

## Intraindividual Assessment of Retinal Pigment Epithelial Atrophy in Eyes with Bilateral Involvement Due To Exudative Age-Related Macular Degeneration and Treated By Anti –Vascular Growth Factor (Aflibercept and Ranibizumab)

Nathalie Massamba MD<sup>1,2\*</sup>, Gisele Soubrane PhD<sup>3</sup>, Emmanuelle Champion MD<sup>1</sup>, Nathalie Butel MD<sup>1</sup>,  
Violaine Caillaux MD<sup>1</sup> and Bahram Bodaghi MD, PhD<sup>1</sup>

<sup>1</sup>PITIE SALPETRIERE Hospital, University of Pierre Marie Curie; DHU: Handicaps and Vision, Paris, France.

<sup>2</sup>Department of ophthalmology, Moran Eye Center, University of Utah. UTAH. USA.

<sup>3</sup>INSERM, Paris, France.

### \*Correspondence:

Nathalie Massamba, Department of Ophthalmology, Moran Eye Center, University of UTAH, USA, Tel: 801 205 9989; E-mail: nathalie.massamba@gmail.com.

**Received:** 04 December 2018; **Accepted:** 30 January 2019

**Citation:** Nathalie Massamba, Gisele Soubrane, Emmanuel Champion, et al. Intraindividual Assessment of Retinal Pigment Epithelial Atrophy in Eyes with Bilateral Involvement Due To Exudative Age-Related Macular Degeneration and Treated By Anti –Vascular Growth Factor (Aflibercept and Ranibizumab). Ophthalmol Res. 2019; 1(2): 1-9.

### ABSTRACT

**Purpose:** The aim of this study was to evaluate intra individual the size of atrophy in both eyes and compare progression with the fellow eye which developed a CNV.

**Design:** Retrospective case series

**Material and Methods:** This study is a retrospective consecutive case series of 110 eyes undergoing exudative and non-exudative AMD. Overall two groups were identified. The first group included 55 eyes that presented with Geographic atrophy (GA) and Choroidal Neovascularization (CNV) during the follow-up. The second group consisted of the fellow eye of the same patient which presented with GA only. The study was conducted between January 2016 to June 2017 at Department of Ophthalmology, PITIE SALPETRIERE University Hospital.

Group A. EYE with GA

Group B. Eye with GA associated with CNV and received Anti VEGF therapy.

In Group B, was divided into two group

B1: patients who received Ranibizumab

B2: patients who received Aflibercept

GA areas were quantified based on fundus auto fluorescence images using manually image-processing and progression. The hyper auto florescence border was also analyzing.

**Results:** GA was observed in 55/100 patients, 18 eyes of 9 patients were excluded. Overall we analyzed 92 eyes of 46 patients. 46 eyes of 46 patients had GA, the fellow eyes of 46 eyes remained with atrophy associated with GA at baseline, and which developed CNV during the follow -up, treated by anti-VEGF treatment. Mean follow-up after initiation of anti-VEGF therapy was 12.8 months. Mean age 76 year- old for 26 females and 20 males (SD=0.06). Mean VA at baseline was 0.6 Log Mar vs 0.6 Log Mar at the end of the study. Central Macular thickness was 350.50 um at baseline which was reduced to 260 um at M12 (SD =91). The mean size of atrophy for both Anti VEGF group was 5.2 mm<sup>2</sup> at baseline, 7.4 mm<sup>2</sup> at M6 and 8.7 mm<sup>2</sup> at the end of the study specially from 5.4 mm<sup>2</sup> at baseline to 7.1 mm<sup>2</sup> at the end of the study for Ranibizumab group and 5.2 mm<sup>2</sup> to 8.2 mm<sup>2</sup> in Aflibercept group. Overall, the size of atrophy increased from 5.2 mm<sup>2</sup> to 8.79 mm<sup>2</sup> for the group atrophy with CNV vs 3.28 mm<sup>2</sup> to 6.88 mm<sup>2</sup> of

eyes with GA (FIG 1). Moreover, for groups with anti-VEGF therapy, the size of geography atrophy in Ranibizumab group increased from 5.4 mm<sup>2</sup> to 7.1 mm<sup>2</sup> ( $p = 0.4$ ) (FIG 2), while eyes treated with Aflibercept increased from 5.2 mm<sup>2</sup> to 8.2 mm<sup>2</sup> after 12 months ( $p = 0.04$ ) (Figure 3).

**Conclusion:** Previously publications have confirmed the evolution or presence of atrophy in patients treated by Anti VEGF. This cases series has confirmed these findings. Furthermore, to our knowledge, there is currently no intra-individual study comparing atrophy, because this situation is very infrequent. Our results showed that atrophy evolves in a progressively comparatively with which found in patients treated with Anti VEGF especially Aflibercept. We believed that a multicenter study with genetic analysis could still shed light on this subject.

## Keywords

Age-related macular degeneration, Geography atrophy, Choroidal neovascularization, Fundus auto florescence, Optical coherence tomography, Aflibercept, Ranibizumab.

## Introduction

Age-related macular degeneration (AMD), is a leading cause of visual impairment over 65 years of age, and is also the major cause of blindness in people. Clinically, AMD is divided into two major types, which are the non-neovascular or dry type and the more severe neovascular or wet exudative type. Choroidal neovascularization (CNV) is a common feature of neovascular AMD, however, the wet type of AMD with CNV is the major cause of severe loss of vision [1,2].

