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The Double Layer Sign Is Highly Predictive of Progression to Exudation in Age-Related Macular Degeneration

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Purpose: The presences of a double layer sign (DLS) and a shallow irregular retinal pigment epithelium (RPE) elevation (SIRE) were investigated using spectral domain-OCT (SD-OCT) imaging to determine their ability to predict progression to exudative macular neovascularization (eMNV) in the unaffected fellow eyes (study eye) of participants with age-related macular degeneration (AMD) with newly diagnosed unilateral eMNV.

Design: Retrospective, reanalysis of SD-OCT scans of study eyes from the Early Detection of Neovascular AMD (EDNA) study with 3 years follow-up (FU).

Participants: The EDNA study repository of SD-OCT scans was assessed for inclusion. Cases with incomplete data sets, low quality scans, or exhibiting other pathology were excluded, which resulted in 459 eligible cases.

Methods: Spectral domain-OCT volume scans of study eyes were graded for irregular elevation of the RPE (IE), with length, and height measurements made on the most affected B-scan. Eyes with heterogeneous reflectivity within the IE were classified as exhibiting the DLS. Eyes with DLS where the length of separation between RPE and Bruch's membrane was $\geq$ 1000 μm in length and $<$ 100 μm in height were subclassified as SIRE.

Main Outcome Measures: Hazard of progression to eMNV for DLS and SIRE.

Results: Of the 459 eyes, 268 had IE, of which 101 were DLS-like and 51 also fulfilled criteria for SIRE. Over the 3 years FU period, 104 (23%) eyes progressed to eMNV. After an FU of 18 months, a significantly higher proportion of study eyes ($P < 0.001$) with IE, DLS, and SIRE developed eMNV compared with those without these features (IE: 17% vs. no IE 6.3%; DLS: 23% vs. no DLS 9.9%; SIRE: 22% vs. no SIRE 11%). In the adjusted Cox regression models, a significantly greater hazard of progression ($P < 0.001$) was associated with the presence of IE (adjusted hazard ratio [HR], 3.01; 95% confidence interval [CI], 1.88–4.82), DLS (adjusted HR, 3.41; 95% CI, 2.26–5.14), or SIRE (adjusted HR, 2.83; 95% CI, 1.68–4.75).

Conclusion: The DLS is a highly sensitive predictor of progression to eMNV, and the use of SIRE does not improve predictability.

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Exudative age-related macular degeneration (eAMD) is a sight-threatening condition that commonly affects older adults. Despite the introduction of highly effective therapies that can control the exudation from the abnormal blood vessels that invade the macular tissues and lead to tissue damage, early treatment is critical for the prevention of permanent visual loss.[1,2] As eAMD can remain undetected when it occurs in the first eye and even when it begins in the second eye, patients can be unaware of changes in central visual function.[2] Therefore, identifying eyes at high risk of progression to exudation is of paramount importance. The most robust noninvasive technology for identifying eAMD is spectral domain-OCT (SD-OCT).[3] However, the burden associated with screening large numbers of older adults repeatedly with SD-OCT is substantial; therefore, it is clearly necessary to define the patient population of highest risk of progression to eAMD. Over the past decade, both on SD-OCT[4] and on swept source-OCT

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(SS-OCT),[5] dormant networks of blood vessels lying between the retinal pigment epithelium (RPE) and its basement membrane were found to exist without overt exudation. This type of nonexudative macular neovascularization (neMNV) has been detected using OCT angiography (OCTA) and is associated with a low-lying elevation of the RPE seen on structural OCT captured on either SD or SS instruments.[5–7] Initially termed a double layer sign (DLS), this low-lying elevation of the RPE was defined as the presence of a highly reflective layer consisting of the RPE and a further highly reflective layer composed of Bruch's membrane (BM) on its outer aspect.[8]

Reports from longitudinal studies of neMNV detected using OCTA showed that these lesions are predictive for progression to exudative MNV (eMNV).[9–11] While OCTA is a noninvasive imaging strategy and shows good sensitivity and specificity for the detection of neMNV, the instrumentation is expensive, not widely available, and requires specific algorithms and an expertise in B-scan segmentation to reliably identify the presence of neMNV. By contrast, SD-OCT imaging is universally used, and most retina physicians have the expertise and skills to identify the presence of a DLS.

The characterization of the DLS on structural OCT B-scans was further refined by Narita et al.[6] They assigned a length requirement to the DLS with the goal of distinguishing the DLS from other, nonneovascular causes of RPE detachments. By placing dimensional requirements on the DLS ($\geq$ 1000 μm in length on a horizontal B-scan and $<$ 100 μm in height), Narita et al[6] coined the term shallow irregular RPE elevation (SIRE). The SIRE sign consistently agreed with the presence of neMNV detected by OCTA with both high sensitivity and specificity.[6] However, it remains to be determined if the SIRE sign provided any added benefit for predicting the onset of exudation compared with the DLS which has no size requirement.

The predictive values of the DLS and SIRE sign were explored in the Early Detection of Neovascular AMD (EDNA) study. The EDNA was a prospective diagnostic accuracy trial of participants with unilateral eMNV to detect the onset of eMNV in the unaffected fellow eye (study eye). Although, the primary aim of grading was to confirm the onset of eMNV in the high-risk EDNA study eye, a variety of OCT features of intermediate AMD were also systematically graded.[2,3] In the current study, SD-OCT images from the EDNA repository were reanalyzed for the DLS and SIRE sign, and their predictive ability of progression to eMNV was assessed.

Methods

This was a retrospective analysis of SD-OCT scans from the Early Detection of Neovascular AMD (EDNA) study. The EDNA was a diagnostic accuracy study with $>$ 552 participants enrolled from 24 clinical sites in the United Kingdom (UK), all of whom provided informed consent. The study was performed in accordance with the tenets of the Declaration of Helsinki and was approved by the Office for Research Ethics Committees in Northern Ireland (14/NI/1120). The protocol, design, and findings from EDNA have been reported in detail.[2,3]

The EDNA imaging data set consisted of color fundus, fluorescein angiography, and SD-OCT images, which were graded at the network of UK ophthalmic reading centers (netWORC UK) for progression to exudative neovascular AMD using a multimodal approach. For the purposes of the present study, regrading of SD-OCT scans to detect DLS and SIRE was undertaken at the Belfast Ophthalmic Reading Centre by certified graders.

OCT Grading

Of the 548 SD-OCT scans of the study eye from the full EDNA data set, 89 were excluded (see flowchart; Fig 1), resulting in 459 eligible for use in the present analysis. Scans had been captured on a variety of acquisition systems including the Heidelberg Spectralis (Heidelberg Engineering), 72%, Topcon (Topcon Healthcare), 15%, Zeiss (Zeiss Meditec, Inc), 9.4%, and Nidek (Nidek Co., Ltd.), 3.3% of the cases (details of scan dimensions and the number of B-scans in volume scans shown in supplementary material Table S1, available at www.ophthalmologyretina.org).

