Diagnosis, Treatment and Prevention of Age-Related Macular Degeneration

Edited by

Laurent Kodjikian

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Contents

Editorial

Age-Related Macular Degeneration: New Insights in Diagnosis, Treatment, and Prevention

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Age-related macular degeneration (AMD) is an aging-related ocular disease that can be responsible for severe loss of visual acuity and loss of autonomy in patients. Although there has been a dramatic reduction in legal blindness resulting from AMD since the introduction of anti-vascular growth factor (VEGF) therapy in 2006 [1], the disease still causes a number of patients to progressively and irrevocably lose their vision despite treatment. However, the advent of the first approved intravitreal therapy for AMD has revolutionized AMD management, as well as our understanding of its pathophysiology and risk factors [2,3]. These findings have contributed to the emergence of major technological innovations in ophthalmology in recent years, particularly in terms of retinal imaging techniques. Multimodal examination now means very early, and extremely precise diagnoses can be made. From a therapeutic point of view, extensive research has been undertaken to investigate new molecules for the treatment of neovascular AMD developed in phases 1, 2, or 3. Atrophic AMD, which could be seen as the poor relation to the neovascular form, has also benefited from more research in these areas. This Special Issue on AMD is divided into three sections: (1) new insights into diagnosis and pathophysiology revealed by modern multimodal imaging; (2) current knowledge regarding treatment to improve visual outcomes, and the future therapeutic molecules being developed; (3) preventive medicine to control risk factors and limit vision loss.

The advent of multimodal imaging in ophthalmology has not simply revolutionized the diagnosis and management of retinal disease but has also led to advances in our understanding of the pathophysiology of the disease and the mechanisms leading to vision loss. The introduction of optical coherence tomography angiography (OCTA) was a great leap forward in terms of our ability to study the microvasculature of the macula and the changes brought about by AMD. Firstly, OCTA enables the noninvasive detection of the neovascular network by showing aberrant flow above or below the retinal pigment epithelium layer. Secondly, it can show modifications to the normal macular vascularization resulting from the disease itself or the aging of the retinal and choroidal tissue [4]. As demonstrated in diabetic macular edema, it appears that intraretinal fluid, which is associated with poorer visual prognosis in AMD, is related to worse vascular reperfusion when treated, in comparison to subretinal fluid [5]. Moreover, deep exploration inside the macular vascularization, at the choriocapillaris level, has demonstrated that the fellow-eyes in cases of unilateral neovascular AMD also showed vascular abnormalities such as nonexudative AMD (known as quiescent neovascularization, sometimes seen on invasive indocyanine green angiography), or choriocapillaris flow deficit, as already described in cases of high blood pressure or diabetes [4,6,7].

The earliest possible diagnosis of AMD and its neovascular complication allows for prompt treatment of the disease with intravitreal injections of anti-VEGF. Many studies have proved the efficacy and safety of bevacizumab, ranibizumab, and aflibercept in treating neovascular AMD [2,3,8] and other related diseases [9,10]. However, some differences in

visual gain and anatomical outcomes exist between series, which appear to be associated with the baseline patient and disease characteristics and the treatment regimen used. Firstly, eyes presenting high visual acuity at baseline had a lower functional response despite maintaining higher final vision than other eyes [8,11]. Moreover, some other factors such as larger macular neovessels [12], increased age [8], or cardiovascular disease [13] were found to be independent risk factors of poor functional outcomes following anti-VEGF therapy. Secondly, the frequency of anti-VEGF injections seems to be one of the most relevant factors influencing functional outcomes [14]. This is supported by the results of randomized clinical trials (RCTs) that typically show better visual gain in comparison to real-life studies [8]. This is explained by the higher number of injections and better follow-up of patients in RCTs, thereby motivating frequent retreatment. Therefore, a proactive regimen usually leads to better outcomes in comparison to an as-needed regimen, as it maintains a high number of injections and reduced treatment burden with visits required. However, the follow-up of a patient with neovascular AMD treated under a proactive regimen is not always plain sailing and can bethrown off course by unforeseen events, such as the onset of another age-related disease. One example of this is the onset of cataracts, which contributes to vision loss. Although cataract surgery is a well-known risk factor for exudative recurrence, special care should be taken before surgery to controlling the neovascular process, and real-world data have suggested a minimum of 6 months of anti-VEGF treatment before surgery. Patients should also be advised of the uncertainty concerning the visual improvement they can expect following surgery [15]. Another example is the recurrence of neovascular exudation despite a short interval between two injections. The recurrence or persistence of retinal fluid in the long term, and macular thickness fluctuations, in particular, have been shown to be predictive factors of vision loss under treatment [16]. In order to minimize exudation, new therapeutic approaches have been developed or are currently under investigation. Brolucizumab is a novel 26 kDa single-chain antibody fragment targeting anti-VEGF and was approved by the FDA in 2019, following the completion of the HAWK and HARRIER RCTs [17]. Its small molecular size allows for a higher molecular concentration in a standard 0.05 mL volume dose. This results in an increased interval between injections in the RCTs and significantly improved control of exudation. The first real-world studies of brolucizumab for AMD seem to confirm these data [18,19], both in treatment-naïve eyes and when switching from another therapy [18,20]. However, the increased, although still rare, the occurrence of intraocular inflammatory adverse events (which can lead to vascular occlusion and vision loss) for this new molecule means a change in the treatment paradigm toward injections that are more spaced out is likely, helped by its improved efficacy [21]. In the coming years, the therapeutic arsenal will be totally transformed by numerous molecules in development [22], and the discovery of new therapeutic targets [23], with the aim of reducing the therapeutic burden and improving visual outcomes.

Generally speaking, the best way of preventing vision loss is to detect and diagnose the disease at an early stage, especially for the exudative form. Observational studies show that nearly one-fourth of patients are diagnosed and begin anti-VEGF treatment with visual acuity of 20/40 or better. However, it is well known that the patients with the best visual acuity at baseline retain better vision. It is therefore necessary to frequently monitor eyes with early or intermediate AMD. Nevertheless, requiring a large, aging population to attend clinics for frequent and regular ocular examinations brings with it its own set of difficulties. Identifying the patients at risk of disease progression at an early stage would be extremely helpful, as it means treatment can be initiated promptly, leading to better visual outcomes. A number of risk scores have been described, most of which include genetic polymorphism assessment and fundus examination [24–26]. However, they are not used in routine practice due to their complexity. The simplified test AMD risk-assessment scale (STARS®) is an easy-to-compile self-administered 13-item questionnaire evaluating individual risk for AMD in daily practice, focusing on demographic, cardiovascular, and lifestyle risk factors. A group of AMD specialists, using the Delphi method, has put forward recommendations

to optimize the preventive care of patients in different geographical areas at risk of AMD based on STARS® scoring. [27]. Another approach to preventing vision loss is to detect progression earlier by developing an at-home self-monitoring method. To date, only the Amsler grid is widely used in a number of different countries, but it has demonstrated poor sensitivity and specificity in detecting the disease at an early stage. Different self-monitoring devices have been tested, such as the hyperacuity test, to proactively monitor visual status and immediately alert the ophthalmologist in the event of a drop in vision. Although these tests have demonstrated good efficacy in clinical trials, and recent publications have begun to compile a growing body of evidence on their effectiveness, their use in clinical practice still needs to be studied further [28].

The diagnostic and therapeutic options currently available are only able to slow down the progression of macular degeneration and cannot stop it. Therefore, the identification of risk factors, which are known to contribute to disease progression, is critical, and many studies have focused on dietary habits, lifestyle, and light exposure. Although the association between smoking and AMD is widely accepted, the conclusions of studies evaluating the dietary intake of nutrients such as omega-3 fatty acids, beta carotene, lutein, and zeaxanthin seem less convincing. However, in the absence of new therapies capable of improving the clinical course of AMD, implementing preventive strategies could be an alternative, as well as advising a Mediterranean diet, which has also been shown to be effective in preventing the onset of AMD. Lastly, the role of light, ultraviolet rays, and blue light, in particular, needs to be further investigated since, to date, retinal toxicities associated with these wavelengths have only been demonstrated in experimental models [29].

In this Special Issue, we hope to review the basics and highlight the latest developments in AMD. To be sure, many other studies have been published elsewhere and have produced interesting data. This demonstrates the benefits of the international scientific community working on this disease, to limit its negative impacts, the most vital of which is the loss of visual function, leading to a loss of autonomy and a decrease in patients' quality of life.

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Article

Initial Pro Re Nata Brolucizumab for Exudative AMD: The PROBE Study

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Abstract: The present study aimed to determine the efficacy and safety of pro re nata (PRN) intravitreal brolucizumab therapy for neovascular age-related macular degeneration (AMD) without a loading dose in the real-world setting. The PROBE study (Pro Re Nata Brolucizumab for Exudative AMD) is a retrospective, observational, multicentric study that included 27 treatment-naïve patients (27 eyes) with neovascular AMD who received PRN brolucizumab therapy with the treatment interval being at least 8 weeks, should the need for a second consecutive injection arise. The primary outcome measure was changed to best-corrected visual acuity (BCVA) over time. Secondary outcome measures included the determination of change in central subfield thickness (CST) and complications. The mean follow-up was 11.2 ± 1.2 months. The mean baseline and final BCVA were 57.4 ± 4.5 letters and 65.3 ± 3.12 letters, respectively ($p = 0.014$). The mean gain in letters at the end of follow-up was 7.8 ± 3.5 letters. There was a significant decrease in CST at the end of the follow-up period ($p = 0.013$). Patients received a mean of 2.2 ± 0.9 injections (in addition to the first mandatory injection) during the follow-up period. There were no adverse events noted. In conclusion, initial PRN brolucizumab for exudative AMD without a loading dose demonstrated significant visual improvement and no adverse events.

Keywords: age-related macular degeneration; anti-vascular endothelial growth factor; brolucizumab; exudation; treatment-naive

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly in industrialized countries. There are two forms of the disease—atrophic or neovascular—the latter being characterized by the formation of new blood vessels either under or above the retinal pigment epithelium (RPE). Before the advent of anti-vascular endothelial growth factor (VEGF) therapy, thermal laser, intravitreal steroid injections, and photodynamic therapy, or a combination of these, were considered the standard of care. Anti-VEGF agents have revolutionized therapy for neovascular age-related macular degeneration (nAMD) [1,2]. Although these molecules provide excellent results when injected every month, visual loss is observed when the treatment is given less frequently [3]. A decade's experience of anti-VEGF therapy has taught us to minimize therapy and to maximize visual gains, thereby sparing patients the physical and psychological burden of multiple treatment visits [4] and the potential threat of geographic atrophy (although this

rarely manifests, if ever) [5]. Pro re nata injections and other less frequent injection protocols [6–10] attempt to achieve this without compromise on visual outcomes. An alternative approach would be to look at more potent and durable formulations that obviate the need for intense therapy.

The latest development in anti-VEGF therapy has been the introduction of brolucizumab, a 26 kDa anti-VEGF antibody that is far smaller than currently available agents such as ranibizumab, bevacizumab, or aflibercept. This allows the manufacturer to pack a higher molecular concentration into the standard 0.05 mL volume, in the hope of increasing the durability of the molecule in the intravitreal compartment. The HAWK and HARRIER studies have established the non-inferiority of the new molecule brolucizumab compared to aflibercept, with some analyses suggesting a superior anatomic outcome [11]. Nearly 50% of enrolled patients could receive 12 weekly injections, considerably reducing the treatment burden. However, concerns about safety with special reference to intraocular inflammation and vasculitis have dampened the initial enthusiasm for the drug [12]. As the data evolves, the risk of serious adverse events is continuously updated (www.brolucimab.info, accessed on 29 May 2021) [13]. The reported predisposing factors for intraocular inflammation following brolucizumab injection include female gender, multiple past treatments, and frequent injections, among others [14].

The current analysis investigated the efficacy and safety of pro re nata brolucizumab for nAMD in a real-world setting.

2. Materials and Methods

The PROBE (Pro Re Nata Brolucizumab for Exudative AMD) study is an observational, retrospective, multicenter study conducted at the Sudhalkar and Raghhudeep group of hospitals in India. A database search was performed for patients with treatment-naïve macular neovascularization (MNV) who received brolucizumab as intravitreal therapy. This study complied with the tenets of the Declaration of Helsinski and was approved by the ethics committee for the Raghudeep Eye Hospital, Ahmedabad, India. Patients provided informed consent for participation in the study.

2.1. Eligibility

The PROBE study examined the outcomes in treatment-naïve patients with nAMD who received PRN intravitreal brolucizumab therapy. Patients needed to complete a minimum of 10 months follow-up for inclusion. Patients with polypoidal choroidal vasculopathy (PCV) or retinal angiomatous proliferation (RAP) were excluded.

2.2. Definitions and Grading

Type I MNV (historically called 'occult' neovascularization) was defined by the presence of a neovascular membrane under the RPE layer. Type II MNV (historically called 'classic' neovascularization) was defined by the presence of a neovascular membrane above the RPE layer. A mixed lesion was defined by the presence of both neovascular components: type 1 and type 2 MNV. Macular fluid was classified as intraretinal (IRF) or subretinal fluid (SRF) according to the recent consensus guidelines [15]. Fluid disappearance post-injection was considered to be a complete response. Pigment epithelial detachment (PED) was noted if present, but it was not considered to be an independent treatment criterion as in the HAWK and HARRIER trials. A recurrence was defined as a complete resolution of fluid in the intraretinal and/or subretinal compartment followed by recurrent fluid in at least one compartment. Baseline images were graded independently by two of the investigators (AS and AB) and adjudicated by a senior colleague (LK). Patients received one mandatory injection at baseline; subsequent injections were administered only if persistent fluid was present more than 8 weeks after the first injection. Even if there was persistent fluid at the end of 4 weeks, patients were followed up until 8 weeks.

2.3. Acquisition of Data

Data retrieved included patient demographics; the best-corrected visual acuity as recorded using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart (also mentioned in the manuscript in Snellen's notations for ease of interpretation); the best-corrected visual acuity (BCVA); intraocular pressure (IOP); the details of the ocular examination and special investigations conducted, such as fluorescein angiography (FA) and/or indocyanine angiography (ICGA) and central subfield thickness (CST) as determined by SD-OCT (Heidelberg Spectralis, Heidelberg Engineering, Heidelberg, Germany); the type of MNV (type 1/type 2/mixed); the size and location thereof; the anti-VEGF agents used; the number of injections administered; the treatment-free interval; and a switch to an alternative anti-VEGF agent, if any. In addition, BCVA, measurement of IOP, slit-lamp examination, fundoscopy, and SD-OCT were documented at each visit.

2.4. Follow-Up

Intravitreal injections were performed using a standardized aseptic technique. Follow-up was performed on days 1, 7, 15, and 30 following the first injection, and was then followed monthly. SD-OCT scans were performed at weeks 2, 4, and 8, and then every 4 weeks.

2.5. Outcome Measures

The primary outcome measure was taken to determine the change in BCVA from baseline with treatment. Secondary outcome measures included the change of CST in SD-OCT, the mean number of injections required to achieve the complete resolution of exudation, and any complications associated therewith.

2.6. Statistical Analysis

This being a real-world study, the number of eyes recruited for analysis was based on past literature that looked at less frequent therapy without compromise on visual outcomes [16]. The description of categorical variables was based on absolute (size) and relative (percentage) frequencies. Quantitative variables were represented as the mean and standard deviation. The comparison of the categorical variables between the groups of different indications was performed using Fisher's exact test. When the pairwise comparisons were subsequently performed, the p-value was adjusted using the Benjamini–Hochberg method, wherever applicable. A p-value < 0.05 was considered to be statistically significant.

3. Results

A total of 27 eyes of 27 patients (15 females and 12 males) have received PRN intravitreal brolucizumab at our centers thus far and have completed at least 10 months of follow-up. Table 1 lists the salient characteristics of these eyes, and this analysis forms the basis for our study.

The mean time to treatment after the beginning of symptoms was 37.2 ± 11.5 days. The baseline BCVA was 57.4 ± 4.5 letters and the mean CST was 398.1 ± 47.2 μm. The most frequent MNV was type 1, and the mean area of the neovascular membrane was 169.4 ± 34.5 μm.

A total of 7/27 eyes (25.9%) showed completely resolved exudation after one injection, 13/27 eyes (48.2%) showed complete resolution of exudation after two injections and the remaining seven eyes (25.9%) needed three or more injections (Figure 1). Recurrence in exudation was seen in 23/27 eyes (85.2%) prior to the end of follow-up. Recurrence was seen a mean of 3.7 ± 1.2 months after the last injection. Four eyes (14.8%) did not show any recurrence in exudation prior to the last follow-up.

Table 1. Baseline characteristics of patients with treatment-naïve nAMD who received brolucizumab therapy.

Characteristic	Treatment Naïve (*N* = 27)
Mean age, years (SD)	65.1 (3.4)
Male:Female, *n*	12:15
Follow-up, months (SD)	11.2 (1.2)
Mean BCVA, letters (SD)	57.4 (4.5)
Mean CST, μm (SD)	398.1 (47.2)
MNV subtype, *n*:	
Type I	16
Type II	8
Mixed	3
Fluid localization, *n* *:	
IRF	18
SRF	8
PED	16

BCVA: best-corrected visual acuity; CST: central subfield thickness; IRF: intraretinal fluid; MNV: choroidal neovascularization; PED: pigment epithelium detachment; SD: standard deviation; SRF: subretinal fluid. * Patients could have fluid in more than one compartment.
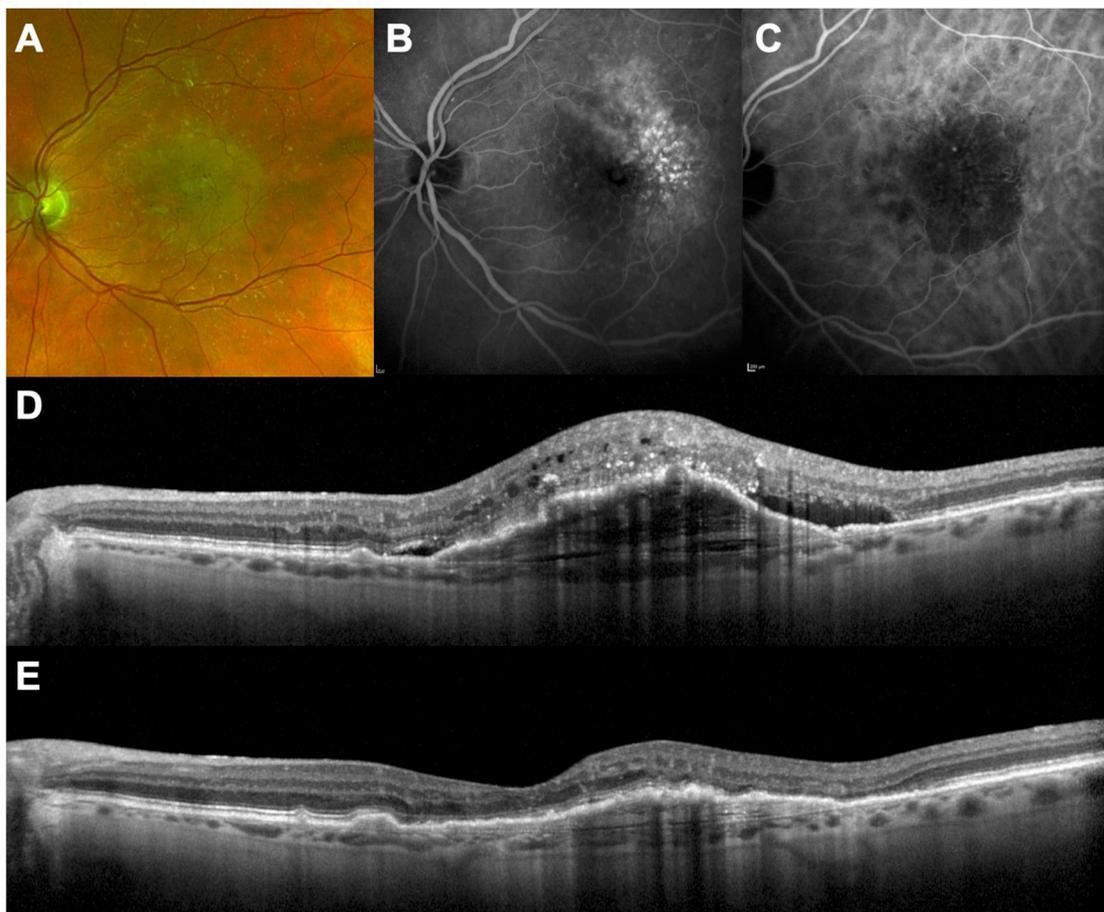
Figure 1. A 72-year-old female with vision loss and metamorphopsia in her left eye beginning 5 days previously. (**A–C**): Multimodal imaging at baseline showing type 1 macular neovascularization in a context of exudative AMD; (**D**) SD-OCT showing pigment epithelial detachment, subretinal fluid, and intraretinal fluid. Visual acuity was 68 letters; (**E**) SD-OCT 1 month after a single intravitreal injection of brolucizumab showing total regression of retinal fluid. Visual acuity increased to 76 letters.

The mean CST decreased significantly to 283.0 ± 57.2 µm at the final visit from the presentation CST of 398.1 ± 47.2 µm ($p = 0.021$). Patients received an average of 2.2 ± 0.9 brolucizumab injections (range: 1–4 injections in addition to the first mandatory injection) over the mean course of 11.2 ± 1.2 months.

3.1. Visual Gain

The mean BCVA significantly increased from the baseline (57.4 ± 4.5 letters) to the final visit (65.3 ± 3.1 letters; $p = 0.014$). The mean letter gain in vision was 7.8 ± 3.5 letters (Figure 2). A total of 5/27 eyes (18.5%) gained 15 letters or more from baseline at one month after the loading dose and another 7/27 eyes (25.9%) showed a 10-letter gain. At the end of the follow-up, 14/27 patients (51.9%) retained a BCVA ≥ 20/30. Moreover, none of the patients lost letters from the baseline.
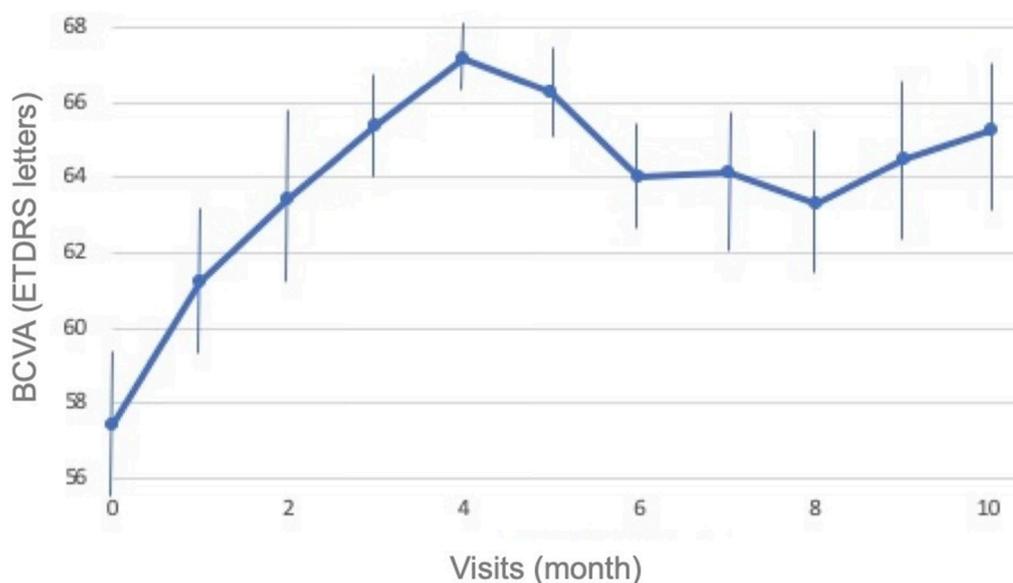
Figure 2. Evolution of best-corrected visual acuity (BCVA) during the follow-up period.

3.2. Adverse Events

We did not note a single case of intraocular inflammation during follow-up in any of the 27 eyes. We also did not note any instance of post-injection endophthalmitis or visual loss in any of the study eyes until the last follow-up. None of the patients was required to be switched to an alternative anti-VEGF agent.

4. Discussion

The present study showed good functional and anatomical results following the intravitreal injections of brolucizumab in a PRN regimen for the treatment of naïve eyes with nAMD. Moreover, almost a half of patients demonstrated a significant vision gain of ≥10 to 15 letters, occurring as early as 1 month after the first injection. Approximately three quarters of eyes demonstrated complete resolution of exudation with two injections, and a quarter showed complete resolution with one injection. The chosen minimum retreatment interval of 8 weeks was based on the findings of the HAWK and HARRIER studies, which showed a low rate of disease activity of patients treated with brolucizumab [11]. No significant adverse events were reported here. Despite the longer injection interval, our visual gains are readily comparable to the findings of the HAWK and HARRIER studies. This demonstrates that the chosen treatment interval may not adversely influence the visual gain while fulfilling our anatomical objectives of a dry macula. In addition to our study, other real-life studies have demonstrated the effectiveness of brolucizumab injections in

naïve, but also switch patients [17–21]. These data, combined with the present study, reinforce the results of the pivotal randomized controlled trial [11].

Since the advent of anti-VEGF therapy, numerous studies have demonstrated that monthly injections provide high and sustained visual gain, but were found to be rather impractical and perhaps even unsustainable in the long term. Past literature is replete with instances of different studies looking at less intensive treatment regimens. The PRN regime is one such protocol, as is the treat-and-extend strategy. Some studies have even questioned the need for a loading dose. Monés et al. have looked at the possibility of combining fixed interval and PRN injections for nAMD, thereby reducing the total number of requisite treatments while maintaining visual acuity gains comparable to historical evidence published for monthly injections [16]. Moreover, it has also been shown that a single dose followed by a PRN strategy provided comparable and sustained visual gains to strategies that incorporated a loading dose followed by PRN therapy [22]. Finally, we recently showed that some patients need only one anti-VEGF injection over the long term, arguing against the historical three loading doses [23]. In the same way, the treat-and-extend regimen was introduced as an alternative to monthly injections, allowing adjustment of the reinjection interval by 2 weeks according to the disease activity. Recently, the ALTAIR study looked at liberalization of the treatment regimen by introducing four weekly extensions to the treatment interval as opposed to the standard two weekly extensions [24].

Brolucizumab was designed to provide better efficacy and a longer duration of action, thereby promoting a longer treatment interval. It follows, then, that every new anti-VEGF molecule that is approved for use in wet AMD should have its own treatment protocol and that past experience and older protocols with similar molecules may guide present protocols but may not always be replicated. In other words, monthly injections or treat-and-extend protocols may actually be considered obsolete as far as brolucizumab is concerned; this is evolving data, however, and only long-term analyses will point towards more appropriate treatment regimens. Moreover, the original concept of loading dose should be revisited, in our opinion, given that it was formulated for molecules (such as ranibizumab and aflibercept) that were far less potent and had a far lower molecular concentration than brolucizumab. The results found herein support this new paradigm of treatment as we demonstrated good outcomes provided by a strategy based on one initial injection of brolucizumab followed immediately by PRN injections. This can be explained by the small size of the molecule, which allows a higher concentration of brolucizumab to be delivered to the vitreous in comparison to other FDA-approved drugs. The large dose of anti-VEGF probably accounts for the improved efficacy and durability, despite a higher rate of hypersensitivity-like reactions that are rarely reported for other agents [25]. The initial enthusiasm for this new molecule has been somewhat offset by reports of an increased propensity to produce inflammatory side effects, such as hyalitis or vasculitis [14,17,26]. However, due to the limited therapeutic arsenal available in the treatment of nAMD, brolucizumab could be an effective alternative anti-VEGF molecule. One explanation for the increased incidence of inflammatory events could probably be the increased ocular concentration of the drug and thereby its degradation products. It has already been hypothesized that it is these degradation products that lead to trabecular meshwork clogging [27] and thus a sustained rise in intraocular pressure. It is probable that this accumulation of degradation products also influences a currently poorly understood inflammatory reaction. However, this needs further analysis. It is with this phenomenon under consideration that we decided to explore PRN brolucizumab for nAMD.

The main limitation of the present study was its retrospective design and relatively small size. Some data may be missing, and some patients may have been lost to follow-up. It is possible that our study was not sufficiently powered to determine the incidence of intraocular inflammation (currently reported to be around 5%, increasing to approximately 9% in the MERLIN trial, NCT03710564). However, we only included patients with a minimum of 10 months follow-up, thus providing useful information on the first year of treatment. Although the eyes included were fewer than in most trials, the follow-

up was adequate. Intraocular inflammation has been observed, overall, after a mean of two injections in most eyes and is more common amongst females. In addition, real-life observational studies allow the analysis of populations with characteristics that are different from those included in randomized studies, such as those with high or low baseline visual acuity. Finally, the aim of this study was to provide a foothold for future alternatives as well as to open up newer avenues for treatment regimens that may signify a break from traditional monthly or treat-and-extend protocols. Indeed, monthly injections are probably an important reason for the high number of instances of intraocular inflammation noted in the recently aborted MERLIN study (NCT03710564). Finally, the loading dose concept need not necessarily entail four weekly injections. For molecules with a longer duration of action, even an eight weekly schedule should be equally effective. This requires further analysis.

To conclude, intravitreal brolucizumab therapy is effective when administered PRN in treatment-naïve patients with nAMD. We did not note any adverse events during the follow-up. Furthermore, the extended treatment interval did not compromise visual gains when compared to historically published literature. The initial PRN regimen with brolucizumab for nAMD thus appears to be a valid alternative.

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Real-World Experience with Brolucizumab in Wet Age-Related Macular Degeneration: The REBA Study

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Abstract: The aim of the present study was to determine the efficacy and safety of intravitreal brolucizumab therapy for neovascular age-related macular degeneration (AMD) in the real-world setting. The REBA study (real-world experience with brolucizumab in wet AMD) was a retrospective, observational, multicentric study that included 78 consecutive patients (105 eyes), with neovascular AMD, who received brolucizumab therapy. Both treatment-naive and switch-therapy patients were included. Switch therapy was based either on fluid recurrence, fluid recalcitrance, or inability to extend beyond q4/q6. All relevant data were collected. The primary outcome measure was change in best-corrected visual acuity (BCVA) over time. Secondary outcome measures included determination of change in central subfield thickness (CST) and complications. The mean baseline BCVA was 49.4 ± 5.4 letters and 40 ± 3.2 letters, and corresponding mean BCVA gain was $+11.9 \pm 3.9$ letters ($p = 0.011$) and $+10.4 \pm 4.8$ letters ($p = 0.014$) in the treatment-naive and switch-therapy groups, respectively. The change in CST was significantly decreased in the treatment-naive ($p = 0.021$) and the switch-therapy ($p = 0.013$) groups. The mean follow-up was 10.4 months in both groups. One patient in the switch-therapy group developed vascular occlusion and another a macular hole after the fifth brolucizumab injection. Both patients recovered uneventfully. In conclusion, patients showed a very good anatomical and functional response to brolucizumab therapy in the real world, regardless of prior treatment status, until the end of the follow-up period. Two significant untoward events were noted.

Keywords: age-related macular degeneration; anti-vascular endothelial growth factor; brolucizumab; exudation; switch therapy

1. Introduction

Anti-vascular endothelial growth factor (VEGF) agents have been established as the treatment of choice for neovascular age-related macular degeneration (AMD) [1,2]. Monthly injections are rather impractical and negate functional benefits through intense follow-up schedules. There are psychological and physical consequences [3] due to the strict schedules and the potential threat of geographic atrophy (although it rarely, if ever, manifests) [4]. One way to reduce the number of treatments is infrequent injections (e.g., pro re nata injections, or some manner of treat-and-inject regimen) [5–9] without compromise

on visual outcomes. Another approach would be to look at more potent formulations that obviate the need for intense therapy.

The HAWK and HARRIER studies established the non-inferiority of the new molecule brolucizumab vis-a-vis aflibercept, with some analyses suggesting a superior anatomic outcome [10]. Nearly 50% of enrolled patients could receive 12-weekly injections, considerably reducing the treatment burden. However, little is known in patients already treated for neovascular AMD who have been switched to brolucizumab therapy. Moreover, concerns about safety with special reference to intraocular inflammation and vasculitis dampened the initial enthusiasm for the drug [11]. As data continues to evolve, the risk of serious adverse events is currently fixed at 4.6% and continues to be updated [12,13].

