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The role of retinal fluid location in atrophy and fibrosis evolution of patients with neovascular age-related macular degeneration long-term treated in real world

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ABSTRACT.

Purpose: **To assess the effect of clinical factors on the development and progression of atrophy and fibrosis in patients with neovascular age-related macular degeneration (nAMD) receiving long-term treatment in the real world.**

Methods: **An ambispective 36-month multicentre study, involving 359 nAMD patients from 17 Spanish hospitals treated according to the Spanish Vitreoretinal Society guidelines, was designed. The influence of demographic and clinical factors, including the presence and location of retinal fluid, on best-corrected visual acuity (BCVA) and progression to atrophy and/or fibrosis were analysed.**

Results: **After 36 months of follow-up and an average of 13.8 anti-VEGF intravitreal injections, the average BCVA gain was +1.5 letters, and atrophy and/or fibrosis were present in 54.8% of nAMD patients (OR = 8.54, 95% CI = 5.85–12.47, compared to baseline). Atrophy was associated with basal intraretinal fluid (IRF) (OR = 1.87, 95% CI = 1.09–3.20), whereas basal subretinal fluid (SRF) was associated with a lower rate of atrophy (OR = 0.40, 95% CI = 0.23–0.71) and its progression (OR = 0.44, 95% CI = 0.26–0.75), leading to a slow progression rate (OR = 0.34, 95% CI = 0.14–0.83). Fibrosis development and progression were related to IRF at any visit ($p < 0.001$). In contrast, 36-month SRF was related to a lower rate of fibrosis (OR = 0.49, 95% CI = 0.29–0.81) and its progression (OR = 0.50, 95% CI = 0.31–0.81).**

Conclusion: **Atrophy and/or fibrosis were present in 1 of 2 nAMD patients treated for 3 years. Both, especially fibrosis, lead to vision loss. Subretinal fluid (SRF) was associated with good visual outcomes and lower rates of atrophy and fibrosis, whereas IRF yields worse visual results and a higher risk of atrophy and especially fibrosis in routine clinical practice.**

Key words: macular atrophy – retinal fluid location – subretinal fibrosis – nAMD

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Introduction

Age-related macular degeneration (AMD) is a chronic progressive neurodegenerative disease and its advanced forms, such as neovascular AMD, can lead to severe irreversible vision loss. Neovascular AMD (nAMD) is characterised by macular neovascularization (MNV) that can progress to subretinal fibrosis and macular scarring (Ferris et al. 2013; Spaide et al. 2020). Subretinal macular fibrosis is a result of excessive wound healing following MNV in nAMD and can facilitate the local destruction of photoreceptors, retinal pigment epithelium (RPE) and choroidal vessels (Ishikawa, Kannan & Hinton 2016). Macular atrophy is characterised by the presence of atrophic lesions of the outer retina, RPE and underlying choriocapillaris, and it is a frequent finding in patients with long-term nAMD (Bhisitkul et al. 2015). Both atrophy and fibrosis can cause permanent and devastating macular dysfunction and lead to legal blindness or the inability to perform routine activities, such as reading, driving, recognising faces and seeing colour (Sadda et al. 2020).

Advances in diagnostic techniques and the introduction of anti-vascular endothelial growth factor (anti-VEGF) therapy have helped, in some countries, to reduce legal AMD-related blindness by up to 50% and its growing social and emotional impact (Ruiz Moreno et al. 2016; Mehta et al. 2018). However, the need for frequent intravitreal injections and ophthalmological visits implies a significant burden for patients, family members and healthcare professionals (Spooner et al. 2018). In addition, several patients do not achieve a satisfactory response in the long term with current anti-VEGF treatment, and they develop atrophy and fibrosis. Therefore, it is essential to identify biomarkers that facilitate a better understanding of the clinical differences between good and poor responders and the factors that may increase the risk or protect against the development or progression of atrophy and fibrosis (Schmidt-Erfurth & Waldstein 2016; Ashraf, Souka & Adelman 2018; Lai et al. 2019).

Recently, some clinical and imaging biomarkers have been reported to be associated with the anatomical and functional prognoses of patients with nAMD. Furthermore, they can help in the planning of individualised anti-VEGF therapies (Regillo et al. 2015; Schmidt-Erfurth & Waldstein 2016; Ashraf, Souka & Adelman 2018; Guymer et al. 2019; Lai et al. 2019). Central subfield thickness (CST) has been used as a biomarker to guide treatment in clinical trials and routine practice. In recent years, special importance has been given to the location of retinal fluid to evaluate the anatomical and functional prognoses and different responses to anti-VEGF treatment (Saenz-De-Viteri et al. 2021). Intraretinal fluid (IRF) appears to negatively impact best-corrected visual acuity (BCVA), whereas subretinal fluid (SRF) has been associated with better BCVA (Schmidt-Erfurth & Waldstein 2016; Sadda et al. 2020).

However, controversies on the role of fluid location in the long-term development and progression of atrophy and fibrosis have persisted. It can be considered that the worst visual prognosis usually accompanies these sequelae of AMD. Therefore, we carried out this study to evaluate the effect of clinical factors, especially retinal fluid location, on the development and progression of atrophy and fibrosis in nAMD patients receiving long-term anti-VEFG treatment in the real world, regardless of the treatment regimen and drug used.

Materials and methods

Study design

An ambispective (retrospective and prospective) 36-month multicentre study including a cohort of nAMD patients of 17 Spanish hospitals was designed. A total of 359 patients were included between September 1, 2016 and February 28, 2020.

All procedures carried out in this study were in accordance with the guidelines of the Declaration of Helsinki. The Institutional Review Board and the Ethics Committee of Clínica Universidad de Navarra (CUN-RAN-2016-01) and Government of Navarra, Spain (EO16/19), approved the protocols used in this study. All patients were fully informed of the purpose and procedures, and written consent was obtained from each patient.

All cases underwent a detailed ophthalmologic examination including automatic objective refraction, visual acuity assessment, slit-lamp biomicroscopy with pupillary dilation, colour fundus photography and macular optical coherence tomography (OCT): swept source (SS) DRI OCT Triton (Topcon Corporation, Tokyo, Japan), Cirrus HD-OCT 5000 (Carl Zeiss Meditec AG, Oberkocken, Germany), Spectralis spectral domain (SD) OCT (Heidelberg Engineering, Heidelberg, Germany). Each patient was assessed with the same OCT device throughout the study.

