as good as or better than ICGA for the detection of these lesions.[2-6, 10, 12, 14, 30, 32] However, it is possible that ICGA might have detected a small subclinical neovascular lesion that was not detected using SS-OCTA, but since ICGA is not routinely performed or reimbursed when used to screen for subclinical MNV in eyes with non-exudative AMD, this limitation isn't clinically relevant since ICGA imaging would not have been performed. Moreover, if SS OCTA were available, then ICGA would be riskier, more time consuming and more expensive for patients with non-exudative AMD. Another limitation of this study is that we used SS OCT images to exclude cases in which subtle leakage might have been detected using fluorescein angiography (FA). Even if FA is the golden standard for detecting leakage from MNV, it is not routinely performed in the absence of intra- or sub-retinal fluid seen on structural OCT imaging. We don't routinely perform FA to detect subtle leakage in the absence of OCT structural changes, and a recent study has shown that FA provides no additional benefit for the management of exudative AMD compared with OCT imaging alone.[33] In the worse case scenario, our study correlates the presence of a double-layer sign seen on structural OCT B-scans with the presence of subclinical MNV detected using SS-OCT angiographic technology. In the best-case scenario, SS-OCTA

imaging is the gold standard for the detection of these lesions, and while the correlations between structural B-scans and SS-OCTA images are good, SS-OCTA imaging is superior to structural B-scans for the detection of subclinical MNV.

In summary, we found that the double-layer sign on structural OCT B-scans could predict the presence of subclinical MNV in most eyes with non-exudative AMD identified by SS-OCTA imaging. Confirmatory prospective natural history studies of eyes with iAMD and late AMD are ongoing and the development of a machine-learning algorithm to identify subclinical MNV based on structural OCT is underway.

References

1. Sato T, Kishi S, Watanabe G, et al. Tomographic features of branching vascular networks in polypoidal choroidal vasculopathy. Retina 2007;27(5):589-94.
2. Querques G, Srour M, Massamba N, et al. Functional characterization and multimodal imaging of treatment-naive "quiescent" choroidal neovascularization. Invest Ophthalmol Vis Sci 2013;54(10):6886-92.
3. de Oliveira Dias JR, Zhang Q, Garcia JMB, et al. Natural History of Subclinical Neovascularization in Nonexudative Age-Related Macular Degeneration Using Swept-Source OCT Angiography. Ophthalmology 2018;125(2):255-66.
4. Roisman L, Zhang Q, Wang RK, et al. Optical Coherence Tomography Angiography of Asymptomatic Neovascularization in Intermediate Age-Related Macular Degeneration. Ophthalmology 2016;123(6):1309-19.
5. Capuano V, Miere A, Querques L, et al. Treatment-Naive Quiescent Choroidal Neovascularization in Geographic Atrophy Secondary to Nonexudative Age-Related Macular Degeneration. Am J Ophthalmol 2017;182:45-55.
6. Carnevali A, Cicinelli MV, Capuano V, et al. Optical Coherence Tomography Angiography: A Useful Tool for Diagnosis of Treatment-Naive Quiescent Choroidal Neovascularization. Am J Ophthalmol 2016;169:189-98.
7. Hanutsaha P, Guyer DR, Yannuzzi LA, et al. Indocyanine-green videoangiography of drusen as a possible predictive indicator of exudative maculopathy. Ophthalmology 1998;105(9):1632-6.
8. Guyer DR, Yannuzzi LA, Slakter JS, et al. Classification of choroidal neovascularization by digital indocyanine green videoangiography. Ophthalmology 1996;103(12):2054-60.
9. Schneider U, Gelisken F, Inhoffen W, Kreissig I. Indocyanine green angiographic findings in fellow eyes of patients with unilateral occult neovascular age-related macular degeneration. Int Ophthalmol 1997;21(2):79-85.
10. Novais EA, Adhi M, Moult EM, et al. Choroidal Neovascularization Analyzed on Ultrahigh-Speed Swept-Source Optical Coherence Tomography Angiography Compared to Spectral-Domain Optical Coherence Tomography Angiography. Am J Ophthalmol 2016;164:80-8.