The current analysis offers a real-world perspective on patients with wet AMD who received brolucizumab either as primary or as switch therapy.

2. Materials and Methods

The REBA study (real-world experience with brolucizumab in wet AMD) was an observational, retrospective, multicentric study conducted at Alphavision Augenarztpraxis, Bremerhaven, Germany and the Raghudeep and Sudhalkar group of eye hospitals, Ahmedabad and Baroda, India. A database search was made for patients with macular neovascularization (MNV) who received brolucizumab as intravitreal therapy. This study complied with the tenets of the Declaration of Helsinski and was approved by an international review board (Ethics Committee of the French Society of Ophthalmology, IRB 00008855 Société Française d'Ophtalmologie IRB#1) and by the ethics committee for the Raghudeep Eye Hospital, Ahmedabad, India. Patients provided informed consent for participation in the study.

2.1. Eligibility

We looked at the outcomes in treatment-naive patients with neovascular AMD who received intravitreal brolucizumab as well as those who received brolucizumab as switch therapy. Patients who received switch therapy had to have received prior therapy in accordance with the treat-and-extend protocol with either ranibizumab or aflibercept to ensure that they had been treated with current guidelines. Therapy switch was based on previously published recommendations [14]. Patients with polypoidal choroidal vasculopathy (PCV) or retinal angiomatous proliferation (RAP) were excluded.

2.2. Definitions

Standard definitions in compliance with the latest consensus guidelines [15] are reiterated here for ease of interpretation:

- Intraretinal fluid (IRF): accumulation of the fluid in retinal thickening and formation of cystoid spaces.
- Subretinal fluid (SRF): leakage in excess of the local capability of removal leading to accumulation of the fluid under the retina.
- Retinal pigment epithelial detachment (PED): a clinically evident separation of the retinal pigment epithelium (RPE) monolayer from the underlying Bruch's membrane.

Fluid at the macula was identified as intraretinal or subretinal fluid, whereas its disappearance post-injection was considered to be a 'fluid-free macula or a complete response' [7]. PED fluid was noted if present, but it was not considered to be an independent treatment criterion as per the HAWK and HARRIER trials. Baseline images were graded independently by two of the investigators (F.M.R. and A.B.) and adjudicated by a senior colleague (L.K.).

2.3. Grouping

Treatment-naive patients with macular neovascularization were assessed separately from those who had received brolucizumab as switch therapy. Switch patients did not receive the loading phase of three injections; therapy was based on clinical signs, symptoms,

and spectral-domain optical coherence tomography (SD-OCT) scans. Patients in the switch-therapy group were divided into three subgroups according to the reason for the switch:

- Recurrent group (subgroup 1): good effectiveness of the previous therapy (>100 μm reduction in fluid on SD-OCT at day 15 with recurrence at day 30).
- Recalcitrant group (subgroup 2): no reduction (<100 μm reduction in fluid on SD-OCT at days 15 and 30) or worsening disease.
- Inability-to-extend group (subgroup 3): patients for whom the therapy could not be extended after q4/q6 without fluid recurrence.

2.4. Acquisition of Data

Data retrieved included patient demographics, the best corrected visual acuity as recorded using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart (also mentioned in the manuscript in Snellen's notations for ease of interpretation), the best-corrected visual acuity (BCVA), intraocular pressure (IOP), the details of the ocular examination and special investigations conducted, such as fluorescein angiography (FA) and/or indocyanine angiography (ICGA) and central subfield thickness (CST) as determined by SD-OCT, the type of MNV (type 1/type 2/mixed), the size and location thereof, the anti-VEGF agents used, the number of injections administered, the treatment-free interval, and the need for a therapy switch, if any. BCVA, measurement of IOP, slit-lamp examination, fundoscopy, and SD-OCT were documented at each visit.

2.5. Follow-Up

Follow-up was performed according to an in-house protocol for the follow-up of patients under brolucizumab and in accordance with the recommendation for patients under anti-VEGF injections (Stellungn./Empfehlungen DOG). Eyes were examined for detection of ocular-adverse events on days 7, 15, and 30 following the first injection (including OCT scans at day 15) and every month thereafter. After the loading dose, all treatment-naïve patients were assessed at 8 weeks: (i) If no disease activity (i.e., presence of fluid) was observed the patient still had an injection at 12-weeks, and was then reassessed every 12-weeks with another systematic injection (q12w). In cases of disease activity in a follow-up visit, the delay between injections was shortened to 8 weeks. (ii) If fluid was observed, the patient had an injection at 8 weeks and was then reassessed 8-weekly with a systematic injection (q8w). If no fluid was detected at an 8-week follow-up, extension of injections and follow-up could be done at 12 weeks. For the switch-therapy group, patients were followed monthly after the first injections for possible reinjection in case of disease activity. Once the macula was dry on SD-OCT, all switch patients were assessed at 8 weeks and were reinjected following the above recommendations (Figure 1). Retreatment criteria were based on comparisons with the previous month's examination and retreatment administered if any one criterion of disease activity was considered to be positive [7,10]. Intravitreal injections were performed using a standardized aseptic technique.

Patients had to be followed for a minimum of 9 months to be included in this study. Moreover, for the treatment-naïve group, eyes had to have received at least four intravitreal injections of brolucizumab.
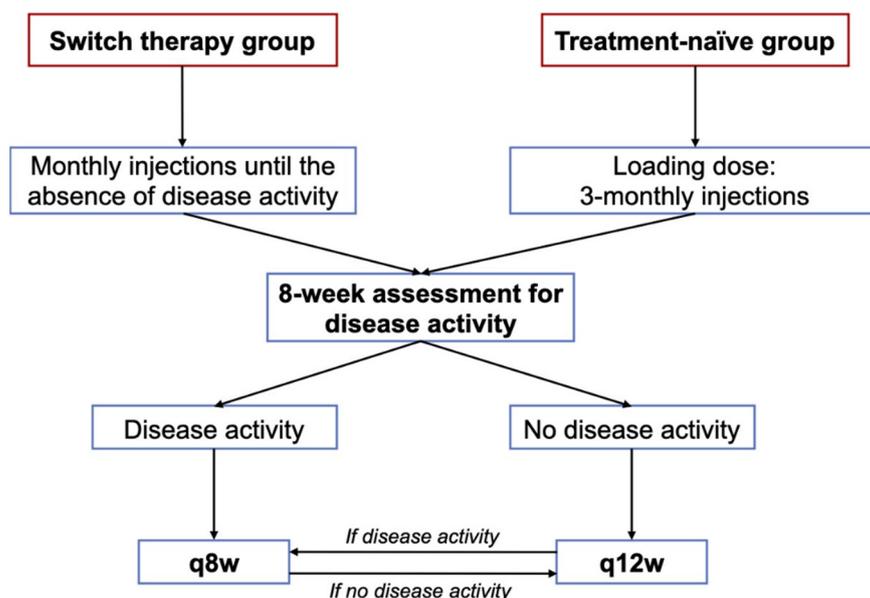
Figure 1. Our in-house protocol for the treatment of wet AMD with intravitreal injections of brolucizumab.

2.6. Outcome Measures

The primary outcome measure was to determine the change in BCVA from baseline with treatment. Secondary outcome measures included change in CST in SD-OCT, the mean number of injections required to achieve repeat resolution, and the complications associated therewith. We also attempted to determine which factors independently influence resolution of exudation with a single injection.

2.7. Statistical Analysis

The description of categorical variables was based on absolute (size) and relative (percentage) frequencies. Quantitative variables were represented as mean and standard deviation. The comparison of the categorical variables between the groups of different indications was performed using Fisher's exact test, and when the pairwise comparisons were subsequently performed, the *p*-value was adjusted using the Benjamini–Hochberg method, wherever applicable. When more than two groups were compared, ANOVA was used. A *p*-value < 0.05 was considered to be statistically significant. Quarterly visits were considered to make up for possible lost follow-ups.

3. Results

A total of 85 patients (112 eyes) have received intravitreal brolucizumab at our centres thus far, of which 78 patients (105 eyes) have completed at least 9 months of follow-up. The remaining seven patients (seven eyes; all switch-therapy patients) did not complete 9 months of follow-up. These 78 patients form the basis for our analysis (68 patients were of Caucasian ethnicity and included in Germany centers and 10 patients were of south Asian ethnicity and included in Indian centers). Of these 78 patients, 23 patients (25 eyes) were treatment-naïve at baseline whereas the rest received switch therapy. The total number of intravitreal procedures carried out in these patients was 572. Table 1 lists salient characteristics of patients in both groups i.e., treatment-naïve and patients who received switch therapy.

Table 1. Characteristics of patients who received brolucizumab therapy.

Characteristic	Treatment-Naïve (N = 25)	Switch Therapy (N = 80)
Mean age, years (SD)	69.2 (4.4)	74.3 (5.8)
Male:female	9:14	26:29
Mean follow-up, months (SD)	10.4 (1.5)	10.4 (2.2) *
Mean baseline BCVA, letters (SD)	49.4 (4.5)	40.0 (3.2)
Mean baseline CST, μm (SD)	428.1 (7.4)	483.2 (59.2)
MNV subtype, *n*:		
Type 1	14	56
Type 2	7	14
Mixed	4	10
MNV localization, *n*:		
Subfoveal	15	56
Juxtafoveal	4	12
Extrafoveal	1	2
Interpapillomacular	5	10
Fluid localization, ** *n*:		
IRF	23	57
SRF	12	31
PED	10	24
None	2	0

BCVA, best corrected visual acuity; IRF, intraretinal; MNV, choroidal neovascularization; PED, pigment epithelium detachment; SRF, subretinal fluid. * After initiation of brolucizumab therapy; ** patients could have fluid in more than one compartment.

3.1. Treatment-Naïve Group

A total of 23 patients (25 eyes) were treated for the first time with brolucizumab (all of Caucasian/European ethnicity). The mean time to presentation after onset of symptoms in the treatment-naïve group was 43.2 ± 12.5 days. Baseline BCVA was 49.4 ± 4.5 letters and the mean retinal thickness was 428.1 ± 73.4 μm. Type 1 MNV was the most frequent neovascular lesion (56.0%). The mean neovascular membrane size was 188.4 ± 44.5 μm.

Overall, 19/25 (76.0%) eyes showed completely resolved exudation at the end of the loading phase and were planned to undergo injections every 12 weeks (q12w), 13/19 (68.4%) eyes were still treated with q12w dosing at the end of follow-up, and 6/13 (46.1%) eyes did not show any recurrence with the q12w regimen (i.e., no disease activity detected at any time). A total of 6/25 patients were injected at 8-weeks (q8w) after the loading phase. For these patients, none of them experienced fluid at the 4-weeks SD-OCT. In this q8w subgroup, 3/6 eyes were finally extended to q12w dosing at the end of follow-up.

The mean final CST decreased significantly to 278.0 ± 47.2 μm ($p = 0.021$) at the final follow-up. The mean follow-up in the treatment-naïve group was 10.4 ± 1.5 months. Patients received a mean of 2.1 ± 0.8 brolucizumab injections after the three loading-dose injections.

3.2. Visual Gain

The mean letter gain was 11.9 ± 3.8 letters in this group ($p = 0.011$). A total of 15/25 eyes (60%) gained more than 15 letters from baseline at 8 weeks after the loading phase. Of these eyes, 14/25 maintained the 15-letter gain until the end of the follow-up period. Overall, 16/25 (64.0%) patients retained a BCVA of 20/30 or better at the end of the follow-up period. None of the treatment-naive group lost letters from baseline.

3.3. Recurrence

A total of 17/25 (68.0%) eyes demonstrated a recurrence in exudation during the whole follow-up period; 7/17 demonstrated a recurrence when treated at 12 weeks whereas 10/17

eyes demonstrated fluid at 8 weeks. Patients who showed a recurrence tended to have type 1 MNV, higher MNV size (mean 287.4 ± 42.5 μm; *p* = 0.03) and peripapillary MNVs. The presence of IRF was more likely to indicate recurrence (OR-2.31, CI 1.7–4.18; *p* = 0.012).

3.4. Switch-Therapy Group

A total of 55 patients (80 eyes; 45 patients were of Caucasian/European, and 10 of Asian ethnicity) received switch therapy from either aflibercept or ranibizumab to brolucizumab. All neovascular AMD patients receiving brolucizumab as switch therapy had been initiated on and received aflibercept injections as primary therapy. According to the cause of the switch, 25 eyes were classified in the subgroup 1 (recurrent), 44 eyes in the subgroup 2 (recalcitrant), and 11 in the subgroup 3 (inability to extend). The mean number of injections prior to switch was 32.4 (±3.4; range 12–96). The minimum number of injections prior to the switch was 12.

Baseline BCVA was 40.0 ± 3.2 letters and the mean retinal thickness was 483.2 ± 59.2 μm. Type 1 MNV was the most frequent neovascular lesion (70.0%). Out of the 80 eyes included in this group, 27 (33.7%) were considered to have no disease activity after the first injection, 38 (47.5%) after the second injection, and 15 (18.8%) after the third injection. None of the patients required a fourth monthly injection before the extension at 8 weeks.

The treatment interval was extended to 12 weeks (q12w) in 25/80 (31.2%) eyes at the end of the follow-up period and 55/80 (68.8%) eyes were maintained at 8 weeks (q8w). The mean CST decreased significantly to 297.5 ± 53.4 μm (*p* = 0.01) at the final follow-up. The mean follow-up in the switch-therapy group was 10.4 ± 2.2 months and patients received a mean of 4.2 ± 2.1 brolucizumab injections during the follow-up period (Figure 2).
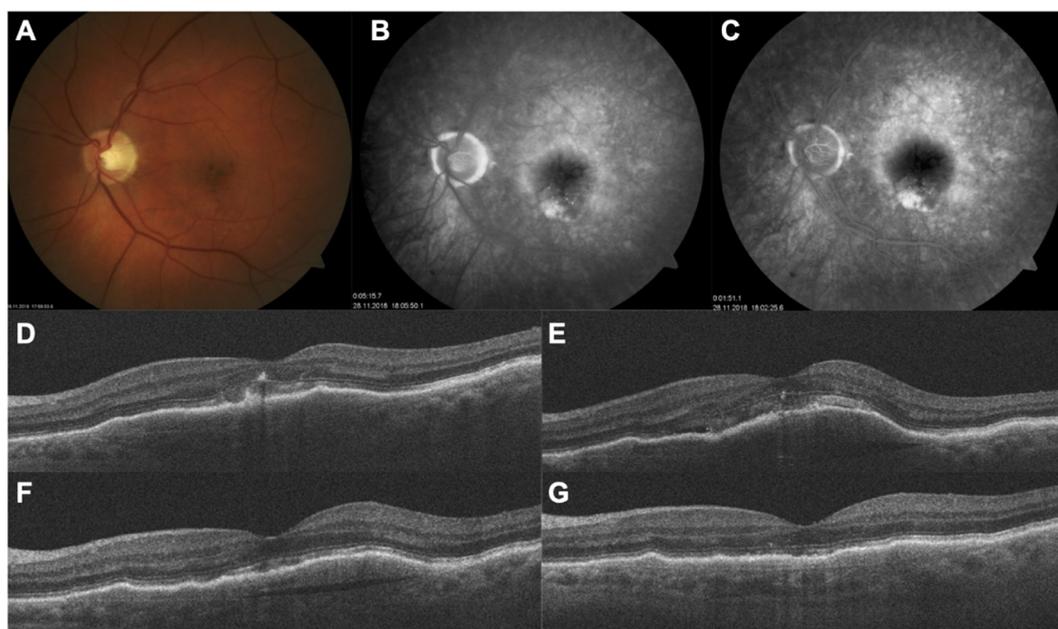
Figure 2. A 74-year-old male with neovascular AMD was switched to brolucizumab after demonstrating worsening of anatomical changes despite six consecutive injections of aflibercept followed by six consecutive injections of ranibizumab. (**A–D**) Multimodal imaging showing the baseline situation before any anti-VEGF therapy. (**E**) SD-OCT showing the condition of the retina immediately prior to initiation of brolucizumab therapy. Large pigment epithelium detachment (PED) was present, associated with persistent subretinal fluid (SRF). (**F**) SD-OCT demonstrating significant improvement after the first intravitreal brolucizumab injection and further resolution of PED and SRF. (**G**) SD-OCT after the second injection. The patient has continued to receive therapy in the q12w dosing regimen.

3.5. Visual Gain

The mean letter gain was 10.4 ± 4.8 letters in this group ($p = 0.014$). A total of 8/80 eyes (10%) gained more than 15 letters from baseline at the end of follow-up. Overall, 9/80 (11.2%) patients retained a BCVA of 20/30 or better at the end of the follow-up period, and 2/80 (2.5%) eyes in the switch-therapy group lost letters from baseline.

According to the cause of switch, the visual gain was 10.9 ± 3.3 letters, 8.6 ± 1.2 letters, and 7.2 ± 2.1 letters for the subgroup 1, 2, and 3, respectively ($p = 0.051$; ANOVA).

3.6. Adverse Events

A total of two patients were noted to have had untoward events after switching to brolucizumab. A 78-year-old male patient (Figure 3) developed a macular hole 15 days after receiving his 5th brolucizumab injection (and 55th injection overall). The patient underwent uneventful macular hole surgery with C_2F_6 intraocular gas tamponade and recovered well; his BCVA was 20/80 prior to the development of macular hole. The BCVA had dropped to 20/200 when he was detected to have a macular hole. Post vitrectomy and gas tamponade, his BCVA recovered to 20/100 and has been stable for 4 months (until the last follow-up) after surgery. He has been switched to aflibercept therapy (six injections overall) and has been maintained on q8w dosing.
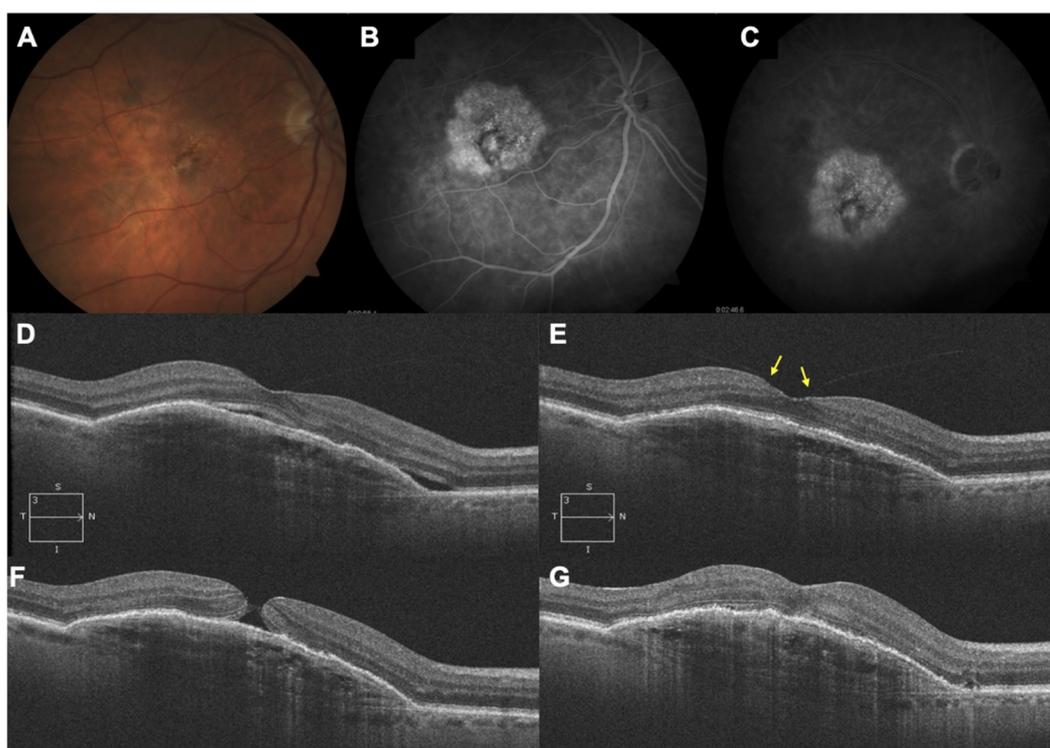
Figure 3. A 78-year-old male patient had been treated for neovascular AMD with aflibercept and ranibizumab and had received 50 injections overall prior to switch to brolucizumab. (**A–C**) Multimodal imaging at baseline showing a large subfoveal lesion. (**D**) SD-OCT immediately prior to initiation of brolucizumab therapy. (**E**) SD-OCT 8 weeks after the first brolucizumab injection showing a total resorption of the subretinal fluid but also an evident vitreomacular adherence (arrows). (**F**) SD-OCT 15 days after receiving his 5th brolucizumab injection, showing macular hole. SD-OCT after the macular hole surgery. (**G**) C-late phase FFA.

A 71-year-old female developed branch arterial occlusion 7 days after receiving her 5th brolucizumab injection and presented within 4 h of onset of symptoms. Her BCVA prior to this event was 20/60; it dropped to 20/200 secondary to the arterial occlusion. Her perimetry analysis showed corresponding field defects. She was immediately referred to the stroke clinic in Bremerhaven, Germany and received low molecular weight hep-

arin. She showed complete recovery, as confirmed with her restoration of BCVA (20/60) and complete disappearance of all field defects (Figure 4). The indication for switch to brolucizumab was an inability to extend the treatment-free interval beyond 4 weeks. With initiation of brolucizumab therapy itself, the interval could be extended to 8 weeks. She has not had any adverse event since and has been continued on aflibercept therapy on a q4w schedule. She has been asked to maintain a close watch on her vision. We did not note any instance of intraocular inflammation, such as anterior chamber reaction, vitritis, or vasculitis, nor vasculitic occlusion in any of the patients.
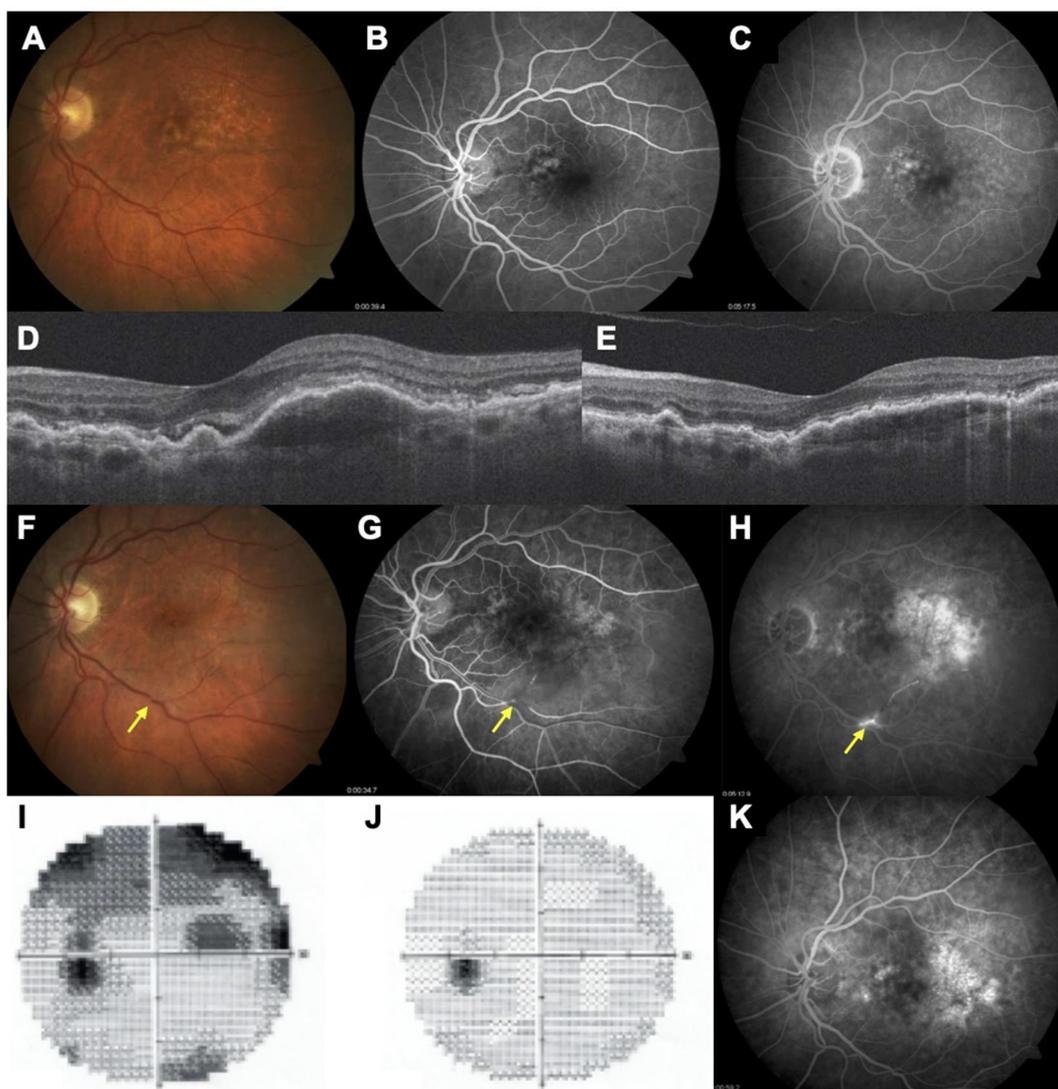
Figure 4. A 71-year-old woman with neovascular AMD had received 70 injections prior to initiation of brolucizumab therapy. (**A**–**C**) Multimodal imaging showing the baseline situation before any anti-VEGF therapy. (**D**) SD-OCT immediately prior to initiation of brolucizumab therapy. (**E**) SD-OCT after the first brolucizumab injection showing total resorption of subretinal fluid and decrease of the pigment epithelium detachment. The patient complained of loss of vision 7 days after the fifth brolucizumab injection. (**F**–**I**) Multimodal imaging showing an retinal arterial occlusion in her left eye (arrow); perimetry analysis demonstrates a corresponding superior scotoma. She had presented within 4 h of onset of symptoms and was immediately referred to the emergency room and received low molecular weight heparin. (**J**,**K**) Post-heparin analysis showing perimetry recovering and arterial reperfusion.

27 patients (27 eyes) receiving switch therapy were on IOP-lowering medications at the time of switch to brolucizumab. The switch in therapy did not adversely affect IOP control in any of these 27 patients. None of the treatment-naïve patients were on prior

therapy with IOP-lowering medications either at the time of first intravitreal brolucizumab injection or during the follow-up period. There was no rise in IOP noted at any point in time during the course of follow-up in any of the patients included in the study.

The seven eyes excluded from the analysis received a mean of 3.2 ($\pm$0.8) injections after switch therapy and did not report any untoward event until the end of follow-up. None of the eyes were switched from brolucizumab to aflibercept (except for the two patients with ocular-adverse events described above).

4. Discussion

The current analysis demonstrates very good anatomical, and to a significant extent, visual outcomes following the administration of intravitreal brolucizumab in treatment-naïve or switch-therapy patients in routine practice. Therefore, the primary aim of the study was to describe a real-world cohort of patients with lower baseline BCVA (in comparison to randomized control trials), that showed significant letter gain despite having undergone several treatments in the past. A significant proportion of treatment-naïve and switch patients demonstrated excellent visual and anatomical outcomes that were maintained until the end of the follow-up period. A sizeable proportion of eyes in the treatment-naïve group and the switch-therapy group were maintained on q12w dosing.

The visual gains reported in the present study are higher than what was reported in the HAWK or HARRIER protocols [10]. In our study, treatment-naïve patients had a significantly lower baseline BCVA compared to the HAWK and HARRIER studies, and this could explain the greater visual gain. The 'ceiling effect' may thus account for the difference of visual gain when baseline BCVA are not similar. The switch-therapy groups demonstrated very good outcomes, and this could also be explained by the lower baseline BCVA than other series described in the literature. A recent paper on real-world evidence with brolucizumab reported no significant changes in BCVA at final follow-up for a cohort of patient who were mainly switched from another anti-VEGF therapy. However, the baseline BCVA reported in the study was higher than ours at 64.1 letters [11]. Another explanation for the high visual gain in the switch group is the presence of fluid, both IRF and SRF, within the retina at baseline in the majority of patients in this group. Brolucizumab injections efficiently treated the fluid in all cases with one to three injections before the total disappearance of the fluid, making an increase in visual acuity possible. Despite a proactive and intensive protocol with historical anti-VEGF molecules, this subgroup of patients had no or low or an ill-sustained response to the previous therapy. The visual gain provided by brolucizumab injections is probably explained by a greater drying effect on the retina and most importantly, it was also sustained in most patients at 8 weeks. However, it should be noted that the comparison in visual gain, according to the cause of switch, did not show any differences, although there was a trend to better visual gain in eyes with fluid at the time of switch in comparison to patients switched for the inability to extend beyond q6/q8 with previous therapy. This point should be investigated further in a larger cohort of patients.

The smaller size of the brolucizumab molecule means a larger concentration of the drug can be delivered intraocularly. This probably accounts for improved efficacy and durability but may account for a higher incidence of hypersensitivity-like reactions. The incidence of vascular occlusion has been estimated to be 3.4/10000 injections [16]. Biological agents are known to cause hypersensitivity reactions [17]. Overall, enthusiasm for the drug on account of its increased efficacy has been offset somewhat by reports of increased propensity towards inflammatory adverse events [18,19]. Notwithstanding, the drug is a useful alternative to currently available anti-VEGF agents for wet AMD and indeed can eventually be the drug of choice given its potency. As regards complications, both adverse events were noted in patients receiving switch therapy and occurred after the loading phase. Treatment-naïve patients did not demonstrate adverse events until the last follow-up. One patient developed vascular occlusion after the fifth brolucizumab injection and this was reversed through timely intervention. There were no residual deficits noted,

either in terms of visual acuity or perimetric changes. The time to development of this adverse event is contrary to past reports on vasculitis associated with brolucizumab that suggest that such adverse events are noted later (day 30) in the course of the follow-up. Additionally, this occurred after the fifth injection of brolucizumab and the 75th injection overall (including past therapy). Whereas a recently published study comprising 172 eyes receiving brolucizumab and 6 months of follow-up reported 14 instances of intraocular inflammation, only one serious adverse event (vasculitic occlusion) was reported [12]. In the remaining 13 eyes in the study, the vast majority of eyes showed spontaneous resolution whereas some eyes needed intervention in the form of topical or subtenon steroid therapy. The incidence of serious adverse events matches our analysis. Moreover, we report for the first time, to the best of our knowledge, macular hole formation after brolucizumab therapy. The patient responded well to surgery. Macular hole formation has been noted earlier with ranibizumab and bevacizumab [20,21]. A combination of alterations in retinal tractional forces and contracture of the choroidal neovascular membrane consequent to VEGF withdrawal along with altered vitreous dynamics consequent to repeat violations of the milieu interior of the vitreous cavity leads to macular hole formation. Whether the higher molecular concentration of brolucizumab plays any role in its incidence merits further investigation.

The retrospective nature of our study is an obvious limitation in that follow-ups are liable to be missed. This is evolving data as it is a new drug. We attempted to analyze response by ethnicity but the numbers are too small (especially for south Asian ethnicity) to permit meaningful analysis. Notwithstanding, this current analysis is, in our opinion, an accurate portrayal of the current status of intravitreal brolucizumab therapy for neovascular AMD and the challenges associated therewith in the real world as opposed to the setting of a randomized trial. Brolucizumab was effective both in treatment-naïve patients and those receiving switch therapy in terms of visual gain and anatomical resolution of MNV associated exudation and in extension of the treatment interval. There was significant improvement in vision from baseline in both groups, thereby demonstrating its efficacy even in patients who had received multiple injections earlier and in patients with low baseline BCVA. Our findings correspond to early reports on the use of brolucizumab [22] but additionally, we also report on patients receiving switch therapy. The current general consensus the world over is one of reduction in the treatment burden. The FLUID study, for example also looks at how much fluid can be tolerated without much compromise of final vision [23] whereas our recent publication looks at a particular subset of patients who do well with one injection over a considerable period of time [24]. This is also important in reducing treatment costs. Finally, our short case series on the efficacy of brolucizumab in patients with MNV and RPE rip reaffirms to some extent the visual gains reported in the present analysis, albeit a very small number of patients [25].

