

Review

The Safety of Anti-VEGF Treatment, in the Context of the Retinal Nerve Fibre Layer, in Patients with Wet Age-Related Macular Degeneration: A Review

Małgorzata Wichrowska^{1,2,*}, Ewa Goździewska¹, Jarosław Kocięcki¹

¹Department of Ophthalmology, Poznan University of Medical Sciences, 61-701 Poznań, Poland

²Doctoral School, Poznan University of Medical Sciences, 61-701 Poznań, Poland

*Correspondence: malgorzata.wichrowska@skpp.edu.pl (Małgorzata Wichrowska)

Academic Editor: Adrian Gericke

Submitted: 11 May 2023 Revised: 29 June 2023 Accepted: 17 August 2023 Published: 25 September 2023

Abstract

Anti-vascular endothelial growth factor (VEGF) drugs are widely used in modern ophthalmology, especially in treating macular disorders like age-related macular degeneration or diabetic macular edema. Protocols for such treatments include repeated administration of intravitreal injections, with the volume of drug injected into the vitreous chamber seemingly high enough to cause an increase in intraocular pressure. Hence, questions might arise if such therapeutic approaches are safe for ocular tissue. Moreover, anti-VEGF compounds may theoretically harm the retinal nerve fibers due to the inhibition of VEGF and its neuroprotective effects. Thus, this manuscript aims to review the literature regarding studies evaluating the retinal nerve fiber layer (RNFL) in eyes receiving anti-VEGF treatment due to age-related macular degeneration. The RNFL was chosen as a subject of this review, as it is the innermost retinal layer exposed to the direct action of intravitreally administered drugs. The results of the available studies remain inconclusive. Most researchers seem to confirm the safety of the anti-VEGF treatment in wet age-related macular degeneration, at least regarding the retinal nerve fiber layer. However, some authors noticed that the influence of anti-VEGFs on RNFL could become apparent after more than thirty injections. Nonetheless, the authors of all studies agree that further, long-term observations are needed to help clinicians understand the effect of anti-VEGF treatment on the dynamics of changes in the thickness of retinal nerve fibers in patients with the wet form of age-related macular degeneration.

Keywords: age-related macular degeneration; anti-VEGF; aflibercept; bevacizumab; ranibizumab; retinal nerve fiber layer

1. Introduction

Age-related macular degeneration is still one of the leading causes of blindness in the elderly worldwide. The disease affects the retina, mainly its macular region, with affected patients exhibiting symptoms such as central vision deterioration, vision distortion (metamorphopsia), decreased contrast sensitivity, and abnormal color vision. The retina is a relatively delicate, highly organized tissue comprising three types of optic neurons (photoreceptors, bipolar cells, and ganglion cells). All these neurons participate in detecting light, converting it into an electrical signal, and sending it through the optic nerve to the central nervous system [1]. The high oxygen demand of the retina is met by the rich network of vascular plexuses of the retina itself, derived from the branches of the central retinal artery, supplying the inner retinal layers, and the choriocapillaris that supplies the outer layers of the retina, mainly retinal pigment epithelium (RPE) and photoreceptors [2,3]. The disturbances in the regularity of the retinal layers and their respective vascular supply are the key to pathophysiology of age-related macular degeneration (AMD).

Of the two primary forms of the disease, only wet (also known as exudative or neovascular) AMD (wAMD)

can be treated at present [4]. There is still no cure for the dry form of age-related degeneration (dAMD). However, recently Food and Drug Administration (FDA) approved pegcetacoplan (SYFOVRE™) as the drug which can slow down the atrophy progression in patients with geographic atrophy (GA) resulting from AMD, up to 20%. The safety and efficacy of the drug were confirmed in phase 2 (FILLY) and phase 3 (OAKS and DERBY) trials [5,6]. Before being used in ophthalmology, Pegcetacoplan, a complement protein C3 inhibitor, was introduced to treat paroxysmal nocturnal hemoglobinuria to increase hemoglobin stabilization and control hemolysis in these patients [7]. Currently, in the field of ophthalmology, another complement inhibitor, avacinaptad pegol (inhibiting complement C5 protein) is being investigated in a phase 3 clinical trial (GATHER 2), after promising results obtained in phase 2/3 GATHER 1 study. In GATHER 1, avacinaptad pegol reduced the GA progression, even up to 28%, in the twelfth month of the study, compared to the control group [8-10].

Anti-vascular endothelial growth factor (VEGF) drugs, such as bevacizumab, aflibercept, and ranibizumab, turned out to be highly effective tools in the fight to preserve the eyesight of wet AMD patients. Such treatment, however, requires repeated intravitreal injections, with no definite end of therapy defined. This may raise concerns about the consequences of adding extra volume to a compact, rigid organ, such as the eyeball, and the side effects of the administered drugs on the retina and all its fibers, not only those examined in the area of the macula. The innermost layer of the retina comprises retinal nerve fibers, which are the axons of the retinal ganglion cells, converging within the optic disc. Retinal nerve fiber layer (RNFL) changes are one of the basic parameters used in the progression monitoring of glaucoma and other neuropathies. Due to the excellent visibility of RNFL in optical coherence tomography-a fundamental examination tool used in agerelated macular degeneration, it can be easily assessed in routine clinical practice. The basic assumptions of the potential negative impact of anti-VEGF treatment on this layer of nerve fibers are described later in the article. Hence, this manuscript aims to familiarize the reader with the basics of the pathophysiology and treatment of age-related macular degeneration and, above all, to review the literature on the safety of anti-VEGF drugs on the retinal nerve fiber layer in patients treated for wAMD.

2. Age-Related Macular Degeneration (AMD)

2.1 AMD Epidemiology and Burden

According to the World Report on Vision, October 2019, the World Health Organization estimates that in 2030, 243.4 million people will suffer from age-related macular degeneration. Therefore, the number of cases will increase 1.2 times compared to 2020, primarily due to population aging. Of the nearly 200 million cases of AMD in 2020, at least 10 million people have experienced severe visual impairment or blindness due to more advanced stages of the disease. The prevalence of AMD in people aged 70-79 is estimated at nearly twenty percent, whereas at 80-85, it is estimated to reach almost thirty percent of the total population [11–13]. These data should be seen from the perspective of not only vision loss but also all the other aspects related to vision deterioration, such as difficulties in independent living, limited social life, a decrease in well-being, increased risk of depression, reduced life quality and an increased risk of falls and injuries (with an increased rate of mortality related to injuries in this age group) [14–16]. One prospective study found that falls resulting in injury in patients with various stages of age-related macular degeneration affected more than half of the study group and most often occurred in their homes [8]. Surprisingly, the study's most potent visual predictor of falls was not decreased visual acuity but reduced contrast sensitivity resulting from AMD.

2.2 Clinical Symptoms and Classification of AMD

The clinically visible fundus changes characteristic of the dry form of the age-related macular degeneration include retinal pigment epithelial abnormalities, drusen (yel-

lowish extracellular debris between the Bruch membrane and retinal pigment epithelium), and/or plaques of chorioretinal atrophy [12,16]. If macular neovascularization can also be observed, manifesting as macular elevation or subor intramacular hemorrhage, the clinical image prompts a diagnosis of wet age-related macular degeneration. All of these changes may vary in severity between patients and in each eye of the same patient. There are several approaches to classify this condition. The most popular systems are based on color fundus photography and mainly assess the size of the drusen observed and the presence or absence of pigmentary abnormalities. Manifestations of geographic atrophy or neovascularisation within the foveal region prompt classification as the last stage of the disease- the late AMD (according to epidemiological classification and Classification Committee of the Beckman Initiative for Macular Research) or advanced AMD (AREDS classification) [12,17].