11. Lane M, Moult EM, Novais EA, et al. Visualizing the Choriocapillaris Under Drusen: Comparing 1050-nm Swept-Source Versus 840-nm Spectral-Domain Optical Coherence Tomography Angiography. Invest Ophthalmol Vis Sci 2016;57(9):OCT585-90.

12. Miller AR, Roisman L, Zhang Q, et al. Comparison Between Spectral-Domain and Swept-Source Optical Coherence Tomography Angiographic Imaging of Choroidal Neovascularization. Invest Ophthalmol Vis Sci 2017;58(3):1499-505.

13. Zhang Q, Zhang A, Lee CS, et al. Projection artifact removal improves visualization and quantitation of macular neovascularization imaged by optical coherence tomography angiography. Ophthalmol Retina 2017;1(2):124-36.

14. Nehemy MB, Brocchi DN, Veloso CE. Optical Coherence Tomography Angiography Imaging of Quiescent Choroidal Neovascularization in Age-Related Macular Degeneration. Ophthalmic Surg Lasers Imaging Retina 2015;46(10):1056-7.

15. Palejwala NV, Jia Y, Gao SS, et al. Detection of Nonexudative Choroidal Neovascularization in Age-Related Macular Degeneration with Optical Coherence Tomography Angiography. Retina 2015;35(11):2204-11.

16. Huang Y, Zhang Q, Thorell MR, et al. Swept-source OCT angiography of the retinal vasculature using intensity differentiation-based optical microangiography algorithms. Ophthalmic Surg Lasers Imaging Retina 2014;45(5):382-9.

17. Wang RK, An L, Francis P, Wilson DJ. Depth-resolved imaging of capillary networks in retina and choroid using ultrahigh sensitive optical microangiography. Opt Lett 2010;35(9):1467-9.

18. Zhang Q, Chen CL, Chu Z, et al. Automated Quantitation of Choroidal Neovascularization: A Comparison Study Between Spectral-Domain and Swept-Source OCT Angiograms. Invest Ophthalmol Vis Sci 2017;58(3):1506-13.

19. Zhang A, Zhang Q, Chen CL, Wang RK. Methods and algorithms for optical coherence tomography-based angiography: a review and comparison. J Biomed Opt 2015;20(10):100901.

20. Yin X, Chao JR, Wang RK. User-guided segmentation for volumetric retinal optical coherence tomography images. J Biomed Opt 2014;19(8):086020.

21. Ferris FL, 3rd, Wilkinson CP, Bird A, et al. Clinical classification of age-related macular degeneration. Ophthalmology 2013;120(4):844-51.
22. Schaal KB, Rosenfeld PJ, Gregori G, et al. Anatomic Clinical Trial Endpoints for Nonexudative Age-Related Macular Degeneration. Ophthalmology 2016;123(5):1060-79.
23. Fleiss JL. Statistical methods for rates and proportions. 2nd ed. New York, NY: John Wiley & Sons; 1981 1981:Pp 212-25.
24. Moult E, Choi W, Waheed NK, et al. Ultrahigh-speed swept-source OCT angiography in exudative AMD. Ophthalmic Surg Lasers Imaging Retina 2014;45(6):496-505.
25. Zhang A, Zhang Q, Wang RK. Minimizing projection artifacts for accurate presentation of choroidal neovascularization in OCT micro-angiography. Biomed Opt Express 2015;6(10):4130-43.
26. Fleiss JL. Statistical Methods for Rates and Proportions. 2nd ed. New York, NY: John Wiley and Sons; 1981 1981:Pp 4-8.
27. Hage R, Mrejen S, Krivosic V, et al. Flat irregular retinal pigment epithelium detachments in chronic central serous chorioretinopathy and choroidal neovascularization. Am J Ophthalmol 2015;159(5):890-903 e3.
28. Dansingani KK, Balaratnasingam C, Klufas MA, et al. Optical Coherence Tomography Angiography of Shallow Irregular Pigment Epithelial Detachments In Pachychoroid Spectrum Disease. Am J Ophthalmol 2015;160(6):1243-54 e2.
29. Bousquet E, Bonnin S, Mrejen S, et al. Optical Coherence Tomography Angiography of Flat Irregular Pigment Epithelium Detachment in Chronic Central Serous Chorioretinopathy. Retina 2018;38(3):629-38.
30. Inoue M, Jung JJ, Balaratnasingam C, et al. A Comparison Between Optical Coherence Tomography Angiography and Fluorescein Angiography for the Imaging of Type 1 Neovascularization. Invest Ophthalmol Vis Sci 2016;57(9):OCT314-23.
31. Maguire MG, Daniel E, Shah AR, et al. Incidence of choroidal neovascularization in the fellow eye in the comparison of age-related macular degeneration treatments trials. Ophthalmology 2013;120(10):2035-41.