Over the past few years, the use of anti-VEGF compounds in treatment of wet AMD has become a standard practice. The current accepted anti-VEGF agents include bevacizumab (Avastin<sup>®</sup>; Genentech, San Francisco, CA, USA), ranibizumab (Lucentis<sup>®</sup>; Novartis, Basel, Switzerland; and Genentech Inc., South San Francisco, CA), pegaptanib sodium (Macugen<sup>®</sup>; Eyetech Pharmaceuticals/Pfizer, NY), and aflibercept (VEGF Trap-Eye, Eylea<sup>®</sup>, Regeneron Pharmaceuticals, Inc., Tarrytown, NY, USA and Bayer, Basel, Switzerland). Aflibercept was released most recently and was approved for the treatment of wet AMD by the United States Food and Drug Administration (FDA) in November 2011 and the European Medicines Agency (EMA) in November 2012. The recommended dose of aflibercept is 2 mg (0.05 ml), administered by intravitreal injection and the recommended treatment regimen is by induction, with administration of 2 mg every 4 weeks (monthly), for 3 months followed by administration every 8 weeks (2 months), thereafter (Eylea [package insert]. Tarrytown, NY: Regeneron Pharmaceuticals, Inc; 2011. Available at: [http:// www.regeneron.com/Eylea/eylea-fpi.pdf](http://www.regeneron.com/Eylea/eylea-fpi.pdf). Accessed December 1, 2014). [3].

## RPE dysfunction

Impairment of RPE cell function, is an early and crucial event in the development of AMD. A driving force of RPE dysfunction is an age-dependent, phagocytic and metabolic insufficiency of postmitotic RPE cells. These RPE cell deficiencies lead to progressive accumulation of lipofuscin granules, composed of mostly lipids and proteins [4]. Lipofuscin accumulation imposes an ever-increasing burden on RPE cell function. Increased lipofuscin in the RPE cells can lead to apoptosis, resulting in accumulation of debris on and within Bruch's membrane, and an

eventual loss of photoreceptor function. Accumulated lipofuscin absorbs light, which damages the RPE and attracts macrophages, thereby evoking a local autoimmune response that leads to chronic inflammation [5]. Products of lipofuscin photo-oxidation in RPE cells also serve as triggers for complementary activation, further contributing to the chronic localized inflammation [6].

A possible association between the buildup of lipofuscin in RPE cells and drusen formation may exist, but such a relationship has not been firmly established [6]. (Drusen and age-related changes that occur in the vicinity of Bruch's membrane, may lead to dysfunction and/or degeneration of the RPE and retina by inducing ischemia and/or restricting the exchange of nutrients and waste products between the neural retina and choroid. Drusen themselves may have a detrimental effect on vision, particularly with respect to contrast sensitivity (Mullins et al. 2000). The presence of inflammatory constituents within drusen, such as complement activators, activation-specific complement fragments, and membrane attack complex, may contribute to local inflammation [7-8].

VEGF-A is a primary driver of angiogenesis and plays a critical role in the pathogenesis of neovascular AMD through its effects on angiogenesis and vascular permeability [9]. VEGF-A expression is increased in pigment epithelial cells during the early stages of AMD (Kliffen et al. 1997), [10], suggesting that VEGF-A may play a role in the initiation of neovascularization rather than being secondary to it [11]. High concentrations of VEGF-A have been observed in excised choroidal neovascular membranes from AMD patients [12], and increased retinal and vitreous VEGF-A levels are found in both animals and humans with ischemic retinopathies, induced by oxidative damage or hypoxia [13]. Thus, VEGF has become a frequent target for the development of new treatments for ocular neovascular [11].

Several anti-VEGF-A therapies for AMD are available and others are currently in development (Kaiser 2006a). Current treatment guidelines discuss options for anti-VEGF-A therapy, including pegaptanib, ranibizumab, and off-label bevacizumab. Although bevacizumab is not approved by the FDA for use in neovascular AMD, the AAO Retina Panel does present the drug as an optional therapy for neovascular AMD, with the caveat that the treating physician should provide appropriate informed consent with respect to the off-label status of bevacizumab (AAO 2006).

Ranibizumab, an effective ocular antiangiogenic therapy: local

deliverability into the eye, ability to penetrate through the retina to the sub-RPE space, high affinity for its target (all forms of VEGF-A), are safety and tolerability with minimal systemic exposure. In order to achieve these characteristics for a neovascular AMD treatment, a humanized anti-VEGF-A Fab was created. Ranibizumab was generated after several rounds of molecular modification that significantly increased binding affinity for its target, VEGF-A. Because of the location on VEGF-A to which ranibizumab binds, it has the ability to bind all VEGF-A forms with high affinity and inhibit their biologic activities (Genentech, Inc. unpublished data).

The safety and efficacy of aflibercept, have been demonstrated by two clinical trials known as VIEW 1 and VIEW 2 (VEGF Trap-Eye: Investigation of efficacy and safety in wet AMD [14]. The VIEW 1 and VIEW 2 studies were pivotal phase 3, randomized, multicenter studies, comparing the efficacy of aflibercept and ranibizumab in patients with wet AMD. VIEW 1 was conducted in the United States and Canada, while VIEW 2 was performed in the European Union, Asia-Pacific region, Japan, and Latin America. The VIEW study regimen for aflibercept, consisted of 3 injections at monthly intervals followed by one of the three different regimens (0.5 mg every 4 weeks, 2 mg every 4 weeks, or 2 mg every 8 weeks) [14].

Over the past few years the VEGF has experienced a lot of gained with the use of anti-VEGF drugs, possible side effects and complications of these therapeutic agents have been recognized including cataract, retinal detachment, endophthalmitis, retinal pigment epithelial (RPE) tear, and others., atrophy and fibrosis [15-16-17-10]. To date, it remains unclear whether; treatment with anti-VEGF could increase the risk of thromboembolic event [11] as recent work under taken by Saint-Geniez et al. [18] demonstrated, that in mice, the absence of soluble VEGF isoforms led to the development of focal choroidal atrophy and RPE attenuation and loss which, on their turn, culminated in photo receptor cell death and reduced Visual function.