For clinicians, the most factor is the presence or absence of macular neovascularization, as the exudative, neovascular (wet) AMD, characterized by the presence of this characteristic, can currently be treated with satisfactory effect. According to the Consensus on the Neovascular AMD Nomenclature Study Group (CONAN), macular neovascularization (MNV) may also develop from different origins, including choroid (choriocapillaris), retina itself (mostly the deep capillary plexus), and aberrant retinochoroidal anastomosis [17].

2.3 AMD Pathophysiology

AMD development is known to have a complex etiology, which has still not been fully elucidated. The mechanisms contributing to the disease onset are compound and based on retinal and uveal homeostasis disturbance. Metabolic factors, impaired lipid metabolism, hypoxia, oxidative stress, inflammation, and genetic and environmental factors are also thought to be involved in this process [12,18-20]. One of the hypotheses claims that the cause of the development of AMD lies in the aging of the Bruch's membrane, with disturbances in metabolism and accumulation of lipids and lipoproteins within the membrane, which leads to its thickening. This, in turn, causes disturbances in the flow of ions, nutrients, and oxygen through the external blood-retinal barrier between the retinal pigment epithelium and the choriocapillaries. These disturbances, through the mechanisms associated with hypoxia, stimulate the retina to produce growth factors, including vascular endothelial growth factor (VEGF). The VEGF family includes several VEGF subtypes (A-F) and placental growth factors (PIGF-1 and PIGF-2), described in more detail in the previous work of our team [11]. VEGF-A is the family's main proangiogenic factor, initiating a cascade of abnormal new vessel creation in wAMD [12,21,22].

The second theory is focused on local inflammation. The aging cells can produce many cytokines and chemokines (e.g., Il-8, Il-6, TNF, MCP-1, MCP-2, Il-1 α , Il-1 β), which can stimulate, among others, the excessive activation of the complement system [12,21].

There is also more and more information that the etiology of AMD development may also be explained at the level of the internal blood-retinal barrier. It could be associated with disorders within the retinal vascularity itself, which also result in hypoxia and a secondary defense reaction of the retina, aimed at forming new vessels [2,16,23,24]. However, the exact course of events in the development of AMD requires further research.

3. Treatment of Wet Age-Related Macular Degeneration

3.1 Anti-VEGF Drugs

Several researchers contributed to the discovery of the glycoprotein (~23 kDa) known nowadays as the vascular endothelial growth factor (VEGF), with its story starting in the 1980s [25,26]. Previously, it was known as vascular permeability factor (VPF), increasing microvascular permeability in carcinoma tissues [25,26]. The term "vascular endothelial growth factor" was first used by the team of Napoleone Ferrara in 1989, as they confirmed the proangiogenic potential of this molecule [27,28]. A few years later, in 1994, a study on the production of VEGF by the retina in hypoxic conditions showed that VEGF might play a role in ocular neovascular disorders, which turned out to be a significant step toward the development of ophthalmic therapies for neovascular diseases [25]. Although anti-VEGF drugs were primarily intended for cancer treatment (bevacizumab was first approved by the U.S. Food and Drug Administration (FDA) in 2004 for colon cancer treatment), they are nowadays widely used in various vascular disorders of the eye [25,27,29]. Bevacizumab, used in ophthalmology since 2005 as an off-label therapy, is a humanized monoclonal antibody with a molecular weight of 149 kDa and an affinity to all isoforms of VEGF-A. The first anti-VEGF drug used with FDA approval (on December 2004) for wAMD treatment was pegaptanib, a single-stranded ribonucleotide oligonucleotide (an RNA aptamer), designed to selectively bind VEGF165 (as well as VEGF 188) [25]. Another drug, ranibizumab, contains the Fab fragment of a humanized immunoglobulin G1 (IgG1) kappa isotype murine monoclonal antibody with a molecular weight of 48 kDa, almost three times smaller than bevacizumab. It was approved by the FDA in 2006 for wAMD treatment [12,21].

Aflibercept, approved by FDA in 2011, was created as a fusion protein with domains of both VEGFR1 and VEGFR2 receptors and the Fc fragment of the human IgG1, also known as the VEGF-trap, with a molecular weight of 115 kDa. Due to its more complex structure, it can bind all VEGF-A isoforms (as the previously mentioned anti-VEGFs), as well as VEGF-B and PIGF [21,30].

More recently introduced drugs for wAMD treatment include brolucizumab and faricimab. Brolucizumab is a single-chain humanized antibody fragment, the smallest functional unit of the antibody, designed to bind VEGF-A [12,31]. Furthermore, faricimab seems to be a promising tool in wAMD treatment, as it has a bigger range of targets: the VEGF-A pathway and the Ang/Tie-2 pathway. Because of its more complex structure (bispecific heterodimeric monoclonal human antibody), faricimab is a bigger molecule than bevacizumab (150 kDa), approved by FDA for wAMD (and DME) treatment in 2022 [21]. Newer drugs (both broluciumab and faricimab), due to their shorter presence on the market, have not yet been extensively studied, especially in the context of their safety concerning RNFL in real-life clinical practice.

3.2 The Side Effects of the Anti-VEGF Treatment 3.2.1 Systemic Side Effects

Serious systemic adverse events seem rare and comparable for the most frequently used anti-VEGF drugs [30,32-34]. Anti-VEGFs have previously raised concerns regarding thromboembolic events or non-ocular hemorrhages. As was found in the MARINA trial, the 2-year incidence of hemorrhagic events was estimated at 9% for the ranibizumab group compared with 5.5% for the shamtreated control group. Similarly, in the ANCHOR study, the 2-year incidence was estimated at 9% in the group treated with combined therapy: ranibizumab and photodynamic therapy, compared to 4.2% for photodynamic treatment only. Regarding thromboembolic events, there was a lack of statistical difference after two years of ranibizumab treatment compared to control [30,35]. The risk of systemic adverse events in routine clinical practice for three anti-VEGF agents (bevacizumab, ranibizumab, aflibercept) was evaluated in Maloney's retrospective cohort study, with a total of 87,844 patients [34]. The authors estimated 180-day event rates for primary systemic adverse event outcomes (acute myocardial infarction (MI), cerebrovascular disease (CVD)), major bleeding, and all-cause hospital admission. They found MI event rates of 0.62-0.64; and CVD rates of 0.53-0.60, proving a similar good systemic safety profile for all the agents.

3.2.2 Ocular Side Effects

The most common ocular side effects comprised perioperative eye irritation or conjunctival hemorrhages, which, due to their low harmfulness to eyesight, may be underestimated in the literature. Infrequently reported serious ocular adverse include ocular inflammation (0.019–1.6 %) or retinal detachment (0–0.67%) [33,36]. However, they are more likely related to the injection procedure than the active substance. In turn, in MARINA and ANCHOR trials for ranibizumab, ocular inflammation occurred most frequently, with the incidence of 2.1 and 2.9%, respectively [37,38].

The ocular inflammation events associated with anti-VEGF treatment may be divided into three different entities, as mentioned earlier, including post—injections endophthalmitis (0.008–0.092%), sterile ocular inflammation (0.02–0.37%) and brolucizumab-associated vasculitis (BARV) (0.8% from HAWK and HARRIER study data, but exact incidence is still unknown) [39,40]. All of them could have a similar clinical appearance and be associated with a significant decrease in visual acuity. However, bacterial endophthalmitis appears to be the most painful, as reported by the patients. On the other hand, BARV has a strong female bias (88–100%) and may be suspected in the eye after brolucizumab injections, particularly in the presence of retinal vascular occlusion. BARV most often develops seven to fifty-six days post-injection, while the other two types usually appear within seven days of injection [39,40].

Occasional retinal tear or detachment cases have been reported, mainly in conditions other than AMD, such as neovascularization due to myopia or proliferative diabetic retinopathy [33].

Increases in intraocular pressure appear to be relatively common immediately after anti-VEGF injection, with most studies demonstrating spontaneous normalization of intraocular pressure within half to several hours [33,38,41,42].