Graders were trained to identify the DLS on SD-OCT scans from an independent data set (images provided by P.R. and N.K.W.) of eyes with high-risk intermediate AMD features imaged on both SD-OCT and SS-OCTA comprising an admixture with and without neMNV. Confirmation of the presence of neMNV was based on SS-OCTA grading by the external experts (P.R. and N.K.W.) and used in quality assurance of grader training. All graders completed the training set before grading the EDNA image set.

Graders reviewed the SD-OCT volume scans with particular attention to the outer retinal layers. Any irregularities of the RPE layer with any degree of separation of this layer from BM, regardless of presence of confluent drusen that may have accounted for this feature, were identified as exhibiting irregular elevation of the RPE (IE). The number of B-scans involved by regions of IE was counted and recorded to capture the vertical extent (extent). The B-scan with the maximum length of the RPE separation in eyes with IE was identified. The horizontal limits of the separation (length) were measured at the nasal and temporal points where RPE and BM are no longer separable. The maximum elevation of the RPE on the same B-scan was also measured (height). At the next stage, graders reviewed the volume scans and made a further categorization of the IE into DLS-like or not. Double layer sign was said to be present if the region of IE showed heterogeneous reflectivity between the low-lying hyperreflective RPE and the BM (Fig 2). When there was disagreement between graders, arbitration was performed (Senior grader K.A.M., clinicians U.C. and T.P.).

Grading for Intermediate AMD Features

Spectral domain-OCT scans were graded for the presence of nodular drusen, subretinal drusenoid deposit (SDD), intraretinal hyperreflective foci, and hypertransmission of the OCT signal into the choroid (HyperTC). Nodular drusen were defined as focal regions of RPE deformation or thickening due to an accumulation of sub-RPE material. Subretinal drusenoid deposit was defined as accumulation of moderately hyperreflective material forming sharp peaks or broad, rounded elevations internal to the RPE. Intraretinal hyperreflective foci was graded as present if there were scattered, punctiform, focal hyperreflective lesions (greater than or equal to the RPE reflectivity) within the neurosensory retina. Hypertransmission of the OCT signal into the choroid was defined as increased signal transmission into the choroid through the RPE/BM complex. A HyperTC defect was said to be present if the diameter of the HyperTC was $\geq$ 40 μm.

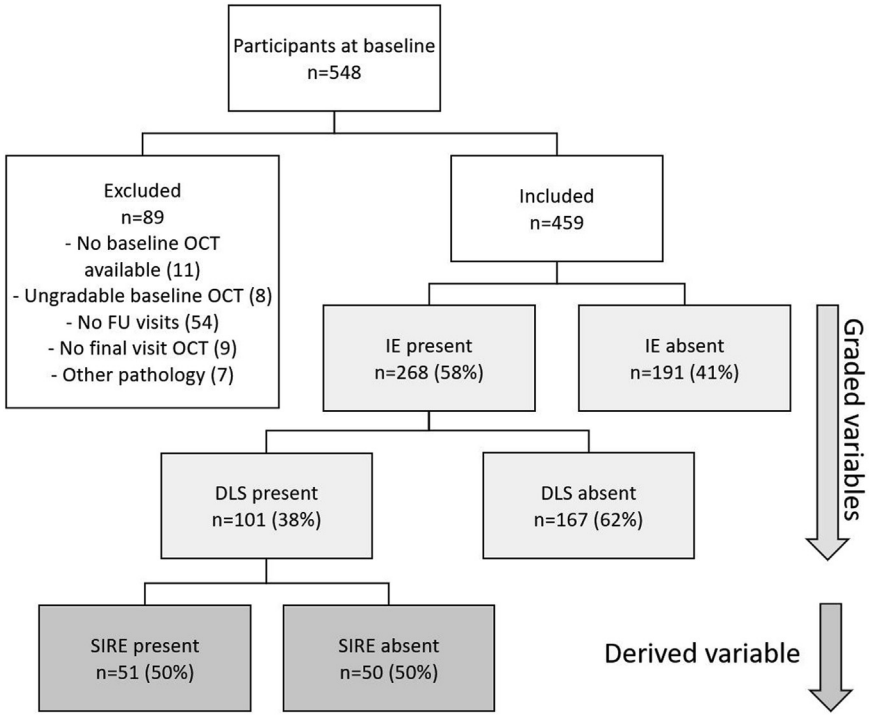

Figure 1. Flow chart showing the excluded and included cases. DLS = double layer sign; FU = follow up; IE = irregular elevation of the retinal pigment epithelium; SIRE = shallow irregular retinal pigment epithelium elevation.