The aim of this study is to evaluate the presence of atrophy in the treated eye as well as in the fellow eye, both with atrophy and to correlate these findings either with Ranibizumab, Aflibercept or to a natural history.

## Methods

### Design and settings

The present study is a retrospective non-comparative case series of consecutive patients with ex AMD and nAMD and were conducted between January 2016 to June 2017, patients were recruited from the Department of Ophthalmology, Groupe Hospitalier PITIE SALPETRIERE (Patients were included if they had been previously treated with Ranibizumab or Aflibercept).

The protocol of treatment for both Anti VEGF was three injections at the beginning, followed by the PRN protocol for both, the decision to treat based on the persistence of exudative signs on Spectral- Domain Optical Coherence Tomography (SD-OCT), and Fluorescein Angiography if needed by the retinal physicians.

The initial protocol used in our routine practice was on pro re nata basis. We used the PrONTO (study) [19], an open-label prospective, single-center, non-randomised clinical study. It's protocol comprised three initial monthly injections, followed by an as-needed (PRN) decision to retreat, or not based on the evolution of VA and the presence or absence of subfoveal fluid as observed by optical coherence tomography (OCT).

The choice of treatment drug was depending on the physician-patient was switched from one drug to another.

### Inclusion/Exclusion

Inclusion criteria were nAMD confirmed by fluorescein angiography and SD-OCT, treated with ranibizumab or aflibercept. Patients were included irrespective of nAMD subtype, including CNV classic CNV, occult CNV mixed with polypoidal choroidal vasculopathy (PCV) and retinal angiomatous proliferation (RAP) lesions, Presence of atrophy or fibro atrophic lesions. Presence of atrophy in the contralateral eye.

Exclusion criteria were age younger than 50 years, other causes associated with choroidal neovascularization (CNV) such as inflammation CNV, pathologic myopia, angioid streaks, and ocular manifestations. The absence of atrophy in the contralateral eye, with follow-up less than 12 months. Presence of only fibrosis lesions.

### Methods

A written informed consent according to the tenets of the Declaration of Helsinki was obtained from each patient enrolled. French Society of Ophthalmology Ethics Committee approval was obtained for this retrospective review of data.

According to the usual protocol of our department, after the initial treatment for every subsequent month, patients underwent an assessment of BCVA using ETDRS charts and ophthalmic examination, which included slit-lamp bio microscopy, fundus bio microscopy, and SD-OCT examination with Fundus Auto Fluorescein. Fluorescein Angiography was performed.

The Region Finder SPECTRALIS , semi automatic quantification of the area of GA based on blue laser autofluorescence imaging was used , also ,in order to avoid the biais , we had determined manually the extent of GA with the Spectral -Domain Optical coherence tomography ( SD-OCT) because, Region Finder usually takes into account the surface of fibrosis. The prevalence of GA in both eyes at baseline, M6 and M12, was first qualitatively. reviewed by two independent observers, (NM and VC) who assessed the presence and the location of GA (appearing hypo auto fluorescent on FAF) and the thinning of the retinal pigment epithelium associated with the presence of hyperreflectivity behind the retina in SD-OCT. Confluent hyper-reflectivity with sharp margins on Fundus autofluorescence, together with corresponding choroidal signal enhancement, the absence of RPE, subsidence of the OPL/Henle layer and loss of the ELM riding to some clinicians.

FAF imaging was obtained using short wavelength (488 nm); this imaging modality has been shown to provide an adequate assessment of the atrophy and the best available tool to define areas of RPE loss. [20]

SD-OCT scans from the baseline to the end of the study; points were reviewed at the same time using [Heidelberg Engineering, Inc., Heidelberg, Germany).

FAF imaging has proven particularly useful for various aspects in the context of geographic atrophy (GA), secondary to AMD due to the absence of RPE cells, and due to the loss of intrinsic LF fluorophores. In patients with GA, atrophic areas appear in dark using the FAF imaging with an excitation wavelength of 488 nm and an emission bandwidth of 500-700 nm with a high contrast between the area of atrophy and the perilesional retina that appears to be hyper auto fluorescent [21], therefore, measurements of the atrophy in both eyes were measured manually.

In case of discordance between the two graders, a third senior grader was mandated to read the images (GS).Thirteen images of 11 patients were discordant and were shared by the senior reader (GS). SD-OCT imaging of GA showed choroidal signal enhancement, owing to the loss of absorbing pigment and thinning of the outer retinal layers, including the outer nuclear layer

### Study outcomes

The primary outcome of the study was the mean change of the atrophy size after 6 months and one year of treatment in the eye treated by Anti VEGF as well as in the fellow eye.

Additional outcomes include- the mean change of atrophy related to Aflibercept, Ranibizumab or natural history at M6 and M12, the number of injections performed and the BCVA at M12 between the two drugs and the percentage of patients who gained five letters or more between M6 and M2.

### Statistical analysis

Qualitative variables were described in percentages and quantitative variables were described by their mean with their standard deviation. Comparisons of qualitative variables were performed using the chi-square or Fisher's exact tests (when  $n < 5$  in the chi-square contingency table). Comparisons of means (visual acuity and central foveal thickness), were performed using the paired t-test or with the Mann-Whitney paired two-sample test if the distribution of the variables was not normal. A value of  $p < 0.05$  was retained as significant. Statistical analyses were performed using STATA 13.0 statistic software (Stata Corp LP, College Station, Texas, USA).

The decrease of the retinal pigment epithelium and the presence of hyperreflectivity behind the retina.