Other very rarely reported events some ischemiarelated cases, such as non-arteritic ischemic neuropathy, retinal vein- or artery occlusion, foveal avascular zone enlargement, or ocular ischemic syndrome [33,35]. An interesting issue that could also be interpreted as an adverse effect of therapy is the recently reported reduction of the density of nerve endings within the cornea in eyes receiving anti-VEGF injections [43].

3.2.3 Basic Assumptions of the Neurodegenerative Potential of Anti-VEGF Drugs

Despite its confirmed role in the development of wAMD, VEGF has other functions in the eye and throughout the human body. Receptors for this factor are found on vascular endothelial cells and, among others, on epithelial cells, fibroblasts, monocytes, macrophages, smooth muscle cells, motor neurons, and peripheral nerve axons [26,33]. It is believed that VEGF is involved in many physiological processes, such as angiogenesis, wound healing, the development of follicles in the ovaries, or regulation of glomerular epithelial cell function in kidneys and alveolar septal endothelial and epithelial cells in lungs [26,33]. Although the role of VEGF in neuronal cells is still not fully understood, scientists proved its neuroprotective role in hippocampal cells and peripheral motor neurons, drawing attention to its potential future use in the treatment of neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) [26,44].

The importance of VEGF in the eye tissues is also an ongoing topic of scientific interest. Expression of VEGF was found in the ganglion cell layer and the inner nuclear layer, Müller cells, and pigment epithelial cells of the retina [21,45]. VEGF-A receptors (VEGFR2, VEGFR1)

are widely expressed within all retinal layers, especially on Müller cells and photoreceptors. One study in the animal model showed that VEGF-inhibition using an adenovirus expressing a soluble form of VEGFR1 (Ad-sFlt1) did not alter normal retinal vasculature but resulted in a decrease of the thickness of both the inner- and outer retinal layer, with signs of neural cell apoptosis confirmed by electron micrographs, as well as by reduction of a- and b- amplitudes in electroretinogram examinations [45]. These findings confirm the hypothesis of the VEGF acting as a neural survival factor. Froger et al. [46] proved that VEGF promotes retinal ganglion cell survival in autocrine and paracrine manners, primarily via VEGFR1 activation. In turn, a study by Foxton et al. [35] demonstrated that VEGF-A inhibition may disrupt the anterograde axonal transport within retinal cells in rodents without disrupted synapse architecture, likely due to phosphorylation of the p38 mitogen-activated protein kinases (p38 MAPK). The authors suggested that anti-VEGF treatment may attenuate axonal transport in the visual pathway, resulting in axonal disruption and cell loss [35].

4. Effects of Anti-VEGF wAMD Treatment on Retinal Nerve Fiber Layer – A Summary of Studies

4.1 Data Sources and Search Parameters

We performed a literature search of the Google Scholar and PubMed databases using the following terms as prompts: age-related macular degeneration, AMD, retinal nerve fiber layer, inner retinal layer, anti-VEGF, bevacizumab, ranibizumab, aflibercept; examining records published between the year 2000 and 2023. All studies included in this summary (Tables 1,2) include patients treated for wAMD with anti-VEGF drugs, without the co-existence of other optic nerve diseases (i.e., glaucoma) or retinal diseases (i.e., vascular diseases including diabetic retinopathy) that could affect the results. In addition, if we found further publications regarding the topic of interest in the bibliography of the included articles, we also took them into account. Due to the obvious ethical concerns associated with the potential refusal or abandonment of active neovascular AMD treatment, there is a notable lack of randomized trials in this area.

Most studies focused on the retinal fiber layer in eyes treated with anti-VEGF, and examined this layer in the peripapillary region (pRNFL) (Table 1, Ref. [47–59]). There are singular studies assessing RNFL thickness changes in the macular area in eyes treated with anti-VEGF due to wAMD (Table 2, Ref. [60–64]). All of the examined studies also assessed other parameters, such as intraocular pressure (IOP) [47–50,52], ganglion cell layer thickness [53,54,60,61], inner plexiform retinal layer thickness, total inner retinal layer thickness [62], and total macular volume [63], which was not the topic in this review.

				(wA	wid), sorted by publ	lication uate.			
_	First author /year of publication	Study design	Follow up (months) (mean ± SD) or mean and 95% CI)	Number of participants/treated eyes; controls	Age of participants (years) (mean ± SD, or median)	Anti-VEGF Drug/mean num- ber of injections	The average RNFL thickness at base- line/at the final fo- llow up visit (μm)	OCT device	Summary of results
1	Michael B. Horsley [56]/2010	retroscpective obser- vational consecutive case series	27 ± 9.7	37/41; lack of con- trol group	79.2 ± 8.7	Pegaptanib,bevacizumab,ranibizumaborcombinationofthese/16.0 \pm 5.5	92.4 ±15.2/93.8 ± 15.2	Stratus (Carl Zeiss)	There were no sta- tistically significant differences in RNFL measurements when comparing individual anti-VEGF treatment groups.
2	2 Jose M. Martinez- de-la- Casa [47] /2012	prospective longitudinal cohort study	12 ± 0	49/49; 27 fellow eyes	78.5 ± 6.9	Ranibizumab/4.8± 1.6	$\begin{array}{rrr} 105.7 \ \pm \ 12.2/100.2 \\ \pm \ 11.0 \ (p < 0.001) \\ \hline \\ \mbox{Control} & \mbox{group:} \\ 101.8 \ \pm \ 11.6/100.5 \\ \pm \ 10.8 \end{array}$	Spectralis (Heidelberg Engineering)	Significant RNFL thinning was noted in the treatment group.
3	Güngör Sobacı [48] /2013	retrospective observational consecutive case series	Ranibizumab group: 13.6 ± 2.1 Bevacizumab group: 14.05 ± 2.6	Ranibizumab group: 35/35; 35 fellow eyes Bevacizumab group: 30/30; 30 fellow eyes	68.0 ± 7.5	Ranibizumab or bevacizumab/6.3 \pm 1.9, 5.1 \pm 1.3, respectively	Ranibizumab group: 105.3 ± 6.9 104.6 ± 8.4 Bevacizumab group: 105.8 ± 8.1 $104.6 + 8$	Stratus (Carl Zeiss)	RNFL thickness values were not statistically different between treated and untreated eyes or groups.
4	Melih Parlak [49] /2015	prospective longitu- dinal cohort study	12 ± 0	22/22; 22 fellow eyes	66.3 ± 8.8	Ranibizumab/4.86 ± 2.18	$101.4 \pm 14.2/99.9 \pm 14.5 (p = 0.009)$ Control group: 99.1 \pm 8.8/96.2 \pm 9.5 (p = 0.022)	Spectralis (Heidelberg Engineering)	Although there was no statistically significant difference in RNFL thickness between the study and control eyes during 12 months of follow-up, a significant thinning was recorded in both groups compared with baseline values.

Table 1. Table of included studies on peripapillary retinal nerve fiber layer (pRNFL) in patients receiving anti-vascular endothelial growth factor (VEGF) therapy due to wet AMD (wAMD), sorted by publication date.