Patients' selection

Patients' inclusion criteria were nAMD with subfoveal and/or juxtafoveal MNV confirmed by OCT and/or fluorescein angiography (FA), treated and followed up according to the Spanish Vitreoretinal Society guidelines (Ruiz Moreno et al. 2012).

Patients' exclusion criteria were: previous photodynamic therapy, macular laser photocoagulation, intravitreal corticosteroids or vitreoretinal surgery in the study eye, basal atrophy and/or fibrosis in more than 50% of the lesion area, tractional maculopathy or epiretinal membrane for the study eye, media opacity, history of uveitis, ocular trauma or high myopia justifying the presence of non-nAMD MNV in the study eye, and central serous choroidopathy in either eye.

After a common loading phase of three monthly anti-VEGF injections, as recommended by the Spanish Vitreoretinal Society (visits: basal/V1, V2 and V3), patients were treated with one of the following different treatment regimens: *pro re nata* or as needed (PRN), treat and extend (T&E) or fixed regimen every 4 weeks (monthly) or 8 weeks (bimonthly) (López-Gálvez et al. 2020). Patients could change their regimen at any time, at the discretion of their centre's researcher. In the PRN treatment regimen, patients underwent monthly follow-up assessments and received treatment when activity criteria were met. In the T&E regimen, treatment and follow-up visits were extended by periods of 2 weeks, if appropriate, to a maximum of 12 weeks. Disease activity was determined by loss of ≥5 Early Treatment Diabetic Retinopathy Study (ETDRS) letters and/or one of the following

criteria: new haemorrhages on fundus examination, persistent or recurrent intraretinal or subretinal fluid on OCT, and leakage from MNV on FA. The study monitored baseline visit (basal/V1), after loading phase visit (V4), at 12 and 36 months visits.

Being an ambispective study, the patient could be recruited at any time during the follow-up, so the health questionnaire was collected only once in any of the visits.

Main outcome measures

The presence of atrophy (A) and fibrosis (F) and their progression during follow-up were the main variables analysed. Atrophy was defined as the presence of an area of hyperfluorescence that persisted throughout the run with identifiable large choroidal vessels in FA, an area of pallor with clearly defined edges and identifiable large choroidal vessels in colour fundus photography and/or increased transmission of the light signal into the choroid and thinning or absence of the outer retinal layers in OCT. Fibrosis was defined as the presence of a yellow-whitish lesion area in colour fundus photography and/or a hyperreflective lesion at the RPE level in OCT. Both characteristics were assessed and measured by each centre, reviewed by two independent ophthalmologists (SLG and MSV) and re-evaluated by AGL in case of discrepancies. Atrophy and fibrosis areas were measured in mm^2 at visits V1, V4, 12 and 36 months, using image analysis software (Adobe Photoshop CS5). Development was defined as the presence of A or F at 36 months, encompassing those patients with such characteristics at the baseline visit and those in whom it appeared at any time during follow-up. Progression (growth) was defined as the increase in the size of the area of A and F at the 36-month visit, compared to the basal visit. Growth rate for A and F was defined as fast if ≥ 2 mm^2/year and slow if <2 mm^2/year.

Retinal fluid was defined as the presence of intraretinal or subretinal fluid on OCT images in any of the monitored visits. For the analysis of anatomical and functional evolution, we divided the patients according to fluid status after the loading phase (V4), as we had found this parameter to be a good biomarker of evolution in previous studies (Saenz-de-Viteri et al. 2021).

Best-corrected visual acuity as measured by ETDRS letter score and mean change from baseline BCVA were also evaluated. Specific anatomical characteristics including CST, lens status, type of MNV classified by OCT (type 1, type 2 or mixed, a combination of both), type and number of anti-VEGF intravitreal injections were collected (Spaide et al. 2020). Based on the average number of intravitreal injections per year reported in the recent literature, it was considered an adequate treatment that each study eye received >15 intravitreal injections in 36 months (assuming an average of 7 injections in the first year, 5 in the second and 4 in the third year), and undertreatment if it received ≤15 injections in 36 months (Monés et al. 2020).

Demographic and clinical factors as age, sex, arterial hypertension (HT), hypercholesterolemia and smoking were collected as well. The smoking variable was compiled with four possible responses and, subsequently, became dichotomous due to the reduction in risk associated with smoking over time (Myers et al. 2014).

Statistical analysis

To evaluate the hypothesis contrast for categorical variables, after assessing application conditions (none of the expected values is less than 5), we used Pearson Chi-square test to compare the proportions. In case that application conditions are not met, we used Fisher exact test.

To evaluate the hypothesis contrast for quantitative variables, we used a *t*-test for two independent samples or one and two way ANOVA (analysis of variance), after verifying that the application requirements were met. To assess normal distribution, we used Shapiro–Wilk test and for variance homogeneity we used Levene's test. For variables not following a normal distribution, we used a Mann–Whitney *U*-test to evaluate the hypothesis contrast. When variance homogeneity criteria were not met, we used a Welch test (or *t*-test for independent samples with variance heterogeneity).

A multivariate logistic regression was also performed to analyse the effect of the main risk and protective factors on development of atrophy and fibrosis.

In all test, p values < 0.05 were considered statistically significant. All the analyses were performed using the free software Python 3.8 (Python Software Foundation, Wilmington, DE, USA) and GraphPad 8.0 (GraphPad Prism Software Inc., San Diego, CA, USA).

Results

General characteristics

A total of 359 patients were recruited, and 1.4% were lost to follow-up (4 cases due to voluntary cause and 1 case due to death). The remaining 354 patients completed the study. The mean age was 76.7 ± 7.1 years at the beginning of the study; 60.7% were women and 39.3% were men. Based on the health questionnaire responses, 63% of the patients had arterial hypertension, 42.4% had hypercholesterolemia, and 21.7% were smokers (or had been in the last 20 years) (Table 1).

The analysis of each of these clinical variables was carried out by separating the patients according to the presence of A or F at 36 months. Patients who developed A or F were older than those who did not. Both A and F were less prevalent among smokers (OR = 0.5, 95% CI = 0.3–0.9, p = 0.025 for atrophy). The other factors (sex, HT and hypercholesterolemia) showed no statistically significant associations with the appearance of A or F (Table 1).