### Results

One hundred and ten eyes of 55 consecutive patients who met the inclusion criteria were included in the analysis. GA was observed

in 55 /110 eyes (50%) of eyes at baseline and 55/110(5%) of eyes following treatment with Anti VEGF. During the analysis, we found 3 eyes of 3 patients were neovascularized, 4 eyes of 2 patients with GA did not partake in the imaging during the follow-up, 8 eyes of 4 patients, were tired of the treatment and stopped the follow-up. Overall, we finally excluded 18 eyes of 9 patients.

In summary, we analyzed 92 eyes of 46 patients. 46 eyes of 46 patients had GA, the fellow eyes of 46 eyes remained with atrophy associated with GA at baseline, and treated by anti-VEGF treatment. Mean follow-up after initiation of anti-VEGF therapy was 12.8 months. Mean age 76 year- old for 26 females and 20 males. Mean VA at baseline was 0.6 Log Mar vs 0.6 Log Mar at the end of the study. Central Macular thickness was 350.50 um at baseline which was reduced to 260 um at M12.The mean size of atrophy for both Anti VEGF group was 5.2 mm<sup>2</sup> 7.4 mm<sup>2</sup> at M6 and 8.7 mm<sup>2</sup> at the end of the study. The p-value from the baseline and M6 was 0.6, M6 to M12 0.03 and the baseline to M12 p-value 0. 007. The enlargement of atrophy increased more after 6 months of Anti-VEGF therapy.

Mean Intravitreal injection of Anti VEGF was 7.0 at six months vs 4.7 at M12 for the group undergoing both, exudative and GA associated.

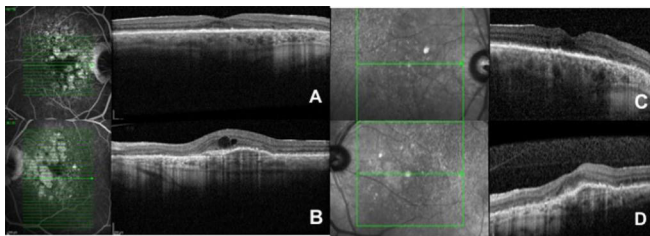
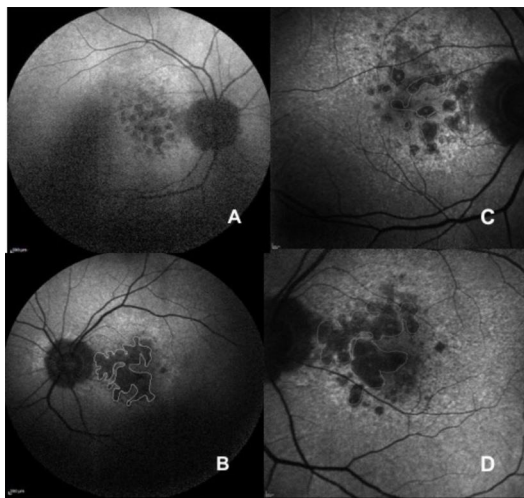
	RANIBIZUMAB			AFLIBERCEPT		
	Baseline	M12	Difference Baseline-M12	Baseline M12	M12	Difference Baseline-
Number of Eyes	20			26		
Mean Age	78			75		
Gender	12F/8M			14F/12M		

**Table 1:** Demographic and clinical characteristics of patients undergoing Mixed AMD and treated by Ranibizumab vs Aflibercept.

### Discussion

We reviewed the charts of all consecutive patients presented for follow-up examination between January 2016 and June 2017 at the University of PARIS VI. PITIE SALPETRIERE Hospital, who had received (anti-VEGF) injections for neovascular AMD; we evaluated these patients for the presence of geography atrophy inside the choroidal neovascularization, and also the fellow eyes which presented geography atrophy. At baseline, geography atrophy was found in both eyes. After an average of 12 months and the initiation of anti-VEGF therapy, the size of atrophy increased from 5.2 mm<sup>2</sup> to 8.79 mm<sup>2</sup> and 3.28 mm<sup>2</sup> to 6.88 mm<sup>2</sup> of eyes with GA (Figure 1). Moreover, for groups with anti-VEGF therapy, the size of geography atrophy in Ranibizumab group increased from 5.4 mm<sup>2</sup> to 7.1 mm<sup>2</sup> ( $p = 0.4$ ) (Figure 2), while eyes treated with Aflibercept increased from 5.2 mm<sup>2</sup> to 8.2 mm<sup>2</sup> after 12 months ( $p = 0.04$ ) (Figure 3).

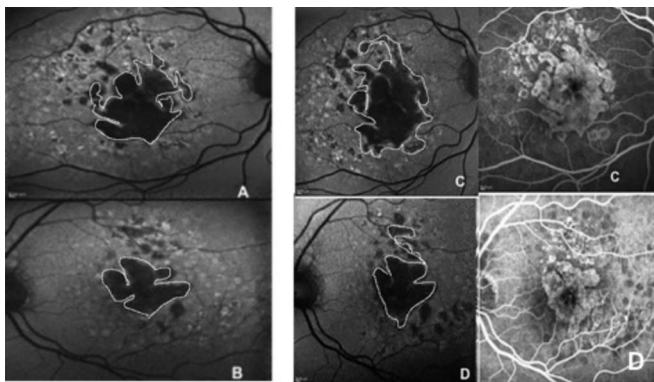
FAF and OCT images seem to be adequate in the evaluation of patients with exudative AMD [22].



**Figure 1:** A 75 years, old, male patient presented in 2015 a GA with a VA 20/400 RE and 20/50 LE.

The FAF and SD-OCT images showed the presence of atrophy well defined with by the hyper auto fluorescence on the lesion 's border showing atrophic evolution.