	Table 1. Continued.								
_	First author /year of publication	Study design	Follow up (months) (mean ± SD) or mean and 95% CI)	Number of participants/treated eyes; controls	Age of participants (years) (mean ± SD, or median)	Anti-VEGF Drug/mean num- ber of injections	The average RNFL thickness at base- line/at the final fo- llow up visit (μm)	OCT device	Summary of results
5	Sibel Demirel [55] /2015	observational, com- parative study	38.96 ± 15.49	29/29; 29 fellow eyes and 27 healthy eyes	Study group: 73.92 \pm 6.1 Control group: 71.87 \pm 4.1	Ranibizumab/13.88 ± 3.81	92.3 ± 7.7/92.46 ± 8.1	Stratus (Carl Zeiss)	There were no statistically significant differences between the mean RNFL thickness in eyes treated with injections and the fellow eyes and between those treated with injections and the healthy control group.
6	Gary L Yau [58]/2015	cross-sectional, paired-eye, compari- son study	47.9 (95% CI 40.6–55.2)	29/29; 29 fellow eyes	81.1 (95% CI 77.9–84.3)	Not given/23.1 (95% CI 19.0– 27.2)	Not given/95.0 (89.8–100.2) in the treated group vs 89.9 (85.5–94.3) in the control group; (85.5–94.3) in the	Cirrus (Carl Zeiss)	The mean peripapillary RNFL in the treated group was significantly thicker than in the non- treated fellow eye.
7	7 Jo Young- Joon [50]/ 2016	prospective cohort study	12 ± 0	20/20; 20 fellow eyes	67.1 ± 8.9	Ranibizumab/5.0 ± 1.0	$\begin{array}{r} 98.0 \pm 6.8/95.5 \pm \\ 4.3 \\ \hline \\ \text{Control group: } 94.5 \\ \pm 7.3/93.6 \pm 7.1 \end{array}$	Cirrus (Carl, Zeiss)	Post-injection differences in total RNFL thickness between the two groups were insignificant at the 12- month follow-up.
8	Ilaria Zuc- chiatti [53]/2017	prospective case se- ries	12 ± 0	24/24; lack of con- trol group	76 ± 7.8	Ranibizumab/5.3 \pm 1,6	82.0 ± 9.9/84.6 ± 15.5	Cirrus (Carl Zeiss)	RNFL thickness did not show statistically signif- icant changes between baseline and month 12.

	Table 1. Continued.									
	First author /year of publication	Study design	Follow up (months) (mean ± SD) or mean and 95% CI)	Number of participants/treated eyes; controls	Age of participants (years) (mean ± SD, or median)	Anti-VEGF Drug/mean num- ber of injections	The average RNFL thickness at base- line/at the final fo- llow up visit (μm)	OCT device	Summary of results	
9	Alicia Valverde- Megías [51] /2019	prospective controll- ed longitudinal study	96 ± 0	20/20; 9 fellow eyes	83 ± 1.4	Ranibizumab/21 ± 2.8	$\begin{array}{c} 105.6 \pm 10.7/96.5 \pm \\ \hline 2.1 \ (p < 0.0001) \\ \hline \\ \hline \\ \text{Control} \\ 103.2 \pm 7.9/92.9 \pm \\ \hline \\ 3.2 \ (p < 0.0001) \\ \hline \\ $	Spectralis (Heidelberg Engineering)	RNFL loss was found to be stati- stically significant during 96 months in all sectors, regardless of receiving ranibizumab injections or not. RNFL loss was comparable	
							$\begin{array}{ccc} \text{ing} \text{RNFL} & \text{loss} \\ \text{in} \text{injected} & \text{and} \\ \text{non-injected} & \text{eyes:} \\ \text{adjusted} p \text{values} \\ \text{above} 0.05 \end{array}$		between the study and control groups.	
10	Liang Wang [57] /2021	retrospective, cross- sectional study	46.8 ± 42.0	54/54; 54 fellow eyes	79.7 ± 7.3	Bevacizumab, ranibizumab, or aflibercept/29.4 \pm 31.5	87.3 ± 9.6 Control group: 89.0 ± 7.5	Cirrus (Carl Zeiss)	No statistically significant differ- ences existed between the mean RNFL thickness in eyes treated with injections and the fellow eyes.	
									The relationship between the dif- ference in the RNFL thickness and the number of injections had a nonlinear dose-response relation- ship that became apparent after approximately 30 injections, and 50 months of injections.	

MR Press

	Table 1. Continued.									
First author /year of publication	Study design	Follow up (months) (mean ± SD) or mean and 95% CI)	Number of participants/treated eyes; controls	Age of participants (years) (mean ± SD, or median)	Anti-VEGF Drug/mean num- ber of injections	The average RNFL thickness at base- line/at the final fo- llow up visit (µm)	OCT device	Summary of results		
11 Jayoung Ahn [52]/2021	retrospective, observational, consecutive case series study	12 ± 0	29/29; 29 fellow eyes	Ranibizumab group:70.86 \pm 8.56 Aflibercept group: 73.00 ± 9.10	Ranibizumab or aflibercept/4.93 \pm 1.39 for ranib- izumab group and 4.69 \pm 1.31 for aflibercept group	Ranibizumab group: $101.03 \pm 15.07/99.32$ ± 14.07 Fellow eyes $98.90 \pm$ $14.3/98.00 \pm 16.16$ Aflibercept group: $97.39 \pm 18.61/94.54$ ± 17.71 Fellow eye: $94.33 \pm$ $17.96/93.58 \pm 18.84$	Spectralis (Heidelberg Engineering)	There was no significant differ- ence in the RNFL thickness be- tween the treated and fellow eyes among patients in both groups.		
12 Maja Zivkovic [54]/2023	prospective inter- ventional study of consecutive patients	24 ± 0	135/NA*	65 ± 15 years	Bevacizumab/12.4 ± 2.4	87.6 ± 12.23/86.23 ± 12.55	Cirrus (Carl Zeiss)	Average RNFL at baseline and 24 months did not differ significantly.		
13 Sung Yeon Jun [59] /2023	retrospective obser- vational case series	3 ± 0	22/19	67.77 ± 5.95	One injection of brolucizumab and earlier: bevacizumab, ranibizumab and/or afliber- cept/16.50 ± 9.81	99.47 ± 14.19/98.86 ± 14.73 Fellow eye: 97.22 ± 12.19	Spectralis (Heidelberg Engineering)	The RNFL thickness did not change in the treated eyes and fellow eyes.		

*NA, not available; *p* values < 0.05 were considered statistically significant. OCT, optical coherent tomography.

	First author /year of publication	Study design	Follow up	Number of parti- cipants/treated eyes; controls	Mean age of participants (years)	Anti-VEGF Used/mean num- ber of injections	The average RNFL thickness at base- line/the final follow- up visit (µm)	OCT device	Summary of results
1	Marco Beck [61] /2016	Retrospective case series with fellow- eye comparison	45.3 ± 10.5	34/34; 34 fellow eyes	76.2 ± 8.2	Bevacizumab, ranibizumab and/or aflibercept/ 31.5 ± 9.8	$\frac{36.4 \pm 81/32.2 \pm 6.4}{\text{Fellow eyes: } 36.2 \pm 65/36.8 \pm 6.9}$	Spectralis (Heidelberg Engineering)	Although the RNFL decrease was not statistically significant, the authors found that RNFL thick- ness in the treated eyes was sig- nificantly thinner than the fellow untreated eyes.
2	ümit übeyt Inan [62] /2019	Prospective consec- utive case series	12.0 ± 0	33/33	72 ± 7.4	Ranibizumab/ 9.08 (range 6–11)	Foveal RNFL 25.0 ± 18.3/23.0 ± 17.5 Parafoveal RNFL 29.3 ± 12.1/26.9 ± 10.1	Spectralis (Heidelberg Engineering)	The thickness of RNFL did not show a significant change in any sector during the follow-up.
3	Seong Woo Lee [60]/2020	Retrospective clini- cal study	19.9 ± 7.1	52/ranibizumab group: 23 afliber- cept group: 29	74.3 ± 8.1	Aflibercept or ranibizumab/ 5.1 ± 2	Ranibizumab group: $42.9 \pm 19.3/33.2 \pm$ $9.4 (p = 0.036)$ Aflibercept group: $42.9 \pm 19.3/37.4 \pm$	Spectralis (Heidelberg Engineering)	There was a significant de- crease in RNFL thickness in the ranibizumab group and when both study groups were com- bined.
4	Jan Niklas Lüke [63] /2023	Retrospective clini- cal study	NA*	120/NA	78.5 ± 7.8	Ranibizumab, bevacizumab and aflibercept/ 10 ± 4.2	$\begin{array}{c} 16.3 \\ \hline \text{Ranibizumab group:} \\ 54.5 \pm 12.2 \\ \hline \text{Bevacizumab group:} \\ 51.2 \pm 9.9 \\ \hline \text{Aflibercept group:} \\ 48.7 \pm 9.4 \\ \hline \text{Total:} 52.1 \pm 11.8 \\ \end{array}$	Spectralis (Heidelberg Engineering)	RNFL thickness remained constant.
5	Małgorzata Wichrowska [64]/2022	Cross-sectional study	21.13 ± 18.37	53/53	73.02 ± 7.42	Bevacizumab, ranibizumab and/or aflibercept/ 11.66 ± 9.10	39.06 ± 8.69/39.66 ± 5.84	Topcon DRI OCT Triton	RNFL was not statistically differ- ent in both groups; there was no correlation between the number of injections and RNFL thickness.