32. Barbazetto IA, Saroj N, Shapiro H, et al. Incidence of new choroidal neovascularization in fellow eyes of patients treated in the MARINA and ANCHOR trials. Am J Ophthalmol 2010;149(6):939-46 e1.

33. Parekh PK, Folk JC, Gupta P, et al. Fluorescein Angiography Does Not Alter the Initial Clinical Management of Choroidal Neovascularization in Age-Related Macular Degeneration. Ophthalmology Retina 2018;2(7):659-66.

FIGURE LEGENDS

Figure 1: Two eyes with intermediate age-related macular degeneration (AMD) and subclinical macular neovascularization (MNV) where all graders agreed on the presence of the double layer sign on structural B-scan images. A, E: 6x6 mm *en face* angiographic image of MNV from a slab with segmentation boundaries extending from the retinal pigment epithelium (RPE) to Bruch's membrane (BM) showing CNV pattern; B, F: 6x6 mm *en face* structural images using the same slab as in panels A and E; C, G: OCT structural B-scans through the subclinical type 1 MNV with color-coded flow using red for the retinal microvasculature and green for flow under the RPE. Yellow dashed lines represent the slab boundaries from the RPE to BM. D, H: OCT structural B-scans through the lesion showing a double layer sign (yellow arrow).

Figure 2: Two eyes with late age-related macular degeneration (AMD) and subclinical macular neovascularization (MNV) where none of the graders identified a double layer sign. A, E: 6x6 mm *en face* angiographic image of MNV from a slab with segmentation boundaries extending from the retinal pigment epithelium (RPE) to Bruch's membrane (BM) showing small foci of CNV pattern; B, F: 6x6 mm *en face* structural images using the same slab as in panels A and E; C, G: OCT structural B-scans through the subclinical type 1 MNV with color-coded flow using red for the retinal microvasculature and green for flow under the RPE. Yellow dashed lines represent the slab boundaries from the RPE to BM. D, H: OCT structural B-scans through the lesion showing small elevation of RPE (yellow arrow).

Figure 3: Two eyes with late age-related macular degeneration (AMD) and subclinical macular neovascularization (MNV) where some graders did not identify a double layer sign. A, E: 6x6 mm *en face* angiographic image of MNV from a slab with segmentation boundaries extending from the retinal pigment epithelium (RPE) to Bruch's membrane (BM) showing CNV pattern; B, F: 6x6 mm *en face* structural images using the same slab as in panels A and E; C, G: OCT structural B-scans through the subclinical type 1 MNV with color-coded flow using red for the retinal microvasculature and green for flow under the RPE. Yellow dashed lines represent the slab boundaries from the RPE to BM.

D, H: OCT structural B-scans through the lesion showing a double layer sign (yellow arrow).