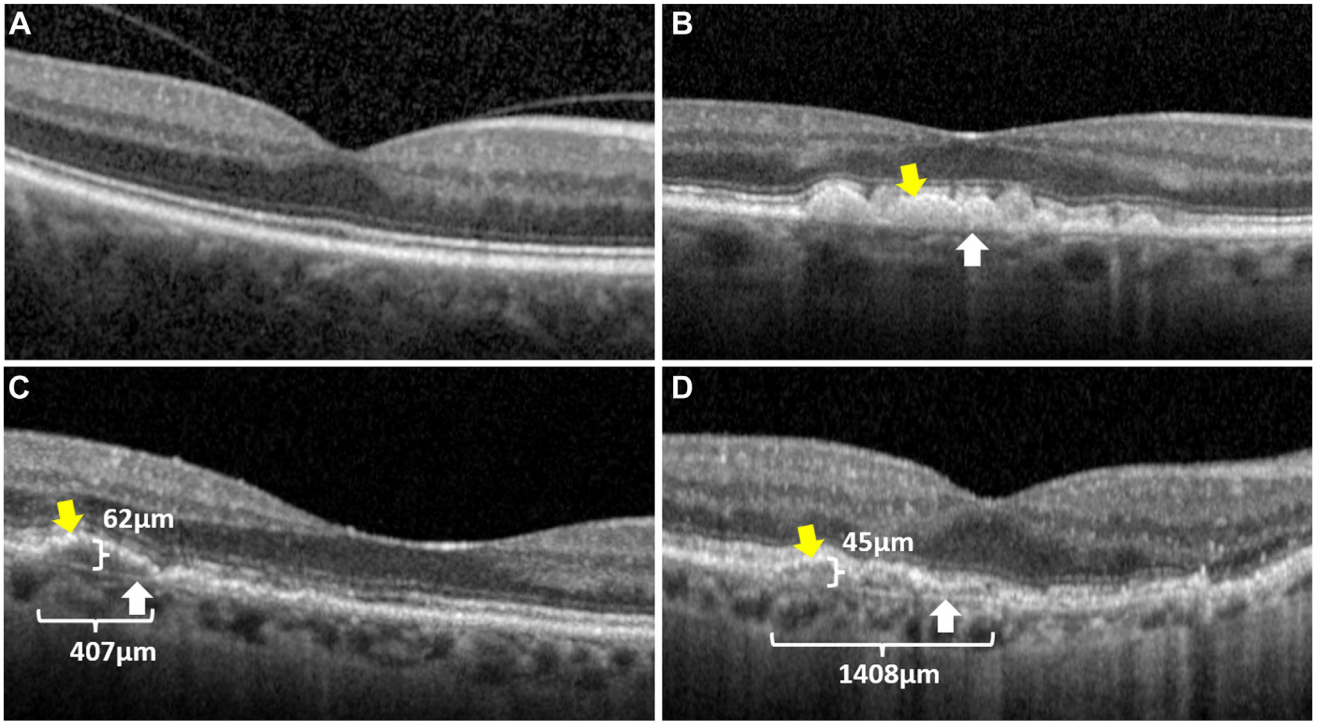

Figure 2. Example of grading categories for irregular elevation of the retinal pigment epithelium (IE), double layer sign (DLS), and shallow irregular retinal pigment epithelium elevation (SIRE). Scan **A** showing no IE. Scan **B** showing IE that is not DLS like (C) due to the homogenous reflectivity of the space between the retinal pigment epithelium (RPE) and Bruch's membrane. Scan **C** showing a low-lying RPE elevation with a heterogenous sub-RPE space, which therefore fulfills criteria for DLS. Scan **D** also showing a low-lying RPE elevation with a heterogenous sub-RPE space (DLS) and with a length > 1000 µm and height < 100 µm which therefore fulfills criteria for SIRE.

Table 2. Baseline Cohort Characteristics

Cohort Characteristics N = 459*		OCT Features N = 459†	
Age	76 (8)	**Overlapping categories**	
Sex		**IE** NO	191 (42%)
Male	194 (42%)	YES	268 (58%)
Female	265 (58%)	**DLS** NO	358 (78%)
Smoking		YES	101 (22%)
Never	183 (40%)	**SIRE** NO	408 (89%)
Ex	222 (48%)	YES	51 (11%)
Current	54 (12%)	**Exclusive categories**	
		No IE	191 (42%)
Hypertension NO	216 (47%)	IE only	167 (36%)
YES	243 (53%)	DLS only	50 (11%)
Cardiovascular NO	356 (78%)	SIRE only	51 (11%)
YES	103 (22%)	**OCT features of intermediate AMD**	
Diabetes NO	384 (84%)	**ND** NO	65 (14%)
YES	75 (16%)	YES	394 (86%)
Supplements NO	320 (70%)	**SDD** NO	186 (40%)
YES	139 (30%)	YES	273 (60%)
Family history NO	389 (85%)	**IHRF** NO	225 (49%)
YES	70 (15%)	YES	234 (51%)
		HyperTC NO	386 (84%)
		YES	72 (16%)

AMD = age-related macular degeneration; DLS = double layer sign; HyperTC = hypertransmission of OCT signal into the choroid; IE = irregular elevation of the retinal pigment epithelium; IHRF = intraretinal hyperreflective foci; ND = nodular drusen; SDD = subretinal drusenoid deposit; SIRE = shallow irregular retinal pigment epithelium elevation.
Table shows the baseline characteristics of the cohort. Overlapping categories: eyes with IE, including eyes with DLS and SIRE; eyes with DLS, including eyes with SIRE; Exclusive categories: eyes with IE only, excluding eyes with DLS and SIRE; eyes with DLS only, excluding eyes with SIRE.
*Mean (standard deviation); n (%).
†n (%).

Progression to eMNV in the study eye and its subtype were based on Consensus on Neovascular Age-Related Macular Degeneration Nomenclature (CONAN) criteria and obtained from the original EDNA grading. Of the 459 included eyes, 104 (22.7%) developed eMNV in the EDNA study eye during the follow-up (FU) period, of which 56 (54%) were type 1, 28 (27%) type 2, and 20 (19%) type 3.

Data Management and Analysis

Study images were double graded for DLS and intergrader agreement was calculated using kappa (κ) statistics and interpreted according to Landis and Koch.[12] For the purposes of analysis, 3 OCT categorizations were created based on gradings that indicated the separation of the RPE from BM: (1) IE; (2) DLS-like; and (3) SIRE. A subset of the DLS-like group (SIRE) was defined based on the length and height of the DLS (length $\geq$ 1000 μm and height $<$ 100 μm; Fig 2).

Demographics, medical history, and OCT features for the 3 categories of IE, DLS, and SIRE were summarized using descriptive statistics. Categorical variables were reported as absolute frequency and percentage, and continuous variables as mean value and standard deviation and/or median value and interquartile range. Data distribution was checked using the Shapiro–Wilk test.

Differences in groups were assessed using Pearson's chi-squared test for categorical and the Wilcoxon rank sum test for continuous variables. Survival probability estimates of progression to eMNV were calculated for each of the 3 categories using Kaplan–Meier (KM) survival and differences assessed using the log-rank test. Cox regressions were applied to calculate hazard ratios (HRs) of progression to eMNV as an outcome variable. In the univariate, unadjusted Cox models, each variable was individually entered as a predictor, drawn from a list which included demographics and medical history. The multivariate fully adjusted model was generated with covariates that included demographics and medical history.

To avoid the effect of overlapping categories, we performed a sensitivity analysis with mutually exclusive groupings that were created using a hierarchical approach. Level 1 included eyes with no IE, DLS, or SIRE (No IE) and was used as the reference arm in the regression models. Level 2 included eyes with IE after excluding those with DLS (IE only). Level 3 consisted of eyes exhibiting DLS, excluding those that fulfilled the criteria for SIRE (DLS only), and level 4 included only eyes classified as SIRE (SIRE only). Sensitivity analyses using KM survival curves with differences assessed using the log-rank test and Cox regressions to calculate HRs of progression to eMNV as an outcome variable were repeated based on the nonoverlapping categories. Data analysis and visualization was performed using R version 4.2.2 (2022-10-31 ucrt with packages including gtsummary, survminer and forestmodel).

Results

Demographic, medical history, and features of intermediate AMD in study eyes are shown in Table 2. The age range of included participants was 50 to 95 (mean 76 ± 8) years and there were slightly more females (265 [58%]; Table 2). Features of intermediate AMD occurred at a frequency of 86% for nodular drusen, 60.0% for SDD, 51% for intraretinal hyperreflective foci, and 16% for HyperTC (Table 2).