Ophthalmological characteristics

In our study, fibrosis was more prevalent (37.8%) than atrophy (31.3%) throughout the follow-up (Tables 1 and 2). We analysed patients with exclusive atrophy (N = 60; 16.9%), exclusive fibrosis (N = 83; 23.5%), or a combination of both (N = 51; 14.4%) at 36 months, and we found that these characteristics (atrophy and/or fibrosis) were present in 54.8% (N = 194) of patients with nAMD treated in the study. Furthermore, the study showed that the risks of both A and F increased eightfold after 36 months of treatment (OR = 8.54, 95% CI = 5.85–12.47, $p < 0.001$), compared with the baseline visit (Fig. 1). Atrophy was the predominant characteristic at diagnosis, and

Table 1. Distribution of atrophy and fibrosis according to demographic and clinical characteristics

	Mean ± SD N = 354 (%)	A 36 N = 111 (31.3%)	No A 36 N = 238 (67.2%)	*OR (95% CI)* p	F 36 N = 134 (37.8%)	No F 36 N = 215 (60.7%)	*OR 95% CI* p
Age (years)	76.7 ± 7.1	**78.0 ± 6.5**	76.1 ± 7.2	**0.023**	77.3 ± 6.3	76.4 ± 7.4	0.22
Female sex	215 (60.7)	74 (66.7)	138 (57.9)	*OR 1.4 (0.9–2.5)* 0.12	80 (59.7)	132 (61.4)	*OR 1.0 (0.6–1.6)* 0.82
Arterial hypertension	223 (63)	76 (68.5)	144 (60.5)	*OR 1.4 (0.9–2.5)* 0.15	83 (61.9)	137 (63.7)	*OR 1.0 (0.7–1.7)* 0.81
Hypercholesterolemia	150 (42.4)	51 (45.9)	97 (40.7)	*OR 1.25 (0.7–2)* 0.42	58 (43.3)	90 (41.8)	*OR 1.1 (0.7–1.7)* 0.82
Smoking	77 (21.7)	**16 (14.4)**	60 (25.2)	***OR 0.5 (0.3–0.9)*** **0.025**	27 (20.1)	49 (22.8)	*OR 0.9 (0.5–1.4)* 0.59

T student was performed to evaluate statistically significant differences ($p < 0.05$).
OR values are shown in italics and $p < 0.05$ values are highlighted in bold.
36 = 36-month visit, 95% CI = 95% confidence interval, A = atrophy, F = fibrosis, N = number of patients, OR = odds ratio.

fibrosis was after the loading phase and at 36 months.

Regarding the ophthalmological examination at the baseline visit (V1), the mean BCVA was 57.1 ± 16.8 letters (ETDRS scale), and the mean CST was 328.7 ± 89.2 microns on OCT. The distribution of MNV was 57.3% for type 1, 27.1% for type 2, and 15.5% for the mixed type (combination of types 1 and 2). Subretinal fluid was present in 79.3% of the patients at the time of diagnosis, and IRF was present in 61%. In addition, A was present in 7.1% of the patients at baseline, and F was present in 5.9%. The average number of intravitreal injections after 36 months of anti-VEGF treatment was 13.8 ± 5.3 (Table 2).

The patients who developed A had a higher percentage of mixed MNV than type 1 MNV, compared with those who did not develop A (Table 2).

Visual outcomes

The patients who did not develop A and F had the best visual outcome (Fig. 2A). The individuals who presented with fibrosis at 36 months had worse BCVA at diagnosis (ETDRS V1) than those who did not ($p < 0.001$); they also had worse BCVA at V4, 12 and 36 months. Meanwhile, the individuals who had atrophy at 36 months did not show a significant difference in the VA measured using the ETDRS chart at any visit (Table 2).

The presence of isolated atrophy at 36 months was related to a minimal reduction in vision at any visit. However, the presence of isolated fibrosis at 36 months was associated with a considerable loss of vision, significant with respect to atrophy at 12 ($p < 0.05$) and 36 months ($p < 0.001$), and highly significant at any visit with respect to the absence of both at 36 months ($p < 0.001$) (Fig. 2A).

After the common loading phase of three monthly anti-VEGF injections (V4), the average vision gain was +7.6 letters. At 12 months, letter gain was maintained with a slight decrease (+6.2 letters), whereas at 36 months, the gain was reduced to +1.5 letters (Table 2 and Fig. 2A,B).

Regarding the functional progression related to the presence or location of retinal fluid in V4, the patients with isolated SRF had similar visual outcomes, during any visit, as those with dry macula, all of which had a higher BCVA than those with isolated IRF ($p < 0.001$ for ETDRS at 36 months) or the combination with SRF (SRF+IRF in V4) ($p < 0.05$ for ETDRS at 36 months) (Fig. 2B).

Atrophy and fibrosis progression according to the retinal fluid after the loading phase

Patients with isolated SRF at V4 showed a significantly lower prevalence of atrophy during any visit than the patients with isolated IRF (36 months: OR = 0.34, 95% CI = 0.15–0.77, p = 0.012) and dry macula (36 months: OR = 0.45, 95% CI = 0.23–0.85, p = 0.017) (Fig. 2C).

The presence of fibrosis from V4 was more frequent in the patients with isolated IRF at V4 than those with isolated SRF (V4: OR = 3.57, 95% CI = 1.28–10, p = 0.02; 12 months: OR = 2.94, 95% CI = 1.25–7.14, p = 0.02; 36 months: OR = 2.63, 95% CI = 1.23–5.55, p = 0.014) or dry macula (V4: OR = 2.56, 95% CI = 1.16–5.88, p = 0.03; 12 months: OR = 2.32, 95% CI = 1.12–5, p = 0.04; 36 months: OR = 2.5, 95% CI = 1.29–5, p = 0.009). Moreover, the presence of fibrosis during any visit was more frequent in patients with a combination of fluids at V4 (SRF + IRF) than in those with isolated SRF (V4: OR = 5.55, 95% CI = 2–14.28, $p < 0.001$; 12 months: OR = 4.34, 95% CI = 1.78–10, p = 0.001; 36 months: OR = 3.44, 95% CI = 1.58–7.14, p = 0.002) or dry macula (V4: OR = 4, 95% CI = 1.82–8.33, $p < 0.001$; 12 months: OR = 3.45, 95% CI = 1.66–7.14, p = 0.001; 36 months: OR = 3.22, 95% CI = 1.66–6.25, $p < 0.001$) (Fig. 2D).