Table 2. Table of included studies on macular retinal nerve fiber layer (RNFL) in patients receiving anti-VEGF therapy due to wAMD, sorted by publication date.

*NA, not available; p values < 0.05 were considered statistically significant.

4.2 Effect on the Peripapillary RNFL

While some studies have shown ranibizumab to be harmful to the retinal nerve fiber layers, they do not prove its inferiority to other anti-VEGF compounds but only indicate that it is the most frequently analyzed anti-VEGF in this type of research. Martinez de-la-Casa et al. [47], in their 2012 study, showed significant RNFL thinning (p <(0.001) in a group treated with ranibizumab (n = 49) during a twelve-month of follow-up (mean number of injections: 4.8; SD = 1.6), compared to fellow untreated eyes (n = 27). However, the continuation of this trial revealed, in 2019 (after ninety-six months of follow-up; mean number of injections: 21; SD = 2.8), that RNFL loss, although present, was comparable between the study (n = 20) and control group (n = 9) (p > 0.05) [51]. A significant thinning in both groups (treated with ranibizumab and control fellow eyes; p = 0.009, p = 0.022, respectively) was also shown in the study of Parlak et al. [49], who included twenty-two patients with unilateral wAMD, also during twelve months of follow up. These findings may indicate that the passage of time is involved in thinning. However, the progression of the disease itself (AMD), or a potential effect of the drug on the untreated second eye via the systemic route, cannot be ruled out. Among all examined studies, only one by Demirel et al. [55] included twenty-seven healthy eyes of age-matched patients as the control group. The authors examined twenty-nine patients with unilateral wAMD treated with ranibizumab (mean number of injections: 13.88; SD = 3.81). After at least twelve months of follow-up (mean time of follow-up: 38.96; SD = 15.49 months), they found a lack of RNFL thinning or other statistically significant differences between treated, contralateral, and healthy eye groups.

Lack of RNFL thinning due to ranibizumab monotherapy during twelve months of follow-up was also noted by Jo *et al.* [50] and Zucchiatti *et al.* [53] in their studies (with the mean number of injection: 5.0; SD = 1.0 and 5.3; SD = 1.6, respectively).

Furthermore, aflibercept monotherapy safety regarding RNFL thickness was assessed in the study of Ahn *et al.* [52]. The authors evaluated groups of patients treated with aflibercept (IVA) or ranibizumab (IVR), compared to contralateral untreated eyes during twelve months of follow-up (mean number of injections: 4.69, SD = 1.31 and 4.93; SD = 1.39, respectively). Although they found a decrease in RNFL thickness in both treated groups (IVA p = 0.023, IVR p = 0.038), no significant difference occurred between the treated and control eyes, and no significant correlation was detected between the number of injections given and RNFL thinning.

Bevacizumab monotherapy was also recently assessed in the study of Zivkovic *et al.* [54], published in 2023. In their prospective interventional study of one hundred thirtyfive patients, with a twenty-four-month follow-up, the authors found a lack of statistically significant difference in RNFL thickness from the baseline value (p = 0.126) after the mean number of 12.4 injections; SD = 2.4. Similarly, Sobacı *et al.* [48] did not find changes in RNFL during bevacizumab or ranibizumab treatment, with the mean number of 5.1 (SD = 1.3) and 6.3 (SD = 1.9) injections, respectively.

The first study conducted in patients treated with a combination of different anti-VEGF drugs due to wAMD was published by Horsley *et al.* [56] in 2010. Fortyone eyes of thirty-seven patients were treated with pegaptanib, bevacizumab, ranibizumab, or a combination of these, with a mean number of 16.0 injections, SD = 5.5. Researchers found no statistically significant difference in RNFL thickness between the groups treated with all anti-VEGFs, ranibizumab alone, and the combination of ranibizumab and bevacizumab, during at least twelve months of follow-up (average 27; SD = 9.7 months).

A more extended follow-up period was assumed in the study conducted by Wang *et al.* [57], published in 2021 (mean length of injection treatment in months: 46.8; SD = 42.0). The authors included fifty-four eyes of patients with unilateral wAMD treated with bevacizumab, ranibizumab, or aflibercept (mean number of injections: 29.4; SD = 31.5), finding no statistically significant differences in RNFL thickness between the study and fellow eyes. However, the study revealed a relationship between the changes in RNFL thickness and the number of injections given, which was more apparent after at least thirty injections and fifty months of treatment. These results emphasize the need to continue monitoring the safety of treatment with anti-VEGF drugs during long-term therapy.

In one cross-sectional study [58], researchers even found thickening of the RNFL layer in the group of patients treated with anti-VEGF during the mean follow-up of 47.9 months (95% CI 40.5–55.2), compared to the fellow untreated eyes (p = 0.01). However, they did not specify the anti-VEGF used.

Moreover, Sung Yeon Jun [59] recently published their work regarding the effect of brolucizumab injection on RNFL in patients previously receiving other anti-VEGFs (bevacizumab, ranibizumab and/or aflibercept). In their 3-month follow-up, they did not notice RNFL thinning in treated and contralateral untreated eyes [59].

4.3 Effect on the Macular Retinal Nerve Fiber Layer

Studies on the macular retinal nerve fiber layer could not be simply compared, as they assessed different parts of the macular area (Table 2). Two studies included in this review evaluated RNFL in the outer ring of the ETDRS grid [45,48], one study only in temporal sectors [50], and two studies in all the macular area [62,64]. The study of Beck *et al.* [61] included thirty-four patients with unilateral wAMD, with the second eye treated as control, with a minimum follow-up of twenty-four months. Although they found that RNFL thickness in eyes receiving anti-VEGF injections was thinner than in the fellow eyes, the decrease in RNFL from baseline at the final visit did not reach statistical significance. Similarly, studies of Inan et al. [62] and Lüke et al. [63] did not show significant changes in macular RNFL thickness after administration of a mean number of 9.08 (SD = 1.63) (ranibizumab) and ten injections (SD = 4.2) (ranibizumab, bevacizumab, and aflibercept), respectively. One study compared aflibercept and ranibizumab safety regarding the RNFL, during an average of 19.9 (SD = 7.1) months of follow-up and 5.1 (SD = 2.0) injections given, and found that only in the ranibizumab group (or both groups combined) the RNFL thinning was statistically significant. In turn, a previous study of our team evaluated RNFL thickness in the macular area in patients treated with anti-VEGF (bevacizumab, ranibizumab, and/or aflibercept) compared to the fellow untreated eye. We found a lack of differences and a lack of correlation between the number of injections given and RNFL thickness [64].

4.4 Risk of Bias

Overall, the cited studies may be hindered by several factors that make it difficult to compare their results. The first is the small size of study groups, which can significantly affect statistical analysis results. The second bias factor is the difference among the anti-VEGFs drugs used. Their molecular structure and physicochemical and pharmacokinetic properties may be associated with their different impact on the tested parameters. The third bias factor is the length of the follow-up period. As it seems to be confirmed in the study, the differences in RNFL thickness may become apparent only in long-term observation after more injections have been made [57].