To conclude, intravitreal brolucizumab therapy is effective in both treatment-naïve patients and patients who receive the drug as switch therapy. There is a small but definite risk associated with the drug (1.9% overall in our series; 0.95% as far as vascular occlusion is concerned). Notwithstanding the small risk of untoward events, intravitreal brolucizumab therapy is a useful addition to the retinal surgeon's armamentarium and should establish itself as an effective form of primary therapy that can do well with infrequent administration. A high index of suspicion maintained both by the physician and the patient can help avert disastrous complications.

Institutional Review Board Statement: This study complied with the tenets of the Declaration of Helsinski and was approved by an international review board (Ethics Committee of the French Society of Ophthalmology, IRB 00008855 Société Française d'Ophtalmologie IRB#1). Patients gave their informed consent to participate in the study.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data are available upon request to the corresponding author.

Conflicts of Interest: Laurent Kodjikian is a consultant for Allergan/Abbvie, Bayer, Horus, Novartis, Roche and Théa; Thibaud Mathis is a consultant for Allergan/Abbvie, Bayer, GSK, Horus and Novartis. Other authors have no conflicts of interest to declare.

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Comparative Effectiveness of Intravitreal Anti-Vascular Endothelial Growth Factor Therapies for Managing Neovascular Age-Related Macular Degeneration: A Meta-Analysis

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Abstract: Intravitreal injections (IVI) of anti-vascular endothelial growth factor (anti-VEGF) have become the standard of care for age-related macular degeneration (AMD). Although most pivotal trials have used monthly injections, alternative strategies that enable the injections to be administered on a more flexible schedule, including pro re nata (PRN) and treat-and-extend (T&E) regimens, are being applied more frequently. This review sought to provide further scientific evidence about the visual outcomes and treatment burden among the currently available anti-VEGF agents and regimens, including aflibercept, ranibizumab, abicipar and brolucizumab. To this end, a systematic review of published randomized studies was conducted from the MEDLINE and EMBASE databases and the Cochrane library, and a meta-analysis was applied to the obtained data using single-means modeling to compare the efficacy and maintenance among the different available treatments and regimens at Years 1 and 2. Quality analysis identified the best-informed data for modeling purposes. Overall, 47 relevant publications were retrieved for the analysis. Superior efficacy, meaning that there were observed improvements in visual acuity (VA) and central retinal thickness (CRT), occurred with monthly versus PRN regimens, yet a higher IVI number was also observed. Conversely, the

T&E regimens displayed similar efficacy to the monthly regimens, but with a reduced IVI number. Aflibercept T&E exhibited similar efficacy to ranibizumab T&E, but with significantly lower IVI numbers at both Year 1 ($p < 0.0001$) and Year 2 ($p = 0.0011$). Though all of the regimens resulted in maintained efficacy between Years 1 and 2, the required IVI number varied. The retrieved data did not enable other regimens or newer anti-VEGF agents such as brolucizumab to be compared. In conclusion, the T&E regimens were shown to be the most efficient, optimizing durable effectiveness whilst minimizing the IVI number in newly diagnosed exudative AMD, with aflibercept requiring the lowest IVI number.

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1. Introduction

Neovascular age-related macular degeneration (nAMD) is a progressive degenerative disease of the retinal macula that can lead to permanent central vision impairment and blindness [1]. The overall prevalence of the condition in developed countries is estimated to be approximately 8.7% [2]. Risk factors for developing AMD include smoking, a higher body mass index and hypertension, as well as soft drusen and pigment abnormalities within the macula [3,4]. Over the past decade, substantial improvements have been made in the treatment of nAMD, following the introduction of new molecules and treatment regimens. These comprise several agents that are targeted at vascular endothelial growth factor (VEGF), including ranibizumab, bevacizumab, aflibercept, abicipar pegol and brolucizumab.

Indeed, VEGF upregulation, which is assumed to be induced by retinal and choroidal hypoxia, has been shown to drive angiogenesis and increase vascular permeability, which has been observed in nAMD [2]. The visual acuity (VA) gain reported in clinical trials using anti-VEGF agents during the first two years of treatment [5] resulted in a marked upgrade in disease prognosis [6], thereby establishing anti-VEGF agents as the standard of care for this condition [7]. Despite considerable improvements in legal blindness related to nAMD following anti-VEGF introduction, real-world studies have failed to confirm these encouraging data, which is primarily due to under-treatment and a lack of long-term results [2]. A study using the Fight Retinal Blindness observational registry showed that it was possible to achieve good visual outcomes in eyes managed in routine clinical practice with a treat-and-extend (T&E) regimen while also decreasing the treatment burden and number of clinic visits at the same time [8]. Additionally, the monthly intravitreal anti-VEGF injections that are required for the standard of care have been proven to be burdensome for both patients and healthcare systems, often leading to poor treatment adherence in real-life practice [9]. Thus, other regimens that administer anti-VEGF agents at various fixed intervals, such as every 8 or 12 weeks (q8w and q12w, respectively) or as-needed (pro re nata [PRN]), as well as T&E regimens with treatment intervals based on individual disease activity, have been investigated [1,10].

To compare different anti-VEGF agents and regimens, several meta-analyses have been performed [11–14] with the goal of better assisting physicians in their therapeutic decision-making process. Indeed, meta-analyses primarily seek to provide more accurate outcomes and comprehensive conclusions than individual studies through the use of a pool of previously published studies that allow for data comparison [15]. It was in this context that a Cochrane library meta-analysis demonstrated the similar effectiveness and safety of aflibercept versus ranibizumab for managing nAMD patients [12]. Another Cochrane library meta-analysis by Solomon et al. indicated that anti-VEGF agents such as pegaptanib, ranibizumab and bevacizumab had similar effectiveness [11]. A recent network meta-analysis indirectly compared aflibercept and ranibizumab administered

via T&E regimens and revealed a similar efficacy between the treatment arms, but for aflibercept, this was associated with a significantly reduced number of injections over a two-year period [14]. Nevertheless, it must be stressed that no meta-analysis conducted on nAMD has compared all of the anti-VEGF agents and treatment regimens that are currently available. It must similarly be stressed that 10 to 35% of patients either failed to respond to anti-VEGF agents, referred to as non-responders, or developed resistance to anti-VEGF agents over time, a condition known as tachyphylaxis [16,17]. Given this background, our review primarily sought to provide further scientific evidence regarding the visual outcomes and treatment burden among all of the available anti-VEGF agents and regimens. To this end, we performed a meta-analysis that included data from all of the randomized clinical trials conducted in the nAMD field that were designed to (1) compare the effectiveness of the available treatment regimens at Years 1 and 2, (2) assess the efficacy maintenance of the various regimens over time and (3) compare the effectiveness of various anti-VEGF agents using the same treatment regimen at Years 1 and 2.

2. Methods

2.1. Selection of Studies

A systematic literature review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and the Cochrane Collaboration statement [18,19]. We looked for studies using a sensitive search strategy pertaining to anti-VEGF agents used in AMD management in the PubMed/Medline®, Embase® (access via Ovid platform) and Cochrane CENTRAL databases from the earliest available time up to 16 March 2020. Moreover, studies from ClinicalTrial.gov (accessed on 15 March 2022). with detailed results that had not been published elsewhere were retrieved, as well. The individual search terms applied for the searches are listed in Table 1. The selection of search terms was based on a two-expert consultation. Studies from PubMed were limited to "clinical trials" and "human" studies, with animal and basic science studies being excluded from the analysis, whereas studies from ClinicalTrials.gov were limited to those that were either "terminated" or "completed". After the database search, two of the authors of this review independently selected articles, and selection was followed by a double-check and validation process. The manual selection process for eligible publications comprised a review of the titles and abstracts; studies that did not match the previously established criteria were excluded. Next, the abstracts and full texts of the retrieved publications were read by the authors in full, thereby confirming their eligibility. The selected publications reported results of prospective, randomized controlled studies related to exudative nAMD and its synonyms and acronyms. The Cochrane risk of bias tool was applied to further assess the quality of the included studies. All of the selected studies had at least one treatment arm involving first-line treatment, thus involving treatment-naïve patients undergoing first-line treatment with one of the following agents: aflibercept 2 mg, ranibizumab 0.5 mg, bevacizumab 1.25 mg, brolucizumab 6mg or abicipar pegol 2 mg. Phase 1 studies, in addition to subgroup or post hoc analyses, were excluded. Moreover, to meet the eligibility criteria, these studies needed to include primary or secondary outcome measures assessing the best-corrected visual acuity (BCVA) and BCVA at $\geq$12 months after the baseline. In contrast, studies involving previously treated patients and those with a follow-up duration of <12 months or >24 months without any available intermediate data were not considered. Based on these criteria, a total of 69 studies were retrieved.

Table 1. Search terms used in the systematic literature review.

Queried Database	Filter	No. Studies	Comment
PubMed	(visual acuity) AND (randomised OR randomized) AND (wet age-related macular degeneration [Title/Abstract]) OR (exudative age-related macular degeneration [Title/Abstract]) OR (neovascular age-related macular degeneration [Title/Abstract]) OR (wet AMD [Title/Abstract]) OR (exudative AMD [Title/Abstract]) OR (neovascular AMD [Title/Abstract]) OR (wet-AMD [Title/Abstract]) OR (exudative-AMD [Title/Abstract]) OR (neovascular-AMD [Title/Abstract]) OR (wAMD [Title/Abstract]) OR (eAMD [Title/Abstract]) OR (nAMD [Title/Abstract])) AND (aflibercept [Title/Abstract]) OR (ranibizumab [Title/Abstract]) OR (bevacizumab [Title/Abstract]) OR (abicipar [Title/Abstract]) OR (brolucizumab [Title/Abstract]) **Filters: Clinical Trial; Humans**	207	No filter was applied for the doses, times at which visual acuity was assessed or the nature of the prospective design, and these aspects were treated manually
Cochrane Central Register for Controlled Trials [a]	"age related macular degeneration" in Title Abstract Keyword AND "Aflibercept" OR "Ranibizumab" OR "Bevacizumab" OR "Brolucizumab" OR "Abicipar" in Title Abstract Keyword AND randomized in Title Abstract Keyword AND visual acuity in Title Abstract Keyword—in Trials (Word variations were searched)	229	The selection process was as carried out on studies found in Embase but not referenced in PubMed. No filter was applied for the doses, times at which visual acuity was assessed or prospective nature, and these aspects were treated manually
CliniclTrials.gov	"age related macular degeneration" in Title Abstract Keyword AND "Aflibercept" OR "Ranibizumab" OR "Bevacizumab" OR "Brolucizumab" OR "Abicipar" in Title Abstract Keyword AND randomized in Title Abstract Keyword AND visual acuity in Title Abstract Keyword—in Trials (Word variations were searched) **Filters: Terminated OR Completed**	68	Of these 68 trials, 60 were retrieved from PubMed or Embase. No filter was applied for the doses, times at which visual acuity was assessed or the nature of the prospective design, and these aspects were treated manually

[a] Only studies referenced in Embase were selected.

2.2. Data Extraction and Outcome Analyses

Relevant data were manually extracted from each selected publication and were transcribed into an Excel sheet, and these data were subsequently verified by a second reader. The following data were included: population-related criteria, such as the number of patients, patients lost to follow-up and demographic data; choroidal neovascularization (CNV) features such as the type and presence of polypoidal choroidal vasculopathy (PCV); treatment criteria, including the therapeutic regimen, proportion of patients receiving treatment every 4, 6, 8, 10, 12 or 16 weeks, proportion of patients receiving treatment every ≥8, 12 or 16 weeks, average treatment interval at month 12 or 24 and the IVI number; functional criteria, including the BCVA at the baseline and at month 12 or 24, mean gain in BCVA from the baseline or from randomization depending on the trial design, proportion of patients with BCVA gain ≥15 letters, proportion of patients with BCVA gain ≥5 letters

and proportion of patients with VA loss $\geq$15 letters; anatomical features such as central retinal thickness (CRT) at the baseline and at month 12 or 24, mean reduction in CRT and the intra-retinal fluid (IRF) or subretinal fluid (SRF) at the baseline and at month 12 or 24.

To define the follow-up duration, the outcomes that were assessed at 52 $\pm$ 4 weeks were considered one-year data, and those assessed at 104 weeks $\pm$ 4 weeks were considered two-year data; study arms with other follow-up durations were excluded from the meta-analysis. For better homogeneity within each protocol type and to ensure relevant comparisons, only study arms with an induction phase consisting of the administration of at least two monthly loading doses were considered. In the event of a change in the treatment/regimen during the second year, two-year data were excluded from the analysis. For every six-weekly (q6w) dosing regimen, study arms that were in the induction phase or that took place throughout the treatment period were excluded, meaning loading dose and maintenance treatment regimens could be distinguished during the second year. When the studies were selected, it is important to note that bevacizumab was being used off-label for the treatment of wAMD in many European countries, and the question of whether the use of this off-label therapy was responsible or not went unanswered, with a benefit–risk assessment under investigation [20]. Therefore, study arms involving the off-label use of bevacizumab alone were not retained for the final analysis. Of note, the European Medicines Agency (EMEA) recently documented that it did not approve a marketing authorization for a bevacizumab-based drug due to the lack of appropriate safety and efficacy that had been demonstrated and concerns about the risks outweighing the benefits. Instead, a temporary-use authorization (TUA) was granted [21].

2.3. Data Synthesis and Analysis

Studies were assessed for clinical and methodological heterogeneity and for the risk of bias, as well as to determine their suitability for inclusion in the model. Single-means modeling based on fixed- and random-effect models and heterogeneity tests were applied to compare the anti-VEGF regimens, as well as agents within the same regimen. Next, "2 to 2" modeling was applied to further refine the comparisons of various anti-VEGF regimens and agents, aiming to retain the most relevant and robust data. A meta-analysis of the data was conducted to compare the study arms. All of the study arms with means, standard deviations and counts were entered into the model to attain significant statistical power. Intra-study comparisons between randomized study arms were not carried out. Visual outcome data were analyzed following conversion into ETDRS letters if not already available, similar to in [22].

Data quality analysis was conducted to identify the best-informed data at Years 1 and 2, as well as the best-informed clinical outcomes at Year 1, for inclusion in the model. For illustration, the age criterion was very well-informed, whereas other criteria such as the number of visits were poorly informed, meaning that the latter were unable to provide statistical power. The best-informed data at Years 1 and 2 were comparisons of monthly, T&E and PRN regimens, as well as comparisons of aflibercept and ranibizumab, with 75% of the eye of concern being included. Moreover, the best-informed clinical outcomes at Year 1 were improvements in BCVA, reductions in CRT and the IVI number. All of the statistical analyses were conducted using R statistical software version 3.6.0 (R Core Team 224 2019).

2.4. Compliance with Ethical Guidelines

This meta-analysis was a secondary statistical analysis that combined data collected by eligible clinical trials. The data sources primarily included data from the public domain, and one clinical trial contained limited patient data. All of the clinical data were anonymized before they were entered into the model, with the analysts having no access to any personal information that would enable them to identify individual patients. For this reason, ethical committee approval was not required for this meta-analysis.

3. Results

3.1. Included Studies

The literature searches across all of the databases and the references of systemic reviews returned a total of 5389 references. After further filtering, a total of 207 publications were retrieved from PubMed, and 330 were retrieved from Embase. The removal of 187 duplicates yielded 350 unique results. After further evaluation, 68 publications and one additional completed clinical trial with relevant results identified from ClinicalTrials.gov were selected. Following the application of previously mentioned inclusion and exclusion criteria to these 69 reports, 47 were retained for the final analysis (Figure 1), and the details of these 47 reports are provided in Table 2. Overall, the included publications provided one-year data from 78 treatment arms (12,689 eyes) and two-year data from 35 treatment arms (8560 eyes).
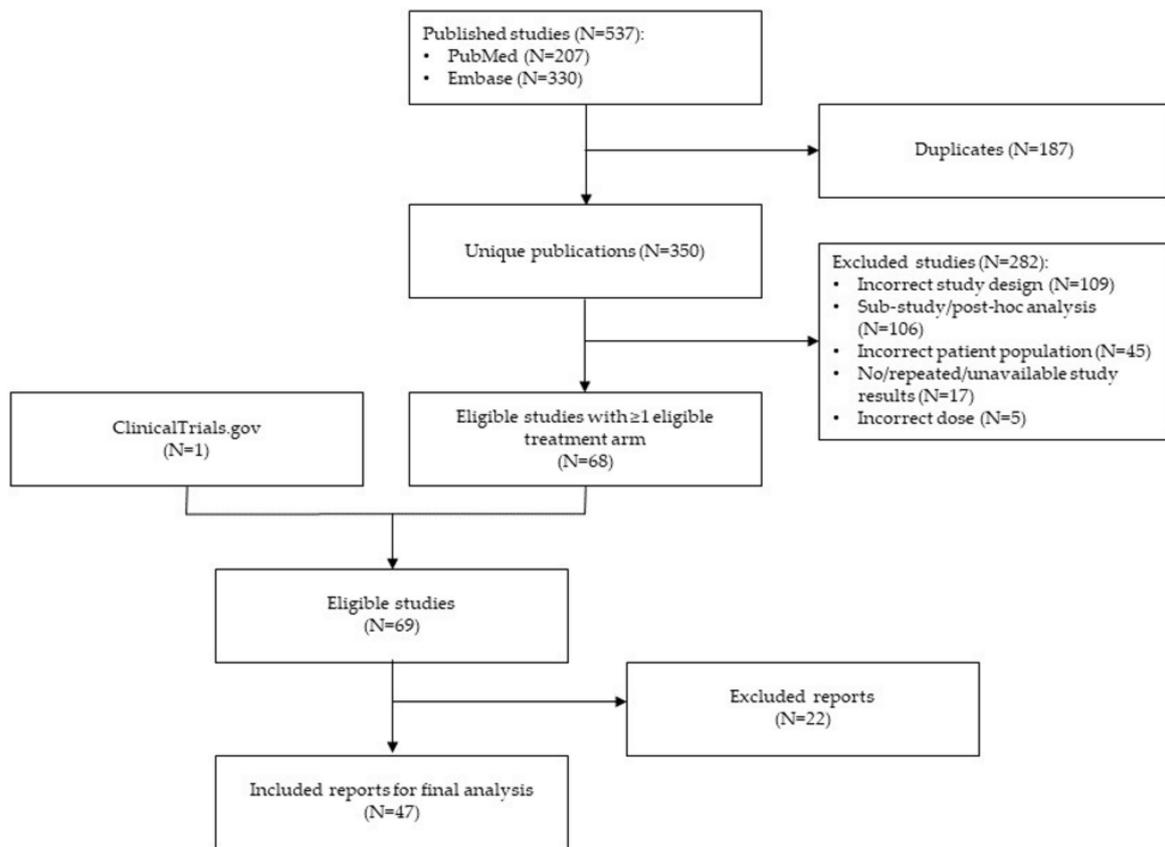
Figure 1. Flow chart of included reports.

Table 2. Included reports.

Reference	Study Name	Phase	N	Treatment Arm (s) of Interest	Regimen Comparisons of Interest	Outcomes of Interest
Dugel PU et al. 2020b [23] (Dugel, Koh et al. 2020)	HAWK, HARRIER	III	1459	Aflibercept 2 mg q8w vs. brolucizumab 3 or 6 mg q12/8w	q8 vs. q12/q8	• BCVA • CRT • IVI (for brolucizumab only)
Kertes PJ et al. 2020 [24] (Kertes, Galic et al. 2020)	CANTREAT	III	466	Ranibizumab	Monthly vs. T&E	• BCVA • CRT • IVI

Table 2. *Cont.*

Reference	Study Name	Phase	N	Treatment Arm (s) of Interest	Regimen Comparisons of Interest	Outcomes of Interest
Ohji M et al. 2020 [14] (Ohji, Takahashi et al. 2020)	ALTAIR	IV	458	Aflibercept	T&E (2w or 4w intervals)	• BCVA • CRT • IVI
Staurenghi G et al. 2020 [25] (Staurenghi, Garweg et al. 2020)	OCTAVE	III	305	Ranibizumab	PRN (VA guided vs. VA and/or OCT guided)	• BCVA • CRT • IVI
Gillies MC et al. 2019 [26] (Gillies, Hunyor et al. 2019)	RVAL	IV	559	Ranibizumab 0.5 mg vs. aflibercept 2.0 mg	T&E	• BCVA • CRT • IVI
Guymer RH et al. 2019 [27] (Guymer, Markey et al. 2019)	FLUID	IV	690	Ranibizumab 0.5 mg	T&E (intensive vs. relaxed retinal fluid treatment regimen)	• BCVA • CRT • IVI
Kertes PJ et al. 2019 [28] (Kertes, Galic et al. 2019)	CANTREAT	IV	526	Ranibizumab	Monthly vs. T&E	• BCVA • IVI
Mitchell P et al. 2019 [29] (Mitchell, Souied et al. 2019)	ARIES	IV	271	Aflibercept	q8w vs. T&E	• BCVA • CRT • IVI
Nunes RP et al. 2019 [30] (Nunes, Hirai et al. 2019)	–	III	30	Ranibizumab vs. bevacizumab	PRN	• BCVA • CRT • IVI
Semeraro F et al. 2019 [31] (Semeraro, Gambicordi et al. 2019)	–	Pilot	20	Aflibercept	PRN	• BCVA • CRT • IVI
Wykoff CC et al. 2018 [32] (Wykoff, Ou et al. 2018)	TREX-AMD	III	60	Ranibizumab	Monthly vs. T&E	• BCVA • CRT • IVI
Russo A et al. 2018 [33] (Russo, Scaroni et al. 2018)	–	Pilot	29	Ranibizumab	PRN	• BCVA • CRT • IVI
Silva R et al. 2018 [34] (Silva, Berta et al. 2018)	TREND	III	650	Ranibizumab	Monthly vs. T&E	• BCVA • CRT • IVI
Dugel PU et al. 2017 [35] (Dugel, Jaffe et al. 2017)	OSPREY	II	99	Aflibercept 2 mg vs. brolucizuamb 6 mg	q8w or q8w/q12w	• BCVA • CRT • IVI
Feltgen N et al. 2017 [36] (Feltgen, Bertelmann et al. 2017)	RABIMO	IV	40	Ranibizumab 0.5 mg	q8w vs. PRN	• BCVA • CRT • IVI
Gallemore RP et al. 2017 [37] (Gallemore, Wallsh et al. 2017)	RADICAL	II	82	Ranibizumab 0.5 mg		• BCVA • CRT • IVI
Li K et al. 2017 [38] (Li, Chen et al. 2017)	SIGHT	III	228	Aflibercept	q8w	• BCVA • IVI
Mori R et al. 2017 [39] (Mori, Tanaka et al. 2017)	–	IV	58	Aflibercept	q8w vs. PRN	• BCVA • CRT • IVI

Table 2. *Cont.*

Reference	Study Name	Phase	N	Treatment Arm (s) of Interest	Regimen Comparisons of Interest	Outcomes of Interest
Weingessel B et al. 2016 [40] (Weingessel, Mihaltz et al. 2016)	–	NR	16	Ranibizumab	PRN	• BCVA • CRT • IVI
Berg K et al. 2016 [41] (Berg, Hadzalic et al. 2016)	LUCAS	NR	339	Ranibizumab 0.5 mg vs. bevacizumab 1.25 mg	T&E	• BCVA • CRT • IVI
Berg K et al. 2015 [42] (Berg, Pedersen et al. 2015)	LUCAS	NR	371	Ranibizumab 0.5 mg vs. bevacizumab 1.25 mg	T&E	• BCVA • CRT • IVI
Eldem BM et al. 2015 [43] (Eldem, Muftuoglu et al. 2015)	SALUTE	IV	77	Ranibizumab 0.5 mg	PRN	• BCVA • CRT • IVI
Semeraro F et al. [44] (Semeraro, Russo et al. 2015)	–	Pilot	25	Ranibizumab	PRN	• BCVA • CRT • IVI
Wykoff CC et al. 2015 [45] (Wykoff, Croft et al. 2015)	TREX-AMD	IIIb	60	Ranibizumab	q4w vs. T&E	• BCVA • CRT • IVI
Ho AC et al. 2014 [46] (Ho, Busbee et al. 2014)	HARBOR	III	1100	Ranibizumab	q4w vs. PRN	• BCVA • CRT • IVI
Dugel PU et al. 2013 [47] (Dugel, Bebchuk et al. 2013)	CABERNET	III	155	Ranibizumab 0.5 mg	PRN	• BCVA • CRT • IVI (1-year data only)
Kodjikian L et al. 2013 [48] (Kodjikian, Souied et al. 2013)	GEFAL	III	374	Ranibizumab vs. bevacizumab	PRN	• BCVA • CRT • IVI
Krebs I et al. 2013a [49] (Krebs, Vecsei Marlovits et al. 2013)	–	NR	24	Ranibizumab	PRN	• BCVA • CRT • IVI
Krebs I et al. 2013b [50] (Krebs, Schmetterer et al. 2013)	MANTA	III	317	Ranibizumab vs. bevacizumab	Q4w	• BCVA • CRT • IVI
Ranchod TM et al. 2013 [51] (Ranchod, Ray et al. 2013)	LUCE-DEX	II	20	Ranibizumab	PRN	• BCVA • CRT • IVI
Heier JS et al. 2012 [52](Heier, Brown et al. 2012)	VIEW 1, VIEW 2	III	1815	Aflibercept vs. ranibizumab	q4w vs. q8w	• BCVA • CRT
Kaiser PK et al. 2012 [53] (Kaiser, Boyer et al. 2012)	DENALI	IIIb	112	Ranibizumab	PRN	• CRT • IVI
Larsen M et al. 2012 [54] (Larsen, Schmidt-Erfurth et al. 2012)	MONT-BLANC	II	133	Ranibizumab	PRN	• BCVA • CRT • IVI
Soderberg AC et al. 2012 [55] (Soderberg, Algvere et al. 2012)	–	III	44	Ranibizumab	PRN	• BCVA • CRT • IVI

Table 2. *Cont.*

Reference	Study Name	Phase	N	Treatment Arm (s) of Interest	Regimen Comparisons of Interest	Outcomes of Interest
Williams PD et al. 2012 [56] (Williams, Callanan et al. 2012)	–	Pilot	27	Ranibizumab	PRN	• BCVA • CRT • IVI
Holz FG et al. 2011 [57] (Holz, Amoaku et al. 2011)	SUSTAIN	III	513	Ranibizumab	PRN	• BCVA • CRT • IVI
Martin DF et al. 2012 [58] (Comparison of Age-related Macular Degeneration Treatments Trials Research, Martin et al. 2012)	CATT	III	778	Ranibizumab vs. bevacizumab	q4w vs. PRN	• BCVA • CRT • IVI
Schmidt-Erfurth U et al. 2011 [59] (Schmidt-Erfurth, Eldem et al. 2011)	EXCITE	IIIb	88	Ranibizumab	q12w	• BCVA • CRT • IVI
Vallance JH et al. 2010 [60] (Vallance, Johnson et al. 2010)	–	Pilot	9	Ranibizumab	PRN	• BCVA • CRT • IVI
Dugel PU et al. 2020c [23]	HAWK/ HARRIER	III	1459	Aflibercept vs. brolucizumab	q8w vs. q8w/q12w	• BCVA • CRT • IVI
Heier JS et al. 2011 [61]	CLEAR-IT	II	31	Aflibercept	PRN	• BCVA • CRT • IVI
Kunimoto D et al. 2020 [62]	CEDAR/ SEQUOIA	III	1648	Ranibizumab vs. abicipar	q4w vs. q8w vs. q12w	• BCVA • CRT • IVI
Khurana RN et al. [63]	CEDAR/ SEQUOIA	III	1411	Ranibizumab vs. abicipar	q4w vs. q8w vs. q12w	• BCVA • CRT • IVI
The CATT Research Group, 2011 [64]	CATT	NR	1185	Ranibizumab vs. bevacizumab	q4w vs. PRN	• BCVA • CRT • IVI
Taipale C et al. 2020 [65]		NR	52	Aflibercept	T&E	• BCVA • CRT • IVI
Mitchell P et al. 2019 [29]	ARIES	IV	135	Aflibercept	T&E	• BCVA • CRT • IVI
Li K et al. 2016 [66] (Li, Zhu et al. 2016)	DRAGON	IV	499	Ranibizumab	q4w vs. PRN	• BCVA • CRT • IVI

Subgroup of treatment-naïve patients; Selected study arm only (i.e., ranibizumab 0.5 mg q4w after ranibizumab induction). Abbreviations: NR, not reported; PRN, as-needed regimen; q4w, every 4 weeks; q8w, every 8 weeks; q12w, every 12 weeks; T&E, treat-and-extend regimen; BCVA, best-corrected visual acuity; CRT, central retinal thickness; IVT, intravitreal injections.

3.2. Evaluated Treatments

Among the studies included in the analysis, the following anti-VEGF agents were evaluated: ranibizumab 0.5 mg (74% of studies), aflibercept 2 mg (20% of studies) and brolucizumab 6 mg (4% of studies), whereas none of the included studies evaluated abicipar pegol 2 mg. Three studies using bevacizumab 1.25 mg alone were not retained.

For seven other studies using bevacizumab along with a comparative arm, the latter arm was considered for analysis, whereas the bevacizumab arm was not.

3.3. Comparison of Monthly versus Pro Re Nata (PRN) Regimens

For the comparison of monthly versus PRN regimens, at Year 1, the mean gains in the BCVA (8.82 vs. 6.36 letters; $p = 0.0018$) and the reductions in the CRT (mean -146.25 vs. -118.68 μm; $p = 0.0194$) were significantly higher with the monthly regimens than they were with the PRN regimens (Figure 2A, Figure 2B, respectively), and the IVI number was significantly higher with the monthly regimens (mean 10.6 vs. 7.3; $p < 0.0001$) (Figure 2C). At Year 2, the IVI number was significantly higher for the monthly regimens versus the PRN regimens (22.9 vs. 13.3; $p < 0.0001$), and the monthly regimens were associated with significantly greater reductions in the CRT (mean -185.94 vs. -158.28 μm; $p = 0.0332$) (Figure 2E,F). Concerning gains in the BCVA (mean 7.89 vs. 6.30 letters; $p = 0.2973$), no significant differences were identified between the monthly and PRN regimens (Figure 2D).
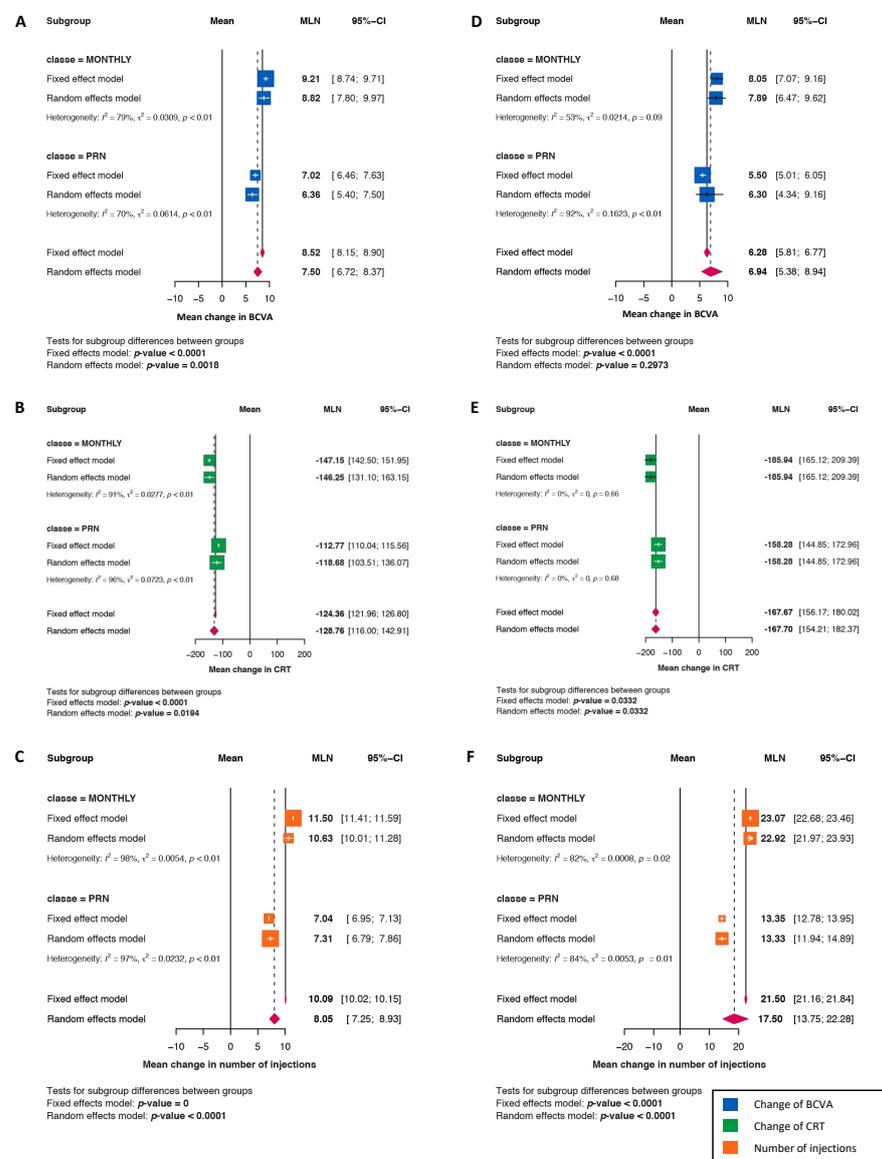
Figure 2. As needed (PRN) versus Monthly regimens. (**A–C**): Mean change at 1 year, in best corrected visual acuity ((**A**), blue squares), in CRT ((**B**), green squares) and in the number of injections ((**C**), orange squares). (**D–F**): Mean change at 2 years, in best corrected visual acuity ((**D**), blue squares), in CRT ((**E**), green squares) and in the number of injections ((**F**), orange squares).