Table 4. Differences in Baseline Characteristics by IE, DLS, and SIRE

		IE			DLS			SIRE		
	N	NO, n = 191*	YES, n = 268*	P Value†	NO, n = 358*	YES, n = 101*	P Value†	NO, n = 408*	YES, n = 51*	P Value†
Age	459	76 (8)	77 (7)	0.5	76 (8)	77 (7)	0.5	76 (8)	77 (7)	0.5
Sex	459			< 0.001			0.002			0.023
Male		109 (57%)	85 (32%)		165 (46%)	29 (29%)		180 (44%)	14 (27%)	
Female		82 (43%)	183 (68%)		193 (54%)	72 (71%)		228 (56%)	37 (73%)	
Smoking	459			0.7			> 0.9			0.5
Never		80 (42%)	103 (38%)		144 (40%)	39 (39%)		164 (40%)	19 (37%)	
Ex		88 (46%)	134 (50%)		172 (48%)	50 (50%)		194 (48%)	28 (55%)	
Current		23 (12%)	31 (12%)		42 (12%)	12 (12%)		50 (12%)	4 (7.8%)	
Hypertension	459	101 (53%)	142 (53%)	> 0.9	190 (53%)	53 (52%)	> 0.9	218 (53%)	25 (49%)	0.6
Cardiovascular	459	42 (22%)	61 (23%)	0.8	75 (21%)	28 (28%)	0.15	86 (21%)	17 (33%)	0.048
Diabetes	459	26 (14%)	49 (18%)	0.2	57 (16%)	18 (18%)	0.6	68 (17%)	7 (14%)	0.6
Supplements	459	48 (25%)	91 (34%)	0.043	105 (29%)	34 (34%)	0.4	122 (30%)	17 (33%)	0.6
Family history	459	25 (13%)	45 (17%)	0.3	60 (17%)	10 (9.9%)	0.090	65 (16%)	5 (9.8%)	0.3
ND	459	133 (70%)	261 (97%)	< 0.001	295 (82%)	99 (98%)	< 0.001	344 (84%)	50 (98%)	0.008
SDD	455	99 (53%)	174 (65%)	0.007	206 (58%)	67 (67%)	0.11	234 (58%)	39 (76%)	0.011
IHRF	459	62 (32%)	172 (64%)	< 0.001	151 (42%)	83 (82%)	< 0.001	191 (47%)	43 (84%)	< 0.001
HyperTC	459	15 (7.9%)	57 (21%)	< 0.001	40 (11%)	32 (32%)	< 0.001	53 (13%)	19 (37%)	< 0.001

DLS = double layer sign; HyperTC = hypertransmission of OCT signal into the choroid; IE = irregular elevation of the retinal pigment epithelium; IHRF = intraretinal hyperreflective foci; ND = nodular drusen; SIRE = shallow irregular retinal pigment epithelium elevation; SDD = subretinal drusenoid deposit.
*Mean (standard deviation); n (%).
†Wilcoxon rank sum test (continuous variable); Pearson's chi-squared test (categorical variables); differences in baseline characteristics between eyes exhibiting (YES) IE, DLS, or SIRE and those do not (NO).

Baseline Characterization of OCT Features of IE, DLS, and SIRE

Of the 459 study eyes eligible for analysis, IE was present in 268 (58%) and DLS in 101 (22%) at baseline (Table 2). After extracting the subset of study eyes with DLS into those that fit the criteria for SIRE, 51 (11%) fulfilled these requirements. Kohen's κ statistics showed almost perfect agreement when intergrader agreement was calculated for DLS ($\kappa = .934$, $P < 0.001$).

Mutually exclusive recategorization resulted in the following groupings: no IE, 191 (42%), IE only, 167 (36%), DLS only, 50 (11%), and SIRE only, 51 (11%; Table 2).

Table S3 (available at www.ophthalmologyretina.org) shows the mean, median, and range of the measurements for IE, DLS, and SIRE by overlapping and mutually exclusive categories for IE, DLS, and SIRE. The extent (number of B-scans involved) and the length (the width of the horizontal separation) showed an increasing gradient; SIRE > DLS > IE. The differences between DLS and SIRE remained but were less marked when assessed within the mutually exclusive categories (Table S3). The height of the RPE elevation (measured on the most affected B-scan) was similar ($P = 0.10$) for IE, DLS, and SIRE regardless of whether categorized into overlapping or mutually exclusive groups (Table S3).

Associations of IE, DLS, and SIRE with Clinical Findings and Intermediate AMD Features

Table 4 shows the differences in baseline characteristics by IE, DLS, and SIRE. All 3 OCT characteristics were seen more frequently in female participants (IE: 68%, $P < 0.001$; DLS: 71%, $P = 0.002$; SIRE: 73%, $P = 0.023$). Participants who reported taking nutritional supplements were represented at a higher frequency among those with IE compared with no IE ($P = 0.043$). Those with a history of cardiovascular disease were represented in a slightly higher proportion among eyes with SIRE ($P = 0.048$). Features of intermediate AMD were also more likely to be observed in eyes with IE ($P < 0.001$), DLS ($P < 0.001$) and SIRE ($P < 0.01$) compared with eyes without these features. The association between SDD and DLS was not significant ($P = 0.11$; Table 4).

Association of IE, DLS, and SIRE with the Onset of eMNV

Of the 459 included eyes, 104 (23%) developed eMNV over the 3 years FU (Table S5, available at www.ophthalmologyretina.org) of which 25 (13%) did not have features of IE, DLS, or SIRE at baseline. The proportions of eyes that developed eMNV by IE, DLS, or SIRE are shown in Table S5. Compared with study eyes without IE, DLS, or SIRE, significantly higher proportions of eyes with those features ($P < 0.001$) converted to eMNV (IE vs. no IE [13% vs. 29%], DLS vs. no DLS [18% vs. 41%], SIRE vs. no SIRE [21% vs. 37%]; Table S5). Table S5 also shows the frequencies of angiographic eMNV subtypes at detection of exudation for all eyes that progressed to eMNV, and by presence of IE, DLS, and SIRE. Numerical differences are seen in the proportions when classified by type of eMNV and IE, DLS, or SIRE. Eyes with IE or DLS at baseline had a higher proportion of type 1 membranes when eMNV was detected, with type 2 and 3 occurring at lower proportions. Eyes with SIRE had similar proportions of types 1, 2, and 3 eMNV at onset.