The presence of IRF at baseline increased the proportion of mixed MNV and decreased the proportion of type 1 MNV (p = 0.011), whereas basal SRF markedly increased the proportion of type 1 MNV and decreased the proportion of mixed MNV (p = 0.015) (Fig. 3A,B).

Macular atrophy

Figure 4 shows the progression of atrophy (development, growth and growth

Table 2. Distribution of atrophy and fibrosis according to ophthalmological characteristics

	Mean ± SD N = 354 (%)	A 36 N = 111 (31.3 %)	No A 36 N = 238 (67.2%)	*OR (95% CI)* p	F 36 N = 134 (37.8%)	No F 36 N = 215 (60.7%)	*OR 95% CI* p
ETDRS V1 (letters)	57.1 ± 16.8	55.5 ± 18.3	57.6 ± 16.6	0.27	**48.7 ± 19.7**	60.5 ± 14.7	**<0.001**
CST V1 (micron)	328.7 ± 89.2	348.3 ± 85.3	333.4 ± 79.5	0.895	346.3 ± 92.8	323.7 ± 87.7	0.099
MNV V1: 1	203 (57.3)	58 (52.2)	141 (59.2)	***OR 2.4***	72 (53.7)	127 (59.1)	*OR 1.5*
2	96 (27.1)	26 (23.4)	70 (29.4)	***(1.3–4.5)***	44 (32.8)	52 (24.2)	*(0.9–2.4)*
Mixed	55 (15.5)	**27 (24.3)**	27 (11.3)	**0.005**	18 (13.4)	36 (16.7)	0.12
SRF V1	279 (79.3)	75 (68.1)	**201 (84.4)**	***OR 0.4***	106 (79.1)	170 (79.1)	*OR 0.9*
				(0.2–0.7)			*(0.6–1.6)*
				<0.001			1.0
IRF V1	214 (61)	**74 (67.2)**	138 (57.9)	***OR 1.87***	**96 (71.6)**	116 (53.9)	***OR 2.2***
				(1.1–3.2)			***(1.4–3.6)***
				0.021			**0.001**
ETDRS V4 (letters)	64.6 ± 14.5	62.6 ± 14.2	65.4 ± 14.7	0.09	**59.8 ± 16.8**	67.4 ± 12.2	**<0.0001**
SRF V4	125 (35.3)	30 (27.5)	**95 (40.0)**	***OR 0.6***	52 (39.3)	73 (33.9)	*OR 1.2*
				(0.3–0.9)			*(0.8–2.0)*
				0.03			0.35
IRF V4	93 (26.3)	34 (31.2)	59 (24.7)	*OR 1.4*	**53 (40.1)**	40 (18.6)	***OR 2.9***
				(0.8–2.3)			***(1.8–4.8)***
				0.24			**<0.0001**
ETDRS 12 (letters)	63.3 ± 16.6	61.3 ± 17.2	64.0 ± 16.4	0.16	**56.1 ± 19.9**	67.5 ± 12.4	**<0.0001**
ETDRS 36 (letters)	58.5 ± 20.7	56.1 ± 20.5	59.5 ± 20.8	0.17	**48.3 ± 24.0**	64.7 ± 15.5	**<0.001**
Injections 36	13.8 ± 5.3	13.1 ± 4.5	14.3 ± 5.4	0.56	13.1 ± 4.7	14.5 ± 5.4	0.42

T student was performed to evaluate statistically significant differences ($p < 0.05$). OR values are shown in italics and $p < 0.05$ values are highlighted in bold.

OR values are shown in italics and $p < 0.05$ values are highlited in bold.

12 = 12-month visit, 36 = 36-month visit, 95% CI = 95% confidence interval, A = atrophy, CST = central subfield thickness, F = fibrosis, IRF = intraretinal fluid, mixed = combination of MNV type 1 and 2, MNV 1 = macular neovascularization type 1, MNV 2 = macular neovascularization type 2, N = number of patients, OR = odds ratio, SRF = subretinal fluid, V1 = baseline visit, V4 = visit after loading phase.

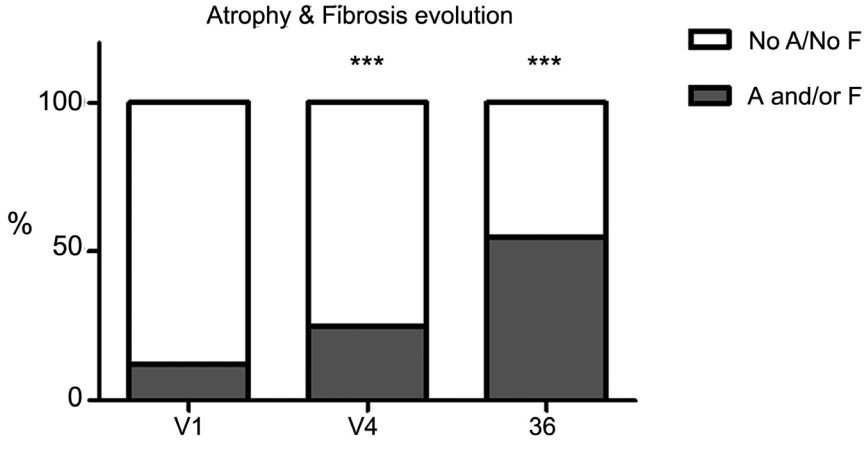

Fig. 1. Evolution of patients with atrophy and/or fibrosis compared to those who do not develop any of these characteristics. V1: baseline visit; V4: visit after loading phase; 36: 36-month visit; A: atrophy; F: fibrosis. Pearson Chi-square test was performed to evaluate statistically significant differences ($p < 0.05$); ***$p < 0.001$.

rate), based on the presence, compared with the absence, of each type of retinal fluid. Since patients may present with a combination of both fluids, we wanted to evaluate the predominance of the effect of each during follow-up and have larger groups for evaluation.