Figure 4: Two eyes with intermediate age-related macular degeneration (AMD) without macular neovascularization (MNV). A – D shows one eye in which all the graders identified the presence of a double layer sign; E – H shows one eye in which junior and senior graders did not reach an agreement as to whether a double layer sign was present. A, E: 6x6 mm *en face* angiographic image using a slab with segmentation boundaries extending from the retinal pigment epithelium (RPE) to Bruch's membrane (BM) showing no CNV pattern; B, F: 6x6 mm *en face* structural images using the same slab as in panels A and E; C, G: OCT structural B-scans with color-coded flow using red for the retinal microvasculature and green for flow under the RPE. Yellow dashed lines represent the slab boundaries from the RPE to BM. D, H: OCT structural B-scans showing double layer sign (yellow arrow).

Figure 5: Two eyes with late age-related macular degeneration (AMD) and subclinical macular neovascularization (MNV) where all graders agreed on the presence of the double layer sign on structural B-scan images. A, F: 6x6 mm *en face* structure image using a slab with segmentation boundaries extending from 64 microns to 400 microns under Bruch's membrane (BM) showing geographic atrophy (GA); B, G: 6x6 mm *en face* angiographic image using a slab with segmentation boundaries extending from the retinal pigment epithelium (RPE) to BM showing CNV pattern; C, H: 6x6 mm *en face* structural images using the same slab as in panels B and G; D, I: OCT structural B-scans through the subclinical type 1 MNV with color-coded flow using red for the retinal microvasculature and green for flow under the RPE. Yellow dashed lines represent the slab boundaries from the RPE to BM. E, J: OCT structural B-scans through the lesion showing double layer sign adjacent to GA (yellow arrow).

Figure 6: Two eyes with late age-related macular degeneration (AMD) and evidence of double layer signs on structural B-scans, but no evidence of subclinical macular neovascularization (MNV) on swept-source optical coherence tomography angiography

(SS-OCTA). A – D shows one eye in which all the graders incorrectly identified as subclinical MNV based on the presence of double layer sign; E – H shows one eye in which junior and senior graders did not reach an agreement as to whether a double layer sign was present. A, F: 6x6 mm *en face* structure image using a slab with segmentation boundaries extending from 64 microns to 400 microns under Bruch's membrane (BM) showing geographic atrophy (GA); B, G: 6x6 mm *en face* angiographic image showing no CNV pattern using a slab with segmentation boundaries extending from the retinal pigment epithelium (RPE) to BM; C, H: 6x6 mm *en face* structural images using the same slab as in panels B and G; D, I: OCT structural B-scans with color-coded flow using red for the retinal microvasculature and green for flow under the RPE. Yellow dashed lines represent the slab boundaries from the RPE to BM. E, J: OCT structural B-scans showing double layer sign adjacent to GA (yellow arrow).

Table 1: Presence of subclinical macular neovascularization in test eyes with non-exudative age-related macular degeneration

Group	Intermediate AMD N = 64	Late AMD N = 36
Presence of subclinical MNV N = 33	20	13
Absence of subclinical MNV N = 67	44	23

MNV = macular neovascularization; AMD = age-related macular degeneration

Table 2: Identification of a double layer sign in test eyes with non-exudative age-related macular degeneration