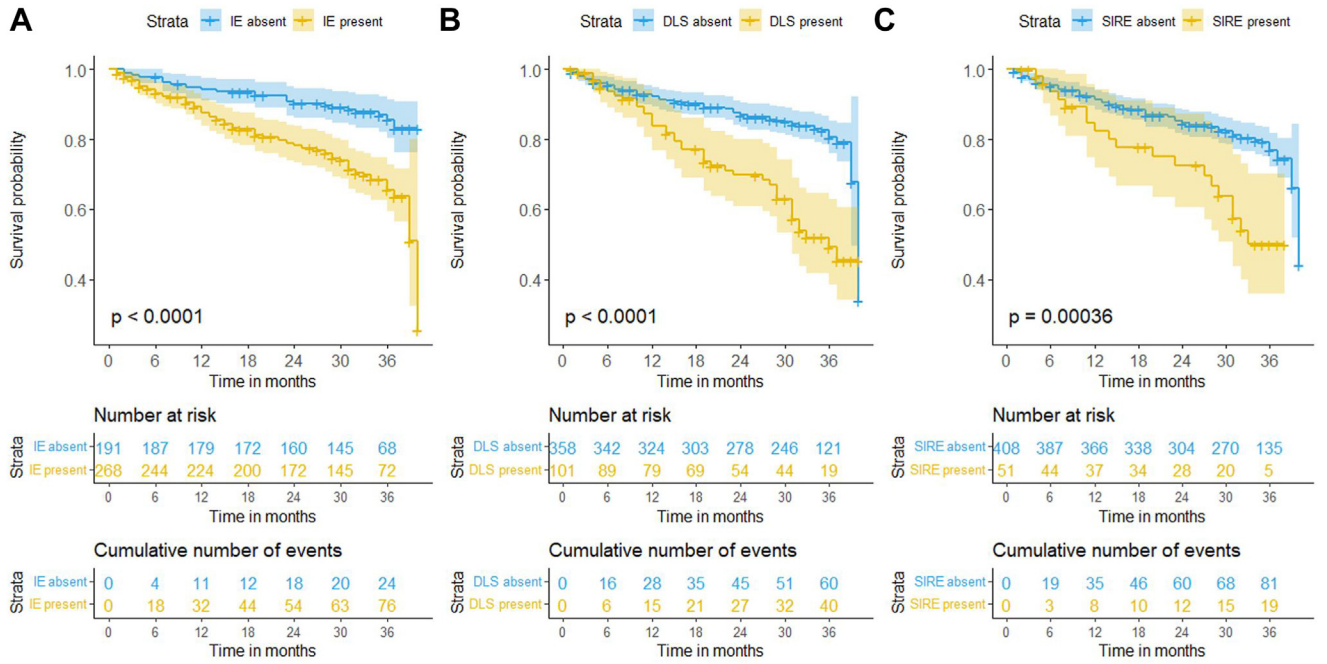

Figure 3. Kaplan–Meier survival plots comparing time to development of exudative macular neovascularization by overlapping categories of irregular elevation of the retinal pigment epithelium (IE), double layer sign (DLS), and shallow irregular retinal pigment epithelium elevation (SIRE). **A,** Eyes with no IE vs. eyes with IE. **B,** Eyes with DLS vs. eyes without DLS. **C,** Eyes with SIRE vs. eyes without SIRE. The numbers at risk by strata are shown. *P* values were calculated using the log-rank test.

There were no significant differences in extent, length, or height of IE, DLS, or SIRE when comparing eyes that developed eMNV to those that did not during FU (Table S6, available at www.ophthalmologyretina.org).

Survival Analysis

Kaplan–Meier survival curves comparing IE to no IE, no DLS to DLS, and no SIRE to SIRE are shown in Figure 3. The development of eMNV occurred significantly earlier in eyes with IE when compared with eyes without IE (Fig 3A). Comparing eyes without DLS to those with DLS, and eyes without SIRE to eyes with SIRE, eMNV events accumulated rapidly with clear separation of the KM plots (Fig 3B, C). The estimates of the cumulative rate of incidence of eMNV with 95% confidence intervals (CIs) at 18 and 36 months by presence of IE, DLS, or SIRE are shown in Table 7. Eyes that did not exhibit any IE had lower incidences that ranged from 6.3% at 18 months to 14% at 36 months of FU (Table 7). By month 36, the incidence of eMNV in eyes with DLS reached 51% (95% CI, 37.0%–61.0%, $P < 0.001$). The incidence of eMNV in eyes with SIRE did not exceed that of the DLS (Table 7).

Survival times at 90% and 75% survival probabilities are shown in Table 8. At 90% survival probability, the durations over which study eyes remained event free were 11, 10, and 8 months for IE, DLS, and SIRE, respectively; at 75% probability, these durations were 29, 19 and 23 months.

Sensitivity analysis of KM plots with nonoverlapping, mutually exclusive categories of no IE, IE only, DLS only, and SIRE is shown in Figure 4, and findings are similar to those observed in the main analysis. Pairwise comparisons showed significant differences in progression time between no IE to IE only ($P = 0.009$) and between IE only to DLS only ($P = 0.005$). However, time to progression between DLS only and SIRE was not significantly different ($P = 0.91$).

Hazard Analysis

The univariate Cox regression revealed a significantly greater hazard of progression to eMNV ($P < 0.001$) for those with IE (HR, 2.7; CI, 1.7–4.2), DLS (HR, 2.9; CI, 2–4.3), and SIRE (HR, 2.4; CI, 1.5–4; Table S9, available at www.ophthalmologyretina.org). None of the other variables tested reached significance (Table S9).

In the fully adjusted Cox regression model (Fig 5), the hazard of conversion for IE was 3.0 (95% CI, 1.8–4.8), for DLS 3.4 (95% CI, 2.2–5.1), and for SIRE 2.8 (95% CI, 1.7–4.7). A history of cardiovascular disease was associated with a marginal reduction in the point estimates of risk for DLS (adjusted HR, 0.5; 95% CI, 0.3–0.9; $P = 0.03$) and SIRE (adjusted HR, 0.5; 95% CI, 0.3–0.9; $P = 0.03$), whereas family history of AMD increased this risk for DLS only (adjusted HR, 1.8; 95% CI, 1.1–2.9; $P = 0.03$; Fig 5).

Results of the sensitivity analyses with nonoverlapping, mutually exclusive categories of IE only, DLS only, and SIRE only were no different from the main analysis and shown in Table S9 and Figure 6.