The size of GA at baseline was in FAF was 4.72 and 6.44 mm<sup>2</sup> after 15 months of follow up (Image A and B), the VA decrease from for RE from 20/400 to luminous perception at 1 m, and 20/80 for the LE. The FAF in images C and D increased to 6.72 mm<sup>2</sup> (2.02 mm<sup>2</sup>/year) for the RE and 6.57 (0.13 mm<sup>2</sup>) for the LE.

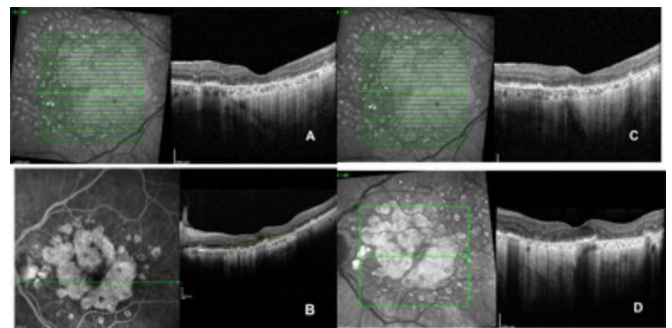
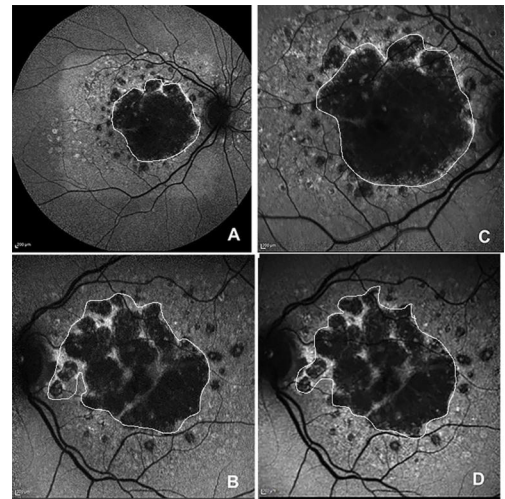


**Figure 2:** A 71 -year-old male patient presented with bilateral atrophy and developed a CNV type I, extra fovea localization in his left eye. VA at baseline was 20/125 LE and 20/400 RE.

The size of atrophy on the RE was estimated to 0.94 mm<sup>2</sup> vs 0.56 mm<sup>2</sup> on the LE (images A and B). After 10 intravitreal injection of RANIBIZUMAB for 14 months, the size of atrophy lesion increased to 1.51 mm<sup>2</sup> on the RE (0.57 mm<sup>2</sup>) vs 0.99 mm<sup>2</sup> on the LE (0.46 mm<sup>2</sup>) (image C and D), the VA remained stable 20/125.

Overall, GA progression rates reported in the literature FAM study from 0.7 mm<sup>2</sup>/year. This means that the atrophy in these two cases has evolved normally.

SD-OCT for all images showed the hyper reflectivity behind the RPE, which is become thickening with an increased minimum intensity corresponded to GA.



**Figure 3:** A 68 -year-old female patient presented with bilateral atrophy and developed a CNV type I, sub fovea localization in her Left eye. VA at baseline was 20/125 vs 20/32 on the RE. The size of atrophy was 2.0 8 mm<sup>2</sup> in the RE and 2.77 mm<sup>2</sup> in the LE.

After 9 intravitreal injection of AFLIBERCEPT, the VA decrease on the RE TO 20/40 and increase on the LE to 20/63, however the size of atrophy increased from 2.08 mm<sup>2</sup> to 2.9 mm<sup>2</sup> (0.82 mm<sup>2</sup>) from the RE and from 2.08 to 6.37 mm<sup>2</sup> (4.29 mm<sup>2</sup>) for the LE.

The SD-OCT for all images 1.2 and 3 showed the hyper reflectivity behind the RPE, which is become thickening with an increased minimum intensity corresponded to GA.

Age macular-related degeneration is characterized by the degeneration of the retinal pigment epithelium (RPE), which is situated between the retinal photoreceptors and the choroidal capillaries [7]. RPE dysfunction disrupts both photoreceptors and choroidal vasculature; this tissue disruption leads to atrophy and neovascularization. Though both are subsets of advanced AMD, GA and CNV are different phenomena; GA is usually defined as a sharply circumscribed area of pigment epithelial atrophy, through which choroidal vessels can be seen, whereas CNV is defined as neovascularization under the retina, in the choroidal area. Several studies have described the natural history and progression of eyes with geographic atrophy and AMD, but only limited data are available concerning the analysis of the progression of atrophy in both eyes, simply because it is rare. This also explains the

availability of on a limited sample in this review. The mechanisms by which atrophy demise takes place under these circumstances are not clear. However, the GA associated with CNV has been described previously. Sarks et al, reported a group of 20 eyes that had CNV before the advent of anti-VEGF therapy and developed GA [23].

We also agree with the findings of the research group of CATT study for two years [24], that the vast majority of patients with GA had extra-foveal GA, this finding corroborated our data 54.5% of eyes in Ranibizumab group were extrafoveal in contrast with 60.60% in Aflibercept group at the end of the study (Table 1).

Candidate risk factors for GA included demographic characteristics, cigarette smoking, hypertension, diabetes, dietary supplement use, cancer, hypercholesteremic, were not analyzed. We analyzed the impact and the evolution of atrophy in eyes who received Ranibizumab as well as eyes treated with Aflibercept with the same regimen treatment on pro re nata (PRN) injection regimen. And we correlated these data with the fellow eye undergoing GA (Tables 2 and 3).

Ranibizumab is a highly effective treatment for neovascular age-related macular degeneration (AMD) [25]. It is an antibody fragment that binds and inhibits all identified VEGF isoforms. It has been shown to be effective in neovascular AMD and has changed the standard of care and raised treatment expectations beyond simple slowing of vision loss, as patients now expect stabilization or improvement of their vision. It is now the reference treatment for neovascular AMD. As such, two experimental studies have shown that bevacizumab and Ranibizumab injected into the vitreous tissues of rats and primates, reduces the fenestrations in the chorizo capillaries [26].