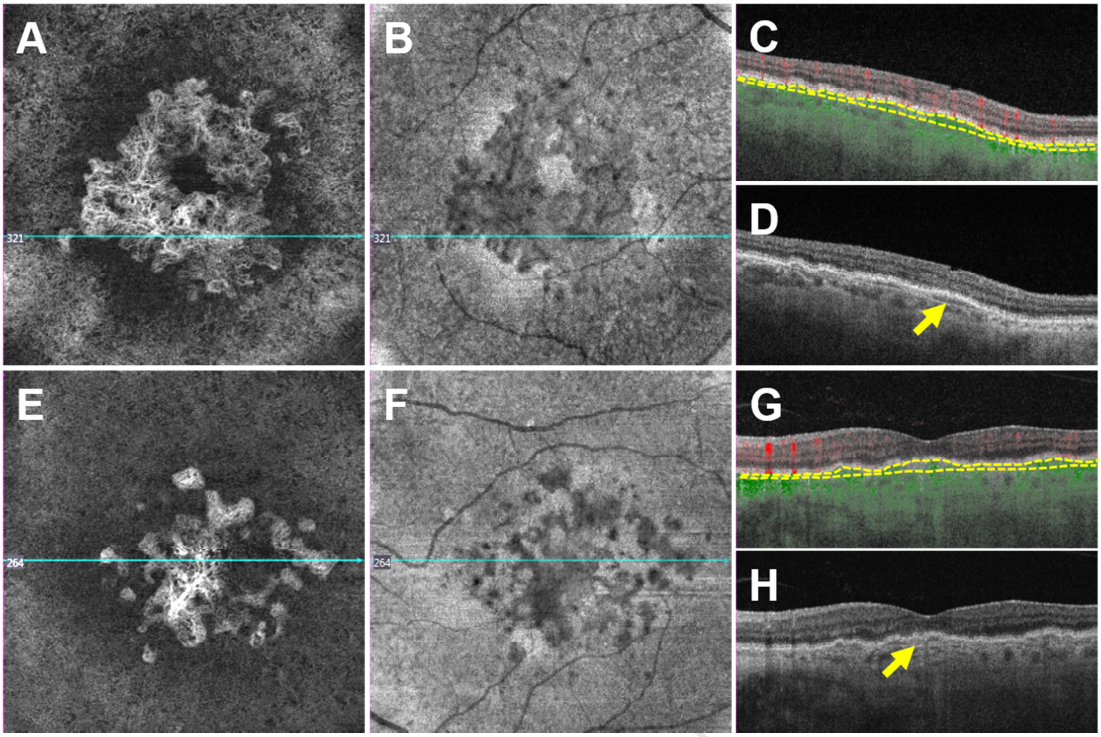
Group	Double layer sign present for junior and senior graders	Double layer sign absent for junior and senior graders	No agreement between junior and senior graders
Presence of Subclinical MNV N = 33	24	4	5
Absence of Subclinical MNV N = 67	5	52	10