Discussion

We analyzed a large, longitudinal data set from the EDNA study that enrolled participants with new-onset, unilateral eMNV. Three SD-OCT-defined characteristics, namely IE, DLS, and SIRE, were analyzed as predictive biomarkers for

Table 7. Cumulative Rate of Progression to eMNV at 18 and 36 Months by IE, DLS, and SIRE

	N	18-Months Incidence (95% CI)	*P* Value*	36-Months Incidence (95% CI)	*P* Value*
IE			< 0.001		< 0.001
NO	191	6.3% (2.8%, 9.7%)		14% (8.6%, 20%)	
YES	268	17% (12%, 22%)		34% (27%, 41%)	
DLS			< 0.001		< 0.001
NO	358	9.9% (6.7%, 13%)		19% (15%, 24%)	
YES	101	23% (14%, 31%)		51% (37%, 61%)	
SIRE			< 0.001		< 0.001
NO	408	11% (8.3%, 15%)		23% (18%, 28%)	
YES	51	22% (9.0%, 33%)		50% (30%, 64%)	

CI = confidence interval; DLS = double layer sign; eMNV = exudative macular neovascularization; IE = irregular elevation of the retinal pigment epithelium; SIRE = shallow irregular retinal pigment epithelium elevation.
Table shows the cumulative rate of incidence (progression to eMNV) estimates with 95% CI at 18 and 36 months for each category of IE, DLS, and SIRE.
*Log-rank test.

progression to eMNV in the contralateral, high-risk, intermediate AMD eyes (study eye). We also report the proportions of eyes with other features of intermediate AMD, including nodular drusen, SDD, HRF and HyperTC by IE, DLS, and SIRE. The presence of the DLS was associated with a higher and faster rate of progression to eMNV compared with eyes that exhibited any IE, or in those in which the RPE was fully apposed to the BM (no IE in any scan). The presence of the SIRE sign, a derivative of the DLS, was not shown to increase the hazard of progression to eMNV compared with the DLS. Our findings strengthen the hypothesis that the DLS, a pragmatic, easily defined SD-OCT sign, is a sensitive and important biomarker of risk for progression to eMNV.

When study eyes were grouped by presence of IE, DLS, and SIRE we found that all 3 SD-OCT biomarkers increased the risk of progression to eMNV, with the highest rate of progression in eyes with DLS. The proportions rose from 13% for eyes without any of the aforementioned SD-OCT biomarkers, to 29% when IE was present, and increased further to 41% when the DLS was present, and remained around this level (37%) when the criteria for SIRE were fulfilled. Our findings support the view that the dimensions of the DLS are less important in terms of the predictability of progression to eMNV. In a recent retrospective cohort study of 458 eyes with intermediate AMD and 2-year FU, 96 eyes (21%) had a DLS at baseline.[13] These authors subclassified DLS to thin (11.4%) and thick DLS (9.6%) and found that only thick DLS was associated with a higher risk (odds ratio, 4.339; 95% CI, 2.178−8.644; $P < 0.001$) of progression to eMNV over the 2 years. Thin DLS was believed to correspond to basal laminar deposit, whereas thick DLS represents neMNV more accurately. Because no information was provided on the proportions that progressed to eMNV in the study by Wakatsuki, we are unable to directly compare their data with our groups of IE, DLS, and SIRE. Also, in our study we specifically searched for signs of heterogeneous reflectivity in the space generated by IE from the BM, which was our criterion for the diagnosis of DLS. Our protocols for grading and definitions of DLS were prespecified and quality-assured during the grading process, and we

Table 8. Survival Time at 90% and 75% Survival Probability by IE, DLS, and SIRE

		Survival Time at 90% Survival Probability		Survival Time at 75% Survival Probability	
	Number	Months (95% CI)	*P* Value*	Months (95% CI)	*P* Value*
IE	459		< 0.001		< 0.001
NO		28 (19, —)		— (—, —)	
YES		11 (7.0−15)		29 (22−34)	
DLS	459		< 0.001		< 0.001
NO		19 (12−28)		39 (37, —)	
YES		10 (6.0−14)		19 (14−29)	
SIRE	459		< 0.001		< 0.001
NO		15 (11−22)		37 (36, —)	
YES		8.0 (6.0−20)		23 (11−32)	

CI = confidence interval; DLS = double layer sign; IE = irregular elevation of the retinal pigment epithelium; SIRE = shallow irregular retinal pigment epithelium elevation.
Table shows the survival time at 90% and 75% survival probability for each category.
*Log-rank test.