Patients with IRF at baseline had a higher prevalence of atrophy at 36 months than those without IRF (OR = 1.87, 95% CI = 1.09–3.2, p = 0.021) (Fig. 4A). The persistence of IRF at V4 and 36 months did not show statistically significant differences related to the progression of atrophy (Fig. 4B–G). Meanwhile, the patients with basal SRF had a lower prevalence of atrophy at 36 months (OR = 0.4, 95% CI = 0.23–0.71, p = 0.002) (Fig. 4H), less frequent progression of atrophy (OR = 0.44, 95% CI = 0.26–0.75, p = 0.002) (Fig. 4K), and slower growth in the long term (OR = 0.34, 95% CI = 0.14–0.83, p = 0.016) (Fig. 4N) than those without basal SRF. Furthermore, patients with SRF at V4 showed less frequent atrophy

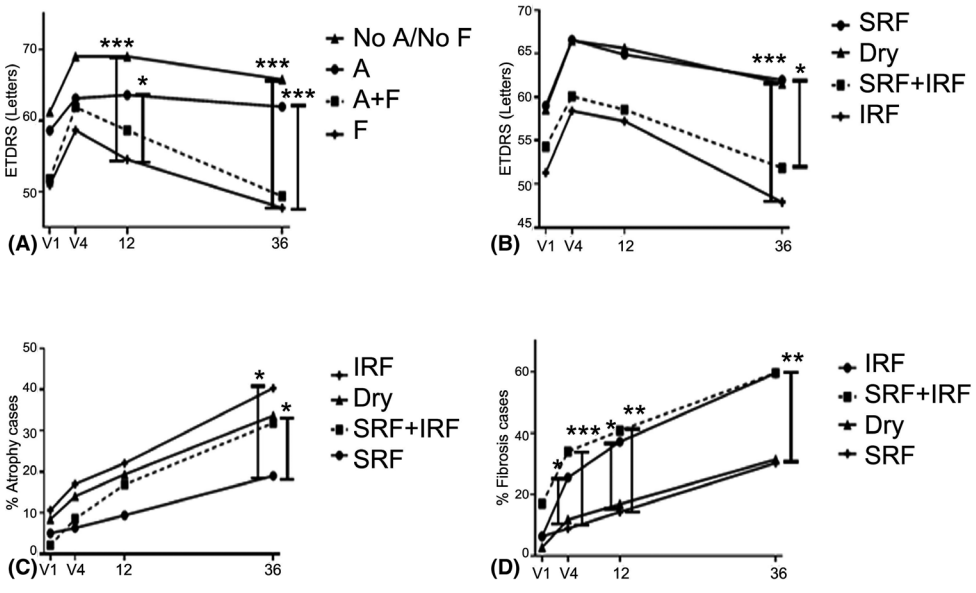

Fig. 2. (A) Evolution of ETDRS at each visit according to presence of atrophy and/or fibrosis at 36 months. (B) Evolution of ETDRS at each visit according to presence and location of retinal fluid at V4. (C) Distribution of patients with atrophy at each visit according to presence and location of retinal fluid at V4. (D) Distribution of patients with fibrosis at each visit according to presence and location of retinal fluid at V4. IRF = intraretinal fluid, SRF = subretinal fluid, V1 = baseline visit, V4 = visit after loading phase, 12 = 12-month visit, 36 = 36-month visit, F = fibrosis, A = atrophy. Two way ANOVA and Pearson Chi-square or Fisher exact test were performed to evaluate statistically significant differences ($p < 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

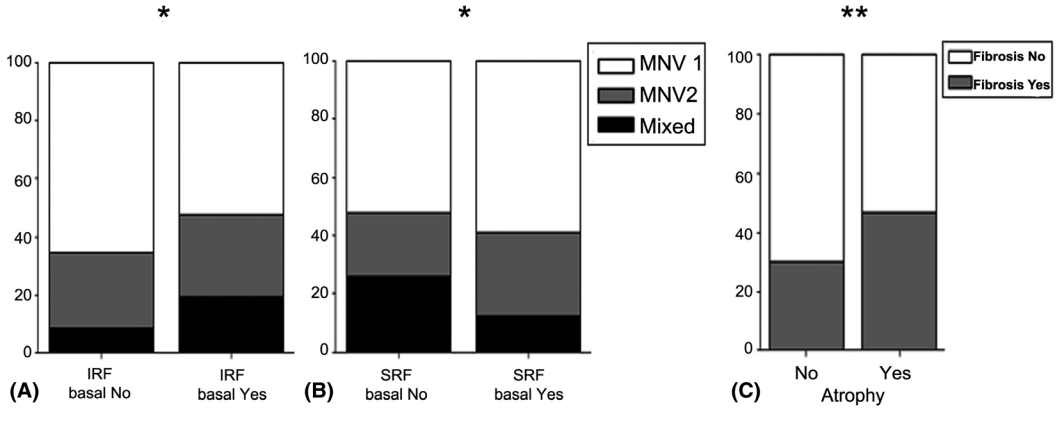

Fig. 3. (A) relationship between intraretinal fluid at baseline and type of macular neovascularization. (B) relationship between subretinal fluid at baseline and type of macular neovascularization. (C) relationship between atrophy and fibrosis at 36 months. IRF = intraretinal fluid, SRF = subretinal fluid, MNV 1 = macular neovascularization type 1, MNV 2 = macular neovascularization type 2, mixed = combination of MNV type 1 and 2. Pearson Chi-square or Fisher exact test were performed to evaluate statistically significant differences ($p < 0.05$); *$p < 0.05$; **$p < 0.01$.

progression (OR = 0.56, 95% CI = 0.34–0.94, p = 0.026) (Fig. 4L). Finally, the patients with a 36-month persistence of SRF showed a lower prevalence (OR = 0.37, 95% CI = 0.21–0.66, $p < 0.001$) (Fig. 4J) and less frequent progression of atrophy (OR = 0.38, 95% CI = 0.22–0.65, $p < 0.001$) (Fig. 4M).

There was no statistically significant difference in the mean number of intravitreal injections received by patients who developed atrophy and those who did not (Table 2).