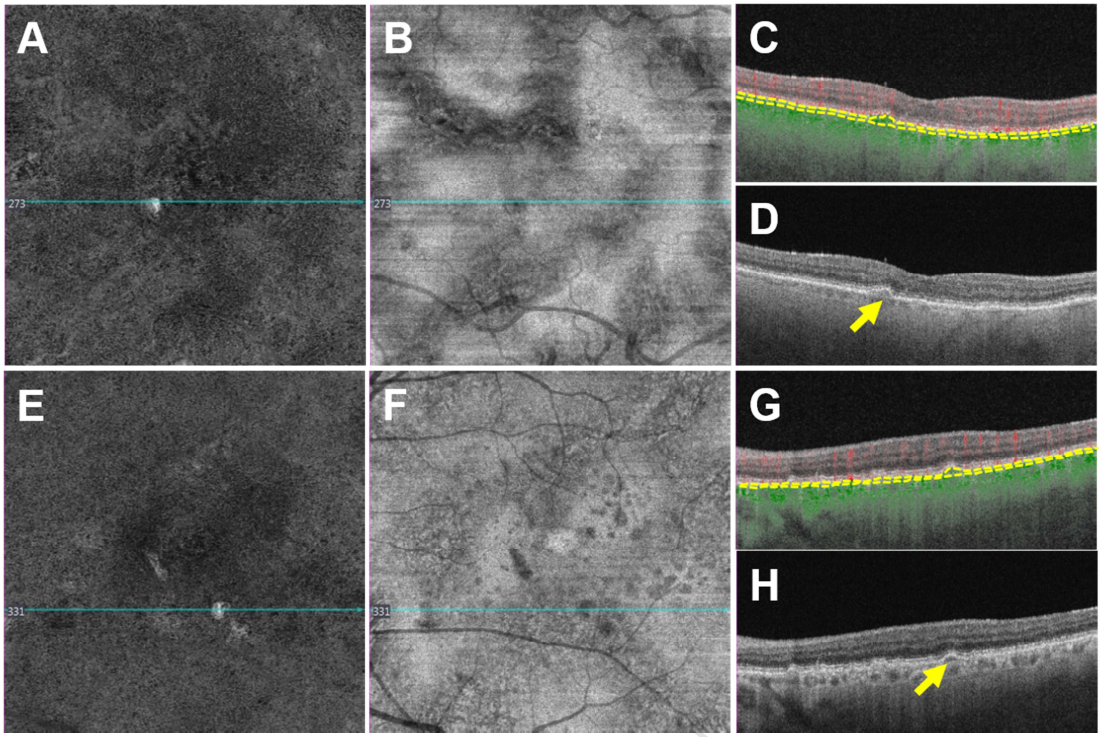
MNV = macular neovascularization

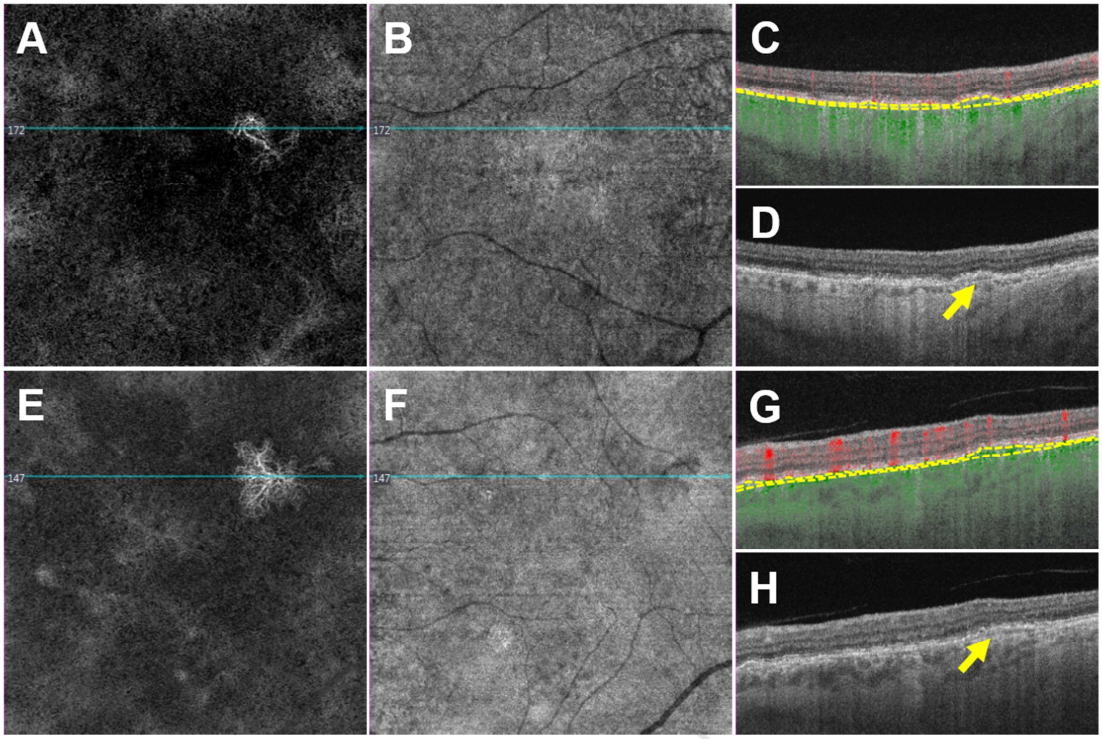
Table 3: Sensitivity, specificity, and predictive values of double layer sign for identifying subclinical macular neovascularization in non-exudative age-related macular degeneration