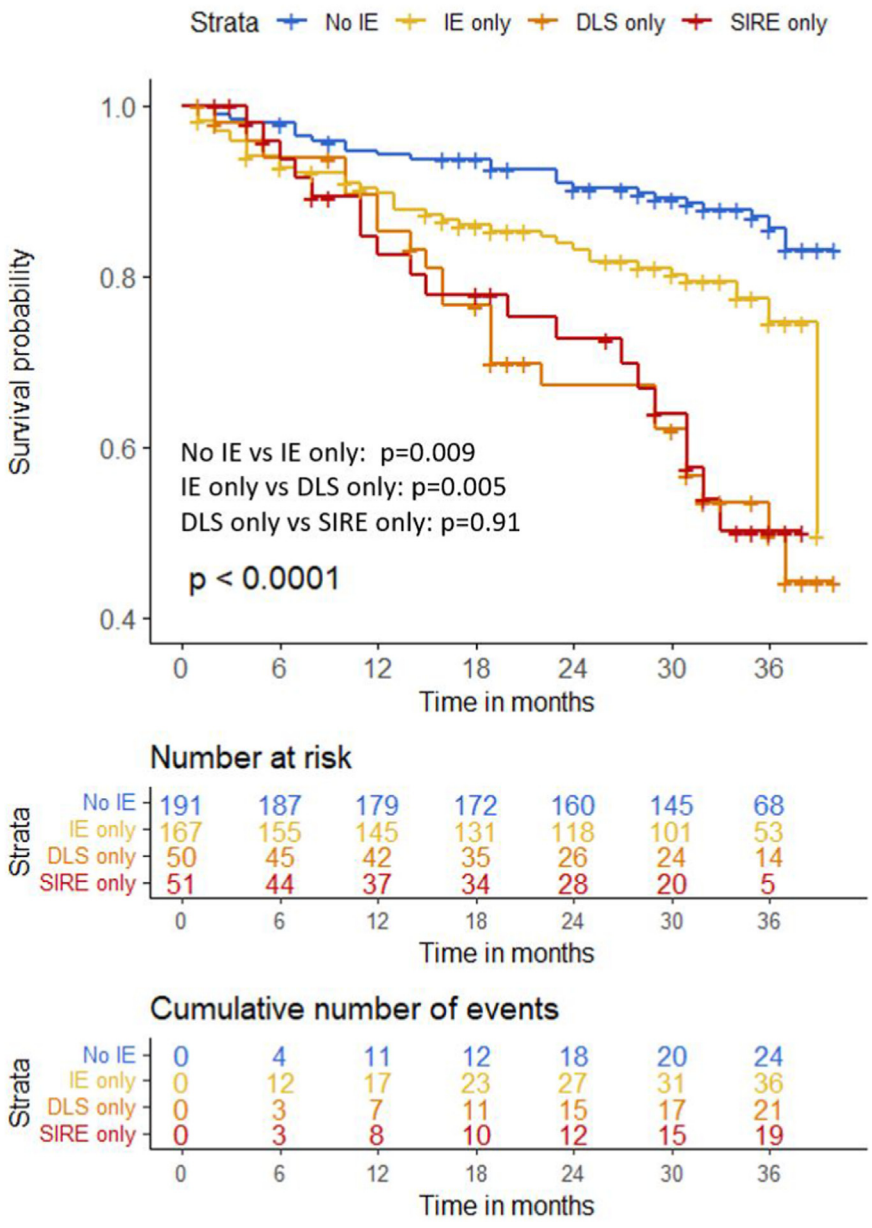

Figure 4. Kaplan–Meier survival plots comparing time to development of exudative macular neovascularization by nonoverlapping categories of irregular elevation of the retinal pigment epithelium (IE) only, double layer sign (DLS) only, and shallow irregular retinal pigment epithelium elevation (SIRE) only. The tables below show number at risk and cumulative number of events by the mutually exclusive categories of no IE (blue), IE only (yellow), DLS only (orange) and SIRE only (red). *P* values were calculated using the log-rank test.

therefore contend that a subclassification by thin and thick DLS is of less importance.

Because we also classified groups by nonoverlapping, mutually exclusive categories, we were able to differentiate the impact of IE, DLS, and SIRE at baseline to the risk of progression to eMNV. The sensitivity analyses confirmed that progression to eMNV was significantly faster for DLS and/or SIRE compared with eyes with IE only. Nonetheless, IE by itself also increased the risk of progression to eMNV though the HRs were smaller compared with DLS or SIRE. Because the 75% survival probability drops from over 2 years (29 months) in eyes with IE to 19 months when DLS is present (Table 4), we believe that it would prudent to review persons who show these very high-risk characteristics on a 6-monthly basis if not attending more frequently.

The definition of IE we employed was any irregular separation of the RPE from its basement membrane. The rationale for considering heterogeneous reflectivity as a marker for DLS is to distinguish RPE elevation due to contiguous drusen from eyes containing a potential space in which new vessels are present. It is possible that SD-OCT cannot resolve these outer retinal layers to a sufficient level to always detect regions of heterogeneous reflectivity arising from networks of new vessels. In this context it has

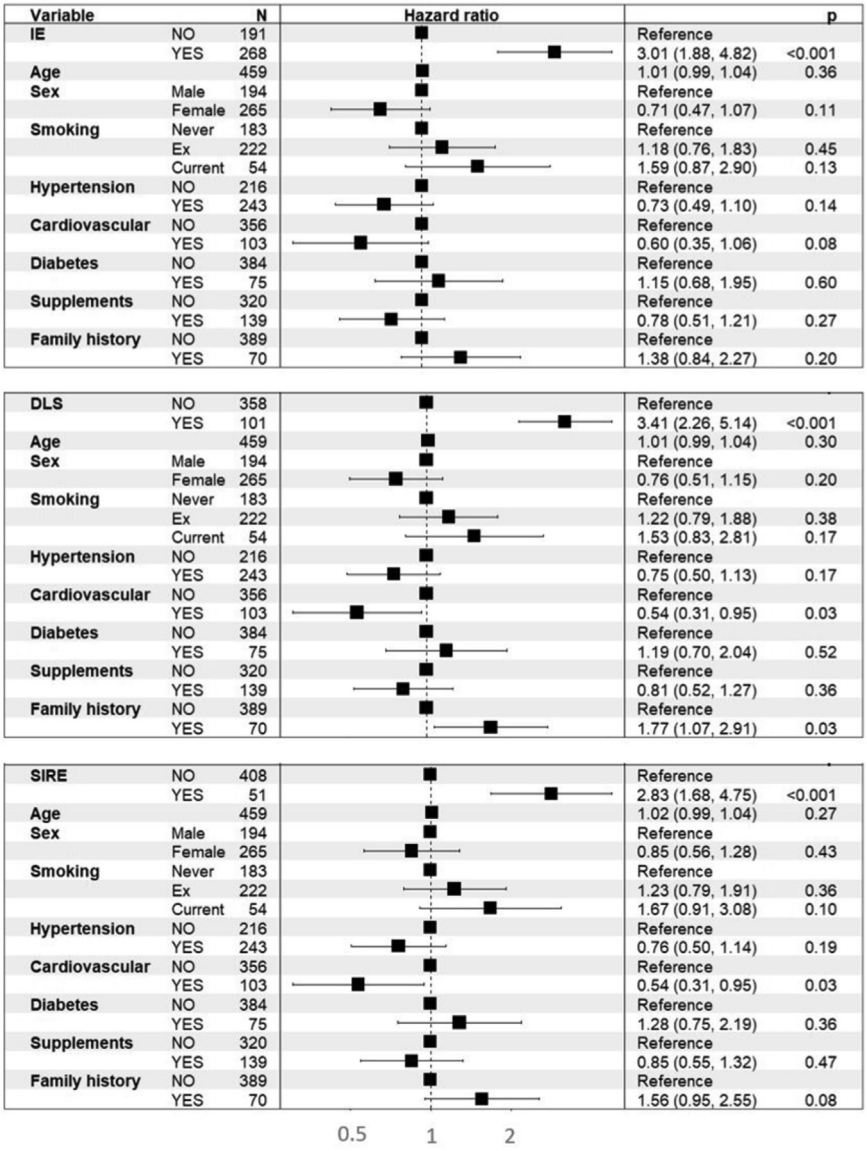

Figure 5. Forest plots and corresponding hazard ratios of progression to exudative macular neovascularization (eMNV) by overlapping categories of irregular elevation of the retinal pigment epitheliuim (IE), double layer sign (DLS), and shallow irregular retinal pigment epithelium elevation (SIRE). Multivariate Cox regression model assessing the hazard of progression to eMNV by the overlapping categories of IE, DLS, or SIRE as predictor variables. Covariates include demographics and medical history. Eyes with no IE (NO) vs. eyes with IE (YES); eyes with no DLS (NO) vs. eyes with DLS (YES); eyes with no SIRE (NO) vs. eyes with SIRE (YES).

been reported that thick basal laminar deposits colocalize with neMNV and can appear as homogeneous areas of hyporeflectivity, rendering detection of DLS difficult[14,15] and resulting in misclassification of eyes.