Some of these results were supported by the outcomes of the multivariate logistic regression analysis. Patients with IRF at V4 were more likely to develop atrophy, whereas those with SRF during any visit showed half the risk of developing it. In addition, atrophy at 36 months was more prevalent in patients with fibrosis (Table 3).

Subretinal fibrosis

The presence of IRF during any visit was strongly associated with the development of subretinal fibrosis (baseline, OR = 2.23, 95% CI = 1.36–3.65, p = 0.001; V4, OR = 2.66, 95% CI = 1.59–4.46, $p < 0.001$; 36 months, OR = 2.49, 95% CI = 1.53–4.04, $p < 0.001$) and the progression of fibrosis (baseline, OR = 2.29, 95% CI = 1.43–3.69, $p < 0.001$; V4, OR = 2.82, 95% CI = 1.73–4.62, $p < 0.001$; 36 months,

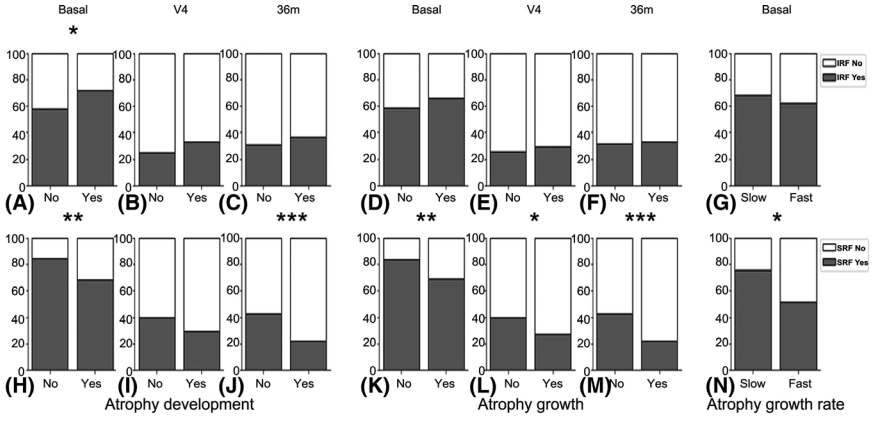

Fig. 4. Association between IRF (upper graphs) and SRF (lower graphs), at each visit, with the appearance of 36-month atrophy in nAMD patients (A–C, H–J), its progression (D–F, K–M) and its growth rate with respect to basal IRF (G) and basal SRF (N). IRF = intraretinal fluid, SRF = subretinal fluid, V4 = visit after loading phase, 36m = 36-month visit. Pearson Chi-square or Fisher exact test were performed to evaluate statistically significant differences ($p < 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 3. Multivariate logistic regression for the development of atrophy and fibrosis was performed to evaluate statistically significant differences ($p < 0.05$)

	ATROPHY 36					
	Coefficient	Standard error	p	Odds ratio	95% confidence interval	
Constant V1	*−0.706*	*0.310*	*0.023*			
Fibrosis 36	0.682	0.283	0.016	1.978	1.136	3.445
SRF V1	−0.899	0.320	0.005	0.407	0.217	0.762
Constant V4	*−1.164*	*0.199*	*0.000*			
IRF V4	0.508	0.296	0.086	1.662	0.931	2.966
SRF V4	−0.623	0.292	0.033	0.536	0.303	0.950
	FIBROSIS 36					
Constant V1	*0.213*	*0.521*	*0.683*			
IRF V1	0.742	0.271	0.006	2.099	1.234	3.570
ETDRS V1	−0.024	0.008	0.002	0.976	0.961	0.991
Constant V4	*0.580*	*0.463*	*0.210*			
IRF V4	0.797	0.274	0.004	2.221	1.297	3.799

36 = 36-month visit, IRF = intraretinal fluid, SRF = subretinal fluid, V1 = baseline visit, V4 = visit after loading phase.
Constant values are shown in italics.

OR = 2.46, 95% CI = 1.54–3.92, $p < 0.001$) (Fig. 5A–F).

In contrast, patients with SRF at 36 months had a lower prevalence (OR = 0.49, 95% CI = 0.29–0.81, $p = 0.005$) and progression (OR = 0.5, 95% CI = 0.31–0.81, $p = 0.005$) of fibrosis (Fig. 5J and M, respectively).

There was no statistically significant difference in the mean number of intravitreal injections received by patients who developed fibrosis and those who did not or those who developed atrophy and fibrosis (Table 2).

On the other hand, patients with fibrosis at 36 months also had a higher prevalence of atrophy (OR = 1.66, 95% CI = 1.02–2.71, $p = 0.04$) (Fig. 3C).

Multivariate logistic regression analysis showed that patients with IRF during any visit had twice the risk of developing fibrosis. In addition, patients with better ETDRS from baseline showed a lower risk of fibrosis development than those with worse baseline ETDRS (Table 3).

Discussion

In this study, we found that atrophy and/or fibrosis were present in 1 of 2 individuals (54.8% of patients develop one or both) after 36 months of follow-up based on the evaluation and treatment criteria for nAMD used in routine clinical practice. Both conditions explain the poor average gain of +1.5 letters at 36 months, whereas the average gain was +7.6 letters after the loading phase and +6.2 letters after 12 months. Other studies reporting vision gains maintained for 4 or more years with adequate anti-VEGF intravitreal injections frequency, showed atrophy rates of 20–30% at 2 years (CATT and IVAN) and 100% at 7 years (SEVEN-UP) (Monés et al. 2020).

Furthermore, we found statistically significant differences between the anatomical and functional results depending on the presence and location of the retinal fluid and the presence of atrophy and fibrosis. Atrophy development was associated with basal IRF, whereas SRF during any visit decreased the risk of atrophy development and progression; basal SRF led to a slow progression. Fibrosis development and progression were related to IRF at any visit. However, the 36-month SRF decreased the risk of fibrosis development and progression (Fig. 6).

Our AMD group had already shown in a previous randomised clinical trial involving a year of follow-up that retinal fluid location (at baseline and after the loading phase of anti-VEGF treatment) could be associated with the development and progression of atrophy and/or fibrosis (Saenz-De-Viteri et al. 2021). The In-Eye study reported that IRF (at baseline and V4) was associated with a greater risk of fibrosis at 12 months. On the contrary, baseline SRF reduced the risk of this adverse anatomical outcome. Interestingly, neither IRF nor SRF had a significant effect on the risk of developing atrophy throughout the study.