Intravitreal Aflibercept, a fusion protein of key domains from human VEGF receptors 1 and 2, with the constant region (Fc) of human immunoglobulin G, was recently approved for the treatment of neovascular AMD [27]. As a designed molecule, featuring optimal pharmacologic characteristics to inhibit intraocular VEGF, intravitreal Aflibercept injection offers improved binding affinity and superior pharmacokinetics in an iso-osmotic formulation [28-29].

The VIEW1 and VIEW2 clinical trial, demonstrated that intravitreal aflibercept groups were clinically groups were clinically equivalent to monthly ranibizumab in maintaining visual acuity at week 52. This result was true when drug was administered every 2 months, which allowed a substantially reduced monitoring and treatment frequency, thus, this study introduced a novel treatment strategy to manage neovascular AMD [28].

Recently, Erfurth S, et al. [30], identified the prevalence and progression of macular atrophy (MA), in neovascular age-related macular degeneration (AMD) patients under long-term anti-vascular endothelial growth factor (VEGF) therapy, and to determine the risk factors. Although, the authors switched from

Ranibizumab to Aflibercept in their result, they found 45% of eyes with atrophy vs 73.5% at the last follow up 5.4 ± 1.5 years.

By analyzing all these manuscripts published later, we can suggest that anti-VEGF such as Aflibercept or Ranibizumab increases the risk of atrophy, especially when it is pre-existing as it is in our series (Table 3).

The biology of neovascular AMD is better understood than the atrophic form of the disease.

Both populations, GA and CNV based on related studies have found evidence of sibling correlations with an estimation that genetic factors can account for between 55% and 57% of the total variability in the risk of disease. Recent findings [31], on single nucleotide polymorphism in genes coding for complement factors (CF) H, I, and B, and complement components 2 and 3 implicate the complement system in the pathophysiology of both intermediate and advanced AMD. Many associations and linkage studies have pointed to chromosomal locus 1q3, they are linked with the CFH gene, in conferring substantial AMD risk [32-33].

Recently, more detailed genome-wide association studies have investigated whether the 2 subtypes of advanced AMD, choroidal neovascularization (CNV), and GA, segregate separately in families and associate with different disease variants. The variants in the 10q26 locus confer increased the risk for both advanced AMD sub-types but imparts greater risk for CNV than for GA [31] the loci were detected with suggestive associations that differ for advanced AMD subtypes and deserve follow-up in additional studies.

Frank Holz G, et al. [36] found the mean progression of atrophy ranged up to 2.10 mm<sup>2</sup>, in a natural history, Sunness et al. [37] have calculated mean progression rate with 2.79 mm<sup>2</sup>per years. Those two data were not significant. Their findings confirmed our data, the progression of atrophy per year was 2.80 mm<sup>2</sup> in eyes with GA.

Thus, as described previous studies such as CATT study, GEFAL study, IVAN study [18-38-20], the progression of atrophy in patients treated by anti VEGF more monthly than PRN. We agree with all group study because we had the same result after one year.

However, by focusing on the patients with Anti VEGF therapy the increase of atrophy was 1.7 mm<sup>2</sup> in Ranibizumab group vs 3 mm<sup>2</sup> in Aflibercept group. (respectively = 0.4 and 0.04). We can assert that anti-VEGF is also risk factors in the evolution of AMD, specially Aflibercept than Ranibizumab. Our results attested also that eyes with Ranibizumab presented at the end of the study, 55 % of examined eyes presented fibro- atrophic lesion vs 45 % of only atrophy. While eyes Aflibercept presented 69.23% of atrophy vs 30.76% with fibro- atrophic lesions. These findings were analyzed and well visualized in SD-OCT.

		RANIBIZUMAB			AFLIBERCEPT		
		Baseline	M12	Difference Baseline- M12	Baseline	M12	Difference Baseline- M12
Number of eyes		20			26		
Mean age		78			75		
Gender		12F/8M			14F/12M		
Types of CNV	Type 1	68%			69.60%		
	Type 2	13.60%			12.10%		
	Rape	13.60%			12.10%		
	PCV	0			3.00%		
	Mixed	4.50%			3.00%		
Localization of CNV	Retrofoveal	54.5%	30 %		42.42%	32.6 %	
	Juxtafoveal	40.9%	15.5%		39.39%	27.15%	
	Extrafoveal	4.5%	54.5%	50%*	18.18%	40.25%	22%*
	Hyper reflectivity border on FAF	54.5%	40.9%	13.6%	42.24%	60.60%	18.4%*

\* increase of number at M12.

	46/92 Eyes with CNV related with GA treated by Anti VEGF			46/92 Eyes with GA ( Fellow Eye)		
	Baseline	M12	p value	Baseline	M12	p value
VA LogMar	0.64	0.66	0.76	0.64	0.66	0.76
CMT um	349	260	2.0	349	260	2.0
Mean number of IVT	4.78	7.0	1.0	4.78	7.0	1.0
Size of GA	5.2	8.7	0.007*	5.2	8.7	0.007*

\*=p < 0.05 It has been calculated between either baseline and M12.

		RANIBIZUMAB			AFLIBERCEPT		
		Baseline	M12	Difference Baseline- M12	Baseline	M12	Difference Baseline- M12
Number of eyes		20			26		
Mean age		78			75		
Gender		12F/8M			14F/12M		
Types of CNV	Type 1	68%			69.60%		
	Type 2	13.60%			12.10%		
	RAP	13.60%			12.10%		
	PCV	0			3.00%		
	Mixed	4.50%			3.00%		
Localization of CNV	Subfoveal	54.5%	30 %		42.42%	32.6 %	
	Juxtafoveal	40.9%	15.5%		39.39%	27.15%	
	Extrafoveal	4.5%	54.5%	50%*	18.18%	40.25%	22% *
	Hyper reflectivity border on FAF	54.5%	40.9%	13.6%	42.24%	60.60%	18.4%*

\* increase of number at M12.