3.4. Comparison of Monthly versus Treat-and-Extend (T&E) Regimens

At Year 1, the improvements that were observed in the BCVA (8.82 vs. 7.62 letters; $p = 0.1305$) and CRT (mean -146.25 vs. -137.04 μm; $p = 0.4164$) were proven to be similar to the improvements observed in the monthly and T&E regimens, whereas the IVI number was significantly lower in the T&E regimens (10.6 vs. 8.2; $p < 0.0001$) (Figure 3). At Year 2, no differences in BCVA gains (mean 7.89 vs. 6.38 letters; $p = 0.0901$) were revealed between monthly and T&E regimens, but the reduction that was observed in CRT was significantly higher with the monthly than the T&E regimens (-185.94 vs. -136.82 μm; $p = 0.0003$), and the IVI number was still significantly lower with the T&E regimen (mean 22.9 vs. 14.6; $p < 0.0001$) (Figure 3).
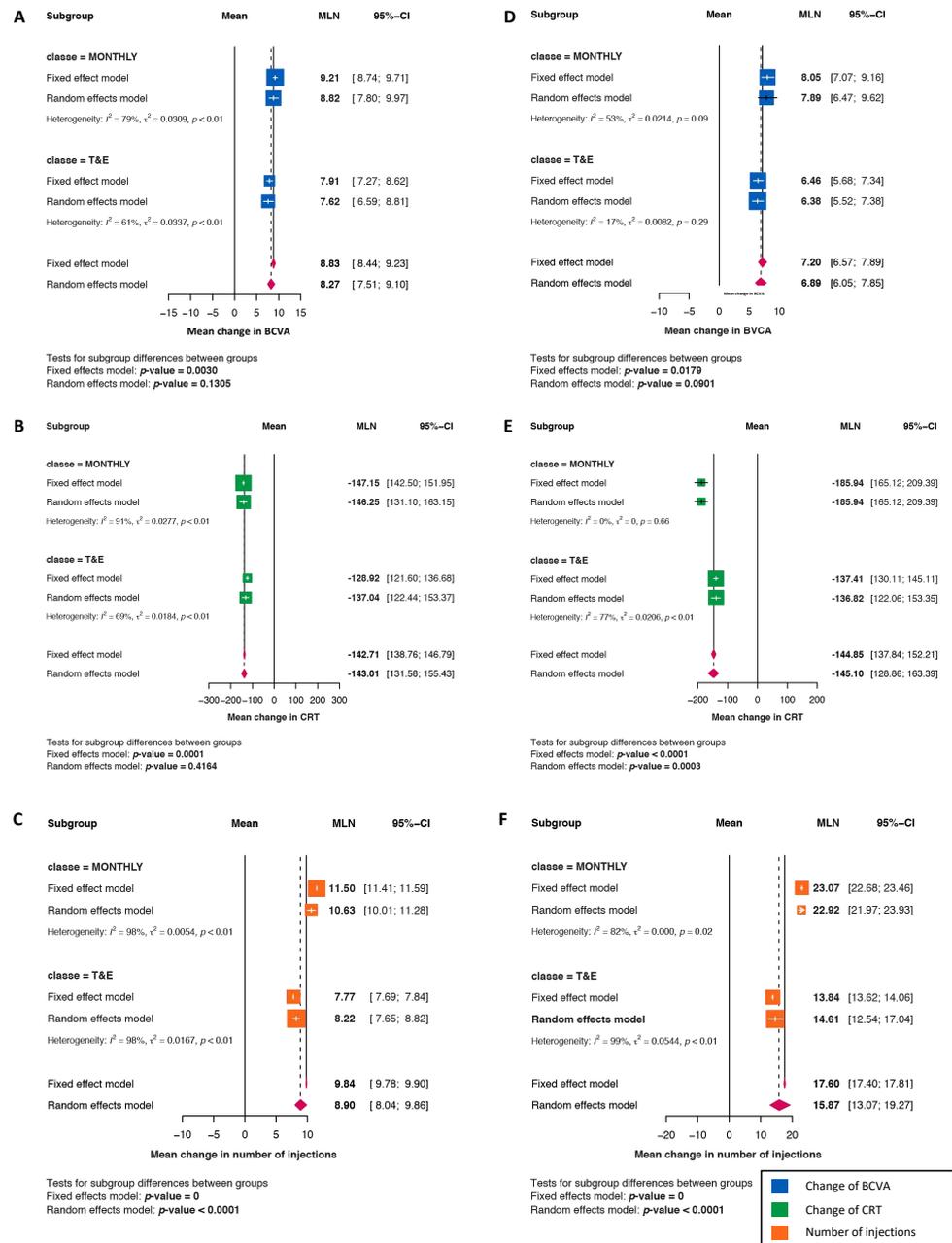
Figure 3. Treat and Extend (T&E) versus Monthly regimens. (**A–C**): Mean change at 1 year, in best corrected visual acuity ((**A**), blue squares), in CRT ((**B**), green squares) and in the number of injections ((**C**), orange squares). (**D–F**): Mean change at 2 years, in best corrected visual acuity ((**D**), blue squares), in CRT ((**E**), green squares) and in the number of injections ((**F**), orange squares).

3.5. Comparison of Pro Re Nata (PRN) Versus Treat-and-Extend (T&E) Regimens

At both Years 1 and 2, the improvements that were observed in the BCVA and CRT, as well as in the IVI number, were similar between the PRN and T&E regimens. The mean gains in the BCVA with PRN regimens versus with T&E regimens at Years 1 and 2 were 6.36 vs. 7.62 letters ($p = 0.1075$) and 6.30 vs. 6.38 letters ($p = 0.9504$), respectively. The corresponding mean reductions in CRT were -118.68 vs. -137.04 μm ($p = 0.1116$) and -158.28 vs. -136.82 μm ($p = 0.048$), whereas the IVI numbers were 7.3 vs. 8.2 ($p = 0.0241$) and 13.3 vs. 14.6 ($p = 0.3409$) at Years 1 and 2, respectively.

3.6. Maintaining Improvements over Time

The monthly, PRN and T&E regimens were all likely to maintain or even improve letter-gaining for BCVA and drying up for CRT between Years 1 and 2 (Table 3). Nevertheless, the mean IVI number differed with each of the regimens over time (10.6 and 22.9 for monthly regimens, 7.3 and 13.3 IVI for PRN regimens and 8.2 and 14.6 for T&E regimens at Years 1 and 2, respectively).

Table 3. Maintenance of changes in BCVA, CRT and IVI numbers over time using monthly, pro re nata and treat-and-extend regimens.

Change in Best-Corrected Visual Acuity (BCVA)					
Regimen	Year	N	Mean (ETDRS Letters)	95% CI	*p* Value
Monthly	1	11	8.8	[7.8; 10]	0.3494
	2	4	7.9	[6.5; 9.6]	
PRN	1	14	6.3	[5.4; 7.5]	0.9634
	2	5	6.3	[4.3; 9.1]	
T&E	1	11	7.6	[6.6; 8.8]	0.0905
	2	9	6.4	[5.5; 7.4]	
Change in central retinal thickness (CRT)					
Regimen	Year	N	Mean	95% CI	*p* Value
Monthly	1	10	146.2	[131.1; 163.1]	0.0036
	2	2	185.9	[165.1; 209.4]	
PRN	1	17	118.7	[103.5; 136.1]	0.0005
	2	3	158.3	[144.8; 173.0]	
T&E	1	9	137.0	[122.4; 153.4]	0.9843
	2	8	136.8	[122.1; 153.3]	
Number of injections					
Regimen	Year	N	Mean	95% CI	*p* Value
Monthly	1	6	10.6	[10.0; 11.3]	<0.0001
	2	2	22.9	[22.0; 23.9]	
PRN	1	18	7.3	[6.8; 7.9]	<0.0001
	2	2	13.3	[11.9; 14.9]	
T&E	1	13	8.2	[7.6; 8.8]	<0.0001
	2	9	14.6	[12.5; 17.0]	

Abbreviations: BCVA, best-corrected visual acuity; CRT, central retinal thickness; ETDRS, Early Treatment Diabetic Retinopathy Study; IVI, intravitreal injections; N, number of treatment arms; PRN, pro re nata (as-needed regimen); T&E, treat-and-extend regimen.

3.7. Comparing Aflibercept and Ranibizumab Treat-and-Extend (T&E) Regimens

The mean gains in the BCVA (Figure 4) and reductions in CRT (Figure 4) were similar between aflibercept and ranibizumab T&E regimens at Years 1 and 2, whereas the mean IVI number was significantly lower with aflibercept than ranibizumab at both Years 1 (7.6 and 9.1, respectively) ($p < 0.0001$) and 2 (12.2 and 16.6, respectively) ($p = 0.0011$; Figure 4).
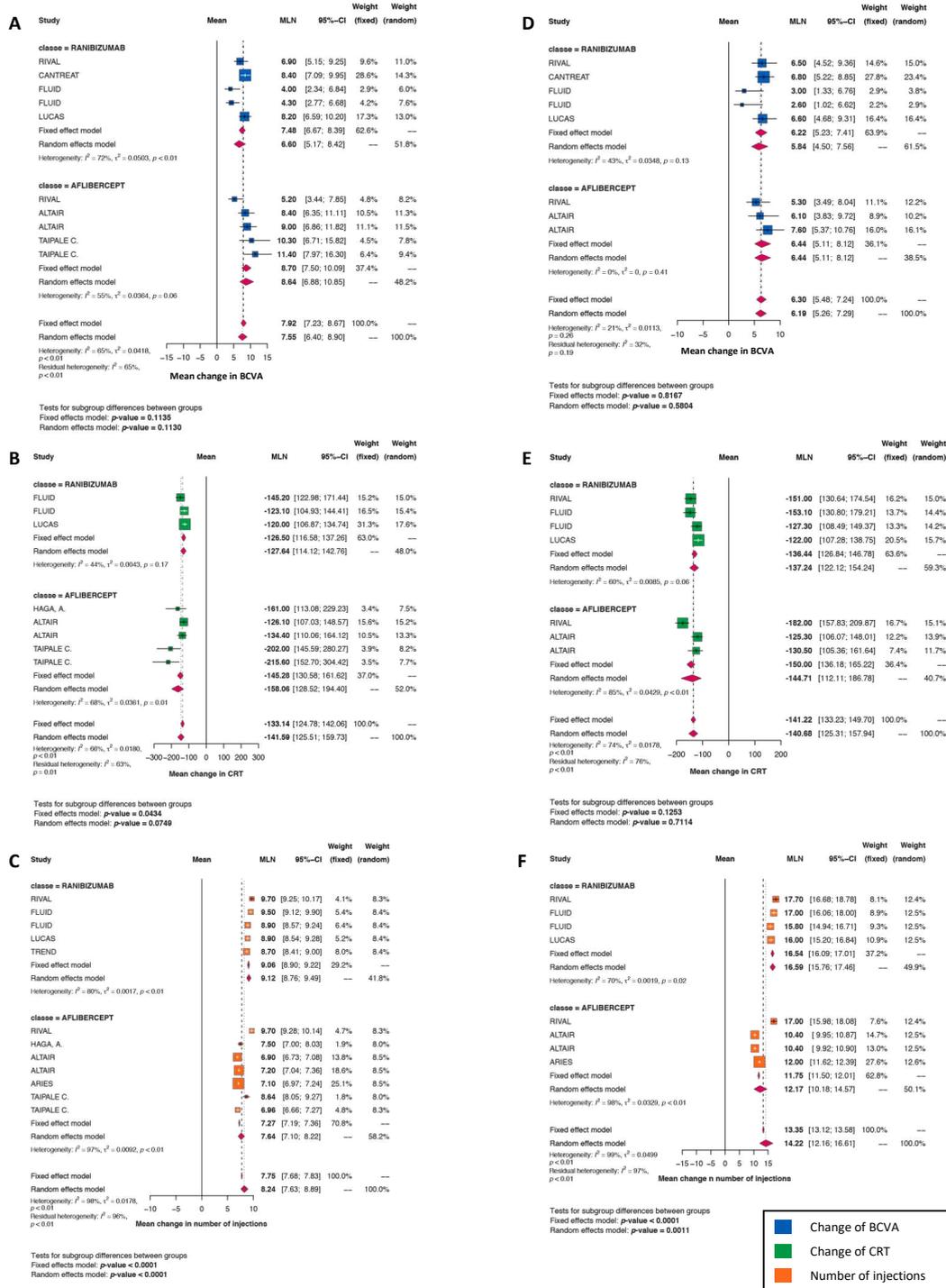
Figure 4. T&E regimen, Aflibercept versus Ranibizumab. (**A**–**C**): Mean change at 1 year, in best corrected visual acuity ((**A**), blue squares), in CRT ((**B**), green squares) and in the number of injections ((**C**), orange squares). (**D**–**F**): Mean change at 2 years, in best corrected visual acuity ((**D**), blue squares), in CRT ((**E**), green squares) and in the number of injections ((**F**), orange squares).

4. Discussion

Anti-VEGF agents are not a curative therapy, but they have become a mainstay and pillar in nAMD management. Indeed, these agents have been proven to slow down disease progression and partially reverse visual impairment [11,66]. Nevertheless, the need for repeated IVI has been a serious burden in AMD management for patients and healthcare systems alike. Hence, alternative administration regimes have been sought, with PRN and T&E regimens the most promising. Under a PRN regimen, the decision to provide an anti-VEGF injection is made at each visit based on the outcomes of the optical coherence tomography measurements. In contrast, when a T&E regimen is applied, the patient undergoes an IVI at each visit, with the between-visit intervals adjusted depending on the disease progression [14]. The primary objective of our meta-analysis was to retrieve further scientific data concerning the visual outcomes and treatment burden with different anti-VEGF agents provided according to either the standard monthly regimen or one of two alternative strategies, PRN or T&E, taking a two-year perspective.

In line with previously published data from the majority of relevant studies, positive outcomes were recorded for all of the anti-VEGF agents and regimens. To summarize our outcome data, at Year 2, both the monthly and T&E regimens resulted in CRT improvements, while the VA outcome improvements were maintained over time with all of the regimens (monthly, PRN, and T&E), and similar improvements in the VA and CRT outcomes were attained with the aflibercept regimens versus the ranibizumab T&E regimens. Hence, our meta-analysis has further confirmed certain previously published observations. Indeed, t significantly improved VA and CRT at Years 1 and 2 were previously reported with monthly versus PRN regimens, similar improvements in VA and CRT were reported at Year 1 with both monthly and T&E regimens and similar improvements were observed in the VA and CRT with PRN and T&E regimens, a finding that was revealed in prior meta-analyses [11,12,66]. In addition to previously published data, our analyses revealed that significantly fewer injections were required for PRN and T&E regimens versus monthly regimens at both time points. All in all, these results clearly suggest that T&E is the best alternative strategy that can be recommended for AMD management compared to the monthly and PRN strategies. Considering this T&E regimen, aflibercept generated similar results compared to ranibizumab, but it required a lower IVI number, meaning that this regimen is also able to provide a longer response. In this context, it must be stressed that a recent survey of retinal specialists found that two-thirds of the respondents in the United States (n = 586) and one-third of the respondents in Europe (n = 424) indicated a clear preference for the T&E regimen with respect to managing their treatment-naïve AMD patients (American Society of Retina Specialists' "2015 global trends in retina" survey results).

The superior efficacy of VA and CRT obtained with monthly versus PRN regimens, as well as the similar efficacy observed with monthly versus T&E regimens in our analysis, are perfectly in line with a recently published Cochrane meta-analysis [67]. These authors revealed that newly diagnosed AMD patients receiving monthly anti-VEGF injections exhibited a slightly better VA at Year 1 versus those receiving PRN injections, whereas no difference in VA was found compared to the T&E regimen. Here, it must be mentioned that, according to the authors, the patients receiving monthly injections actually had a higher IVI number than those receiving injections via PRN or T&E regimens, meaning that patients who were treated monthly were exposed to an increased, although rare, risk of severe undesirable effects such as infections.

An interesting novelty of the current meta-analysis has been its ability to compare the long-term efficacy data of the various anti-VEGF and regimens. For this same purpose, other authors have already focused their research on the outcome data recorded at Years 1 and 2 [11,13,14,50,66]. According to their research, the PRN and T&E regimens displayed VA with decent stability and good control, as well as CRT improvements for up to two years, a finding that is perfectly in line with the monthly standard of care. Moreover, at Year 2, fewer injections were required with both regimens. Here, it must nevertheless be stressed that the PRN regimen appears to be more difficult to handle in real-life settings versus the

T&E regimen due to the required monthly visits. In this context, it must be stressed that PRN regimens have been proven to be effective in prospective randomized studies with carefully selected patients and well-controlled conditions, whereas these regimens were reported to be poorly reproducible in real-life practice [9].

When comparing aflibercept and ranibizumab T&E regimens, their efficacy turned out to be quite similar, whereas this good outcome was achieved with a significantly lower IVI when aflibercept was used at both Years 1 and 2 (7.6 versus 9.1 at Year 1, and 12.2 versus 16.6 at Year 2). In practical terms, this means that aflibercept prolonged the IVI intervals. This outcome is in accordance with the results from a meta-analysis conducted by Ohji et al., which revealed that, at Year 2, aflibercept T&E was associated with six fewer injections on average compared to ranibizumab T&E, providing comparable visual improvements [14]. For clinical practice, this reduced IVI number likely represents a real advantage of aflibercept T&E, especially since anti-VEGF therapy is known to impose a significant additional constraint on patients due treatment-related anxiety and practical problems such as transport burden and common clinic visits [14].

With respect to our analytical methodology, single-means analysis is a new and interesting approach that deserves to be mentioned. This technique enabled us to conduct such a meta-analysis involving large amounts of data to compare three regimens (monthly, PRN and T&E) and two anti-VEGF agents (aflibercept and ranibizumab). Despite the strengths of this statistical methodology, the major limitation was the choice to exclude bevacizumab from the equation. Off-label use of bevacizumab has been discontinued from real-life use. Therefore, our analyses were limited by insufficient data availability. For this reason, several scenarios could not be properly analyzed. Hence, in our literature search, we only came across a few anti-VEGF agent comparisons between monthly versus PRN regimens because most comparisons concerned bevacizumab. It would be interesting to repeat this analysis while including bevacizumab. Likewise, we only identified very few comparisons within the q8w/q12w regimens, given that only brolucizumab has been administered according to the q8w regimen and only very limited data pertaining to the q12w regimen are available. With respect to the q8w regimen, scarcely any data were recorded during Year 2, with several study designs including a regimen change between Years 1 and 2.

We must emphasize several limitations to our report. A major drawback of our meta-analysis, which is rather common, is the failure to obtain data on all patients and from all trials. This may result in an acquisition bias, given that missing studies or patients may not be missing completely at random, resulting in biased outcomes. While meta-analyses represent a powerful tool for the design of future research and to provide evidence for the regulatory process, they may also be controversial, as several conditions are critical to a sound meta-analysis, and small violations of such conditions may lead to misleading results. Another drawback that should be mentioned is that there was a slight discrepancy between the time points at which the Year 2 data were collected.

5. Conclusions

This new meta-analysis revealed superior efficacy, reflected by improvements in VA and CRT with both monthly PRN regimens, but this improved efficacy was achieved with a higher IVI number. On the other hand, the T&E regimens demonstrated similar efficacy to the monthly regimens, but with a reduced IVI number. When comparing the T&E regimens of aflibercept and ranibizumab, aflibercept was associated with a reduced IVI number compared with ranibizumab, but the recorded parameters showed similar efficacy.

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Retinal Vascularization Analysis on Optical Coherence Tomography Angiography before and after Intraretinal or Subretinal Fluid Resorption in Exudative Age-Related Macular Degeneration: A Pilot Study

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Abstract: The aim was to analyze the variations in macular vascularization on optical coherence tomography angiography (OCTA) according to the presence of intraretinal fluid (IRF) induced by exudative age-related macular degeneration (AMD). We included exudative AMD patients with IRF and/or subretinal fluid (SRF) and age-matched control eyes. All patients underwent a macular 6×6 mm swept-source OCTA. The mean perfusion density (MPD) and mean vascular density (MVD) were calculated in the superficial (SCP) and the deep (DCP) capillary plexus at two timepoints: during an episode of exudation (T0) and after its total resorption (T1). A total of 22 eyes in the IRF ± SRF group, 11 eyes in the SRF group and 11 eyes in the healthy group were analyzed. At T0, the IRF ± SRF group showed significantly lower MPD and MVD than healthy eyes in the SCP ($p < 0.001$) and DCP ($p < 0.001$). At T1, MPD and MVD significantly increased from T0 in the SCP ($p = 0.027$ and $p = 0.0093$) and DCP ($p = 0.013$ and $p = 0.046$) but remained statistically lower than in the healthy eyes. For the SRF group, only the DCP showed significantly lower MPD ($p = 0.012$) and MVD ($p = 0.046$) in comparison to the healthy eyes at T0. The present study shows that retinal vascular changes do occur in the case of exudative AMD.

Keywords: age-related macular degeneration; exudation; optical coherence tomography angiography

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness worldwide [1]. Its pathophysiology is multifactorial and remains unclear [2]. Unlike the atrophic form of the disease, neovascular AMD benefits from treatment with intravitreal injections (IVIs) of anti-vascular endothelial growth factor (anti-VEGF). There are two types of exudation: the accumulation of subretinal fluid (SRF) associated with a breakdown of the outer blood–retinal barrier, and the accumulation of intraretinal fluid (IRF) characterized by the presence of intraretinal cysts. The latter is known to be associated with a worse functional prognosis [3–5]. Macular edema (ME) is a common manifestation of several retinal pathologies, its physiopathology is complex and involves the dysregulation of the blood–retinal barrier and hemodynamic phenomenon [6].

Optical coherence tomography angiography (OCTA) is a recent diagnosis tool in ophthalmology for the non-invasive examination of the retinal and choroidal microvasculature [7,8]. It has been shown on OCTA that retinal vascularization can change before and after IRF resorption in patients with retinal vascular disease. For example, in chronic diabetic ME (DME), cystoid spaces co-localized with capillary loss areas and no reperfusion occurred after DME resolution [9]. The same findings have been observed for retinal vein occlusion (RVO) [10]. However, unlike DME and RVO, macular vessel density after the resolution of an acute pseudophakic cystoid ME did not differ from the control eyes [11]. Several studies based on OCTA have analyzed the variations in the choroidal neovessels before and after anti-VEGF treatment [12–14]. However, little is known about changes in retinal vascularization in cases of IRF induced by neovascular AMD.

The aim of the present pilot study was to analyze the variations in retinal macular vascularization on OCTA according to the presence of fluid (IRF and/or SRF) induced by neovascular AMD, in comparison to age-matched control eyes. The secondary objectives included the change in retinal macular vascularization over time after fluid resorption.

2. Materials and Methods

2.1. Subjects and Follow-Up

An observational prospective case–control study was performed in our ophthalmology department at the Croix-Rousse University Hospital in Lyon, France, between January 2019 and December 2019. This study complied with the tenets of the Declaration of Helsinski and was approved on 15 January 2021, by an international review board (Ethics Committee of the French Society of Ophthalmology, IRB 00008855 Société Française d'Ophtalmologie IRB#1). Patients gave their informed consent to participate in the study.

Patients aged 65 years or older with a diagnosis of exudative AMD were included in the study. Best corrected visual acuity (BCVA) was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart. All patients underwent a complete ophthalmological examination including slit lamp and fundus examination, spectral domain– (SD–) OCT, fluorescein angiography (FA), indocyanine green angiography (ICGA) (Heidelberg Spectralis, Heidelberg Engineering, Heidelberg, Germany) and swept source– (SS–) OCTA (PLEX Elite 9000, Carl Zeiss Meditec, Dublin, CA, USA). All the images obtained were analyzed by two retinal specialist graders who confirmed the diagnosis of exudative AMD and determined the neovascular subtype, as described by Freund et al. [15]. The exclusion criteria were the presence of any other eye disease, including myopia $\geq$ 6D, or ophthalmic surgery except for cataract extraction. At the time of inclusion, eyes with retinal hemorrhage were not included due to possible artifact on OCTA. Patients with systemic high blood pressure, diabetes, smokers and patients with other systemic vascular diseases were not included.

Two subgroups of patients were included. The first group was composed only of eyes presenting significant IRF with at least three cystic spaces visualized on SD–OCT with or without SRF (group IRF $\pm$ SRF). The second group of patients with a diagnosis of exudative AMD and SRF without IRF (group SRF) was compared to the first group. Patients in the SRF group were matched for age and disease duration with patients in the IRF $\pm$ SRF group. A third control group was composed of age-matched ($\pm$5 years) healthy individuals with no ocular or systemic diseases, who were recruited and attended a single visit during which they underwent the ophthalmological exams including OCTA. For all patients included in the IRF and SRF groups, the OCTA images were analyzed during an episode of exudation with IRF and/or SRF (T0) and after its total resorption (T1). Any patients who developed another eye disease or underwent eye surgery between T0 and T1 were excluded/withdrawn from the study.

2.2. Data Collection

Demographic data were obtained from the medical charts on the day of inclusion. Data collected were age, sex, duration of the disease, number of IVIs before inclusion, treatment

regimen and molecule injected. The patient, disease and treatment characteristics were recorded in a case report form, filled out by the retinal specialist investigator. The specific disease characteristics reported were the presence of IRF, SRF, atrophy, fibrosis, central retinal thickness (CRT) and the subtype of neovessel according to the recent consensus classification [16] (Supplementary File 1).

2.3. OCTA Acquisition

OCTA volumes were obtained using the swept-source OCTA PLEX Elite 9000 (Zeiss, Dublin, CA, USA). Each patient had a volumetric angiogram of 6×6 mm field of view centered on the fovea, comprised of 500 A-scans per B-scan and 500 B-scans per volume obtained at equally spaced positions. The angiography data were generated by processing two repeated OCT frames per B-scan position and using the eye tracking system (FastTrac, Carl Zeiss Meditec, Dublin, CA, USA). All acquisitions were performed by the same operator. Angiograms presenting motion or blinking artifacts were excluded. En-face angiograms were segmented automatically with the integrated PLEX Elite software, with the manual correction of segmentation errors, especially in cases of pigmented epithelium detachment (PED). Eyes with an OCTA signal strength below 8 (a measurement provided by the instrument software, ranging 0–10 and related to image quality), movement artifacts, or mask effects were not included in the study.

2.4. Image Analysis

Quantitative analysis of the retinal vascular density of the superficial capillary plexus (SCP) and the deep capillary plexus (DCP) was performed on the 6×6 mm OCTA for all patients. The SCP correspond to the capillary network in the ganglion cell layer and the DCP correspond to the capillary network between the outer boundary of the inner plexiform layer and the midpoint of the outer plexiform layer. OCTA volumes were anonymously exported by the software program after the careful verification and correction of possible segmentation errors. Exported OCTA volumes were uploaded to the ARI Network platform (https://arinetworkhub.com), a cloud-based collaborative and processing solution provided by the manufacturer. The vascular densities of the SCP and DCP were computed in the ARI Network platform using the Macular Density algorithm (version 0.7.1), a prototype algorithm provided by the instrument manufacturer (Carl Zeiss Meditec, Inc.) [17]. In short, this algorithm generates density and perfusion maps as follows: firstly, the SCP and DCP angiography images were generated using the segmentation algorithm as provided in the instrument and additional manual edits were made to correct any possible segmentation errors. Angiographic tail projections were corrected from the DCP image using a method equivalent to that available in the software program, removing projections from vessels appearing in the DCP image that were actually located within the SCP [18]. The resulting angiography images were resized to a canonical sampling density and processed using a proprietary algorithm to generate a binary mask indicating the presence of vasculature. This proprietary algorithm consists of the following steps: (1) histogram equalization; (2) hessian filtering (to enhance vessel patterns); (3) global thresholding using a computed "noise floor" (minimum signal level expected as image background) to binarize the image onto pixels belonging to vasculature or background; (4) morphological operations to clean up the resulting binary image from isolated pixels. After this binarization process, the ratio of segmented vessels (white pixels) over the total area gives the perfusion density values. The result is a number ranging from 0 (no perfusion) to 1 (fully perfused). The binarized image was further processed using a skeletonization algorithm (Figure 1). The ratio of the resulting skeleton (vessel length) over the total area constitutes the vessel density values, defined as the total length of perfused vasculature per unit area in a region of measurement. The result is a number with a minimum of 0 (no vessels) and an unbounded maximum. All methods were implemented in C++ and C# and complied into a dll. The workflow was managed using Matlab® software (MathWorks, Natick, MA, USA).
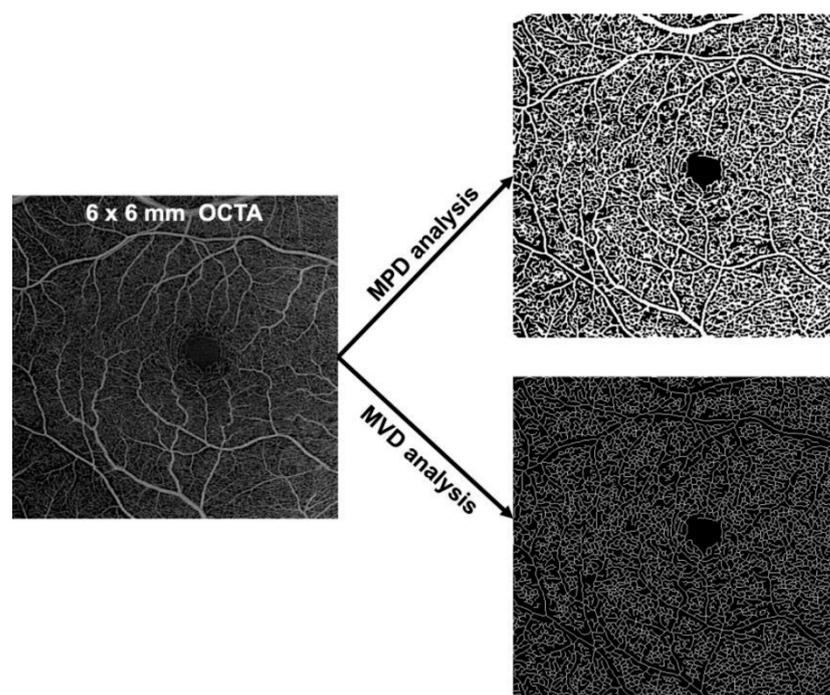
Figure 1. Post-acquisition analysis. MPD: mean perfusion density; MVD: mean vascular density.