Moreover, as noted in the literature, intravitreally administered anti-VEGF could affect the contralateral eye via systemic circulation [65,66]. Only one study included healthy age-matched patients as the control group to avoid this bias [55]. It is also noteworthy that changes in the thickness of the retinal layers in AMD may result from the disease itself and not the implemented treatment. Untreated eyes with dry AMD, treated as control groups, may undermine the results as it has been found that dAMD can be associated with thinning of ganglion cells, the axons of which make up the retinal nerve fiber layer, even in the early stages of the disease [67–69].

An important factor that may also affect the thickness of the RNFL layer is age, which was not included in the statistical analysis in many of the analyzed studies and could have influenced the obtained results. Aging is crucial in older patients, who represent the most AMD cases. As we found in our previous work, patients over 73 had a significantly thinner layer of nerve fibers [64]. Other researchers also emphasize the influence of this factor on the thickness of the retinal nerve fiber layer, which confirms the validity of taking it into account in research on diseases affecting the elderly population [70,71].

Furthermore, there are some concerns regarding the differences between optical coherent tomography (OCT) devices used for examination. They all differ in the diameter of the scanned area of the optic disc, and/or segmentation algorithms [72,73]. The devices also vary in the resolution of the performed tests, with Stratus (time domain OCT) presenting lower sensitivity and specificity in detecting abnormality in RNFL thickness than spectral domain OCT devices (Cirrus, Spectralis, Topcon), so the results of the obtained tests should be evaluated with great caution [74,75]. Moreover, only a part of the studies reported the signal strengths of the results obtained and the potential implementation of manual scan corrections. As it was shown, especially in the case of macular diseases such as age-related macular degeneration, the reproducibility of the automated measurements of macular layers could be affected [76].

5. Conclusions

The results of the available studies remain inconclusive. Most researchers seem to confirm the safety of the anti-VEGF treatment in wet age-related macular degeneration, at least regarding the retinal nerve fiber layer. However, some authors noticed that the influence of anti-VEGFs on RNFL could become apparent after more than thirty injections [60]. Nonetheless, the authors of all studies agree that further, long-term observations are needed to help clinicians understand the effect of anti-VEGF treatment on the dynamics of changes in the thickness of retinal nerve fibers in patients with the wet form of age-related macular degeneration.

Abbreviations

dAMD, dry age-related macular degeneration; wAMD, wet age-related macular degeneration; RNFL, retinal nerve fiber layer; VEGF, vascular endothelial growth factor; GCL, ganglion cell layer; MNV, macular neovascularisation.

Author Contributions

MW—conceptualization, acquiring, analyzing data from the reviewed literature, writing original draft preparation; EG—visualization, acquiring, interpreting data, writing and editing; JK—conceptualization, interpreting data, review and supervision. All the authors approved the final version of the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.



Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Masland RH. The neuronal organization of the retina. Neuron. 2012; 76: 266–280.
- [2] Fruttiger M. Development of the retinal vasculature. Angiogenesis. 2007; 10: 77–88.
- [3] Mulfaul K, Russell JF, Voigt AP, Stone EM, Tucker BA, Mullins RF. The Essential Role of the Choriocapillaris in Vision: Novel Insights from Imaging and Molecular Biology. Annual Review of Vision Science. 2022; 8: 33–52.
- [4] Flaxel CJ, Adelman RA, Bailey ST, Fawzi A, Lim JI, Vemulakonda GA, *et al.* Age-Related Macular Degeneration Preferred Practice Pattern®. Ophthalmology. 2020; 127: P1–P65.
- [5] Liao DS, Grossi FV, El Mehdi D, Gerber MR, Brown DM, Heier JS, et al. Complement C3 Inhibitor Pegcetacoplan for Geographic Atrophy Secondary to Age-Related Macular Degeneration: A Randomized Phase 2 Trial. Ophthalmology. 2020; 127: 186–195.
- [6] Goldberg R, Heier JS, Wykoff CC, Staurenghi G, Singh RP, Steinle N, et al. Efficacy of intravitreal pegcetacoplan in patients with geographic atrophy (GA): 12-month results from the phase 3 OAKS and DERBY studies. Investigative Ophthalmology & Visual Science. 2022; 63: 1500.
- [7] Wong RSM, Pullon HWH, Amine I, Bogdanovic A, Deschatelets P, Francois CG, *et al.* Inhibition of C3 with pegcetacoplan results in normalization of hemolysis markers in paroxysmal nocturnal hemoglobinuria. Annals of Hematology. 2022; 101: 1971–1986.
- [8] Khan H, Aziz AA, Sulahria H, Khan H, Ahmed A, Choudhry N, *et al.* Emerging Treatment Options for Geographic Atrophy (GA) Secondary to Age-Related Macular Degeneration. Clinical Ophthalmology (Auckland, N.Z.). 2023; 17: 321–327.
- [9] Loewenstein A, Trivizki O. Future perspectives for treating patients with geographic atrophy. Graefe's Archive for Clinical and Experimental Ophthalmology = Albrecht Von Graefes Archiv Fur Klinische Und Experimentelle Ophthalmologie. 2023; 261: 1525–1531.
- [10] Jaffe GJ, Westby K, Csaky KG, Monés J, Pearlman JA, Patel SS, *et al.* C5 Inhibitor Avacincaptad Pegol for Geographic Atrophy Due to Age-Related Macular Degeneration: A Randomized Pivotal Phase 2/3 Trial. Ophthalmology. 2021; 128: 576–586.
- WHO. World report on vision. 2019. Available at: https: //www.who.int/publications/i/item/world-report-on-vision (Accessed: 17 Dec 2020).
- [12] Fleckenstein M, Keenan TDL, Guymer RH, Chakravarthy U, Schmitz-Valckenberg S, Klaver CC, et al. Age-related macular degeneration. Nature Reviews. Disease Primers. 2021; 7: 31.
- [13] Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, *et al.* Global causes of blindness and distance vision impairment 1990-2020: a systematic review and metaanalysis. The Lancet. Global Health. 2017; 5: e1221–e1234.
- [14] Wood JM, Lacherez P, Black AA, Cole MH, Boon MY, Kerr GK. Risk of falls, injurious falls, and other injuries resulting from visual impairment among older adults with age-related macular degeneration. Investigative Ophthalmology & Visual Science. 2011; 52: 5088–5092.
- [15] Dave S, Binns A, Vinuela-Navarro V, Callaghan T. What Advice Is Currently Given to Patients with Age-Related Macular Degeneration (AMD) by Eyecare Practitioners, and How Effec-

tive Is It at Bringing about a Change in Lifestyle? A Systematic Review. Nutrients. 2022; 14: 4652.