Narita et al[6] reported a prevalence of 2.58% of neMNV on SS-OCTA imaging in a sample of 101 patients with bilateral intermediate AMD and all of these eyes had lengthy elevations of the RPE with nonhomogenous reflectivity within the potential sub-RPE space. Interestingly, although the height of the elevation was associated with a higher probability of detection of neMNV, for each 10 μm increase in elevation, the odds of neMNV decreased by 25%. Thus, the shallowness of the RPE elevation was considered an important characteristic, and all 6 eyes with neMNV on OCTA had the features of SIRE on SD-OCT. However, the positive predictive value of SIRE was 25%, meaning that around three-quarters of eyes with SD-OCT features of SIRE will not have an neMNV. In the present study, consisting of a sample of eyes at very high risk of progression since the fellow eye already had eMNV at enrollment, we observed that although features of SIRE on SD-OCT marginally increased the risk of progression to eMNV, this was no higher than the risk associated with the presence of the DLS. Interestingly, the prevalence of SIRE in our sample was 11% and almost identical to that of Narita et al[6] who reported an SD-OCT prevalence of 10.3%. These

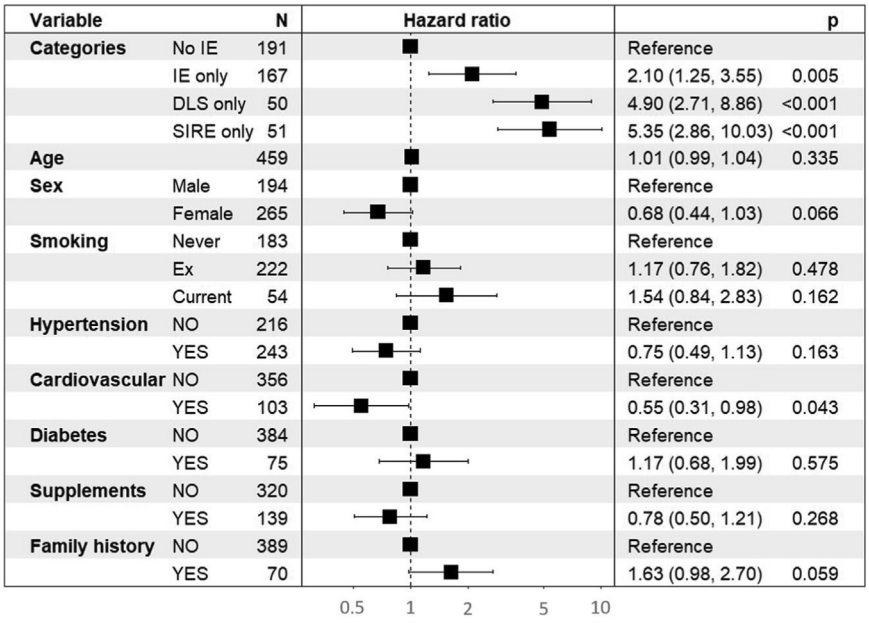

Figure 6. Forest plots and corresponding hazard ratios of progression to exudative macular neovascularization (eMNV) by nonoverlapping categories of irregular elevation of the retinal pigment epithelium (IE) only, double layer sign (DLS) only, and shallow irregular retinal pigment epithelium elevation (SIRE) only (sensitivity analysis). Multivariable Cox regression model assessing the hazard of progression to eMNV by nonoverlapping categories of IE only, DLS only, or SIRE as predictor variable with eyes exhibiting no IE as the reference arm. Covariates include demographics and medical history.

similarities are particularly intriguing as the study by Narita et al[6] included participants with bilateral large drusen, whereas the EDNA study cohort had eMNV in the first-affected eye.

When we examined the relationships between eMNV type at onset in the study eye and baseline categories of IE, DLS, and SIRE, we observed some intriguing findings. Among the 101 study eyes that had DLS at baseline, 59% developed type 1 eMNV. By contrast, among those with SIRE at baseline, 32% developed type 1 eMNV, whereas 37% developed a type 3 lesion. Although the findings with respect to type 1 are in accord with what is known about this type of MNV, as it causes shallow irregular elevations of the RPE monolayer, the higher frequency of type 3 lesions suggests that a more diffuse change due to the accumulation of basal laminar deposits at the level of the RPE/BM may be etiologically associated with type 3 MNV. Nonetheless, these findings should be interpreted with caution, as the numbers in the different eMNV groups were small.

Our study has several strengths. First, EDNA was a prospectively planned diagnostic test accuracy study carried out in accord with Standards for Reporting of Diagnostic Accuracy (STARD) recommendations. It enrolled participants with new-onset eMNV in the first eye from 24 clinical sites across the UK and adhered to protocol based on FU of the fellow eye with systematic collection of clinical and imaging data. Second, a predefined hypothesis and analysis plan was proposed before this revised grading was undertaken and, although the graders were trained to identify IE and DLS, they were unaware of the study hypothesis, thus limiting bias. Progression to eMNV or absence of eMNV at study completion was undertaken at an accredited reading center using multimodal retinal imaging.

Our study is not free of limitations. The original study was necessarily pragmatic; therefore, although sites were provided with a recommended imaging protocol, this protocol was not mandated because the OCT images were obtained during routine National Health Service care visits and therefore were captured using local protocols that had varying OCT scan densities and sizes. Also, OCTA imaging was not mandated as only a limited number of clinical sites had access to this technology during the conduct of EDNA. Therefore, the specificity of DLS, and for that matter SIRE, could not be confirmed due to the lack of OCTA.[6,7]

Our study confirmed on a pragmatic level that eyes exhibiting the DLS have a 4 times greater hazard of progression to eMNV over 3 years. The presence of SIRE, a derivative of the DLS, carried a similar risk of progression to eMNV and did not increase predictive ability. Because IE in itself carried an increased risk of progression to eMNV, we recommend that eyes with any elevation of the RPE are monitored. However, as the presence of the DLS elevates this risk more than twofold, we especially feel that such patients merit closer monitoring with high-resolution imaging, particularly with OCTA.

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Analysis and interpretation: Csincsik, Muldrew, Bettiol, Wright, Empeslidis, De Cock, Yamaguchi, Peto, Chakravarthy

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Abbreviations and Acronyms:
AMD = age-related macular degeneration; **BM** = Bruch's membrane; **CI** = confidence interval; **DLS** = double layer sign; **eAMD** = exudative age-related macular degeneration; **EDNA** = Early Detection of Neovascular AMD; **eMNV** = exudative macular neovascularization; **FU** = follow-up; **HR** = hazard ratio; **HyperTC** = hypertransmission of OCT signal into the choroid; **IE** = irregular elevation of the retinal pigment epithelium; **KM** = Kaplan−Meier; **MNV** = macular neovascularization; **neMNV** = nonexudative macular neovascularization; **OCTA** = OCT angiography; **RPE** = retinal pigment epithelium; **SDD** = subretinal drusenoid deposit; **SD-OCT** = spectral domain-OCT; **SIRE** = shallow, irregular retinal pigment epithelium elevation; **SS-OCT** = swept source-OCT; **UK** = United Kingdom.

Keywords:
Age-related macular degeneration, double layer sign, exudative macular neovascularization, OCT, Shallow irregular RPE elevation.

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