2.5. Outcome Measures

The main outcome measure was the analysis of the SCP and DCP vascularization between groups. Retinal vascularization was assessed using the mean perfusion density (MPD) and mean vessel density (MVD) and computed for the whole 6×6 mm image. The secondary outcome measures were the analysis of variations in these values before and after exudation resolution (T1).

2.6. Statistical Analysis

As we expected more variability in the IRF $\pm$ SRF group due to the cystic areas, we planned to include twice as many patients in this group as in the SRF and control groups. The quantitative variables are presented as the mean and standard deviation (SD) and described as percentages for perfusion density data, and as continuous values for vascular density data. Factors potentially associated with MPD and MVD were studied using a univariable and multivariable linear regression analysis. Factors with $p < 0.1$ in the univariable analysis were entered into a multivariable model. The effect of time on the outcome variables was studied using a linear mixed model with age, fibrosis and atrophy as covariables. All analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

Forty-six eyes (46 patients) were included in the present study. Two eyes were excluded due to poor image quality. A total of 44 eyes (44 patients) were analyzed: 22 eyes in 22 patients in the IRF $\pm$ SRF group, 11 eyes in 11 patients in the SRF group and 11 eyes in 11 patients in the healthy group. In the IRF $\pm$ SRF group, eight eyes were treatment-naive, and 14 eyes were already treated with anti-VEGF under a pro re nata (PRN) or treat-and-extend regimen. The eyes included in the IRF $\pm$ SRF group received a mean of 2.0 ± 1.0 IVIs between the first (T0) and the second (T1) OCTA imaging. The eyes included in the SRF group received a mean of 1.3 ± 0.5 IVIs between the two OCTA exams. Three patients in the SRF group were treatment-naive at the time of inclusion (Table 1). Before inclusion, the non-treatment-naive eyes received a mean of 9.1 ± 11.8 IVIs in the IRF $\pm$ SRF group and 18.6 ± 16.9 IVIs in the SRF group ($p = 0.11$).

Table 1. Patient characteristics.

	IRF ± SRF Group (*n* = 22)	SRF Group (*n* = 11)	Healthy Group (*n* = 11)
Mean age, years (SD)	81.7 (8.3)	79.9 (5.8)	78.6 (2.8)
Sex (male/female), *n*	5/17	5/6	6/5
Laterality (R/L), *n*	10/12	7/4	5/6
Mean disease duration, months (SD)	22.6 (26.1)	33.7 (29.3)	-
Mean duration between T0 and T1, months (SD)	2.7 (1.7)	1.2 (0.4)	-
Mean number of IVI before inclusion, *n* (SD)	9.1 (11.8)	18.6 (16.9)	-
Mean number of IVI between T0 and T1, *n* (SD)	2.0 (1.0)	1.3 (0.5)	-
Treatment regimen, *n* (%)			
Pro re nata	7 (31.8)	1 (9.1)	
Treat and Extend	7 (31.8)	7 (63.6)	-
Treatment-naive	8 (36.4)	3 (27.3)	
Presence of SRF, *n* (%)	6 (27.3)	11 (100)	-
Presence of IRF, *n* (%)	22 (100)	0 (0)	-
Presence of atrophy, *n* (%)	13 (59.1)	2 (18.2)	-
Presence of fibrosis, *n* (%)	8 (36.4)	1 (9.0)	-
Mean BCVA at T0, ETDRS (SD)	56.8 (19.7)	72.0 (15.5)	-
Mean BCVA at T1, ETDRS (SD)	58.1 (19.8)	72.7 (16.0)	-
Type of anti-VEGF, *n* (%)			
Aflibercept	19 (86.4)	11 (100)	-
Ranibizumab	3 (13.6)	0 (0)	

BCVA: best-corrected visual acuity; ETDRS: early treatment diabetic retinopathy study; IRF: intraretinal fluid; IVI: intravitreal injection; R/L: right/left; SD: standard deviation; SRF: subretinal fluid; VEGF: vascular endothelial growth factor.

3.1. Mean Perfusion Density (MPD) and Mean Vascular Density (MVD) at Baseline

In the healthy group, MPD and MVD was $44.4 \pm 2.2\%$ and $19.7 \pm 1.1\%$, respectively in the SCP and $34.1 \pm 6.1\%$ and $16.2 \pm 2.8\%$, respectively in the DCP.

In the SCP at T0, eyes from the IRF ± SRF group showed significantly lower MPD and MVD than the healthy eyes (−7.0 95%CI (−9.6, −4.6), $p < 0.001$ and −3.1 95%CI (−4.3, −1.9), $p < 0.001$, respectively). Eyes from the SRF group showed no difference in MPD ($p = 0.13$) and MVD ($p = 0.2$) compared to the healthy eyes. These results remained significant for the IRF ± SRF group in the multivariable analysis (Tables 2 and 3). Additionally, eyes from the IRF ± SRF group showed significantly lower MPD and MVD than the SRF group ($p = 0.006$ and $p = 0.015$, respectively) (Figure 2). Although lower MPD was found in cases of fibrosis ($p = 0.045$) and lower MVD was found in cases of fibrosis ($p = 0.032$) and atrophy ($p = 0.048$) in the SCP at T0, none of these factors were found to be significantly associated with these parameters in the multivariable analysis.
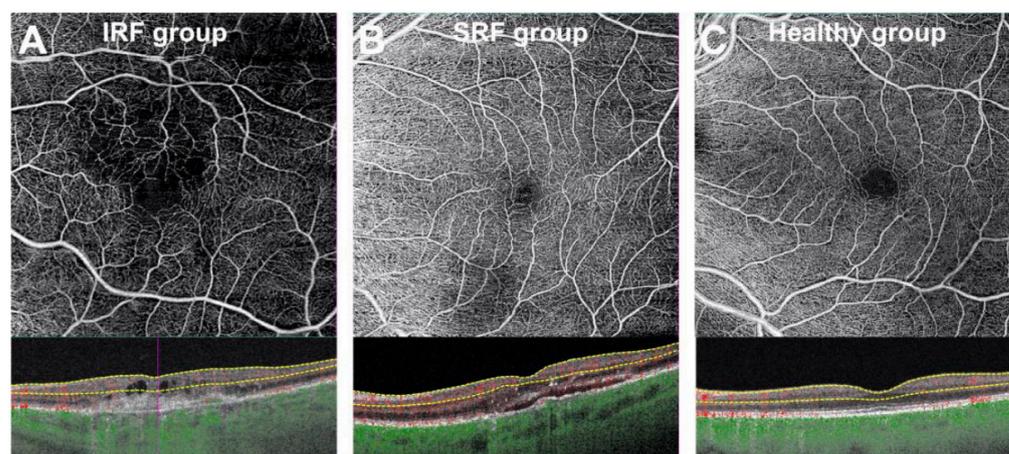
Figure 2. Optical coherence tomography angiography representative images of the superficial capillary plexus at T0 in eyes with intraretinal fluid (**A**) and subretinal fluid (**B**) compared to healthy subjects (**C**).

Table 2. Mean perfusion density (MPD) in the superficial capillary plexus (SCP) according to different factors in the univariate and multivariate analyses.

| | MPD SCP | | | |
| | Univariable | | Multivariable | |
	Estimate (95%CI)	*p*-Value	Estimate (95%CI)	*p*-Value
Fluid group				
Healthy	Ref		Ref	
IRF ± SRF	−7.0 (−9.6, −4.6)	<0.001	−5.7 (−8.7, −2.7)	<0.001
SRF	−2.2 (−5.1, 0.7)	0.13	−1.8 (−4.7, 1.1)	0.2
Age	−0.2 (−0.4, 0.01)	0.061	−0.1 (−0.3, 0.05)	0.2
Sex (Male)	2.1 (−0.7, 4.9)	0.14	-	-
Disease duration	−0.02 (−0.08, 0.04)	0.5	-	-
Number of IVI	0.02 (−0.09, 0.1)	0.7	-	-
Atrophy	−2.8 (−5.7, 0.1)	0.062	−0.7 (−3.4, 1.9)	0.6
Fibrosis	−3.3 (−6.5, −0.07)	0.045	−1.7 (−4.6, 1.2)	0.2
MNV subtype				
Type 1	Ref			
Type 2	−1.5 (−6.3, 3.4)	0.5	-	-
Type 3	1.3 (−2.1, 4.7)	0.4	-	-
Anti-VEGF				
Aflibercept	Ref			
Lucentis	0.4 (−5.0, 5.7)	0.9	-	-
Treatment				
Naive	Ref			
PRN	−0.4 (−4.6, 3.7)	0.8	-	-
TAE	0.8 (−2.8, 4.4)	0.6	-	-

IRF: intraretinal fluid; IVI: intravitreal injection; MNV: macular neovessel; PRN: Pro re nata; SCP: superficial capillary plexus; SRF: subretinal fluid; TAE: treat and extend; VEGF: vascular endothelial growth factor.

Table 3. Mean vascular density (MVD) in the superficial capillary plexus (SCP) according to different factors in the univariate and multivariate analyses.

| | MVD SCP | | | |
| | Univariable | | Multivariable | |
	Estimate (95%CI)	*p*-Value	Estimate (95%CI)	*p*-Value
Fluid group				
Healthy	Ref		Ref	
IRF ± SRF	−3.1 (−4.3, −1.9)	<0.001	−2.4 (−3.8, −0.9)	0.002
SRF	−0.9 (−2.3, 0.5)	0.2	−0.7 (−2.1, 0.7)	0.3
Age	−0.07 (−0.2, 0.02)	0.14	−0.03 (−0.1, 0.04)	0.4
Sex (Male)	1.0 (−0.3, 2.3)	0.14	-	-
Disease duration	−0.01 (−0.03, 0.02)	0.6	-	-
Number of IVI	0.01 (−0.04, 0.07)	0.6	-	-
Atrophy	−1.4 (−2.8, −0.01)	0.048	−0.4 (−1.7, 0.9)	0.5
Fibrosis	−1.7 (−3.2, −0.1)	0.032	−0.9 (−2.3, 0.5)	0.2
MNV subtype				
Type 1	Ref			
Type 2	−0.9 (−3.2, 1.3)	0.4	-	-
Type 3	0.9 (−0.7, 2.5)	0.3	-	-
Anti-VEGF				
Aflibercept	Ref			
Lucentis	0.4 (−2.2, 2.9)	0.8	-	-
Treatment				
Naive	Ref			
PRN	−0.07 (−2.0, 1.9)	>0.9	-	-
TAE	0.8 (−1.3, 2.1)	0.6	-	-

IRF: intraretinal fluid; IVI: intravitreal injection; MNV: macular neovessel; PRN: Pro re nata; SCP: superficial capillary plexus; SRF: subretinal fluid; TAE: treat and extend; VEGF: vascular endothelial growth factor.

In the DCP at T0, eyes from the IRF ± SRF group showed significantly lower MPD and MVD than the healthy eyes (−13.4 95%CI (−19.0, −8.0), $p < 0.001$ and −5.6 95%CI (−8.3, −3.0), $p < 0.001$, respectively). There was also a significant difference for eyes with

SRF in comparison to healthy eyes in terms of MPD (−9.3 95%CI (−16.0, −3.1), p = 0.004) and MVD (−3.0 95%CI (−6.1, −0.05), p = 0.046). These results remained significant in the multivariable analysis except for MVD in the SRF group (p = 0.068) (Tables 4 and 5). Furthermore, there were no differences between the IRF ± SRF and SRF groups in MPD and MVD (p = 0.3 and p = 0.11, respectively) (Figure 3). No other factors were found to be significantly associated with MPD or MVD variation in the univariable or the multivariable analysis.
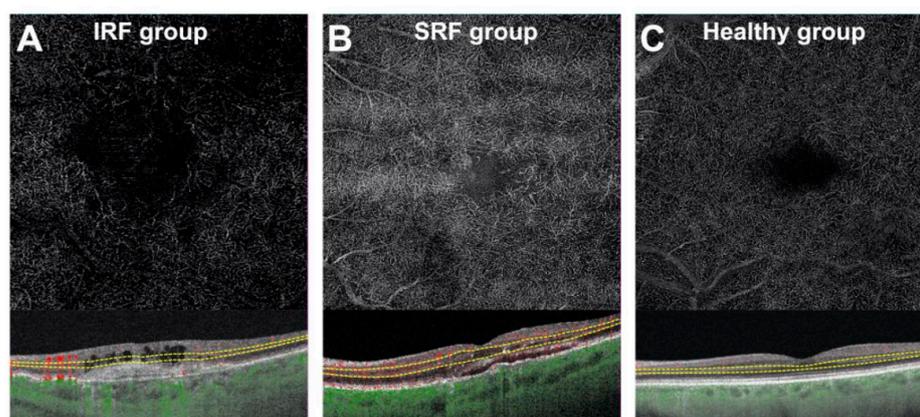
Figure 3. Optical coherence tomography angiography representative images of the deep capillary plexus at T0 in eyes with intraretinal fluid (**A**) and subretinal fluid (**B**) compared to healthy subjects (**C**).

Table 4. Mean perfusion density (MPD) in the deep capillary plexus (DCP) according to different factors in the univariate and multivariate analyses.

	MPD DCP			
	Univariable		**Multivariable**	
	Estimate (95%CI)	*p*-**Value**	**Estimate (95%CI)**	*p*-**Value**
Fluid group				
Healthy	Ref		Ref	
IRF ± SRF	−13.4 (−19.0, −8.0)	<0.001	−13.0 (−19.0, −5.9)	<0.001
SRF	−9.3 (−16.0, −3.1)	0.004	−9.2 (−16.0, −2.7)	0.007
Age	−0.02 (−0.4, 0.4)	>0.9	0.1 (−0.2, 0.5)	0.4
Sex (Male)	4.1 (−1.5, 9.7)	0.15	-	-
Disease duration	−0.06 (−0.2, 0.04)	0.2	-	-
Number of IVI	−0.10 (−0.3, 0.1)	0.3	-	-
Atrophy	−2.9 (−8.3, 2.6)	0.3	−1.1 (−7.1, 4.9)	0.7
Fibrosis	−2.9 (−9.0, 3.2)	0.3	−1.5 (−8.0, 5.0)	0.6
MNV subtype				
Type 1	Ref			
Type 2	−4.4 (−13.0, 4.3)	0.3	-	-
Type 3	2.4 (−3.6, 8.3)	0.4	-	-
Anti-VEGF				
Aflibercept	Ref			
Lucentis	−1.9 (−12.0, 7.7)	0.7	-	-
Treatment				
Naive	Ref			
PRN	−2.8 (−10.0, 4.6)	0.4	-	-
TAE	−1.0 (−7.4, 5.5)	0.8	-	-

DCP: deep capillary plexus; IRF: intraretinal fluid; IVI: intravitreal injection; MNV: macular neovessel; PRN: Pro re nata; SRF: subretinal fluid; TAE: treat and extend; VEGF: vascular endothelial growth factor.

Table 5. Mean vascular density (MVD) in the deep capillary plexus (DCP) according to different factors in the univariate and multivariate analyses.

| | MVD DCP | | | |
| | Univariable | | Multivariable | |
	Estimate (95%CI)	*p*-Value	Estimate (95%CI)	*p*-Value
Fluid group				
Healthy	Ref		Ref	
IRF ± SRF	−5.6 (−8.3, −3.0)	<0.001	−5.3 (−8.6, −2.1)	0.002
SRF	−3.0 (−6.1, −0.05)	0.046	−2.9 (−6.1, 0.2)	0.068
Age	−0.09 (−0.3, 0.1)	0.3	−0.02 (−0.2, 0.1)	0.8
Sex (Male)	1.9 (−0.7, 4.1)	0.15	-	-
Disease duration	−0.02 (−0.07, 0.03)	0.4	-	-
Number of IVI	−0.01 (−0.1, 0.09)	0.8	-	-
Atrophy	−1.3 (−4.1, 1.4)	0.3	−0.5 (−3.4, 2.4)	0.7
Fibrosis	−0.8 (−3.9, 2.2)	0.6	−0.1 (−3.0, 3.3)	>0.9
MNV subtype				
Type 1	Ref			
Type 2	−0.3 (−4.7, 4.2)	0.9	-	-
Type 3	0.4 (−2.6, 3.5)	0.8	-	-
Anti-VEGF				
Aflibercept	Ref			
Lucentis	−2.0 (−6.7, 2.8)	0.4	-	-
Treatment				
Naive	Ref			
PRN	−1.2 (−4.8, 2.4)	0.5	-	-
TAE	−1.1 (−2.1, 4.3)	0.5	-	-

DCP: deep capillary plexus; IRF: intraretinal fluid; IVI: intravitreal injection; MNV: macular neovessel; PRN: Pro re nata; SRF: subretinal fluid; TAE: treat and extend; VEGF: vascular endothelial growth factor.

3.2. Effect of Treatment/Time on Mean Perfusion Density (MPD) and Mean Vascular Density (MVD)

In the SCP, at T1 there was a significant increase in MPD (2.7 95%CI (0.2, 5.2), $p = 0.048$) and MVD (1.5 95%CI (0.5, 2.4), $p = 0.008$) for the IRF ± SRF group (Tables 6 and 7). Although MPD and MVD increased after treatment up until the point of fluid resorption (T1), these values still remained lower than in the healthy eyes both for MPD (−4.3 95%CI (−7.8, −0.96), $p = 0.013$), and MVD (−1.6 95%CI (−2.9, −0.31), $p = 0.017$). Eyes with SRF did not show any significant changes in MPD ($p = 0.36$) or MVD ($p = 0.33$) at T1 in comparison to heathy eyes. Additionally, eyes from the IRF ± SRF group did not show any significant changes in MPD ($p = 0.4$) or MVD ($p = 0.7$) at T1 in comparison to eyes from the SRF group.

Table 6. Effect of time on mean perfusion density (MPD) according to the patient group and plexus analyzed. The analyses were performed using a multivariable linear mixed effects model including age, fibrosis and atrophy as covariates. The estimate gives the increase or decrease in the variable between T0 and T1 independently of age, fibrosis and atrophy.

	T0	T1	Estimate (95%CI)	*p*-Value
Healthy group				
SCP	44.4 ± 2.2		-	-
DCP	34.1 ± 6.1			
IRF ± SRF group				
SCP	37.4 ± 4.1	40.1 ± 5.8	2.7 (0.2, 5.2)	0.048
DCP	20.7 ± 7.8	27.5 ± 8.4	6.8 (2.1, 11.4)	0.009
SRF group				
SCP	42.2 ± 2.3	41.4 ± 3.0	−0.8 (−2.6, 0.9)	0.36
DCP	24.8 ± 6.9	23.8 ± 6.3	−1.0 (−5.7, 3.8)	0.64

DCP: deep capillary plexus; IRF: intraretinal fluid; SCP: superficial capillary plexus; SRF: subretinal fluid.

Table 7. Effect of time on mean vascular density (MVD) according to the patient group and plexus analyzed. The analyses were performed using a multivariable linear mixed effects model including age, fibrosis and atrophy as covariates. The estimate gives the increase or decrease in the variable between T0 and T1 independently of age, fibrosis and atrophy.

	T0	T1	Estimate (95%CI)	*p*-Value
Healthy group				
SCP	19.7 ± 1.1		-	-
DCP	16.2 ± 2.8			
IRF ± SRF group				
SCP	16.6 ± 1.9	18.1 ± 2.1	$1.5\ (0.5, 2.4)$	0.008
DCP	10.6 ± 3.7	12.7 ± 3.6	$2.1\ (-0.02, 4.34)$	0.066
SRF group				
SCP	18.8 ± 1.3	18.3 ± 1.5	$-0.5\ (-1.2, 0.4)$	0.33
DCP	13.2 ± 2.9	12.2 ± 2.7	$-1.0\ (-3.4, 1.6)$	0.49

DCP: deep capillary plexus; IRF: intraretinal fluid; SCP: superficial capillary plexus; SRF: subretinal fluid.

In the DCP, there was a significant increase in MPD (6.8 95%CI (2.1, 11.4), $p = 0.009$) at T1 in the IRF ± SRF group, but not in MVD (2.1 95%CI (-0.02, 4.34), $p = 0.066$). These retinal perfusion parameters at T1 still remained lower than in the healthy group for MPD (-6.6 95%CI (-12.0, -0.96), $p = 0.021$), and for MVD (-3.5 95%CI (-5.9, -1.1), $p = 0.006$). For eyes with SRF, no significant changes in MPD ($p = 0.64$) or MVD ($p = 0.49$) were found at T1. However, in eyes with SRF these values remained lower than in the healthy eyes for both MPD (-10.0 95%CI (-17.0, -3.9), $p = 0.002$), and MVD (-4.0 95%CI (-6.8, -1.2), $p = 0.006$). Additionally, eyes from the IRF ± SRF group did not show any significant changes in MPD ($p = 0.2$) or MVD ($p = 0.7$) at T1 in comparison to eyes from the SRF group.

4. Discussion

In the present study, we analyzed the retinal vascular changes visualized on OCTA in exudative AMD according to the location of the fluid. We showed that patients with IRF ± SRF have lower MPD in both the SCP and the DCP in comparison to healthy individuals, and MPD was only lower in the DCP in eyes with SRF. After exudation resorption, eyes with IRF showed a significant increase in MPD in both plexuses, in comparison to eyes with SRF in which MPD remained constant. However, at the time of fluid resorption (T1), eyes with previous IRF ± SRF still had lower MPD than healthy eyes in both the SCP and DCP, whereas eyes with SRF only had lower MPD in the DCP. In this study, the same results were found for MVD. However, unlike for MPD, all vessels are treated equally. Regarding MPD, larger vessels influence the measurement more than smaller capillaries and can therefore hide the loss of individual capillaries. MVD is more sensitive to the loss of individual capillaries as it measures all of the retinal plexus vasculature equally. The downside of this method is its lower signal to noise ratio.

Several studies have reported abnormal vascular changes in exudative or atrophic AMD [19,20]. This is not surprising as high blood pressure, smoking and obesity are risk factors of the disease [21]. Some authors found arteriolar narrowing and arteriovenous crossing in patients suffering from AMD and suggested a possible association with disease progression [19,22]. Toto et al. found a decrease in perfusion density in the SCP in patients with a high risk of developing late stage atrophic AMD [23]. Although the presence of ischemic areas in the choriocapillaris is a well-known feature of atrophic AMD [24], some studies have also found reduced retinal vessel density in this form of the disease [25,26]. For exudative AMD, same observation has been made for choriocapillaris [27], however, the present study is the first to describe an alteration in retinal perfusion. Surprisingly, there is a significant increase in MPD in eyes with IRF after exudation resorption, meaning that a reperfusion of the cystic area does occur, in contrast to vaso-occlusive diseases. Indeed, in DME and RVO, it has been shown that MPD does not increase after IRF resolution [9,10].

The physiopathology of IRF in exudative AMD is unclear [28]. Gass hypothesized that the extension of choroidal neovessels beneath the retinal capillary-free zone might disrupt the photoreceptors–outer retinal membrane complex. This might lead to the intraretinal migration of subretinal exudate if there are not enough retinal capillaries in this area to remove it [29]. It has been shown that AMD patients with IRF experience worse visual outcomes than patients with SRF alone [30]. In the present study, although MPD and MVD increased between T0 and T1 in eyes with IRF, they remained lower than in the healthy eyes. This could explain the worse visual outcomes associated with IRF. In contrast, eyes with SRF only had significantly lower MPD and MVD in the DCP and there was no significant increase at the time in fluid resorption (T1). This might be explained by the longer duration of disease found for eyes with SRF in the present cohort, which could potentially lead to chronic alterations to the retinal vascular plexus. It should be noted that fluid exhibits some layer-dependent properties in exudative AMD. IRF normally appears above the outer plexiform layer, thus involving DCP and sometimes SCP. In contrast, SRF is accumulated beneath the outer segment layer and do not affect both retinal capillary plexus [31].

Taken together, our analysis of OCTA images showed only a partial reperfusion and vascular reorganization of cystic areas after exudation resorption in eyes with IRF. Knowing that patients with IRF experienced worse visual outcomes than patients with SRF [32,33], our results suggest that patients presenting IRF secondary to exudative AMD should be treated rapidly, as the reperfusion capacity of the cystic areas appears to be overstretched and areas of non-perfusion persist despite edema resolution.

Some studies have reported an association between anti-VEGF treatment and retinal ischemic phenomenon, and hypothesized a possible arteriolar vasoconstriction inducing a decrease in blood flow after injection [34–36]. Recently, Mastropasqua et al. have observed a significant decrease in macular vascular density on OCTA one month after anti-VEGF injection in patients with exudative AMD without IRF [37]. In the present study, the secondary analysis comparing treatment naïve and non-naïve patients did not find any association between anti-VEGF treatment and MPD or MVD. Although no conclusion can be made at this stage, further studies are needed to evaluate if the vascular alterations occur in the early stages of the disease, before the first treatment injections. This hypothesis argues for fast and intensive pro-active treatment, but this assumption needs to be clinically verified. Finally, no differences were found between neovascular subtypes in terms of retinal plexus reperfusion.

The main limitation of the present study was the small number of eyes included in the different fluid groups. Despite matching the control patients for age and duration of disease, several other factors including atrophy, fibrosis or treatment regimen were also present. Moreover, the time from the last injection to OCTA imaging was not the same for all patients and could have modified the capillary alterations observed in this study. In order to limit these biases, we used a univariable and multivariable linear regression analysis to take into account potential confounding factors. However, due to the lack of data, we did not integrate ocular axial length or refractive error into this model, knowing that they can change retinal vessel density measurement [38]. In this case, MPD and MVD values might be changed by an optical magnifying effect caused by the edema itself. Further studies are needed to analyze the impact of such an optical effect on MPD and MVD measurements. Another limitation was the management of segmentation artifacts on OCTA in cases where IRF disorganizes the retinal architecture [39]. For instance, some studies excluded patients with IRF, particularly when analyzing choriocapillaris vasculature [7,39,40]. Pigmented epithelium detachment could also have disorganized retinal segmentation on OCTA in the IRF and SRF groups and could thereby have modified the retinal vascular analyses. However, all OCTA images and segmentation were systematically reviewed, and any segmentation errors were modified manually before imaging analysis.

5. Conclusions

The present study shows that retinal vascular changes occur in patients with IRF in cases of AMD. However, cystic areas can be partially re-perfused after edema resolution. These results could partially explain the worse visual outcomes in eyes presenting IRF induced by macular neovascularization. Further large-scale studies are needed to confirm our hypothesis.

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Review

Managing Neovascular Age-Related Macular Degeneration in Clinical Practice: Systematic Review, Meta-Analysis, and Meta-Regression

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Abstract: The use of anti-vascular endothelial growth factor (VEGF) agents has profoundly changed the prognosis of neovascular age-related macular degeneration (nAMD). As clinical experiences have accumulated, it has become mandatory to summarize data to give information that can be useful in everyday practice. We conducted a systematic review to identify randomized controlled trials (RCTs) and observational studies that reported 12-month changes in best-corrected visual acuity (BCVA) in patients with nAMD on anti-VEGF monotherapy. Data were analyzed in a random-effects meta-analysis with BCVA change as the primary outcome. Meta-regression was conducted to evaluate the impact of multiple covariates. Four hundred and twelve heterogeneous study populations (109,666 eyes) were included. Anti-VEGFs induced an overall improvement of +5.37 ETDRS letters at 12 months. Meta-regression showed that mean BCVA change was statistically greater for RCTs ($p = 0.0032$) in comparison with observational studies. Populations following a proactive regimen had better outcomes than those following a reactive treatment regimen. Mean BCVA change was greater in younger populations, with lower baseline BCVA and treated with a higher number of injections ($p < 0.001$). Our results confirm that anti-VEGFs may produce a significant functional improvement at 12 months in patients with nAMD.

Keywords: aflibercept; age-related macular degeneration; anti-VEGF; bevacizumab; brolucizumab; meta-analysis; meta-regression; ranibizumab

1. Introduction

Neovascular age-related macular degeneration (nAMD) is the leading cause of irreversible vision loss among the over-50s living in developed countries, with a prevalence rate between 5.8% and 15.1% of the population, which constantly increases with age [1].

In recent times, intravitreal anti-vascular endothelial growth factor (anti-VEGF) therapy has become the treatment of choice for nAMD, supported by evidence from randomized clinical trials (RCTs) as well as routine clinical practice, demonstrating efficacy in preventing visual loss and improving vision [1,2]. Currently, three anti-VEGF drugs (ranibizumab, aflibercept, and brolucizumab) are authorized for the treatment of nAMD, whilst bevacizumab, developed and approved for different types of tumors, is widely employed in an off-label fashion in many countries. The magnitude of effect of anti-VEGF drugs on visual acuity was evident from the early monthly dosing trials. Later, studies based on a pro re nata (PRN) or a treat and extend (TAE) dosing strategy led to results that in some cases emulated those obtained with monthly dosing [2]. However, the published outcomes of

real-world experiences show large variability, making it challenging to incorporate this evidence into clinical decision making. Treatment outcomes in routine practice may be different from what is obtained in RCTs. This can reflect the fact that study populations in RCTs are highly selective and may not entirely represent real-world patients. Moreover, patients in real-world clinical settings may be treated with dosing and/or regimens that differ from what recommended in the product's label, mainly due to logistic problems and economic considerations [3–6]. Consequently, it is not clear to what extent the outcomes from RCTs can be replicated in everyday clinical practice. The objective of this study was to synthesize the evidence available about the efficacy of intravitreal anti-VEGFs for the treatment of nAMD based on a systematic review and a meta-analysis of published RCTs and observational/real-life studies. Moreover, we intended to identify clinical and study factors that may have an impact on the reporting of outcomes through a meta-regression model. Specifically, the aim of this work is to give an answer to the following ten questions:

Are results between RCTs and real-life/observational studies different?

1. Are results between RCTs and real-life/observational studies different, when analyzing each anti-VEGF agent?
2. Is the outcome influenced by the treatment regimen?
3. Is the outcome influenced by the treatment regimen, when considering only real-life/observational studies?
4. If proactive regimens produce better results, is this accurate when considering each anti-VEGF agent?
5. Is the outcome influenced by the frequency of treatments?
6. If the number of treatments has an effect on the results, is this accurate when considering each anti-VEGF agent?
7. Comprehensively, which agent shows more favorable results?
8. In real life/observational studies, which agent produces better results?
9. Are real-life visual results influenced by baseline characteristics?

2. Materials and Methods

A stepwise procedure, which includes a systematic literature review (SLR), a meta-analysis, and a meta-regression, was utilized to assess the efficacy/effectiveness of intravitreal therapy in patients affected by nAMD.

2.1. Systematic Literature Review

A SLR of available studies, which include patients affected by naïve nAMD and treated with intravitreal ranibizumab, aflibercept, bevacizumab, or brolucizumab with 52-week follow-up, was conducted. The present review was completed according to the protocols reported in the Cochrane Handbook for Systematic Review of Interventions (v5.1.0). The outcomes are expressed as reported in the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) [7]. In brief, EMBASE, PubMed, and Cochrane databases were searched for papers until March 2021 independently by 3 authors (VSa, VSo, and CD). The research strategy was focused on a mix of medical subject headings and the keywords: "age-related macular degeneration", "choroidal neovascularization", "anti-VEGF", "AMD", "CNV", "aflibercept", "bevacizumab", "ranibizumab", and "brolucizumab".

The review was restricted to clinical studies available in peer-reviewed, English language publications, and those published until March 2021. Conference abstracts/papers, editorials, proposals, reviews, notes, letters to authors, news, and commentaries were not included in the review. The reference lists from selected articles were inspected for additional publications. The risk of bias was estimated both quantitatively and qualitatively with the Downs and Black checklist.