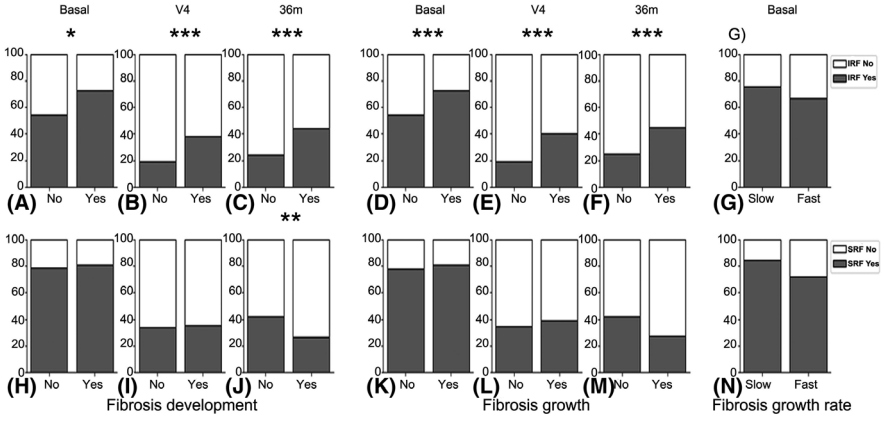

Fig. 5. Relationship between IRF (upper graphs) and SRF (lower graphs) at each visit, with the appearance of 36-month fibrosis in patients with nAMD (A–C, H–J), its progression (D–F, K–M) and growth rate with respect to baseline IRF (G) and SRF (N). IRF = intraretinal fluid, SRF = subretinal fluid, V4 = visit after loading phase, 36m = 36-month visit. Pearson Chi-square or Fisher exact test were performed to evaluate statistically significant differences; **p < 0.01; ***p < 0.001.

Considering these results, we decided to design a more ambitious study, which would be based on routine clinical practice (often far from the strict conditions of clinical trials) with a longer follow-up period, to determine the retinal fluid location and the effect of certain clinical characteristics on the development and progression of macular atrophy or subretinal fibrosis.

It may be obvious that the development of advanced forms of AMD leads to worse VA in the long term, and several studies have considered VA changes from baseline as prognostic and indicative of treatment efficacy (loss or gain of letters on the ETDRS scale) (Regillo et al. 2015). However, our patients with worse ETDRS scores from the beginning most frequently developed atrophy or fibrosis after 3 years, especially fibrosis probably associated with the presence of IRF (Fig. 2A,B and 6).

Usually, the presence of any type of fluid on OCT is considered reflective of disease activity, and it may discourage the extension of treatment and visit intervals in a T&E regimen or advocate for a new PRN anti-VEGF intravitreal injection. However, recent clinical trials have shown that a significant number of patients have persistent retinal fluid, even after very frequent anti-VEGF injections (53% in the CATT trial and 39.6% in the VIEW studies) (Schmidt-Erfurth et al. 2014), and there is not always a correlation between fluid and visual outcomes. Some authors have found that up to 17% of eyes have an episode of vision loss with no associated fluid on OCT, and 38% of visits with stable vision or better have IRF or SRF on OCT (Wickremasinghe et al. 2016).

In the same way, the presence of fluid (basal or persistent) has been considered a valuable prognostic factor. In our study, SRF halved the risk of developing atrophy (basal and 36-month SRF) or fibrosis (36-month SRF) and the risk of the progression of atrophy and fibrosis (SRF at any visit for atrophy and 36-month SRF for fibrosis), with a slower atrophy growth rate (basal SRF): SRF could therefore be considered a nondeleterious factor for both conditions. However, IRF increased twice the risk of developing atrophy (basal IRF) and the risks of the development and progression of fibrosis (IRF at any visit), with a predominant effect even in the presence of SRF (Fig. 2C,D and 6). Furthermore, our study showed a good anatomical–functional correlation, since patients with SRF at V4 showed significantly better functional progression (similar to dry macula at V4), whereas those with IRF at V4 had significantly worse visual outcomes (Fig. 2B). In addition, patients with atrophy and/or fibrosis showed worse visual outcomes than those without, with a particularly significant loss of letters for fibrosis (Fig. 2A and 6).

Similar findings have been described by other authors. Intraretinal fluid (IRF) leads to poorer anatomical and functional outcomes, whereas SRF is associated with a better visual acuity and a lower risk of developing macular atrophy or fibrosis (Ashraf, Souka & Adelman 2018; Ying et al. 2018; Lai et al. 2019). A recently published study confirmed these observations and affirmed that it is possible to achieve good visual outcomes despite the presence of SRF and with fewer injections, with the consequent reduction in injection risk for patients and greater savings for the health system, from the reduced visits and treatment modifications (Guymer et al. 2019).

The exact effect of retinal fluid on the development of macular atrophy or fibrosis is unknown. In our study, SRF (more frequent in type 1 MNV) was associated with lower risks of the development and progression of atrophy and a slower growth rate. Some studies have linked a lower risk of atrophy to SRF, which could be explained by its protective effect or neuroprotective factors that facilitate access to nutrients by photoreceptors (Sadda et al. 2018; Jaffe et al. 2019). Subretinal fluid (SRF) at 36 months also reduces the risks of development and progression of fibrosis in our patients. This has been indirectly suggested in some studies that observed that classic choroidal neovascularization lesions are associated with a higher risk of developing subretinal fibrosis than occult lesions. A possible interpretation is that MNV types that predominantly present with IRF, such as type 2 and mixed, are associated with a greater risk of developing fibrosis than those that predominantly present with SRF, such as type 1 MNV (Ishikawa, Kannan & Hinton 2016). In our