- [16] Salehi MA, Mohammadi S, Gouravani M, Rezagholi F, Arevalo JF. Retinal and choroidal changes in AMD: A systematic review and meta-analysis of spectral-domain optical coherence tomography studies. Survey of Ophthalmology. 2023; 68: 54–66.
- [17] Spaide RF, Jaffe GJ, Sarraf D, Freund KB, Sadda SR, Staurenghi G, *et al.* Consensus Nomenclature for Reporting Neovascular Age-Related Macular Degeneration Data: Consensus on Neovascular Age-Related Macular Degeneration Nomenclature Study Group. Ophthalmology. 2020; 127: 616–636.
- [18] Myers CE, Klein BEK, Gangnon R, Sivakumaran TA, Iyengar SK, Klein R. Cigarette smoking and the natural history of agerelated macular degeneration: the Beaver Dam Eye Study. Ophthalmology. 2014; 121: 1949–1955.
- [19] Chakravarthy U, Wong TY, Fletcher A, Piault E, Evans C, Zlateva G, *et al.* Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. BMC Ophthalmology. 2010; 10: 31.
- [20] Pugazhendhi A, Hubbell M, Jairam P, Ambati B. Neovascular Macular Degeneration: A Review of Etiology, Risk Factors, and Recent Advances in Research and Therapy. International Journal of Molecular Sciences. 2021; 22: 1170.
- [21] Liberski S, Wichrowska M, Kocięcki J. Aflibercept versus Faricimab in the Treatment of Neovascular Age-Related Macular Degeneration and Diabetic Macular Edema: A Review. International Journal of Molecular Sciences. 2022; 23: 9424.
- [22] Rakic JM, Lambert V, Devy L, Luttun A, Carmeliet P, Claes C, *et al.* Placental growth factor, a member of the VEGF family, contributes to the development of choroidal neovascularization. Investigative Ophthalmology & Visual Science. 2003; 44: 3186–3193.
- [23] Toto L, Borrelli E, Mastropasqua R, Di Antonio L, Doronzo E, Carpineto P, *et al.* Association between outer retinal alterations and microvascular changes in intermediate stage agerelated macular degeneration: an optical coherence tomography angiography study. The British Journal of Ophthalmology. 2017; 101: 774–779.
- [24] Trinh M, Kalloniatis M, Nivison-Smith L. Radial Peripapillary Capillary Plexus Sparing and Underlying Retinal Vascular Impairment in Intermediate Age-Related Macular Degeneration. Investigative Ophthalmology & Visual Science. 2021; 62: 2.
- [25] Kim LA, D'Amore PA. A brief history of anti-VEGF for the treatment of ocular angiogenesis. The American Journal of Pathology. 2012; 181: 376–379.
- [26] Breen EC. VEGF in biological control. Journal of Cellular Biochemistry. 2007; 102: 1358–1367.
- [27] Ferrara N. Vascular endothelial growth factor and age-related macular degeneration: from basic science to therapy. Nature Medicine. 2010; 16: 1107–1111.
- [28] Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science (New York, N.Y.). 1989; 246: 1306–1309.
- [29] Pożarowska D, Pożarowski P. The era of anti-vascular endothelial growth factor (VEGF) drugs in ophthalmology, VEGF and anti-VEGF therapy. Central-European Journal of Immunology. 2016; 41: 311–316.
- [30] Plyukhova AA, Budzinskaya MV, Starostin KM, Rejdak R, Bucolo C, Reibaldi M, *et al.* Comparative Safety of Bevacizumab, Ranibizumab, and Aflibercept for Treatment of Neovascular Age-Related Macular Degeneration (AMD): A Systematic Review and Network Meta-Analysis of Direct Comparative Studies. Journal of Clinical Medicine. 2020; 9: 1522.
- [31] Dugel PU, Koh A, Ogura Y, Jaffe GJ, Schmidt-Erfurth U, Brown DM, et al. HAWK and HARRIER: Phase 3, Multicenter, Randomized, Double-Masked Trials of Brolucizumab for Neovascu-

lar Age-Related Macular Degeneration. Ophthalmology. 2020; 127: 72–84.

- [32] Nguyen CL, Oh LJ, Wong E, Wei J, Chilov M. Anti-vascular endothelial growth factor for neovascular age-related macular degeneration: a meta-analysis of randomized controlled trials. BMC Ophthalmology. 2018; 18: 130.
- [33] Tolentino M. Systemic and ocular safety of intravitreal anti-VEGF therapies for ocular neovascular disease. Survey of Ophthalmology. 2011; 56: 95–113.
- [34] Maloney MH, Payne SR, Herrin J, Sangaralingham LR, Shah ND, Barkmeier AJ. Risk of Systemic Adverse Events after Intravitreal Bevacizumab, Ranibizumab, and Aflibercept in Routine Clinical Practice. Ophthalmology. 2021; 128: 417–424.
- [35] Foxton R, Osborne A, Martin KR, Ng YS, Shima DT. Distal retinal ganglion cell axon transport loss and activation of p38 MAPK stress pathway following VEGF-A antagonism. Cell Death & Disease. 2016; 7: e2212.
- [36] Falavarjani KG, Nguyen QD. Adverse events and complications associated with intravitreal injection of anti-VEGF agents: a review of literature. Eye (London, England). 2013; 27: 787–794.
- [37] Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, et al. Ranibizumab for neovascular age-related macular degeneration. The New England Journal of Medicine. 2006; 355: 1419–1431.
- [38] Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T, et al. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. Ophthalmology. 2009; 116: 57–65.e5.
- [39] Cox JT, Eliott D, Sobrin L. Inflammatory Complications of Intravitreal Anti-VEGF Injections. Journal of Clinical Medicine. 2021; 10: 981.
- [40] Monés J, Srivastava SK, Jaffe GJ, Tadayoni R, Albini TA, Kaiser PK, et al. Risk of Inflammation, Retinal Vasculitis, and Retinal Occlusion-Related Events with Brolucizumab: Post Hoc Review of HAWK and HARRIER. Ophthalmology. 2021; 128: 1050–1059.
- [41] de Vries VA, Bassil FL, Ramdas WD. The effects of intravitreal injections on intraocular pressure and retinal nerve fiber layer: a systematic review and meta-analysis. Scientific Reports. 2020; 10: 13248.
- [42] Levin AM, Chaya CJ, Kahook MY, Wirostko BM. Intraocular Pressure Elevation Following Intravitreal Anti-VEGF Injections: Short- and Long-term Considerations. Journal of Glaucoma. 2021; 30: 1019–1026.
- [43] Goldhardt R, Batawi HIM, Rosenblatt M, Lollett IV, Park JJ, Galor A. Effect of Anti-Vascular Endothelial Growth Factor Therapy on Corneal Nerves. Cornea. 2019; 38: 559–564.
- [44] Nishijima K, Ng YS, Zhong L, Bradley J, Schubert W, Jo N, et al. Vascular endothelial growth factor-A is a survival factor for retinal neurons and a critical neuroprotectant during the adaptive response to ischemic injury. The American Journal of Pathology. 2007; 171: 53–67.
- [45] Saint-Geniez M, Maharaj ASR, Walshe TE, Tucker BA, Sekiyama E, Kurihara T, *et al*. Endogenous VEGF is required for visual function: evidence for a survival role on müller cells and photoreceptors. PLoS ONE. 2008; 3: e3554.
- [46] Froger N, Matonti F, Roubeix C, Forster V, Ivkovic I, Brunel N, et al. VEGF is an autocrine/paracrine neuroprotective factor for injured retinal ganglion neurons. Scientific Reports. 2020; 10: 12409.
- [47] Martinez-de-la-Casa JM, Ruiz-Calvo A, Saenz-Frances F, Reche-Frutos J, Calvo-Gonzalez C, Donate-Lopez J, et al. Retinal nerve fiber layer thickness changes in patients with age-related macular degeneration treated with intravitreal ranibizumab. Investigative Ophthalmology & Visual Science. 2012; 53: 6214–6218.