2.2. Meta-Analysis

A meta-analysis of the outcomes obtained from the SLR was performed. Inclusion criteria for the meta-analysis consisted of studies including naïve nAMD patients treated

with ranibizumab, aflibercept, bevacizumab, or brolucizumab in monotherapy and reported 1-year (±4 weeks) effectiveness outcomes. The main aim of this meta-analysis was to extract a pooled estimate for effectiveness (best-corrected visual acuity (BCVA) change from baseline to week 52 in Early Treatment Diabetic Retinopathy Study (ETDRS) letters). Visual acuities expressed in LogMAR unit or decimal scale were converted to ETDRS letters before performing statistical analysis. Randomized controlled trials, real-life prospective, and retrospective clinical studies were considered. Papers that investigated specific populations affected by retinal angiomatous proliferation, polypoidal choroidal vasculopathy, or fibrovascular pigment epithelial detachment were excluded from the analysis. Studies in which a specific type of anti-VEGF could not be extracted from the results were also not considered. Publications from the same author/organization that included duplicated data were not included.

The treatment strategy was categorized into one of three groups. Populations treated on a fixed protocol such as monthly or bimonthly were codified as fixed. Those being injected under a PRN interval were categorized as PRN and in the same manner TAE approaches constituted the TAE group.

Fixed-effects and random-effects models were utilized to obtain estimates. Heterogeneity was determined with the I^2 statistic. Egger's linear regression was used to evaluate publication bias along with visualization of funnel plots.

2.3. Meta-Regression and Moderators Selection

We performed a meta-regression analysis. Pre-selected primary moderators were chosen on the basis of existing evidence. Moderators of interest were age at baseline, baseline BCVA, study type (RCT, real-life/observational study), drug, number of injections, and treatment schedule. The output variable considered was mean BCVA change in ETDRS letters at 52 weeks (±4 weeks).

2.4. Compliance with Ethics Guidelines

The present study is based on previously published articles and does not imply any new studies of human participants. This work did not necessitate ethical approval as it did not include human participants or animal subjects.

3. Results

3.1. Study Selection

The primary search produced 7709 reports. After screening of titles and abstracts and removal of duplicates, 683 potentially relevant papers were identified, and the full texts were extracted and individually screened for eligibility. Two hundred and seventy-six studies with 412 heterogeneous populations fulfilled inclusion criteria and were included in the analysis. [8–283]. The flowchart of selection steps is illustrated in Figure 1.
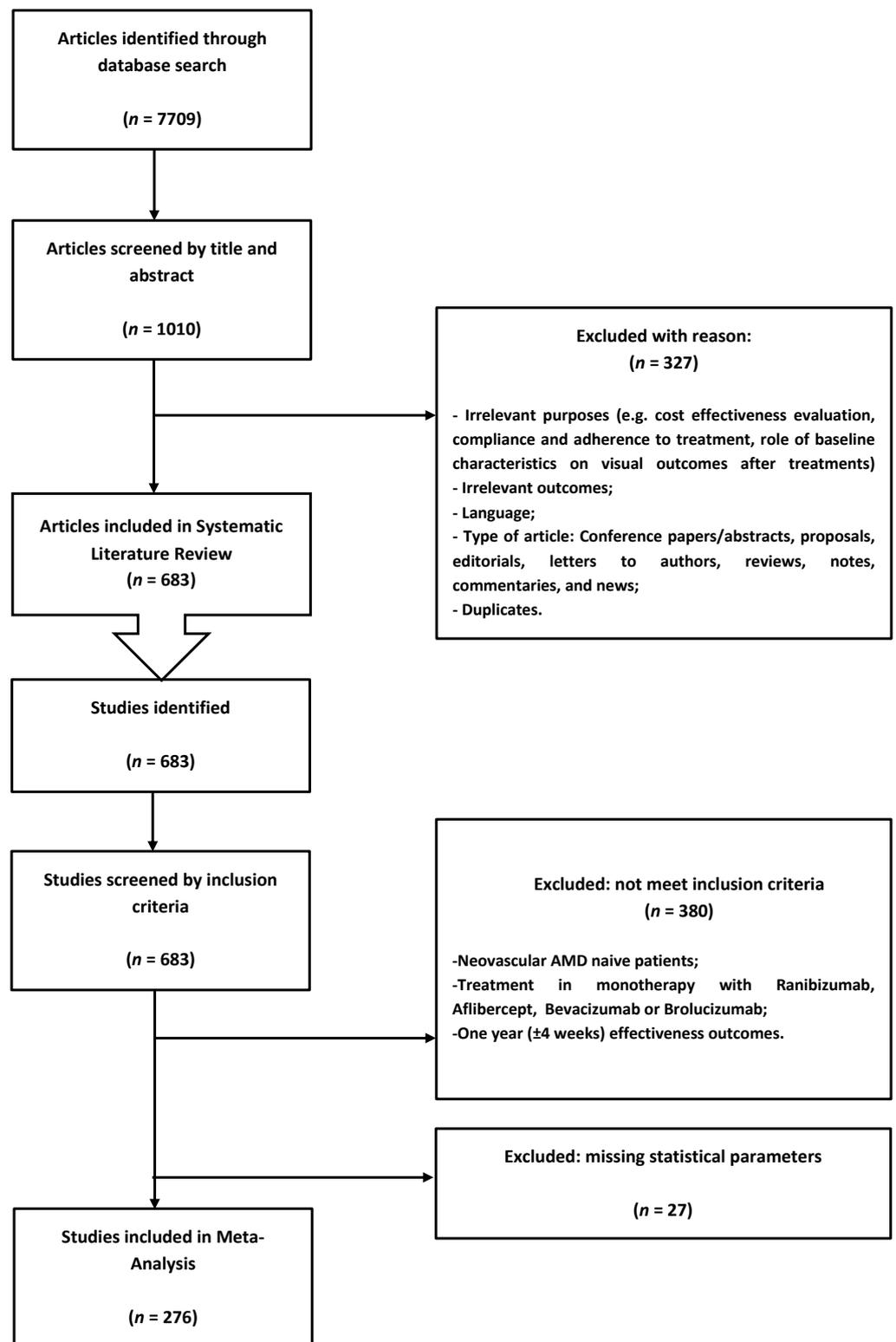
Figure 1. Flowchart of selection of studies and reason for exclusion.

3.2. Study Characteristics

Attributes of the 276 analyzed studies are detailed in Table 1. Some studies consist of different heterogeneous study groups, which were considered as individual study populations in the present analysis. Most studies were real-life/observational, which was defined as single-arm interventional designs and retrospective chart reviews. Of

the 276 studies included, 81 were randomized clinical trials, 95 were prospective cohort studies, and 100 were retrospective cohort studies. A total of 73 trials were conducted in Asia and Australia; 161 were conducted in Europe; and 42 in the United States. Less than half of the included studies (36%) were comparative. Four hundred and twelve heterogeneous study populations were found. The numerosity of the study populations ranged from 9 to 8598 eyes, with a total of 109,666 eyes enrolled. Specifically, 24,517 eyes were treated with aflibercept, 65,591 eyes with ranibizumab, 1038 eyes with brolucizumab and 18,520 eyes with bevacizumab. The relatively low number of subjects treated with brolucizumab is a consequence of the limited number of eligible studies available in literature so far. Therefore, this imbalance in sample size impacts the meta-regression analysis, limiting the sense of a comparison between brolucizumab and other anti-VEGFs. Nevertheless, brolucizumab data were included in the meta-analysis and in the meta-regression processes that did involve a comparison among drugs. Overall, the mean age varied from 63 to 90 years. Mean baseline BCVA ranged from 31 to 77 ETDRS letters. Two hundred and seventy-six studies were separately scored for their methodological quality using the Downs and Black checklist. Methodological quality ranged from 13 to 20, with a mean overall score of 17.3. For the most part, reduced quality across studies can be ascribed to poor reporting of blinding, loss to follow-up and characteristics of subjects lost to follow-up, randomization process, adjustment for confounding variables, and estimates of random variability.

Table 1. Study Characteristics.

Study Type	Randomized Controlled Studies	Observational/ Real-Life Studies	Prospective Studies	Retrospective Studies
Eyes (populations)	27,785 (81)	81,881 (331)	39,008 (202)	70,288 (210)
Drug	Aflibercept	Ranibizumab	Brolucizumab	**Bevacizumab**
Eyes (populations)	24,517 (102)	65,591 (230)	1038 (3)	18,520 (77)
Regimen	Fixed	Pro-re-nata	Treat and Extend	
Eyes (populations)	13,318 (74)	81,651 (270)	7285 (57)	

3.3. Meta-Analysis

We found a high heterogeneity among studies considered in the analysis ($I^2 = 94.478\%$; $p < 0.0001$), and thereafter we chose a random-effects model. None of studies showed a significant effect on overall effect size, as showed by a leave-one-out sensitivity analysis. The meta-analysis provided an overall gain in BCVA of +5.37 ETDRS letters (95% CI: 5.01–5.72) at 12 months.

3.4. Meta-Regression

Several moderators showed robust effect modification. We applied a meta-regression process to provide answers to the following clinically significant questions.

1. Are results between RCTs and real-life/observational studies different?

A statistically significant ($p = 0.0032$) regression of difference in means on study type showed a coefficient of +1.32 ETDRS letters favoring RCTs over real-life/observational studies (CI 95%: +0.45; +2.20).

2. Are results between RCTs and real-life/observational studies different, when analyzing each anti-VEGF agent?

A statistically significant regression of difference in means on study type for aflibercept and ranibizumab ($p = 0.042$ and $p = 0.0009$, respectively) showed a coefficient of +1.80 (aflibercept) and +1.84 (ranibizumab) ETDRS letters advantaging RCTs over real-life/observational studies (CI 95%: +0.06; +3.53 for aflibercept and CI 95%: +0.75; +2.92 for ranibizumab). The same analysis performed for bevacizumab resulted in a not statistically significant difference ($p = 0.95$).

3. Is the outcome influenced by the treatment regimen?

Regression of difference in means on regimen was statistically significant, higher benefit was seen for fixed and TAE regimen over PRN regimen. Fixed regimen showed a coefficient of +2.23 ETDRS letters over PRN. (CI 95%: +1.32; +3.14; $p < 0.0001$). Treat and extend regimen showed a coefficient of +2.40 ETDRS letters over PRN. (CI 95%: +1.41; +3.39; $p < 0.0001$). No statistically significant difference was found between fixed and TAE regimen ($p = 0.78$).

4. Is the outcome influenced by the treatment regimen, when considering only real-life/observational studies?

Regression of difference in means on regimen was statistically significant, in favor of fixed and TAE regimen over PRN regimen, when including only real-life/observational studies. Fixed regimen showed a coefficient of +1.68 ETDRS letters over PRN. (CI 95%: +0.70; +2.67; $p = 0.0008$). Treat and extend regimen showed a coefficient of +2.02 ETDRS letters over PRN. (CI 95%: +0.98; +3.06; $p = 0.0001$). No statistically significant difference was found between fixed and TAE regimen ($p = 0.61$).

5. If proactive regimens produce better results, is this accurate when considering each anti-VEGF agent?

In patients treated with aflibercept, a statistically significant difference indicating more favorable results for fixed regimen over PRN regimen (coefficient +2.01 ETDRS letters; CI 95%: +0.62; +3.41; $p = 0.005$), and TAE regimen over PRN regimen (coefficient +2.58 ETDRS letters; CI 95%: +1.01; +4.15; $p = 0.001$) was described. Similarly, ranibizumab-treated populations had better outcomes in studies utilizing fixed regimen over PRN regimen (coefficient +2.47 ETDRS letters; CI 95%: +1.06; +3.88; $p = 0.0006$), and TAE regimen over PRN regimen (coefficient +2.33 ETDRS letters; CI 95%: +0.98; +3.69; $p = 0.008$). In patients treated with bevacizumab, regression of difference in means on regimen was not significant ($p > 0.5$).

6. Is the outcome influenced by the frequency of treatments?

A highly statistically significant effect resulted from regression of difference in means on mean number of treatments (coefficient +0.51 ETDRS letters; CI 95%: +0.34; +0.68; $p < 0.0001$).

7. If the number of treatments has an effect on the results, is this accurate when considering each anti-VEGF agent?

When looking at ranibizumab-treated populations, outcomes were significantly influenced by mean number of treatments (coefficient +0.69 ETDRS letters; CI 95%: +0.47; +0.91; $p < 0.0001$). The same analysis was not statistically significant for aflibercept (coefficient +0.32 ETDRS letters; CI 95%: -0.12; +0.76; $p = 0.16$) and bevacizumab (coefficient +0.16 ETDRS letters; CI 95%: -0.23; +0.55; $p = 0.42$).

8. Comprehensively, which agent shows more favorable results?

Regression of difference in means on drug showed that the studies employing aflibercept reported significantly superior results over ranibizumab (coefficient +1.78 ETDRS letters; CI 95%: +0.4; +4.15; $p < 0.0001$). A non-statistically significant trend for better results for aflibercept-treated populations over bevacizumab-treated populations was seen (coefficient +0.97 ETDRS letters; CI 95%: +0.09; +2.04; $p = 0.07$). The comparisons between the results published for brolucizumab-treated populations and the populations treated with other anti-VEGF agents were not statistically significant ($p > 0.3$). However, a non-significant trend towards better outcomes in the brolucizumab studies was detected (coefficient +0.03, +1.00, +1.78 ETDRS letters against aflibercept, bevacizumab, and ranibizumab, respectively).

When the same analyses were performed posing the treatment regimen as a precondition, no statistically significant differences among anti-VEGF drugs were found. In the

populations treated with a fixed regimen, a trend to better outcomes (not statistically significant) was found for aflibercept (coefficient +0.87, +0.25 ETDRS letters over bevacizumab, and ranibizumab, respectively). When considering the populations treated with a PRN regimen, a trend to better outcomes (not statistically significant) was found for aflibercept (coefficient +0.04, +0.85 ETDRS letters over bevacizumab and ranibizumab, respectively). In the populations treated with a TAE regimen, a trend to better outcomes (not statistically significant) was found for aflibercept (coefficient +0.50, +0.89, and +1.01 ETDRS letters over bevacizumab, brolucizumab, and ranibizumab, respectively).

9. In real life/observational studies, which agent produces better results?

Aflibercept reported significantly better results over ranibizumab (coefficient +1.94 ETDRS letters; CI 95%: +1.05; +2.82; $p < 0.0001$), as shown by regression of difference in means on drug in real life/observational studies.

10. Are real-life visual results influenced by baseline characteristics?

Regression of difference in means was significant on age (coefficient −0.17 ETDRS letters; CI 95%: −0.26; −0.07; $p < 0.001$) and baseline BCVA (coefficient −0.11 ETDRS letters; CI 95%: −0.16; −0.07; $p < 0.0001$).

3.5. Publication Bias and Sensitivity Analysis

Funnel plot asymmetry was seen in the present meta-analysis. Egger's linear regression (intercept = 3.11, $p < 0.001$) and by Begg's rank correlation test (Kendall's $\tau = 0.245$, $p < 0.001$) also suggest the existence of publication bias. After imputing missing studies in the funnel plot, adjustment of effect size for possible publication bias using the trim-and-fill correction results in decreased, albeit still highly significant estimate of pooled mean difference (adjusted = +4.35 ETDRS letters; CI 95%: +4.02; +4.68; $p < 0.0001$). A 'one-study-removed' technique and a 'cumulative meta-analysis' technique were used to evaluate the potential influence of a small-study effect. Both techniques express negative results.

4. Discussion

Neovascular AMD is the main cause of vision loss in adult patients in developed countries [1,2]. The present study was conducted to synopsize the clinical evidence from RCTs and real-life/observational studies on functional results of intravitreal anti-VEGF treatment in the management of nAMD, obtaining a pooled estimate for BCVA change from baseline to week 52. This meta-analysis consists of 109,666 eyes and it is the largest and most comprehensive research to date that aim at synthetizing the clinical efficacy of intravitreal ranibizumab, aflibercept, bevacizumab, and brolucizumab in the treatment of nAMD at 12 months. The results obtained from this meta-analysis support the utilization of anti-VEGF agents as an effective therapeutic option for the treatment of nAMD, showing that significant BCVA gain is attainable. The present meta-analysis reports an overall increase in BCVA of approximately +5.3 ETDRS letters after one year of intravitreal anti-VEGF therapy. A high variability was found between studies, as demonstrated by the wide variance in pooled effect size (p heterogeneity, <0.0001). The interpretation of average effect size is increasingly complex as the presence of intertwined modifiers, independent predictors, and confounding variables multiplies. It remains an important goal to identify under what conditions anti-VEGF therapies may unlock their full potential. To elaborate on this matter, a meta-regression was carried out. RCTs showed an overall gain in visual acuity of +6.42 letters (95% CI: 5.50–7.33). Real-life/observational studies were calculated to have an increase of +5.01 letters (95% CI: 4.65–5.38). A statistically significant difference in BCVA was noticed between RCTs and real-life studies ($p < 0.01$) and, as expected, we found a higher variability in real-life results. This is in line with previous reports indicating that outcomes achieved with anti-VEGFs in real-life studies for the treatment of nAMD are not as good as those obtained in RCTs. However, it remains a matter of discussion whether a difference of +1.3 ETDRS letters is clinically meaningful. In the present meta-analysis and meta-regression, we choose a random-effects approach as the observed heterogeneity in the

estimates may be attributed to between-study heterogeneity in true effects and within-study sampling error.

Growing evidence suggests that the regimen employed, and the frequency of anti-VEGF injections, have an impact on the visual outcome when treating a patient affected by nAMD [2]. Data from our analysis confirm this hypothesis. In detail, we found a statistically significant correlation between the number of anti-VEGF administrations and BCVA change ($p < 0.0001$). At month 12, each additional treatment induces a +0.51-letter gain. Yet, these results are not uniform among all anti-VEGF agents. The drug most dependent on the number of injections per year seems to be ranibizumab (coefficient +0.69 ETDRS letters per injection). We believe that this finding can be ascribed to both pharmacological properties and to the characteristics of the studies analyzed. In detail, the variability in the number of injections is much wider in ranibizumab studies than in those using aflibercept and bevacizumab. This is mainly because the larger part of ranibizumab studies apply a PRN regimen that involves a wider variability in the number of injections. Moreover, we investigated the role of the treatment regimen employed in obtaining the most favorable results. Results from the present meta-regression indicate that better outcomes are seen when employing a proactive treatment regimen (fixed or TAE) over a reactive treatment regimen (PRN). These results are also confirmed when analyzing real-life studies alone. Actually, many factors may interfere with the therapeutic efficacy of PRN treatment regimen in a real-life scenario, including administrative and logistic considerations. For example, improper appointment scheduling for treatment and monitoring visits is indeed a real-world factor that may result in unsatisfactory outcomes. Moreover, strict adherence to rigorous retreatment criteria is often difficult to obtain in a real-life scenario, due to inhomogeneity in imaging technologies and physicians' knowledge and skills. This represents a limitation in maximizing visual gains, leading to suboptimal outcomes for the patients.

When analyzing baseline characteristics that may influence visual outcomes, our meta-regression showed that the 12-month BCVA change negatively correlated with baseline BCVA, which is consistent with prior experiences, revealing an inverse correlation between baseline BCVA and long-term BCVA change. Our analysis also revealed a negative correlation in BCVA change with increasing age. This negative correlation may be a consequence of worsened functional results at later age of presentation, when both the advanced stage of the disease and a decreased response to therapy may lead to inferior clinical outcomes. Key results from our work are reported in Table 2.

Table 2. Efficacy of intravitreal anti-VEGFs for the treatment of neovascular AMD at 12 months: key results.

- The use of anti-VEGF agents leads to a significant visual improvement in neovascular AMD patients.

- Randomized clinical trials typically produce higher visual gains over real-life studies.

- Proactive treatment regimen (fixed or treat-and-extend) usually leads to better outcomes over a reactive treatment regimen (pro-re-nata)

- Frequency of anti-VEGF injections is a relevant factor and influences the visual outcome.

- High baseline visual acuity and increased age reduce the functional response to intravitreal anti-VEGF therapy.

Legend: AMD: Age-related macular degeneration; VEGF: Vascular endothelial growth factor.

The main strength of the present work is that it provides an exhaustive and paradigmatic overview of the various therapeutic approaches used in real-life clinical practice and

in RCTs for nAMD patients. We employed a predefined search strategy, three independent reviewers performed data extraction, and subgroup and sensitivity analysis were also conducted.

However, some limitations of the current study should not be ignored. First, the enrolled studies were limited to English language. This may have led to studies not being included, resulting in a not quite comprehensive data set. Second, the quality of included studies is variable. Real-life/observational studies exhibit a higher level of bias than RCT, including publication bias. Third, the heterogeneity among studies was notable, possibly due to confounding variables such as sample sizes, ethnic distribution of the study population, study designs, CNV types, and treatment modalities. Actually, uncontrolled confounding predisposes to bias when comparing observational studies and RCT. Fourth, the data used to establish these results might suffer from sample selection bias.

Finally, our results, from a methodological point of view, are also susceptible to ecological bias and study-level confounding, which means that the observed across-study relationships may not properly mirror the individual-level relationships within trials. In this sense, a network meta-analysis is probably less prone to misinterpretation. For all these motives, care must be exercised in conjecturing any form of quantitative relationship, which may alter over time and with a larger number of reports/studies included in the analysis.

5. Conclusions

In conclusion, the evidence for intravitreal therapy with anti-VEGF agents has been confirmed in this meta-analysis to be highly beneficial in the therapy of nAMD both in clinical trials and in real-life experiences. Frequency of injections and proactive treatment regimens are both factors related to best outcomes with currently available anti-VEGF agents.

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Article

Exploring Consensus on Preventive Measures and Identification of Patients at Risk of Age-Related Macular Degeneration Using the Delphi Process

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Abstract: Background: Early identification of AMD can lead to prompt and more effective treatment, better outcomes, and better final visual acuity; several risk scores have been devised to determine the individual level of risk for developing AMD. Herein, the Delphi method was used to provide recommendations for daily practice regarding preventive measures and follow-up required for subjects at low, moderate, and high risk of AMD evaluated with the Simplified Test AMD Risk-assessment Scale (STARS®) questionnaire. Methods: A steering committee of three experts drafted and refined 25 statements on the approach to be recommended in different clinical situations [general recommendations (*n* = 2), use of evaluation tools (*n* = 4), general lifestyle advice (*n* = 3), and AREDS-based nutritional supplementation (*n* = 5)] with the help of a group of international experts, all co-authors of this paper. Thirty retinal specialists from Europe and the US were chosen based on relevant publications, clinical expertise, and experience in AMD, who then provided their level of agreement with the statements. Statements for which consensus was not reached were modified and voted upon again. Results: In the first round of voting, consensus was reached for 24 statements. After modification, consensus was then reached for the remaining statement. Conclusion: An interprofessional guideline to support preventive measures in patients at risk of AMD based on STARS® scoring has been developed to aid clinicians in daily practice, which will help to optimize preventive care of patients at risk of AMD.

Keywords: age-related macular degeneration; prevention; identification; risk; Delphi; STARS®; food supplement

1. Introduction

Age-related macular degeneration (AMD) is a primary reason for blindness in Western countries [1–3]. The number of individuals with AMD is believed to rise globally by around 40% from 2020 to 2040, highlighting the need for effective preventive measures and therapies [2]. The two late phenotypes of AMD, neovascular and atrophic AMD, are usually preceded by drusen and pigmentary abnormalities.

Considerable progress has been made in the treatment of AMD, and in this regard, intravitreal injections of antiangiogenic agents have greatly changed the management of neovascular AMD [3]. While these agents can provide stabilization or even rapid improvement of visual acuity in the majority of patients at the start of treatment, the long-term results regarding visual acuity and quality of life remain uncertain [4–6]. Moreover, there is still no therapy for geographic atrophy, which represents a considerable proportion of cases of late AMD. This confirms that despite therapeutical progress, late-stage AMD remains a major cause of visual loss. Early identification of patients at risk of AMD is thus of significant clinical relevance, as it can lead to more effective treatment, better outcomes, and better visual acuity [7].

Epidemiological studies have unveiled the existence of several risk factors for AMD, which include smoking, diet, family history of AMD, and cardiovascular disease, as well as both clinical and ocular risk factors. Several of these risk factors have shown strong associations with AMD (especially smoking, ethnicity, and family history of AMD), although for others such as gender and iris color the results have been less consistent [3,8,9]. In addition, more than 50 genetic polymorphisms have been identified that contribute to the disease [9,10].

Conversely, many endogenous and exogenous micronutrients have been implicated in the protection of the retina against AMD through different mechanisms including antioxidant, anti-inflammatory, and blue light absorption [9,11–18]. Animal models and cell culture studies have suggested that oxidative stress to the retina is an important factor contributing to AMD, and large interventional studies have shown that antioxidant nutrients given as supplements appear to exert a protective effect against progression to advanced forms of AMD [18–22]. Altogether, it is now generally accepted that adequate intake of micronutrients is crucial for a healthy retina and vitamin supplementation needs to be considered in the management of patients with late-stage or progressing AMD [23–27].

Early identification of AMD is of significant clinical relevance, as it can lead to prompt and more effective treatment, better outcomes, and better final visual acuity [7]. In this regard, risk scores have been devised to establish the risk for AMD, thereby allowing for individualized management [28–35]. Even if some of these models have good discrimination for AMD, most include assessment of genetic polymorphisms, which are not used on a routine basis in clinical practice. While clinical, lifestyle, and ocular risk factors have been related to AMD, few risk scores have included dietary considerations [14,29,31–33].

Given the need for early identification of individuals at risk and improving disease outcomes, an easy to compile self-administered 13-item questionnaire to evaluate individual risk for AMD in daily practice has been developed (Simplified Test AMD Risk-assessment Scale—STARS®) [36]. The scoring system was derived from an initial sample of 12,639 Italian subjects and subsequently validated on 6897 French subjects. The questionnaire showed good discrimination, allowing stratification of subjects according to the risk of AMD (low, moderate, high). The translational relevance of the STARS® score in predicting macular function in early and intermediate AMD has been recently pointed out: STARS® is able to predict central retinal function with a high degree of accuracy, as assessed by full-field electroretinogram, which suggests that both parameters can be combined to assess the clinical risk for loss of visual function even in the early disease stages [37].

Following the development and validation of the score, a process was activated to develop specific recommendations regarding the preventive measures and ophthalmological follow-up to adopt, tailored according to the individual level of risk for AMD that can complement existing guidelines. This is also important considering that validated

recommendations can help ophthalmologists to individualize consultations with patients and save time.

Herein, the Delphi method [38] has been used to provide recommendations for the preventive measures to adopt in individuals at low, moderate, and high risk of AMD based on STARS®.

2. Materials and Methods

2.1. Delphi Process

The consensus process was carried out with a three-step Delphi method [39,40]. The Delphi method has been used in healthcare settings as a good means of obtaining consensus [38,41–45]. The method is a consecutive process that uses repeated rounds of voting and is effective in obtaining expert consensus for which there is limited evidence [42].

The overall Delphi process adopted is summarized in Figure 1. In March 2019, in the first step, a steering committee composed of three experts drafted a series of statements through a web-based meeting. The scientific committee, a group of international experts and co-authors of this paper, then further refined the statements until final approval of a list of 25 statements, regarding the best approach to recommend in patients with diverse levels of risk for developing AMD based on STARS® score (low, score 0–9; moderate, score 10–19; high, score $\geq$ 20), age (50–70 years, >70 years) and AREDS categories [46]. STARS® is based on patient data (age, sex, and ethnicity), family history of AMD, medical history, and eye characteristics [36].
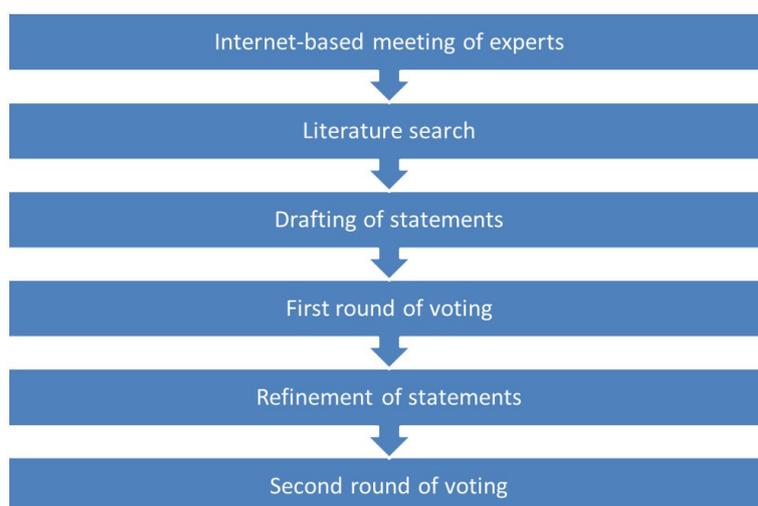
Figure 1. Overview of Delphi process used.

For the second step of the Delphi process, retinal specialists from across Europe and the US were chosen based on relevant publications, clinical expertise in the retina, and experience in AMD. Thirty retinal experts were contacted via email and asked to take part in the consensus project, and 24 participants (75%) agreed to participate (France, 2; Spain, 4; UK, 3; Germany, 1; Portugal, 2; Russia, 3; Ukraine, 1; Italy, 2; Poland 2; Turkey, 2; Belgium, 1; USA, 1). The first round of voting took place in July 2019 via a dedicated, password-protected online platform. The steering committee analyzed the results of voting and modified the statements for which agreement was not obtained, based on feedback from the participants. The second round of voting took place in January 2020.

2.2. Analysis of Voting and Determination of Agreement

Participants were requested to rate their agreement with the statements proposed on a scale from 1 to 9, where 1 is complete disagreement and 9 is complete agreement. Ratings of 1–3 were classified as disagreement, while ratings of 7–9 were classified as agreement. Ratings of 4–6 were classified as neutral.

Disagreement was assessed as follows. First, the value of inter-percentile range (IPR) was calculated, i.e., the range of responses between the 70th and the 30th percentiles. Next, the value of the inter-percentile range adjusted for symmetry (IPRAS) was calculated, which assesses dispersion for asymmetric distributions; finally, the values of IPR and IPRAS were calculated. Disagreement was considered when IPR > IPRAS [46].

Disagreement inevitably produced an uncertain decision. If, on the other hand, there was no disagreement, the median determined if the agreement was positive, negative, or uncertain. If the median was from 7–9, then a positive is obtained, and the statement was considered relevant for the management of AMD. If the median was from 1–3, then the decision was negative, and the statement was not considered relevant for the management of AMD. A median that was within the 4–6 range produced an uncertain decision.

2.3. Ethics Approval

No formal ethics approval was required. All participants agreed to participate, and the data are presented at the group level only. As such, it is not possible to identify an individual participant.

3. Results

The Steering Committee, with the help of the scientific committee, all co-authors of this paper, drafted a total of 25 statements in different areas, comprising general recommendations (n = 2), use of evaluation tools (n = 4), general lifestyle advice (n = 3), and AREDS-based nutritional supplementation (n = 5) (Table 1). In addition, five statements were proposed for subjects at moderate risk of AMD, and six statements for those at high risk (Table 2). Each Delphi panel expert then voted on his/her level of agreement with each statement using an online platform. Participants had no access to the decisions of the other experts. Consensus, considered when IPR < IPRAS, was reached for 24 of the 25 statements in the first round of voting for statements involving general issues, evaluation tools, lifestyle advice, and AREDS-based nutritional supplementation.

Table 1. Consensus statements on general issues, evaluation tools, general lifestyle advice, and AREDS-based supplementation.