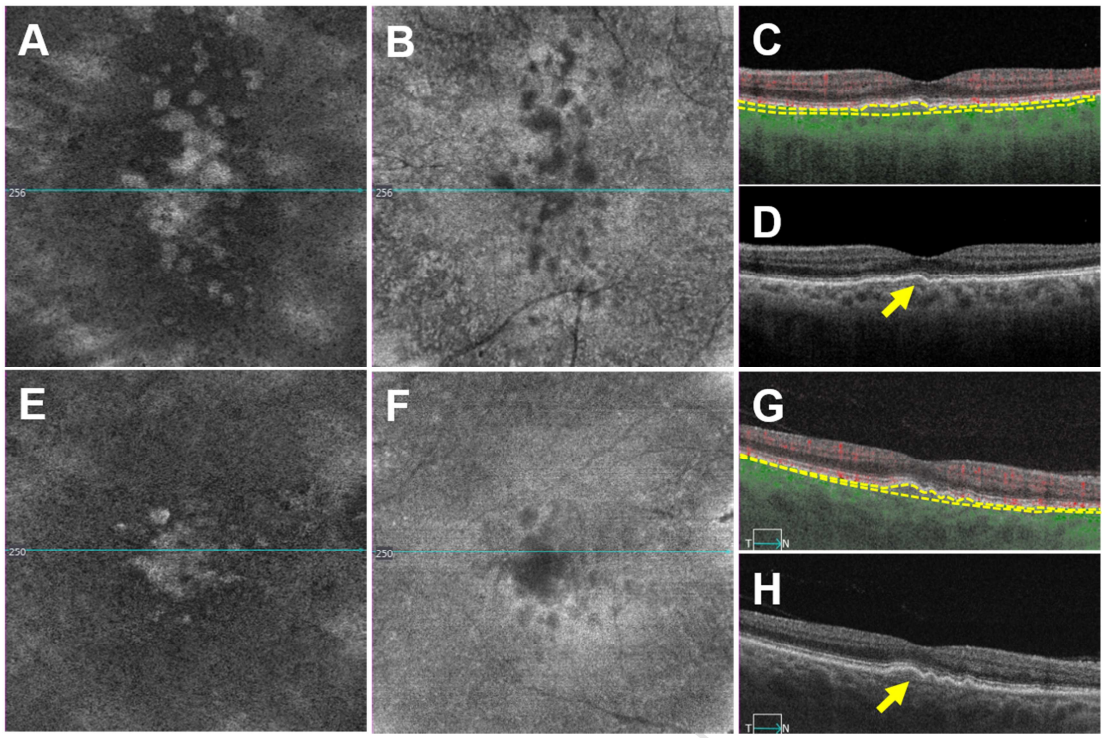
Values	AMD N = 100		Intermediate AMD N = 64		Late AMD N = 36	
	Consensus from junior graders	Results from senior grader	Consensus from junior graders	Results from senior grader	Consensus from junior graders	Results from senior grader
Sensitivity	24/33 (73%)	29/33 (88%)	17/20 (85%)	18/20 (90%)	7/13 (54%)	11/13 (85%)
Specificity	56/67 (84%)	58/67 (87%)	37/44 (84%)	41/44 (93%)	19/23 (83%)	17/23 (74%)
PPV	24/35 (69%)	29/38 (76%)	17/24 (71%)	18/21 (86%)	7/11 (64%)	11/17 (65%)
NPV	56/65 (86%)	58/62 (94%)	37/40 (93%)	41/43 (95%)	19/25 (76%)	17/19 (89%)
P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.056**	**0.001**