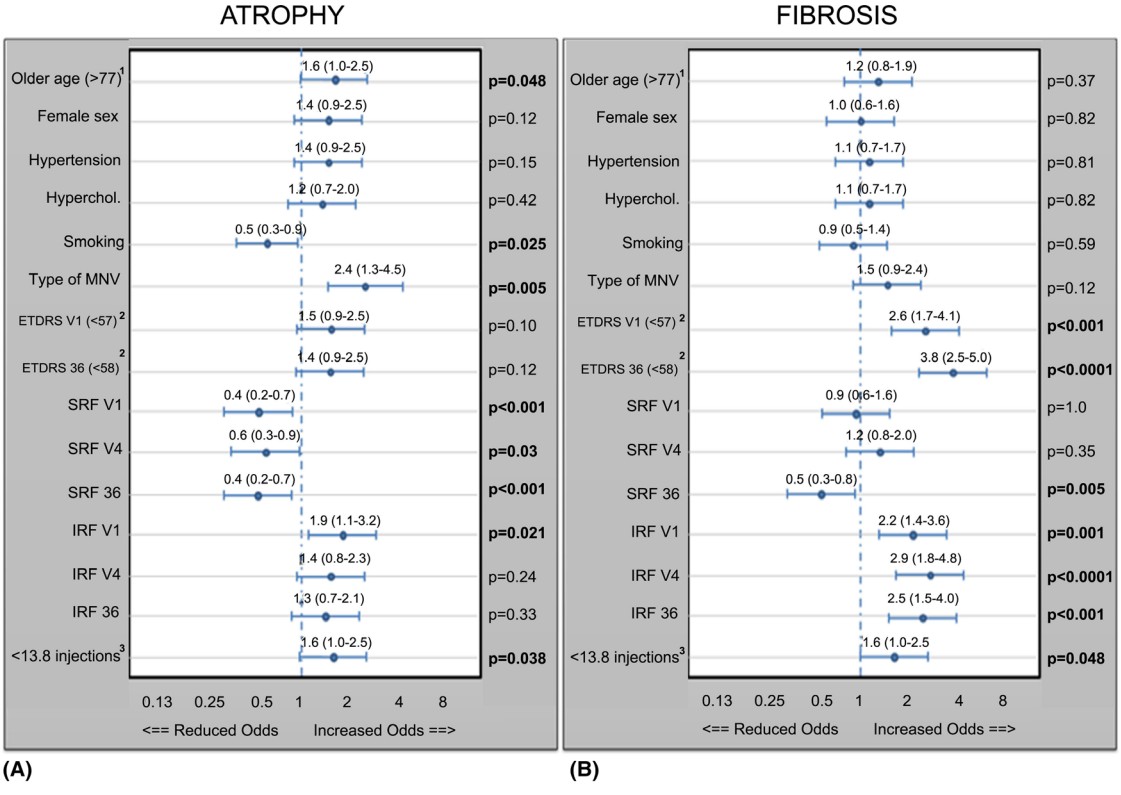

Fig. 6. Relationship between risk factors and the presence of atrophy (A) and fibrosis (B) at 36 months. Age was measured in years at the baseline visit. Visual acuity was measured in letters, according to the Early Treatment Diabetic Retinopathy Study (ETDRS) scale. Patients were separated above and below the mean to calculate the odds ratio for age1, ETDRS2 and number of intravitreal injections3 received. Hyperchol = hypercholesterolemia; MNV = macular neovascularization; SRF = subretinal fluid; IRF = intraretinal fluid. Fisher exact test was performed to evaluate statistically significant differences ($p < 0.05$).

sample, IRF was fundamentally associated with mixed MNV, greater risks of the development and progression of fibrosis, and, less significantly, an increased risk of the development of atrophy. Previous studies have reported similar outcomes (Gianniou et al. 2015; Sharma et al. 2016).

Furthermore, we found that the risk of developing either of the two unfavourable anatomical changes (atrophy or fibrosis) doubles the risk of developing the other. This association has been recently described in an article that highlights the association of fibrosis with RPE atrophy, lower perfusion and greater functional disability. These findings suggest that the maturation of vessels in fibrosis may be a better therapeutic target than their complete abolishment (Querques et al. 2020).

We consider that most patients received fairly adequate treatment, which were slightly fewer in those who develop atrophy and fibrosis (13.8 injections on average), considering that the optimum would have been >15 anti-VEGF injections over 36 months, and that most studies describe an average of 1.6–6.1 injections per year (Monés et al. 2020). It has been considered that undertreatment may promote the progression of these unfavourable anatomical outcomes (Bhisitkul et al. 2015; Monés et al. 2020). However, as this is a real-world study and not a clinical trial, it is also possible that this slight undertreatment with anti-VEGF is not the cause but the consequence of the progression of atrophy and fibrosis; patients who develop extensive foveal atrophy or fibrosis will probably not be candidates for further treatment because they will not obtain any visual benefit.

Interestingly, smoking appeared to be a protective factor for the development of atrophy. This may be attributed to the higher tolerance of smokers to hypoxia, which may be responsible for the development of atrophy and the more severe stages of other macular pathologies, such as those associated with pathological myopia, including myopic choroidal neovascularization (Stone et al. 2001; Bilbao-Malavé et al. 2020).

Although our results are in agreement with others; it should be noted that this is an ambispective study, with limitations including the lack of information on recruitment time and environmental and clinical risk factors and the reduced number of patients after separating them into groups for statistical analysis. Nevertheless, we have designed an interesting study with long-term follow-up, in real-world conditions, of patients with a prevalent and disabling pathology such as nAMD. In addition, we have identified possible clinical and imaging biomarkers that can be used in future prospective studies with a greater number of patients.

In conclusion, this study shows that macular atrophy or subretinal fibrosis is present in 1 of every 2 patients with nAMD treated with anti-VEGF injections in the real world for three years. They are largely responsible for the poor visual acuity of these patients in the long term, especially in the case of fibrosis. In addition, SRF is associated with good visual and anatomical

outcomes, whereas IRF is associated with worse visual outcomes and a higher risk of macular atrophy and, especially, subretinal fibrosis in nAMD patients treated according to the routine clinical practice guidelines. Based on these data, retinal fluid accumulation should be taken into account as a reflection of nAMD activity. Therefore, we should be especially incisive in the treatment of IRF, but we may be more tolerant with SRF, being able to maintain or increase the interval of treatment in T&E regimen, due to its possible protective effect against long-term atrophy and fibrosis. In conclusion, we must guide nAMD treatments towards reducing the development and progression of atrophy and fibrosis, since they are the most frequent factors limiting the visual capacity of patients.

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Appendix 1

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