	General Recommendations	IPR	IPRAS	Median	Consensus
1	Intravitreal injections are the first-choice treatment for patients with wet AMD to stop progression of the disease	0	15.0	9.0	Positive agreement
2	Patients at high risk of AMD should receive nutritional supplements to help reduce the risk of progression at an early phase after diagnosis of AMD	2.2	10.9	9.0	Positive agreement
	Evaluation tools				
3	Early detection with simple tools is desirable to detect patients at risk of AMD and treat promptly if needed	0	15.0	9.0	Positive agreement
4	The STARS® questionnaire is a valid tool to assess risk of AMD in the general population	1	9.4	8.0	Positive agreement
5	Stratification according to risk of AMD is useful in order to plan lifestyle interventions, give dietary advice and plan follow-up using STARS® and the AREDS category score	1	13.1	8.0	Positive agreement
6	Ophthalmologists should use the STARS® and AREDS classifications in daily practice to evaluate the risk of AMD and to define the best prevention strategy and follow-up for patients	2	11.3	8.0	Positive agreement

Table 1. *Cont.*

	General Recommendations	IPR	IPRAS	Median	Consensus
	General lifestyle advice				
7	All subjects at risk of AMD should be advised to stop smoking, adopt a Mediterranean diet, and carry out regular physical activity	0	15.0	9.0	Positive agreement
8	Increased intake of vegetables, fruit and fish should be actively encouraged in the aging population as <4% of individuals $\geq$ 55 years of age achieve adequate intake of these food groups	1	13.1	9.0	Positive agreement
9	If patients are unable or unwilling to follow a Mediterranean diet, nutritional supplements should be recommended in subjects at high risk of AMD	2	11.3	9.0	Positive agreement
	AREDS-based supplementation				
10	An AREDS-based formulation significantly reduces the risk of developing advanced AMD in the long-term	2	11.3	8.0	Positive agreement
11	An AREDS-based formulation decreases the overall risk of moderate vision loss in the long term	2	11.3	8.0	Positive agreement
12A	An AREDS-based formulation has no significant benefit on the progression of dry AMD or development of geographic atrophy in the long term	4.5	0.9	5.0	No agreement
12B	An AREDS-based formulation may have benefit on the progression of dry AMD or development of geographic atrophy in the long term	3	5.6	7.0	Positive agreement
13	The best-validated supplementation therapy for patients suffering from AMD with geographic atrophy without central involvement of the fovea is an AREDS-based formulation	3.5	4.7	7.0	Positive agreement
14	Initiating supplementation with an AREDS-based formulation in patients at high risk of AMD is more cost effective than no use of supplements and should be advocated	2	11.3	8.0	Positive agreement

Table 2. Consensus statements for subjects with moderate and high risk of developing AMD.

	Moderate Risk Subjects (STARS® 10–19)	IPR	IPRAS	Median	Consensus
15	Moderate risk subjects according to STARS® (STARS® score 10–19) and with AREDS category 2 and 55–70 years of age should be asked to carry out self-monitoring (e.g., with Amsler grid)	2.5	10.3	7.5	Positive agreement
16	Moderate risk subjects according to STARS® (STARS® score 10–19) and with AREDS category 2 and 55–70 years should have follow-up every 2 to 3 years	3	3.8	6.5	Uncertain relevance
17	Moderate risk subjects according to STARS® (STARS® score 10–19) and with AREDS category 2 and age > 70 years should be asked to carry out self-monitoring (e.g., with Amsler grid).	2	11.3	8.0	Positive agreement
18	Moderate risk subjects according to STARS® (STARS® score 10–19) and with AREDS category 2 and age > 70 years should be recommended specific nutritional supplements for prevention of AMD	2.5	10.3	8.0	Positive agreement

Table 2. *Cont.*

	Moderate Risk Subjects (STARS® 10–19)	IPR	IPRAS	Median	Consensus
19	Moderate risk subjects according to STARS® (STARS® score 10–19) with AREDS category 2 and age > 70 years should have annual follow-up	2	11.3	9.0	Positive agreement
	High risk subjects (STARS® ≥ 20)				
20	High risk subjects according to STARS® (STARS® ≥ 20), with AREDS category 1 and 55–70 years of age should be asked to carry out self-monitoring (e.g., with Amsler grid)	2.5	10.3	8.0	Positive agreement
21	High risk subjects according to STARS® (STARS® ≥ 20), with AREDS category 1 and age > 70 years should be asked to carry out self-monitoring	3	9.4	8.0	Positive agreement
22	High risk subjects according to STARS® (STARS® ≥ 20), with AREDS category 1 and age > 70 years should be recommended specific nutritional supplements for prevention of AMD	3	9.4	8.0	Positive agreement
23	High risk subjects according to STARS® (STARS® ≥ 20), with AREDS category 2, aged 55 years or more, should be asked to carry out self-monitoring (e.g., with Amsler grid)	1.5	12.2	8.0	Positive agreement
24	High risk subjects according to STARS® (STARS® ≥ 20), with AREDS category 2, aged 55 years or more, should be recommended specific nutritional supplements for prevention of AMD	2	11.3	8.0	Positive agreement
25	High risk subjects according to STARS® (STARS® ≥ 20) with AREDS category 2, independently of age, should have follow up every 6 months	4.5	4.7	7.0	Positive agreement

In the first round of voting, consensus (IPR < IPRAS) was reached for all statements, except for statement 12, which had IPR > IPRAS and reached a median of 5 in voting (Table 1, statement 12A). This statement had been initially worded as "An AREDS-based formulation has no significant benefit on the progression of dry AMD or development of geographic atrophy in the long term". While some experts commented that more evidence is needed, others said that there is sufficient data to recommend the use of an AREDS-based formulation in dry AMD. Based on this, the statement was reworded as "An AREDS-based formulation may have benefit on the progression of dry AMD or development of geographic atrophy in the long term". After a second round of voting, in which 19 (79%) Delphi panel experts participated, consensus was reached for this reworded statement (12B in Table 1). Eventually, all statements received positive agreement (median ≥ 7), except for statement 16 which was judged of uncertain relevance (median of votes 6.5).

4. Discussion

The present Delphi consensus had the main objective of formulating a series of validated recommendations on ophthalmological follow-up and preventive measures to adopt for subjects with a low, moderate, and high risk of developing AMD evaluated with the STARS® questionnaire, which can be useful for ophthalmologists in daily practice. The Delphi process was chosen with the aim of obtaining a consensual response from international panel of experts as there is limited evidence available [43]. While absolute agreement is rarely obtained, the Delphi methodology helps to identify a group consensus [43]. Consensus was reached for 24 of the 25 statements proposed in the first round of voting. The results of voting for each of the three main areas are discussed below.

4.1. General Recommendations

Statement #1 addressed the use of intravitreal injections as the first choice in the treatment of patients with wet AMD to stop disease progression as supported by expert recommendations following diagnosis of choroidal neovascularization (CNV) [47], and fully recommended by the European Society of Retina Specialists (EURETINA) [48]. Some of the experts commented that the utility of intravitreal injections is not only to halt the progression of the disease, but also to increase visual acuity and quality of life as much as possible.

Statement #2 addressed the use of nutritional supplements at an early phase of AMD to help reduce the risk of progression in patients at a high risk of AMD. Indeed, there is now general agreement that the positive effects of the AREDS1 and AREDS2 formulations are a result of their antioxidant properties [49], and, based on the AREDS studies, a number of dietary supplements are currently available. Such formulations, in addition to the diet and healthy lifestyle recommendations below, are now considered to be the standard of care to reduce the risk of reaching advanced AMD among those with an elevated risk of progressing to severe visual loss [50].

4.2. Use of Evaluation Tools

Early identification of high-risk subjects using a simple tool is a highly desirable goal such that appropriate subjects can be offered ophthalmological follow-up (especially for early diagnosis and treatment of neovascular AMD, as needed) (Statement #3). Moreover, the STARS® questionnaire was considered to be a valid tool to assess the risk of AMD in the general population (statement #4). STARS® is a simple and easy-to-use 13-item questionnaire based on the presence of risk factors auto-administered by patients [36]. The STARS® questionnaire has been validated in two large European observational cohorts and shows good discrimination of risk of AMD into low, moderate, and high-risk categories [36]. As such, STARS® would appear to meet the ideal criteria for a simple screening tool for daily practice.

It was further held that stratification according to the risk of AMD is useful in order to plan lifestyle interventions, give dietary advice, and plan ophthalmological follow-up using STARS® and the AREDS category score (statement #5). Both scoring systems provide simple risk categories and are complementary since the AREDS classification relies only on retinal alterations identified at clinical examination, while the STARS® score relies only on an auto-administered evaluation of risk factors [46]. Several recent studies have indicated that consumption of a Mediterranean diet appears to offer some benefits against developing AMD, possibly through increased intake of micronutrients and antioxidants [14,15,51,52]. In addition, physical activity and weight control are receiving increased attention for their possible role in the prevention of AMD [27]. The last statement on evaluation tools considered that ophthalmologists should use the STARS® and AREDS category score in daily practice to evaluate the risk of AMD and to define the best prevention strategy and follow-up (statement #6). This recommendation is based on the above considerations.

4.3. General Lifestyle Advice

Consensus was reached for all the statements on general lifestyle advice, advocating that all subjects who are at risk for AMD should stop smoking, adopt a Mediterranean diet, and carry out regular physical activity (statement #7) as documented in the literature [9,14, 15,27,52,53]. In addition, increased intake of vegetables, fruit and fish should be actively encouraged in the aging population, considering that <4% of individuals $\geq$ 55 years of age had adequate intake of these food groups in an epidemiological study performed in the Netherlands (statement #8) [17,54]. The benefits of dietary omega-3 fatty acid and fish intake in reducing the risk of AMD, for example, appear to be well consolidated as demonstrated by several studies and summarized in a meta-analysis by Chong et al. [55]. This is in contrast to the unexpected, negative results of the AREDS2 study in terms of the benefits of omega-3 fatty acids and AMD [19]. Possible explanations for this, as noted by

other authors, include the complex study design. AREDS2 subjects were already taking AREDS1 supplements, which together with the lack of a placebo group for comparison, may not have allowed for the effects of omega-3 fatty acids to be sufficiently evident [18].

Furthermore, smoking is considered to be an important modifiable risk factor for the development and progression of AMD [56,57]. All patients at risk should therefore be actively encouraged to stop smoking. The last statement on general lifestyle advice considered that if patients are unable or unwilling to follow a diet rich in green leafy vegetables, fruits, and fish, such as the Mediterranean diet, nutritional supplements should be recommended in subjects at high risk of AMD (statement #9).

4.4. AREDS-Based Supplementation

There were five statements on AREDS-based supplementation. The experts considered that a nutritional formulation reduces the risk of developing advanced AMD in the long-term (statement #10) and that it decreases the overall risk of moderate vision loss in the long term (statement #11). Statement #12A was initially worded as "An AREDS-based formulation has no significant benefit on the progression of dry AMD or development of geographic atrophy in the long term", for which agreement was not reached. The statement was then modified to "An AREDS-based formulation may have benefit on the progression of dry AMD or development of geographic atrophy in the long term", and after a second round of voting, agreement was reached.

The Age-Related Eye Disease Study (AREDS)-2 study showed that supplementation with antioxidant nutrients decreases the risk of progression to neovascular AMD [19,58]. The potential benefit of AREDS-based supplementation is also based on several clinical trials with individual supplements [18,22,59,60], and reviewed in [50]. In fact, for many years before AREDS, micronutrition was held to be an integral part of routine management of AMD [17,23]. Moreover, nutritional strategies for secondary prevention of AMD are present in virtually all clinical guidelines [61].

The experts also considered that the best-validated supplementation therapy for patients suffering from AMD with geographic atrophy without central involvement of the fovea is an AREDS-based formulation (statement #13). The last statement on AREDS-based supplementation (#14) held that initiating a supplementation with an AREDS-based formulation in patients at high risk of AMD is more cost effective than no use of supplements and should be advocated. This is largely based on the real-world study by Lee et al. in the UK, wherein initiating AREDS-based supplements in AREDS category 4 patients was found to be both cost-effective and superior to no use of supplements [26].

4.5. Moderate and High-Risk Subjects

The remaining statements (#15–25) involved recommendations for monitoring, nutrition, and follow-up in subjects with moderate (STARS® 10–19) and high (STARS® ≥ 20) risk for AMD. Subjects with moderate risk, AREDS category 2, and 55–70 years of age should be asked to carry out self-monitoring (statement 15) and have follow-up every 2–3 years (statement 16), although the recommendation on follow-up frequency was considered of uncertain relevance. The lengthy follow-up time was made in consideration of the low risk of progression at 5 years, although for some panelists it was held that follow-up might be more frequent and that any patient at risk should be asked to carry out self-monitoring. Those with the same risk profile but with age > 70 years should carry out self-monitoring, be recommended specific nutritional supplements, and have annual follow-up (statements 17–19). Some participants felt that even stricter follow-up might be applied in some cases.

The statements for high-risk subjects (STARS® ≥ 20) were divided into those with AREDS category 1 or 2. All subjects with high-risk and with either AREDS category 1 or 2 over the age of 55 should be asked to carry out self-monitoring (statements 20, 21, and 23). High-risk subjects with AREDS category 1 over the age of 70 should be recommended to take specific nutritional supplements (statement 22), as should high-risk subjects with AREDS category 2 over the age of 55 years (statement 24). Independently of

age, high-risk subjects with AREDS category 2 should undergo follow-up every 6 months. It was, however, noted that a prospective randomized trial would be needed to confirm the utility of these recommendations in this group of subjects. Lastly, it should be highlighted that these recommendations are broadly in line with those issued by several national societies, such as the American Optometric Association, EURETINA [48], NICE [62], and the American Academy of Ophthalmology [63].

The Delphi methodology is a well-known process that aids in achieving group consensus. An advantage of our methodology is the online procedure adopted. During the two rounds carried out online, response rates were high with copious feedback, in line with a previous report [64].

At the same time, the limitations of the Delphi process warrant comment. First, given the internet-based nature of the present study, the panel members were unable to directly discuss specific wording of the statements. Moreover, the success of the Delphi process is further dependent on the panel selected to participate. While there are no established criteria to select experts and no specific guidance regarding the number of participants, we made a concerted effort to obtain a representative distribution of experts across several countries. The high level of consensus achieved suggests that these limitations are minimal.

5. Conclusions

In the present Delphi consensus, statements to support preventive measures in patients at risk of AMD based on STARS® scoring were developed. The recommendations deliver the means to optimize preventive care for patients in different geographical regions. It is hoped that the statements developed will be of benefit to clinicians in daily practice. This is especially relevant given that a proportion of ophthalmologists do not always properly counsel patients on the lifestyle changes to adopt, and, likewise, patients do not always adhere to specific recommendations. Future work should involve translating guidelines into routine clinical practice followed by evaluating their impact on the care of patients.

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Article

Efficacy of Anti-Vascular Endothelial Growth Factor Treatment in Neovascular Age-Related Macular Degeneration and Systemic Cardiovascular Risk Factors

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Abstract: This study evaluates whether the presence of cardiovascular risk factors (CRFs) affects functional and morphological responses to anti–vascular endothelial growth factor (VEGF) therapy in patients with neovascular age-related macular degeneration (nAMD). Retrospective analysis included 98 treatment-naïve eyes followed for at least 12 months. Patients received intravitreal injections of ranibizumab or aflibercept with the dosage and regimen set according to each manufacturer's recommendations for their product. Parameters evaluated at each follow-up visit included best-corrected visual acuity and central retinal thickness. Additionally, the presence of the following CRFs was evaluated: male sex, age of older than 70 years, history of current or past smoking, systemic arterial hypertension, diabetes mellitus, total hypercholesterolemia, low-density lipoprotein hypercholesterolemia, high-density lipoprotein concentration of 45 mg/dL or less, atherogenic dyslipidemia, family history of cardiovascular disease, and chronic kidney disease. A statistically significant better letter gain in visual acuity ($p = 0.012$) and greater percentage of responders ($p = 0.035$)—that is patients in whom best corrected visual acuity was stabilized or improved at 12 months—were noted among patients without a diagnosis of arterial hypertension. A statistically significant better mean visual improvement was also achieved in patients with higher total cholesterol plasma levels ($p = 0.004$), but this finding was not reflected in the significantly higher percentage of responders. The presence of remaining analyzed risk factors did not substantially affect the results of treatment. Systemic arterial hypertension is an independent factor leading to a poor functional outcome following anti-VEGF therapy in patients with nAMD. Effects of anti-VEGF treatment in patients with high total cholesterol levels should be analyzed in further research.

Keywords: age-related macular degeneration; anti–vascular endothelial growth factor; cardiovascular risk factors; arterial hypertension

1. Introduction

Age-related macular degeneration (AMD) is a common retinal disorder typically affecting elderly individuals and one of the leading causes of blindness worldwide in those older than 50 years of age [1–3]. The introduction of intravitreal injections of anti-vascular endothelial growth factor (anti-VEGF) agents has led to a reduction of 50% of patients with the neovascular form of AMD (nAMD) who end up blind; however, the group of patients, whose vision deteriorates despite receiving treatment, remains of great concern for clinicians [4]. It should be noted that, in a large percentage of patients, best-corrected visual acuity (BCVA) declines over the years despite a rigorous treatment regime and good compliance [5–7].

Factors influencing the outcome of anti-VEGF treatment of nAMD have been analyzed in many clinical studies to date [8–10]. Traditionally, the attention of researchers has been

directed toward the efficacy of a specific agent or retinal morphological biomarkers at the beginning of treatment, and the analysis of systemic factors affecting the results of intravitreal therapy has not been of interest to researchers around the world. To the best of our knowledge, only a small number of papers exist that directly seek to elucidate this relationship, including such risk factors as smoking, body mass index (BMI), and systemic hypertension [11–20]. As numerous studies prove, both cardiovascular disorders and AMD show similarities in their etiopathogenesis [21–23]. According to the literature, the prevalence of AMD is more frequent in patients with cardiovascular diseases [24,25]. Moreover, patients with AMD are at greater higher risk for stroke and cardiovascular mortality [26–28]. On the other hand, cardiovascular disorders are frequently listed as risk factors for AMD development [29–31]. These facts inspired us to analyze the relationship between cardiovascular risk factors (CRFs) and the effects of anti-VEGF treatment in patients with nAMD.

2. Materials and Methods

All procedures performed in this study were conducted in accordance with the ethical standards of the institutional research committee and the 1964 Declaration of Helsinki. The study was approved by the local bioethical board (Komisja Bioetyczna at OIL in Gdańsk, approval no. KB-29/18).

The retrospective analysis included 267 eyes of consecutive patients who began treatment for nAMD within the Drug Program of Treatment of nAMD (DP) in the ophthalmological ward and outpatient clinic of Wejherowo Hospital between 2015 and 2018. Written consent for inclusion in the study was obtained in 266 cases. Only treatment-naïve cases were selected from the whole group and included in the study. Patients who joined the program after undergoing treatment previously were excluded. In cases where both eyes of the same patient were treated, only one eye was randomly selected for the study. The study inclusion criteria limited the number of study eyes (patients) to 110, and 98 ultimately completed at least one year of follow-up. The study group consisted of 67 women and 31 men with a mean age of 76.5 ± 7.65 years. The flowchart for patient recruitment into the study is presented in Figure 1.

All study participants were diagnosed and followed up with according to the rules of the DP. A diagnosis of nAMD was established on the basis of the following assessments: BCVA, fluorescein angiography (FA), and spectral-domain optical coherence tomography (SD-OCT). BCVA was assessed on standard Early Treatment Diabetic Retinopathy Study 4-m charts, FA was performed using the Visucam NM/FA system (Carl Zeiss, Oberkochen, Germany), and SD-OCT was performed using the Cirrus 5000 system (Carl Zeiss). The most important inclusion criteria for DP were a BCVA between 0.2 and 0.8 on the Snellen chart (i.e., ETDRS letter score of between 50 and 80) and the presence of active subfoveal neovascularization confirmed by the FA and SD-OCT findings.

Patients were treated with either ranibizumab (Lucentis; Genentech, San Francisco, CA, USA) or aflibercept (Eylea; Regeneron, Tarrytown, NY, USA) according to the dosing regimen recommended by the manufacturer of each product. Ranibizumab was administered in 32 cases, and aflibercept was given in 66 cases. Patients were randomized to receive either aflibercept or ranibizumab, however, at the moment of introduction of DP, only aflibercept was reimbursed. Ranibizumab was introduced to DP later, and that explains the difference in numbers of aflibercept and ranibizumab injections performed in the study.

Treatment with ranibizumab was initiated with one injection given per month until maximum visual acuity was achieved and/or there were no signs of disease activity during ophthalmological examination and SD-OCT. Afterward, the drug was administered in a pro re nata fashion.
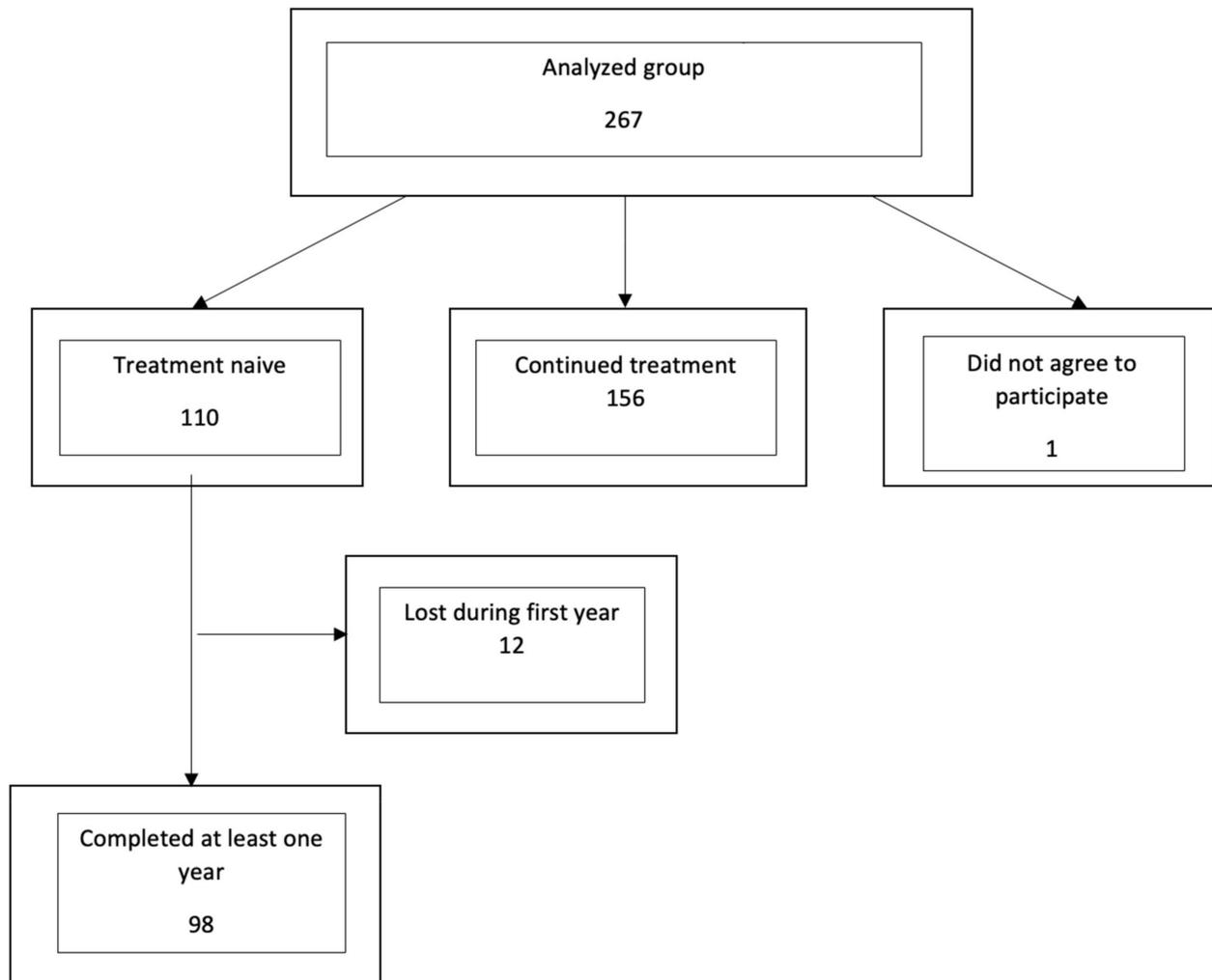
Figure 1. Flowchart for the selection of eyes included in the study.

Treatment with aflibercept was initiated with one injection given per month for five consecutive doses, then one injection given every two months until the end of the first year. Subsequently, the drug was administered in a pro re nata fashion. The retreatment criteria included any loss in BCVA and the presence of disease activity on SD-OCT, which was suggested by any increase in central retinal thickness (CRT), any increase in the amount of pigment epithelial detachment (PED), onset of subretinal or intraretinal fluid, or hemorrhage. Measurements of CRT using the Carl Zeiss Cirrus 5000 system refer to the mean retinal thickness within the central area of the retina measuring 1 mm in diameter.

BCVA assessments and SD-OCT imaging were performed for each participant every month. Statistical analysis included baseline, three-month, and 12-month follow-up results.

During their baseline examination, each of the patients included in the study was interviewed according to the presence of selected CRFs. The following factors were analyzed: male sex, age of greater than 70 years, history of current or past smoking (not applicable if patient had not smoked for at least 20 years), systemic arterial hypertension (AH) (BP $\geq$ 140 mmHg or intake of hypotensive medications), diabetes mellitus (blood glucose concentration > 126 mg/dL or intake of hypoglycemic medications), total hypercholesterolemia (total cholesterol [TCH] $\geq$ 190 mg/dL), low-density lipoprotein (LDL) hypercholesterolemia (LDL $\geq$ 115 mg/dL or intake of cholesterol-lowering medications), high-density lipoprotein (HDL) concentration of 45 mg/dL or less, atherogenic dyslipidemia (triglyceride level > 150 mg/dL and HDL $\leq$ 45 mg/dL or intake of lipid-lowering medications), family history of cardiovascular disease (e.g., stroke, myocardial infarction,

or peripheral atherosclerosis in a close family member younger than 60 years of age), and chronic kidney disease (CHKD) as evaluated by the estimated glomerular filtration rate (<60 mL/min/1.73 m^2).

Apart from using patients' medical history, CRFs were evaluated in laboratory tests conducted before initiation of anti-VEGF treatment for all patients in a certified hospital laboratory; tests included the assessment of plasma levels of total TCH, HDL, LDL, triglycerides, creatinine, and glucose. The estimated glomerular filtration was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula [32]. Blood pressure was measured before and throughout the study.

All of the patients had anthropometric measurements collected during their baseline examination, including their weight (kg) and height (cm), which were used to calculate the body mass index (BMI). A BMI of at least 30 kg/m^2 was considered a CRF. Additionally, the circumferences of the waist and hips were measured using the World Health Organization (WHO) data-gathering protocol, where the waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, and the hip circumference was measured around the widest portion of the buttocks. Results were used to calculate the waist-to-hip ratio (WHR); abdominal obesity was recognized when the WHR value was more than 1 in men or 0.85 in women, according to WHO recommendations [33].

The distribution of analyzed CRFs in the study group is detailed in Table 1.

Table 1. Distribution of risk factors in the study population.

Risk Factor	No.	%
Age ≥ 70 years	77	78.57
Male sex	31	31.63
Smoking	32	32.65
Systemic arterial hypertension	69	70.41
Diabetes	28	28.57
BMI > 30 kg/m^2	36	36.73
WHR > 1 for M and > 0.85 for F	61	62.24
Total hypercholesterolemia	50	51.02
Hypertriglyceridemia (triglyceride level ≥ 150 mg/dL)	21	21.43
LDL hypercholesterolemia	77	78.57
HDL ≤ 45 mg/dL	12	12.24
Dyslipidemia	9	9.18
CHKD	28	28.57
Family history	17	17.35

Abbreviations: BMI, body mass index; CHKD, chronic kidney disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TCH, total cholesterol; WHR, waist-to-hip ratio.

The morphological response was considered good (i.e., the patient was responsive to treatment) if any reduction in CRT after 12 months of treatment was noted. Conversely, the patient was considered a non-responder if their CRT increased after 12 months of treatment.

The response to anti-VEGF treatment was defined as functionally good if the BCVA was stabilized or improved at 12 months (and the patient was deemed a responder to treatment). Non-responders were those who showed a decrease in BCVA at the end of the follow-up period.

Statistical Analysis

The analysis of the gathered data included the following results and correlations:

- Changes in BCVA and CRT at 3 and 12 months of treatment in the whole study group.
- Mean changes in BCVA and CRT in the subgroups of patients with and without specific CRF.
- Proportions of responders and non-responders (functional and morphological) according to the presence of each specific CRF.
- Correlation between the changes in BCVA and CRT at 12 months and the number of coexisting CRFs.

The relationship between the presence of specific CRFs and the response to treatment was evaluated in two ways. First, the changes in BCVA and CRT were compared between patients with and without a specific CRF. Second, the percentages of responders and non-responders were calculated and compared between these groups.

Statistical analysis in this study had to take into account not only mean improvements in BCVA and CRT but also the proportions of responders and non-responders. Statistical significance of the measured changes in mean BCVA and CRT values without a statistically significant difference between the percentages of responders and non-responders may suggest a substantial dispersion of individual results and not reflect the existence of a true trend in the whole study cohort

Statistical analysis was performed using the Statistica version 10.0 software program (StatSoft, Tulsa, OK, USA). Quality variables were presented with the frequency distribution.

For quotative variables, arithmetic mean, standard variation, and minimum and maximum values were calculated. The normalcy of distribution was evaluated with the Shapiro–Wilk test.

As the variables did not fulfill the criteria for the use of parametric methods, nonparametric tests were used to verify statistical hypotheses. The following tests were used for analysis: ANOVA Friedman with the post-hoc Dunn–Bonferroni test (analysis of variance with correction for multiple comparisons), chi-squared test (with the Yates amendment for small samples), Mann–Whitney U test, and Spearman's rank correlation test. The results were regarded as statistically significant when $p < 0.05$.

3. Results

The mean number of intravitreal injections administered per eye in the whole group during 12 months of follow-up was 6.65 ± 1.41 injections, with a median of seven injections, minimum of three injections, and maximum of nine injections, respectively. The mean numbers of injections per eye during the study period were 5.7 in those receiving ranibizumab and 7.54 in those receiving aflibercept. BCVA was improved by a significant mean result of 4.57 ± 14.03 letters at 12 months, while CRT was reduced by a significant mean value of 130.76 ± 173.59 μm; exact data on these changes are presented in Table 2. Functional and morphological improvements occurred in 75.51% and 82.65% of patients, respectively (Table 3).

Table 2. Effects of anti-VEGF treatment at 12 months.

Variable	Before Treatment	At 3 Months	At 12 Months	*p*-Value
BCVA (ETDRS letters), mean	77.5 ± 14.09	81.08 ± 12.18	82.07 ± 13.9	0.000
CRT (μm)	434.6 ± 214.33	340.23 ± 185.67	303.85 ± 129.92	0.000

Abbreviations: BCVA, best-corrected visual acuity; CRT, central retinal thickness; ETDRS, Early Treatment Diabetic Retinopathy Study; VEGF, vascular endothelial growth factor.

Table 3. Stratification of responders and non-responders according to changes in BCVA and CRT at 12 months.

Parameter	Improved or Stable	%	Worsened	%
BCVA change	74	75.51	24	24.49
CRT change	81	82.65	17	17.35

Abbreviations: BCVA, best-corrected visual acuity; CRT, central retinal thickness.

According to our results, there were no significant differences in the functional or morphological outcome between the use of aflibercept or ranibizumab. We noted mean BCVA improvement of 5.09 ± 13.67 letters in case of aflibercept versus 3.5 ± 15.77 for ranibizumab ($p = 0.753$) and CRT reduction of mean 148.5 ± 198.49 μm in case of aflibercept versus 94.16 ± 98.11 μm for ranibizumab ($p = 0.55$).

Data concerning the correlation of the presence of specific CRFs and the efficacy of treatment are provided in Tables 4 and 5. Statistically significantly greater improvements in BCVA were achieved in patients without a diagnosis of AH (10.07 ± 11.91 vs. 2.26 ± 14.7; $p = 0.012$); this result was also true with respect to the higher percentage of responders to anti-VEGF treatment (89.66% vs. 69.57%; $p = 0.035$). Meanwhile, a statistically significant letter gain improvement was also confirmed in patients with higher TCH levels (7.78 ± 14.56 vs. 1.23 ± 13.43 mg/dL; $p = 0.004$), but this result was not reflected in the substantially higher percentage of responders (82.00% vs. 68.75%; $p = 0.127$). An analogous situation was noted in patients without CHKD; however, this result bordered on statistical significance ($p = 0.051$) and was not reflected in a difference between responders and non-responders. There was a tendency toward better mean CRT reduction in patients with LDL hypercholesterolemia, and this result also bordered on statistical significance ($p = 0.048$) and was not apparent in the ratio of responders to non-responders. Neither response group experienced better functional improvement over the other.