- [48] Sobaci G, Güngör R, Ozge G. Effects of multiple intravitreal anti-VEGF injections on retinal nerve fiber layer and intraocular pressure: a comparative clinical study. International Journal of Ophthalmology. 2013; 6: 211–215.
- [49] Parlak M, Oner FH, Saatci AO. The long-term effect of intravitreal ranibizumab on retinal nerve fiber layer thickness in exudative age-related macular degeneration. International Ophthalmology. 2015; 35: 473–480.
- [50] Jo YJ, Kim WJ, Shin IH, Kim JY. Longitudinal Changes in Retinal Nerve Fiber Layer Thickness after Intravitreal Anti-vascular Endothelial Growth Factor Therapy. Korean Journal of Ophthalmology: KJO. 2016; 30: 114–120.
- [51] Valverde-Megías A, Ruiz-Calvo A, Murciano-Cespedosa A, Hernández-Ruiz S, Martínez-de-la-Casa JM, García-Feijoo J. Long-term effect of intravitreal ranibizumab therapy on retinal nerve fiber layer in eyes with exudative age-related macular degeneration. Graefe's Archive for Clinical and Experimental Ophthalmology = Albrecht Von Graefes Archiv Fur Klinische Und Experimentelle Ophthalmologie. 2019; 257: 1459–1466.
- [52] Ahn J, Jang K, Sohn J, Park JI, Hwang DDJ. Effect of intravitreal ranibizumab and aflibercept injections on retinal nerve fiber layer thickness. Scientific Reports. 2021; 11: 5010.
- [53] Zucchiatti I, Cicinelli MV, Parodi MB, Pierro L, Gagliardi M, Accardo A, *et al*. Effect of intravitreal ranibizumab on ganglion cell complex and peripapillary retinal nerve fiber layer in neovascular age-related macular degeneration using spectral domain optical coherence tomography. Retina. 2017; 37: 1314– 1319.
- [54] Zivkovic M, Radosavljevic A, Zlatanovic M, Jaksic V, Davidovic S, Stamenkovic M, *et al.* Influence of Multiple Anti-VEGF Injections on Retinal Nerve Fiber Layer and Ganglion Cell-Inner Plexiform Layer Thickness in Patients with Exudative Age-Related Macular Degeneration. Medicina (Kaunas, Lithuania). 2023; 59: 138.
- [55] Demirel S, Batioğlu F, Özmert E, Erenler F. The effect of multiple injections of ranibizumab on retinal nerve fiber layer thickness in patients with age-related macular degeneration. Current Eye Research. 2015; 40: 87–92.
- [56] Horsley MB, Mandava N, Maycotte MA, Kahook MY. Retinal nerve fiber layer thickness in patients receiving chronic antivascular endothelial growth factor therapy. American Journal of Ophthalmology. 2010; 150: 558–561.e1.
- [57] Wang L, Swaminathan SS, Yang J, Barikian A, Shi Y, Shen M, et al. Dose-Response Relationship between Intravitreal Injections and Retinal Nerve Fiber Layer Thinning in Age-Related Macular Degeneration. Ophthalmology. Retina. 2021; 5: 648–654.
- [58] Yau GL, Campbell RJ, Li C, Sharma S. Peripapillary RNFL thickness in nonexudative versus chronically treated exudative age-related macular degeneration. Canadian Journal of Ophthalmology. Journal Canadien D'ophtalmologie. 2015; 50: 345– 349.
- [59] Jun SY, Hwang DDJ. Short-term effect of intravitreal brolucizumab injections in patients with neovascular age-related macular degeneration on retinal nerve fiber layer thickness. Scientific Reports. 2023; 13: 6685.
- [60] Lee SW, Sim HE, Park JY, Kim JS, Chang IB, Park YS, et al. Changes in inner retinal layer thickness in patients with exudative age-related macular degeneration during treatment with anti-vascular endothelial growth factor. Medicine. 2020; 99: e19955.
- [61] Beck M, Munk MR, Ebneter A, Wolf S, Zinkernagel MS. Retinal Ganglion Cell Layer Change in Patients Treated With Anti-Vascular Endothelial Growth Factor for Neovascular Agerelated Macular Degeneration. American Journal of Ophthalmology. 2016; 167: 10–17.
- [62] Inan ÜÜ, Baysal Z, Inan S. Long-term changes in retinal lay-



ers in patients undergoing intravitreal ranibizumab for neovascular age-related macular degeneration: Retinal layers after anti-VEGF therapy. International Ophthalmology. 2019; 39: 2721– 2730.

- [63] Lüke JN, Alquoqa H, Alsamman A, Aljabary B, Schaub F, Heindl LM. Retrospective analysis of OCT parameters after intravitreal anti-VEGF inhibitors in neovascular AMD patients in a real-world setting. International Ophthalmology. 2023; 43: 13–25.
- [64] Wichrowska M, Wichrowski P, Kocięcki J. Morphological and Functional Assessment of the Optic Nerve Head and Retinal Ganglion Cells in Dry vs Chronically Treated Wet Age-Related Macular Degeneration. Clinical Ophthalmology (Auckland, N.Z.). 2022; 16: 2373–2384.
- [65] Shin HJ, Kim SN, Chung H, Kim TE, Kim HC. Intravitreal Anti-Vascular Endothelial Growth Factor Therapy and Retinal Nerve Fiber Layer Loss in Eyes With Age-Related Macular Degeneration: A Meta-Analysis. Investigative Ophthalmology & Visual Science. 2016; 57: 1798–1806.
- [66] Michalska-Małecka K, Kabiesz A, Kimsa MW, Strzałka-Mrozik B, Formińska-Kapuścik M, Nita M, et al. Effects of intravitreal ranibizumab on the untreated eye and systemic gene expression profile in age-related macular degeneration. Clinical Interventions in Aging. 2016; 11: 357–365.
- [67] Savastano MC, Minnella AM, Tamburrino A, Giovinco G, Ventre S, Falsini B. Differential vulnerability of retinal layers to early age-related macular degeneration: evidence by SD-OCT segmentation analysis. Investigative Ophthalmology & Visual Science. 2014; 55: 560–566.
- [68] Kim JM, Lee MW, Lim HB, Won YK, Shin YI, Lee WH, et al. Longitudinal changes in the ganglion cell-inner plexiform layer thickness of age-related macular degeneration. Acta Ophthalmologica. 2021; 99: e1056–e1062.
- [69] Lee SE, Lim HB, Shin YI, Ryu CK, Lee WH, Kim JY. Characteristics of the inner retinal layer in the fellow eyes of pa-

tients with unilateral exudative age-related macular degeneration. PLoS ONE. 2020; 15: e0239555.

- [70] Tong J, Phu J, Khuu SK, Yoshioka N, Choi AY, Nivison-Smith L, et al. Development of a Spatial Model of Age-Related Change in the Macular Ganglion Cell Layer to Predict Function From Structural Changes. American Journal of Ophthalmology. 2019; 208: 166–177.
- [71] Trinh M, Khou V, Zangerl B, Kalloniatis M, Nivison-Smith L. Modelling normal age-related changes in individual retinal layers using location-specific OCT analysis. Scientific Reports. 2021; 11: 558.
- [72] Saks D, Schulz A, Craig J, Graham S, PROGRESSA Study Group. Determination of retinal nerve fibre layer and ganglion cell/inner plexiform layers progression rates using two optical coherence tomography systems: The PROGRESSA study. Clinical & Experimental Ophthalmology. 2020; 48: 915–926.
- [73] Jeoung JW, Park KH. Comparison of Cirrus OCT and Stratus OCT on the ability to detect localized retinal nerve fiber layer defects in preperimetric glaucoma. Investigative Ophthalmology & Visual Science. 2010; 51: 938–945.
- [74] Sung KR, Kim DY, Park SB, Kook MS. Comparison of retinal nerve fiber layer thickness measured by Cirrus HD and Stratus optical coherence tomography. Ophthalmology. 2009; 116: 1264–1270.e1.
- [75] Mulak M, Cicha A, Kaczorowski K, Markuszewski B, Misiuk-Hojło M. Using Spectralis and Stratus optical coherence tomography devices to analyze the retinal nerve fiber layer in patients with open-angle glaucoma - preliminary report. Advances in Clinical and Experimental Medicine: Official Organ Wroclaw Medical University. 2013; 22: 831–837.
- [76] Shin IH, Lee WH, Lee JJ, Jo YJ, Kim JY. THICKNESS OF THE MACULA, RETINAL NERVE FIBER LAYER, AND GAN-GLION CELL-INNER PLEXIFORM LAYER IN THE AGE-RELATED MACULAR DEGENERATION: The Repeatability Study of Spectral Domain Optical Coherence Tomography. Retina. 2018; 38: 253–262.