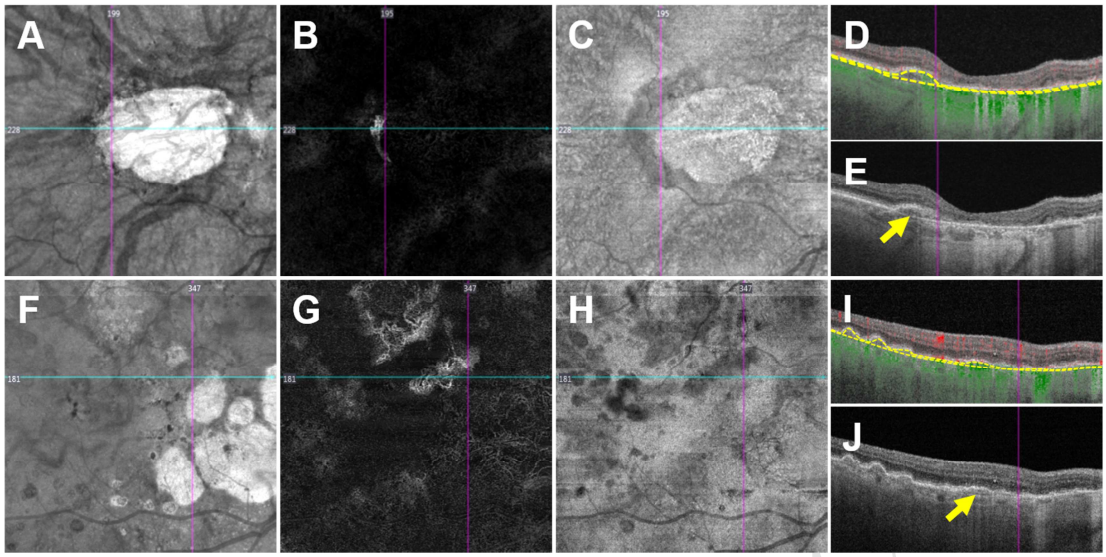
AMD = age-related macular degeneration; PPV = positive predictive value; NPV = negative predictive value

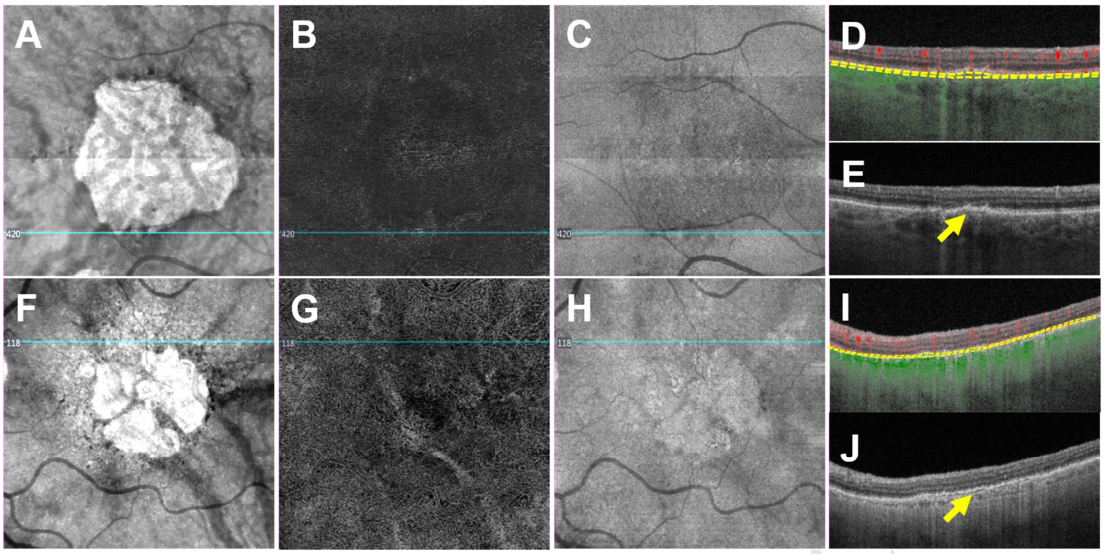












Précis

The double layer sign seen on structural OCT B-scans can predict the presence of subclinical type 1 macular neovascularization in non-exudative age-related macular degeneration, but swept source OCT angiography is better at identifying these lesions.