	RANIBIZUMAB :20/46 eyes			AFLIBERCEPT : 26/46 eyes		
	Baseline	M12	p value	Baseline	M12	p value
VA LogMar	0.58	0.59	0.7	0.65	0.68	0.7
CMT um	368	255.9	0.5	338.5	263.2	0.8
Mean number of IVT	6.1	4.4	0.5	7.5	5.12	0.8
Size of GA mm <sup>2</sup>	5.4	7.1	0.4	5.2	8.2	0.04*

\*=p < 0.05 It hasa been calculated between either baseline and M12.

		RANIBIZUMAB			AFLIBERCEPT		
		Baseline	M12	Difference Baseline- M12	Baseline	M12	Difference Baseline- M12
Number of Eyes		20			26		
Mean Age		78			75		
Gender		12F/8M			14F/12M		
Types of CNV	Type 1	68%			69.60%		
	Type 2	13.60%			12.10%		
	RAPE	13.60%			12.10%		
	PCV	0			3.00%		
	MIXT	4.50%			3.00%		
Localization of CNV	Retrofoveal	54.5%	30 %		42.42%	32.6 %	
	Juxtafoveal	40.9%	15.5%		39.39%	27.15%	
	Extrafoveal	4.5%	54.5%	50%*	18.18%	40.25%	22% *
	Hyper reflectivity border on FAF	54.5%	40.9%	13.6%	42.24%	60.60%	18.4%*

\* increase of number at M12.

	46/92 Eyes with CNV related with GA treated by Anti VEGF			46/92 Eyes with GA ( Fellow Eye)		
	Baseline	M12	p value	Baseline	M12	p value
VA LogMar	0.64	0.66	0.76	NA	NA	
CMT um	349	260	2.0	NA	NA	
Mean number of IVT	4.78	7.0	1.0	NA	NA	
Size of GA mm <sup>2</sup>	5.2	8.7	0.007*	3.28	6.88	0.02*

\*=p < 0.05 ( It has been calculated between either baseline and M12).

We also analyzed the increase in atrophy by FAF around the atrophic patches. As these areas of increased FAF consist sometimes only of small isolated spots of some micrometers, or may be largely distributed around atrophic patches or irregularly shaped, hyperacute fluorescence.

In both cases, 18.4 % of eyes with Afibercept treatment presented a hyperreflective patch on FAF vs 13.6 % of eyes with who received Ranibizumab treatment. Our analysis suggests that Anti VEGF increase in an abnormal enlargement of atrophy, especially Anti VEGF drugs than the natural history of the progression of atrophy respectively (p = 0.07 and 0.02).

We could not confirm the exact role of this drug in the fast evolution of atrophy in patients with exudative AMD and associated geography atrophy.

Based on our study, this could not be due to the number of injection or the size of atrophy in eyes treated by two different drugs. Further studies are recommended to ensure that assessment of the enlargement of atrophy, taking into account, the risk and also the genetic analysis.

Our study had several limitations, mainly due to the retrospective nature of imaging analysis, the measurement and analysis of atrophy progression during the follow-up, the short follow up for analysis assessment. Baseline risk factors were not included and genotype analysis. Prospective investigations multicentric with a large sample, a quality of life questionnaire and a genetic analysis

are needed to improve our knowledge regarding the efficacy and safety of intravitreal

Afibercept in the abnormal enlargement of geographic atrophy (GA) in eyes undergoing anti-vascular endothelial vs fellow eyes with GA.

## References

- Gehlbach P, Li T, Hatef E. Statins for age-related macular degeneration. *Cochrane Database Syst Rev.* 2016; 8.
- Congdon N, O'Colmain B, Klaver CC, et al. Eye Diseases Prevalence Research Group; Causes and prevalence of visual impairment among adults in the United States. *Arch Ophthalmol.* 2004; 122: 477-485.
- Hiroko Okuma, Tatsuya Mimura, Mari Goto, et al. Effect of aflibercept in patients with age-related macular degeneration. *Int Ophthalmol.* 2016; 36:159-169.
- Nowak JZ. Age-related macular degeneration (AMD): pathogenesis and therapy. *Pharmacol Rep.* 2006; 58: 353-363. Review.
- Despriet DD, Klaver CC, Wittman JC, et al. Complement factor H polymorphism, complement activators, and risk of age-related macular degeneration. *JAMA.* 2006; 296: 301-309.
- Zhou J, Jang YP, Kim SR, et al. Complement activation by photooxidation products of A2E, a lipofuscin constituent of the retinal pigment epithelium. *Proc Natl Acad Sci USA.* 2006; 103:16182-16187.
- Mullins RF, Russell SR, Anderson DH, et al. Drusen associated with aging and age-related macular degeneration contain



- proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *FASEB j.* 2000; 14: 835-846.
8. Donoso LA, Kim D, Frost A, et al. The role of inflammation in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol.* 2006; 51:137-152.
  9. Kaiser PK, Do DV. Ranibizumab for the treatment of neovascular AMD. *Int J Clin Pract.* 2007; 6: 501-509.
  10. Kliffen M, Sharma HS, Mooy CM, et al. Increased expression of angiogenic growth factors in age-related maculopathy. *Br J Ophthalmol.* 1997; 8:154-162.
  11. Kaiser PK. Antivascular endothelial growth factor agents and their development: therapeutic implications in ocular diseases. *Am j Ophthalmol.* 2006; 142: 660-668.
  12. Kvant A, Algyere PV, Berglin L, et al. Subfoveal fibrovascular membranes in age-related macular degeneration express vascular endothelial growth factor. *Invest Ophthalmol Vis Sci.* 1996; 37:1929-1934.
  13. Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med.* 1994; 33:1480-1487.
  14. Heier JS, Brown DM, Chong V, et al. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. *Ophthalmology.* 2012; 119: 2537-2548.
  15. Trichonas G, Kaiser PK. Aflibercept for the treatment of age-related macular degeneration. *Ophthalmol Ther.* 2013; 2: 89-98. Milam AH, Jacobson SG. Photoreceptor rosettes with blue cone opsin immunoreactivity in retinitis pigmentosa. *Ophthalmology.* 1990; 97:1620-1631.
  16. Zweifel SA, Engelbert M, Laud K, et al. Outer retinal tubulation: a novel optical coherence tomography finding. *Arch Ophthalmol.* 2009; 127: 1596-602.
  17. DeCraos FC, Toth CA, Stinnett SS, et al. Optical coherence tomography grading reproducibility during the Comparison of Age-related Macular Degeneration Treatments Trials. *Ophthalmology.* 2012; 119: 2549-2557
  18. Joo Yong Lee, Francisco A. Folgar, Maureen G. Maguire, et al. Outer Retinal Tubulation in the Comparison of Age-related Macular Degeneration Treatments Trials (CATT). *Ophthalmology.* 2014; 121: 2423-2431.
  19. Lalwani GA, Rosenfeld PJ, Fung AE, et al. A variable-dosing regimen with intravitreal ranibizumab for neovascular age-related macular degeneration: year 2 of the PrONTO Study. *Am J Ophthalmol.* 2009; 148: 43-58.
  20. Chakravarthy U, Harding SP, Rogers CA, et al. Ranibizumab versus bevacizumab to treat neovascular age-related macular degeneration: one-year findings from the IVAN randomized trial. *Ophthalmology.* 2012; 119: 1399-1411.
  21. Ramkumar HL, Zhang J, Chan CC. Retinal ultrastructure of murine models of dry age-related macular degeneration (AMD). *Prog Retin Eye Res.* 2010; 29:169-190.
  22. Noemi Lois, Vikki Mc Bain, Ehab Abdelkader, et al. Retinal pigment epithelium Atrophy in patients with Exsudative Age-related macula degeneration undergoing anti VEGF therapy. *RETINA.* 2013; 33:13-22.
  23. Sarks J, Tang K, Killingsworth M, et al. Development of atrophy of the retinal pigment epithelium around disciform scars. *Br J Ophthalmol.* 2006; 90: 442-446.
  24. Grunwald JE, Daniel E, Huang J, et al. Risk of geographic atrophy in the comparison of age-related macular degeneration treatments trials. CATT Research Group. *Ophthalmology.* 2014; 121: 150-161.
  25. Saint-Geniez M, Kurihara T, Sekiyama E, et al. An essential role for RPE-derived soluble VEGF in the maintenance of the choriocapillaris. *Proc Natl Acad Sci USA.* 2009; 106:18751-18756.
  26. Rothenbuehler SP, Waeber D, Brinkmann CK, et al. Effects of ranibizumab in patients with subfoveal choroidal neovascularization attributable to age-related macular degeneration. *Am J Ophthalmol.* 2009; 147: 831-837.
  27. Papadopoulos N, Martin J, Ruan Q, et al. Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab. *Angiogenesis.* 2012; 15:171-185.
  28. Heier JS, Brown DM, Chong V, et al. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. *Ophthalmology.* 2012; 119: 2537-2548.
  29. Stewart MW, Rosenfeld PJ, Penha FM, et al. Pharmacokinetic rationale for dosing every 2 weeks versus 4 weeks with intravitreal ranibizumab, bevacizumab, and aflibercept (vascular endothelial growth factor Trap-eye). *Retina.* 2012; 32: 434-457.
  30. Roberts P, Mittermueller TJ, Montuoro A, et al. A quantitative approach to identify morphological features relevant for visual function in ranibizumab therapy of neovascular AMD. *Invest Ophthalmol Vis Sci.* 2014; 55: 6623-630.
  31. Seddon JM, Ajani UA, Mitchell BD. Familial aggregation of age-related maculopathy. *Am J Ophthalmol.* 1997; 123:199-206.
  32. Sobrin L, Ripke S, Yu Y, et al. Heritability and genome-wide association study to assess genetic differences between advanced age-related macular degeneration subtypes. *Ophthalmology.* 2012; 119:1874-1885.
  33. Fritsche LG, Chen W, Schu M, et al. Seven New Loci Associated with Age-Related Macular Degeneration. *The AMD Gene Consortium. Nat Genet.* 2013; 45: 433-439e2.
  34. Schmitz-Valckenberg S, Bindewald-Wittich A, Dolar-Szczasny J, et al. Correlation between the area of increased autofluorescence surrounding geographic atrophy and disease progression in patients with AMD. *Fundus Autofluorescence in Age-Related Macular Degeneration Study Group Invest Ophthalmol Vis Sci.* 2006; 47: 2648-2654.
  35. Sunness JS, Gonzalez-Baron J, Applegate CA, et al. Enlargement of atrophy and visual acuity loss in the geographic atrophy form of age-related macular degeneration. *Ophthalmology.* 1999; 106:1768-1779.
  36. Kodjikian L, Souied EH, Mimoun G, et al. Ranibizumab versus Bevacizumab for Neovascular Age-related Macular Degeneration: Results from the GEFAL Noninferiority Randomized Trial. *GEFAL Study Group.* 2013; 120: 2300-2309.