Table 4. The change of BCVA and CRT at 12 months according to presence or absence of a specific risk factor. *P* values refer to the statistical significance of the difference in that change between patients with a specific risk factor present and absent.

Risk Factor	RF Present BCVA Baseline Mean	RF Present BCVA Mean Change (+)	RF Absent BCVA Baseline Mean	RF Absent BCVA Mean Change (+)	*p* Value	RF Present CRT Baseline Mean	RF Present CRT Mean Change (−)	RF Absent CRT Baseline Mean	RF Absent CRT Mean Change (−)	*p* Value
Age ≥ 70	62.94 ± 14.30	4.39 ± 13.37	60.86 ± 13.57	5.24 ± 17.76	0.307	435.95 ± 219.44	128.49 ± 174.69	429.67 ± 199.46	139.05 ± 173.49	0.778
Male gender (males vs. females)	59.70 ± 12.50	M 6.52 ± 10.98	63.78 ± 14.70	F 3.67 ± 15.63	0.328	587.0 ± 168.29	M 156.26 ± 178.51	452.50 ± 188.26	F 118.96 ± 151.73	0.541
Smoking	64.97 ± 11.98	2.25 ± 16.02	61.28 ± 14.97	5.70 ± 13.42	0.515	433.97 ± 209.64	116.94 ± 162.17	434.91 ± 218.16	137.46 ± 179.68	0.625
Systemic hypertension	63.00 ± 13.55	2.26 ± 14.7	61.28 ± 15.54	10.07 ± 11.91	0.012	332.5 ± 181.75	120.91 ± 157.59	429.55 ± 275.58	154.17 ± 208.01	0.770
Diabetes	63.93 ± 13.05	3.39 ± 10.00	61.91 ± 14.56	5.04 ± 15.77	0.227	411.21 ± 178.45	113.82 ± 165.96	443.96 ± 227.61	137.53 ± 177.26	0.514
Obesity BMI > 30	63.78 ± 12.83	5.42 ± 13.67	61.74 ± 14.85	3.11 ± 15.48	0.526	414.83 ± 181.80	129.50 ± 178.56	446.08 ± 231.77	131.48 ± 172.11	0.979
Obesity WHR > 1 for M and >0.85 for F	62.92 ± 13.99	4.77 ± 13.98	61.78 ± 14.47	4.24 ± 15.07	0.905	394.08 ± 185.22	106.26 ± 155.20	501.40 ± 243.37	171.14 ± 195.84	0.051
Total hypercholesterolemia TCH ≥190 mg/dL	60.44 ± 14.68	7.78 ± 14.56	64.62 ± 13.30	1.23 ± 13.43	0.004	446.36 ± 232.18	140.52 ± 149.58	422.35 ± 195.72	121.69 ± 145.31	0.722
TG ≥ 150 mg/dL	62.48 ± 15.35	4.38 ± 10.97	62.49 ± 13.86	4.62 ± 15.18	0.553	468.38 ± 189.39	176.38 ± 169.26	425.39 ± 220.88	118.31 ± 173.75	0.060
Hypercholesterolemia LDL ≥115 mg/dL	63.82 ± 13.06	3.65 ± 13.93	57.62 ± 16.91	7.95 ± 15.57	0.553	419.45 ± 206.37	113.88 ± 169.48	490.14 ± 238.39	192.62 ± 178.58	0.048
HDL ≤ 45 mg/dL	63.00 ± 14.27	−0.92 ± 19.39	62.42 ± 14.17	5.34 ± 13.44	0.383	353.92 ± 110.40	95.67 ± 103.91	445.86 ± 223.15	135.65 ± 181.09	0.766
Dyslipidemia	61.33 ± 15.18	5.22 ± 8.77	62.60 ± 14.08	4.51 ± 14.81	0.922	413.00 ± 135.08	135.11 ± 111.10	436.79 ± 221.19	131.60 ± 178.39	0.777
Chronic kidney disease	63.54 ± 13.70	2.04 ± 7.73	62.07 ± 14.34	5.59 ± 16.17	0.051	425.50 ± 179.38	129/14 ± 126.60	438.24 ± 227.90	131.40 ± 189.97	0.524
Family history	65.65 ± 14.29	−0.53 ± 18.71	61.83 ± 14.07	5.64 ± 13.12	0.120	356.82 ± 147.59	117.00 ± 139.12	450.92 ± 223.13	133.64 ± 180.60	0.778

BCVA—best corrected visual acuity, CRT—central retinal thickness, M—males F—females, WHR—waist to hip ratio HDL—high density lipoprotein cholesterol LDL—low density lipoprotein cholesterol TCH—total cholesterol RF—risk factor.

Table 5. Difference between percentage of responders to treatment at 12 months according to the presence or absence of a specific risk factor. *p* values refer to statistical significance of that difference between patients with risk factor present versus patients with risk factor absent.

Risk Factor	Responders BCVA (%)		*p* Value	Responders CRT (%)		*p* Value
	RF Present	RF Absent		RF Present	RF Absent	
Age ≥ 70	83.87	80.95	0.513	84.42	90.48	0.725
Male gender (males vs. females)	M 83.87	F 71.64	0.19	M 90.32	F 79.10	0.173
Smoking	68.75	78.79	0.278	78.13	84.85	0.410
Systemic hypertension	69.57	89.66	0.035	81.16	86.21	0.547
Diabetes	67.86	78.57	0.265	82.14	82.86	0.833
Obesity BMI > 30	69.44	79.03	0.287	83.33	82.26	0.892
Obesity WHR > 1 for M and >0.85 for F	73.77	78.38	0.607	77.05	91.89	0.060
Total hypercholesterolemia TCH ≥190 mg/dL	82.00	68.75	0.127	84.00	81.25	0.719
TG ≥ 150 mg/dL	71.43	76.62	0.624	90.48	80.52	0.457
Hypercholesterolemia LDL ≥115 mg/dL	75.32	76.19	0.935	81.82	85.71	0.926
HDL ≤ 45 mg/dL	66.67	76.74	0.688	83.33	82.56	0.733
Dyslipidemia	77.78	75.28	0.810	77.78	83.15	0.955
Chronic kidney disease	75.00	75.71	0.941	89.29	80.0	0.423
Family history	58.82	79.01	0.147	76.47	83.95	0.698

BCVA—best corrected visual acuity, CRT—central retinal thickness, CRF—cardiovascular risk factor M—males F—females, WHR—waist to hip ratio HDL—high density lipoprotein cholesterol LDL—low density lipoprotein cholesterol TCH—total cholesterol RF—risk factor.

As patients with AH generally tend to be older patients free of that disease, we performed additional comparison of AH patients versus non-AH patients in reference to their age to assess the bias of age-factor. Analysis revealed mean age in AH group is 77.20 ± 7.69 versus 75.07 ± 7.47 in non-AH group. The difference is not significant in *t*-test with $p = 0.22$.

Correlation between an increasing number of CRFs and changes in BCVA and CRT were not found; however, there was a tendency toward worse functional improvement in patients with greater numbers of CRFs ($p = 0.052$). Spearman's rank correlation coefficient values are provided in Table 6.

Table 6. Number of risk factors and changes in BCVA and CRT at 12 months (Spearman's rank correlation).

Pair of Variables	R	t(N-2)	*p*-Value
No. of risk factors and change in BCVA	−0.20	−1.97	0.052
No. of risk factors and change in CRT	0.01	0.05	0.959

Abbreviations: BCVA, best-corrected visual acuity; CRT, central retinal thickness; No., number; R, Spearman's coefficient.

4. Discussion

In this section, we present discussion of the results of our study according to each risk factor analyzed.

4.1. Systemic Hypertension

The relationship between elevated systemic blood pressure and AMD has been analyzed in clinical trials; however, research has concentrated on the evaluation of AH as a risk factor for the development of AMD, not the effects of anti-VEGF treatment. Orthostatic

blood pressure behavior (rise after assuming the upright position) was associated with an increased risk of AMD in a recent study [34]. A significant difference between systolic and diastolic pressure values was linked to a greater risk of late AMD in the ALIENOR study [35]. Higher values of systolic blood pressure were also correlated with a higher prevalence of late AMD in the Women's Health Initiative Sight Exam ancillary study [36]. In the Beaver Dam Study, it was also proven that the use of blood pressure–lowering medications, especially beta-blockers, is associated with a higher incidence of nAMD [37]. Other research has argued that the presence of systemic hypertension results in decreased choroidal flow in non-exudative forms of AMD and impaired choroidal perfusion results in insufficient elimination of degradation products from the RPE and the formation of drusen [38]. Moreover, reduced blood flow in the choroid stimulates hypoxia and promotes VEGF upregulation and neovascularization [39]. That mechanism in later exudative stages could explain the insufficient effect of VEGF blockers in nAMD population with accompanying AH. Nevertheless, a direct relationship between the effects of nAMD treatment and the presence of AH has been investigated in only a few trials to date.

One study by Piermarocchi et al. showed that complement factor H risk alleles, smoking history, and AH each independently influenced the patient's response to ranibizumab treatment of nAMD, with worse 12-month BCVA outcomes ($p = 0.036$, $p = 0.037$, and $p = 0.043$, respectively) [11]. These authors recorded a mean improvement of 3.0 ± 8.1 letters in patients without AH versus that of -0.6 ± 9.1 in patients affected by AH.

Better functional results in patients without AH were also reported by Menger et al., [19] who documented BCVA changes at 24 months of treatment of -0.01 logMAR in patients on hypotensive drugs versus $+0.21$ logMAR in patients without AH ($p = 0.045$).

On the other hand, Zhao et al. and van Asten et al. did not find a correlation between the presence of AH and poor outcomes with intravitreal therapy among nAMD patients [12,16].

Our results are consistent with data obtained in the studies by Piermarocchi et al. and Menger et al. In our cohort, the absence of AH was related to significantly better visual gains (10.07 vs. 2.26 letters) and a greater percentage of functional responders (89.66% versus 69.57%); however, reductions in CRT were similar between patients with and without AH. In all of our patients, AH was well-controlled during the study period; however, all study participants experienced systemic blood pressure alterations before their inclusion in the study.

It can be speculated that the presence of AH prior to application of anti-VEGF therapy decreases the potential for BCVA improvement. It might be true that these patients had already worse BCVA compared to the cohort without AH before the development of macular neovascularization, which would explain the worse functional improvement documented despite relatively the good morphological response to anti-VEGF. As proved by research quoted earlier [38,39] AH impairs choroidal flow and nutrition of the RPE and photoreceptors. That theoretically could result in loss of photoreceptors and visual impairment, even in the absence of neovascularization. In our study both, AH and non-AH groups achieved relatively good morphological results (significant reduction of CRT), however functional improvement was much better in cohort free of AH. This fact could also be explained by the lack of potential for improvement due to atrophic retinal alterations preceding the development of the choroidal neovascularization in patients with AH.

Our results suggest, that good control of blood pressure before the onset of nAMD might improve the results of anti-VEGF treatment. Nevertheless, this concept should be confirmed by further studies. Further research is also needed on the impact of the use of different hypotensive drugs on visual outcome during the treatment.

4.2. Plasma Lipids

Results pertaining to the relationship between the efficacy of AMD treatment and plasma lipid levels did not show any unequivocal positive or negative trend. In our cohort, lower LDL levels were associated with a better morphological reaction to anti-VEGF treat-

ment (i.e., larger CRT reduction); however, this result bordered on statistical significance. Moreover, we did not find a correlation between LDL level and functional improvement in our patients. On the other hand, a high TCH level was related to significantly better mean letter gains with strong statistical significance ($p = 0.004$), but not a greater percentage of responders. In other words, there were cases with high TCH levels and spectacular improvements in BCVA, but such was not the rule for the whole group of patients with high TCH. Besides, morphological improvements in patients with high plasma TCH levels were not better than those in patients with lower levels of TCH. Available epidemiological studies suggest there is a lower risk for the early stages of AMD in cases with high TCH levels [40–42]. On the other hand, a relationship between TCH and its fractions levels and the late stages of AMD has not yet been clearly established [43,44]. Moreover, to our knowledge, no relationship between the level of plasma cholesterol and the response to anti-VEGF treatment in AMD has been established so far.

As we know from the literature, the relationship between plasma lipids and AMD is not straightforward. A large meta-analysis of available trials analyzing the risk of AMD and CRFs proved that high HDL levels are associated with a greater risk of progression to AMD, while high LDL and TCH levels play a protective role against such [45,46]. In other words, a systemic factor commonly accepted as protective against atherosclerosis, i.e., a high level of HDL, does not protect against the development of AMD [47–49]. There exists a concept that high HDL levels in the macula, which is constantly exposed to light and, consequently, oxygen stress, result in the production of high levels of reactive oxygen species. These species react with HDL and are converted into pro-inflammatory and pro-oxidant products, which impair cholesterol elimination and promote LDL oxygenation in the retinal pigment epithelium (RPE) [50,51]. In this way, protective HDL properties would be outweighed by the local inflammatory reactions in a manner that leads to an accumulation of residual products in the RPE [52,53]. In the light of these data, HDL appears to be a new target in treatment strategies for AMD [54]. In our study, we did not find any significant relationship between the response to anti-VEGF therapy and HDL levels in patient blood, nor was there such a correlation in reference to triglyceride levels. Interestingly, patients with lower LDL levels tended to achieve better mean CRT reductions after anti-VEGF therapy, but the percentage of responders was not higher here relative to among patients with higher LDL levels. The BCVA improvement was also not significantly greater in patients with lower LDL levels.

Some research suggests that lowering fat levels may improve advanced non-exudative forms of AMD, such as drusenoid epithelial retinal detachments [55]. In a multicenter study of 23 patients treated with 80 mg of atorvastatin daily, Vavvas et al. noted a regression of drusenoid deposits and visual improvement by a mean of 3.3 letters in 10 subjects. Nevertheless, this kind of reasoning cannot be applied to exudative forms of AMD.

A positive relationship between high TCH levels and visual gain following anti-VEGF treatment in selected patients is an interesting phenomenon but one that needs to be confirmed in a larger sample. No matter what future research shows, it is hard to believe that such data will be extrapolated into clinical recommendations.

4.3. CHKD

Patients with CHKD tend to have poorer visual gains after anti-VEGF treatment; however, this relationship borders on statistical significance ($p = 0.051$). Besides, the difference between the percentages of responders and non-responders in this group does not depend upon the presence of CHKD as a risk factor. It has to be emphasized that the presence of CHKP has not been analyzed thus far as a risk factor for poor response in the treatment of nAMD, so we are unable to correlate our findings with other data.

4.4. Other CRFs

We did not find any correlation between the remaining risk factors and the effects of anti-VEGF therapy. Neither age, sex, smoking, obesity, diabetes, dyslipidemia, nor family

history correlated significantly with the outcome of therapy. These findings remain partly consistent with those of other authors; however, it has to be emphasized that, quite often, the results of studies are contradictory. We present a summary of available studies linking CRFs to the efficacy of nAMD treatment in Table 7. Most of the identified studies did not confirm a relationship between age and the effect of anti-VEGF treatment of nAMD. Only Shah et al. and van Asten et al. reported better responses to anti-VEGF treatment in younger patients [14,16], while Bek et al. suggested a better morphological response occurred in older patients. Again, most of the selected studies do not show a relationship between sex and anti-VEGF therapy, and some that explore such a correlation present contradictory conclusions [13–15]. There exists just one study by van Asten et al. that reports a higher risk for non-responders in patients with diabetes [16]. Smoking is an obvious risk factor for AMD and cardiovascular diseases; however, studies that prove a correlation between tobacco intake and the results of anti-VEGF treatment of nAMD offer inconsistent results [11,14,19–21]. Only one study by Zhao et al. documented a relationship between nAMD treatment and BMI [12]. Paradoxically, a higher BMI was linked to a better response to treatment; however, the difference between BMI values among responders and poor responders was not large (26.4 ± 0.4 vs. 24.9 ± 0.5 kg/m^2) if we consider BMI as a range; both values remain in the upper normal range of the BMI scale.

Table 7. Presence or absence of statistically significant correlations between the presence of selected systemic risk factors and the response to anti-VEGF treatment of nAMD.

Study	Age	Sex	DM	HA	Smoking	BMI/ Obesity	Plasma Lipids	CHKD
Present study	No	No	No	Yes (worse VA gains)	No	No	No	No
Bek et al., 2018 [13]	Yes (larger CRT reduction in older age)	Yes (smaller CRT reduction in males)	NA	NA	No	NA	NA	NA
Shah et al., 2016 [14]	Yes (better VA gains in younger patients)	Yes (better VA gains in males)	NA	NA	Yes (better final VA)	NA	NA	NA
Piermarocchi et al., 2014 [11]	NA	NA	NA	Yes (worse VA gains)	Yes (worse VA gains)	No	NA	NA
Guber et al., 2014 [15]	No	Yes (better CRT reduction in males)	NA	NA	NA	NA	NA	NA
Van Asten et al., 2014 [16]	Yes (increasing risk for Non-responders in older age)	No	Yes (risk for Non-responders)	No	No	No	NA	NA
Zhao et al., 2013 [12]	No	No	No	No	No	Yes (BMI higher in responders)	NA	NA
Krebs et al., 2013 [18]	No	No	NA	NA	NA	NA	NA	NA
Lee et al., 2013 [19]	No	No	No	No	Yes (lower VA gains in current smokers)	No	No	NA
Menger et al., 2012 [20]	No	NA	NA	Yes (worse fiNAl VA)	Yes (worse final VA)	NA	NA	NA
Inglehearn et al., 2012 [21]	No	No	NA	NA	Yes (better VA gains in smokers and ex-smokers)	NA	NA	NA

Abbreviations: BMI, body mass index; CHKD, chronic kidney disease; CRT, central retinal thickness; DM, diabetes mellitus; NA, not analyzed; VA, visual acuity.

4.5. Number of Risk Factors

An increasing number of risk factors tended to correlate with poorer BCVA improvement; however, these result only bordered on statistical significance.

4.6. Limitations of the Study

Choosing the 'right points' to evaluate the effects of treatment for a disease such as nAMD (which requires numerous retreatments and a long course with improvements and recurrences) is challenging. In that sense, a 12-month observation period might seem to be a relatively short period of time. On the other hand, some patients who comply with rigorous treatment regimens still lose visual acuity, even in a relatively short period of time. We believe that a 12-month period is long enough to assess factors that influence the final outcome of therapy.

We also realize that the analyzed material is relatively small for assessing as many risk factors as we did; however, statistical significance was possibly achieved in certain areas. Still, a multivariate analysis was not possible due to our relatively small sample in relation to the number of risk factors analyzed. Collecting further material will continue to support our conclusions.

5. Conclusions

Systemic AH is an independent factor leading to a poor functional outcome following anti-VEGF treatment of nAMD. Other cardiovascular risk factors—such as age; sex; smoking; obesity; diabetes; high LDL, HDL, and TG plasma levels; dyslipidemia; and family history—do not have a strong influence on the effects of such treatment. Further research is needed to analyze the effects of anti-VEGF treatment in patients with high TCH levels.

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Article

Assessment of Vascular Changes in Patients after Pars Plana Vitrectomy Surgery Due to Macula-Off Rhegmatogenous Retinal Detachment

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Abstract: The aim of this study was to investigate the changes in the retinal capillary plexuses in patients after pars plana vitrectomy (PPV), which is used for the treatment of rhegmatogenous retinal detachment (RRD). In this study, we included the results of 114 patients who underwent PPV after total retinal detachment (RRD; retinal detachment group). It should be kept in mind that to qualify for the study group, there was a condition that retinal detachment be only present in one eye, allowing the fellow healthy eye to be used for the control group, and the study, therefore, did not include cases where retinal detachment occurred binocularly. Optical coherence tomography (OCT) and OCT-A images were taken at 9 ± 2 months (median 10 months) after the surgery, with the study conducted in the years 2017–2019. OCT was used to examine the external limiting membrane (ELM), central macular thickness (CMT) and retinal nerve fiber layer (RNFL), while OCT-angiography (OCT-A) was used to examine the extent of the foveal avascular zone (FAZ) in the deep and superficial capillary plexuses. Changes in the FAZ area of the superficial plexus (SCP) between the study and control groups were analyzed over 346 ± 50 days. In our study, we observed changes in the FAZ area between the RRD and control groups in the SCP (203.65 ± 31.69 μm^2 vs. 215.30 ± 35.82 μm^2; $p = 0.28733$) and DCP (284.79 ± 35.82 μm^2 vs. 336.84 ± 32.23 μm^2; $p = 0.00924$). Changes in the RNFL thickness between the study and control groups over 346 ± 50 days were as follows: 90.15 μm vs. 82.44 μm; $p = 0.19773$. Disruption of the external limiting membrane was observed in 78.95% (90 eyes) of the study group. In the control group, it was undamaged, and no integrity disorder was observed. In the RRD, changes occurred in the FAZ of both the SCP and the DCP, which reduced the extent of this zone, an effect that was more pronounced in DCPs. A better understanding of the anatomical and hemodynamic changes taking place in the retina after macula-off RRD might be helpful in answering the question as to why BCVA in these cases is "only" or "as much as" from 0.4 to 0.1, namely, that it might be related to changes in the neurosensory retina after macular peeling.

Keywords: macula-off rhegmatogenous detachment; optical coherence tomography angiography; superficial perifoveal capillary plexus; retinal deep perifoveal capillary plexus

1. Introduction

In cases of rhegmatogenous retinal detachment (RRD) with macular plexus, retinal detachment does not always guarantee that anatomical success will equate to functional success, which is highly expected from the patient. In such cases, pars plana vitrectomy is an efficient procedure, which involves removal of the internal limiting membrane (ILM) to relax the central retina and prevent formation of the secondary epiretinal membrane (ERM) [1,2]. The ideal operative outcome is when the anatomical success of retinal reattachment due to RRD is equal to or greater than 90% of the treated cases [3,4].

One of the first reports of visual recurrence after surgery for retinal macular detachment was reported by Burton et al. [5]. It was observed that in 46 out of 87 patients (53%; $p < 0.05$) who had been operated on up to 9 days from the onset of symptoms, the visual acuity after the procedure was in the range of 20/20–20/50. However, when the procedure was performed between 10 and 19 days after symptom onset, the percentage of patients with the same level of visual acuity after the procedure was only 34% ($p < 0.05$), and for surgery following an even longer period after symptom onset, only 29% ($p < 0.05$) [5]. Park et al. [6] distinguished six factors determining visual acuity after surgery. These include ellipsoidal integrity impairment ($\beta = 0.167$; $p < 0.001$), Henle fiber layer and outer nuclear layer (HFL + ONL)/photoreceptor layer ratio ($\beta = 0.199$; $p < 0.001$), photoreceptor outer segment length ($\beta = -0.020$; $p < 0.001$), ratio of photoreceptor inner segment length to photoreceptor outer segment length ($\beta = 0.047$; $p = 0.005$), ratio of photoreceptor layer thickness between the RD and other eye ($\beta = -0.126$; $p = 0.018$) and the ratio of the length of the outer segment of photoreceptors between the RD and the other eye ($\beta = -0.425$; $p < 0.001$) [6].

In turn, Geiger et al. [7] found a visual acuity of at least 20/40 in 81 out of 131 patients (61.8%) receiving surgery 6 months after retinal detachment. These authors emphasized that patients who had better VA logMAR before surgery had better post-operative VA ($p < 0.05$), and that the time from retinal detachment to surgery did not significantly affect post-operative VA [7].

Optical coherence tomography (OCT) is a method that exploits the phenomenon of low-coherence interferometry. As a consequence, it is possible to visualize eye structures in vivo. OCT has become a key component in the clinical evaluation of the cornea and anterior segment of the eye. OCT is used in the diagnosis of cystic swelling of the macula, epiretinal membrane (ERM) and retinal folds [8,9] as well as changes within the external limiting membrane (ELM), which is formed from the combination of photoreceptors and Müller cells [10]. The possibility to measure the central macular thickness (CMT) and the thickness of the retinal nerve fiber layer (RNFL) provides additional prognostic information [11]. Optical coherence tomography angiography (OCT-A) is an examination that may provide further information on the changes occurring in the highly important process of macular vascularization after its reattachment. The retinal vascular plexus of healthy subjects is formed by a superficial capillary plexus (SCP) located in the ganglion cell layer and nerve fiber layer and a deep capillary plexus (DCP) located in the inner nuclear layers [12,13]. OCT-A has been used in the diagnosis and monitoring of treatment effects in the case of macular degeneration, retinal vein obstruction, diabetic retinopathy, posterior uveitis and optic neuropathies [14–17]. McKay et al. [18] used the OCT-A technique to compare the vessel density (VD) and FAZ in the eyes of a group of patients, in which there was one affected eye that had undergone macula-off RRD and the fellow eye for each patient. VD was significantly lower in the RRD eyes compared with the fellow eyes ($p < 0.05$). It was also reported that visual acuity after surgery was poorer for RRD eyes with decreased VD in the DCP compared with other control eyes without VD [18].

Additionally, Bonfiglio et al. [19] reported a reduction in vascular density in the superficial and deep retinal plexuses in RRD eyes after PPV compared with other healthy eyes [19].

Woo et al. [20], in turn, found that, in a group of 34 RRD patients who underwent PPV with gas tamponade, the superficial and deep FAZ areas were significantly larger in

the macula-off group (superficial: 0.374 ± 0.112 mm^2; deep: 0.702 ± 0.193 mm^2) than in the macula-on group (superficial: 0.282 ± 0.105 mm^2; deep: 0.543 ± 0.114 mm^2) following surgery. Post-treatment FAZ enlargement may result from ischemic damage to the retinal capillary plexus in the fovea [20].

In the superficial plexus, the vessels are linear with a centripetal pattern; in the deep plexus, the vessels have a concentric distribution with vertical interconnections. The foveal capillary plexus forms a ring at the margin of the fovea, producing a capillary-free region called the fovea avascular zone (FAZ) [21]. Macular peeling is a surgical technique used in diseases of the retina, which can include primary or secondary diabetic retinopathy, retinal detachment, holes, macular edema or foveal retinoschisis. The technique is based on surgical removal of the preretinal tissue or internal limiting membrane (ILM) in the macula. The term ILM was first used in 1845 by Pacini to describe the boundary between the retina and the vitreous. It is a periodic acid–Schiff (PAS)-positive basement membrane within which astrocytes and the end feet of Müller cells can be distinguished [22,23]. Complications that may occur after macula peeling include cataract progression, intraocular pressure increase, visual field defects, retinal tears, retinal detachment, vitreous hemorrhage, ocular hypotony, dislocation of the intraocular lens in pseudophakic eyes, macular phototoxicity, RPE changes and endophthalmitis [24,25].

Our study aimed to evaluate the changes occurring in superficial and deep perifoveal capillary-free zones in patients after undergoing pars plana vitrectomy (PPV) with macular peeling surgery in which the reason for operation was rhegmatogenous retinal detachment with macular plexus.

2. Materials and Methods

2.1. Patients

The research was approved by the Research Ethics Committee of the Silesian Medical Chamber in Katowice, Poland (Resolution Number ŚIL/KB/100p/17). Patient consent to review their medical records was not required by the bioethical committee due to the retrospective nature of this study and because patient information was sufficiently anonymized. The study was conducted in the years 2017–2019.

In this study, we included the results for 114 patients who underwent PPV after total retinal detachment (RRD; retinal detachment group). It should be kept in mind that to qualify for the study group, there was a condition that retinal detachment be only present in one eye, allowing the fellow healthy eye to be used for the control group, and the study therefore did not include cases where retinal detachment occurred binocularly. OCT-A images were obtained by scanning using a DRI OCT Topcon Triton (Top-con, Tokyo, Japan) with Net 6 imaging by a single trained technician. This device utilizes swept-source technology with a central wavelength of 1050 nm and can capture more than 100,000 scans/second [10]. FAZ was measured using ImageNet (Topcon Medical Systems Inc., Oakland, NJ, USA). The ELM was classified as disrupted when there were signs of any discontinuity, fusion or thickening in the scan OCT. This retrospective study included patients who underwent successful pars plana vitrectomy or combined phacovitrectomy with ILM peeling for idiopathic macula-off rhegmatogenous retinal detachment at St. Barbara Regional Specialist Hospital in Poland. OCT-A was conducted at the Department of Ophthalmology, District Railway Hospital Katowice in Poland.

The exclusion criteria for all participants were as follows: history of trauma; high myopia with an axial length of more than 26.5 mm; other retinal diseases; history of uveitis; diabetic retinopathy; optic disc abnormality; optic nerve disorder; previous intraocular surgery; low-quality OCT-A images; duration of retinal detachment from the uveal membrane longer than 2 months from the appearance of prodromal symptoms.

2.2. Surgical Procedure

All patients signed to indicate their informed consent before undergoing any surgical procedure. All eyes underwent 23-gauge, 3-port pars plana vitrectomy performed by

one vitreoretinal surgeon (A.L.-B.) with the same technique, using the same vitreoretinal machine (Constellation, Alcon, Fort Worth, TX, USA). All phakic patients underwent PPV with phacoemulsification and intraocular lens implantation. First, posterior vitreous detachment was confirmed using Brilliant Blue staining, unless core vitrectomy with increased pressure beginning at the optic disc was performed. Then, peripheral vitrectomy was performed. The next step was the injection of 0.1 mL solution of Brilliant Blue over the retinal surface for 1 min. The internal limiting membrane (ILM) was removed with forceps. The ILM was peeled in a circular fashion with a 2-disc diameter around the macula. Afterwards, fluid–air exchange and 360-degree endolaser photocoagulation were performed. Then, air–gas or air–silicone oil exchange was performed. Oil endotamponade was removed in all patients after 1–1.5 months after PPV; 5000-centistoke silicone oil was used. A corticosteroid and an antibiotic were applied after surgery for four weeks (fludrocortisone acetate + gramicidin + neomycinum, Polfa Warszawa, Warszawa, Poland) according to the recommendation.

2.3. Statistical Analysis

To analyze the results of the first observation, the average RNFL thickness, 4-quadrant RNFL thickness, central macular thickness and FAZ in the superficial and deep plexuses were calculated and displayed, with ELM structures being analyzed. Statistical analysis was performed using STATISTICA 13.3 software (StatSoft, Cracow, Poland) with a statistical significance threshold of $p < 0.05$.

The normality of the data was assessed using the Shapiro–Wilk test. In order to determine whether the differences between the groups were statistically significant, Student's *t*-test was performed for independent groups ($p < 0.05$).

3. Results

We analyzed the OCT results of 228 eyes and the OCT-A results of 114 patients (63 women and 51 men) after an observation period of 346 ± 50 days. The mean age of the group was 63.06 ± 8.96 years. Before surgery, cataracts were removed in 38 (33.33%) eyes that qualified for PPV (RRD group) surgery and in 15 healthy eyes (13.16%). In the remaining eyes, however, an artificial lens was implanted during the PPV procedure. Two types of endotamponade were used depending on the intraoperative evaluation, i.e., silicone oil (removed 1–1.5 months after PPV) in 80 eyes and 20% SF6 in 34 eyes [26,27]. Each patient underwent a complete ophthalmologic examination, including best corrected visual acuity testing using Snellen charts, slit lamp examination and OCT-A, 9 ± 2 months (median 10 months; range 6–17 months) after the operation. The characteristics of the vitrectomized eyes are summarized in Table 1.

Table 1. Characteristics of RRD group and control group.

Parameter	RRD Group	Control Group
BCVA (logMAR)	0.62 ± 0.22	0.83 ± 0.15
Age (years)	63.07 ± 8.96	
Number of eyes	114	114
Oil tamponade	80 (70.18%)	0
20% SF6 endotamponade	34 (29.82%)	0
Follow-up (days)	346 ± 50	
Pseudophakia before surgery	38 (33.33%)	15 (13.16%)
Axial length	23.41 ± 0.81	22.8 ± 0.21

BCVA—best corrected visual acuity; mean ± standard deviation.

In our study, we observed changes in the FAZ area between the RRD and control groups in the SCP ($203.65 \pm 31.69 \ \mu m^2$ vs. $215.30 \pm 35.82 \ \mu m^2$; $p = 0.28733$; non-statistically significant) and the DCP ($284.79 \pm 35.82 \ \mu m^2$ vs. $336.84 \pm 32.23 \ \mu m^2$; $p = 0.00924$; Figure 1).
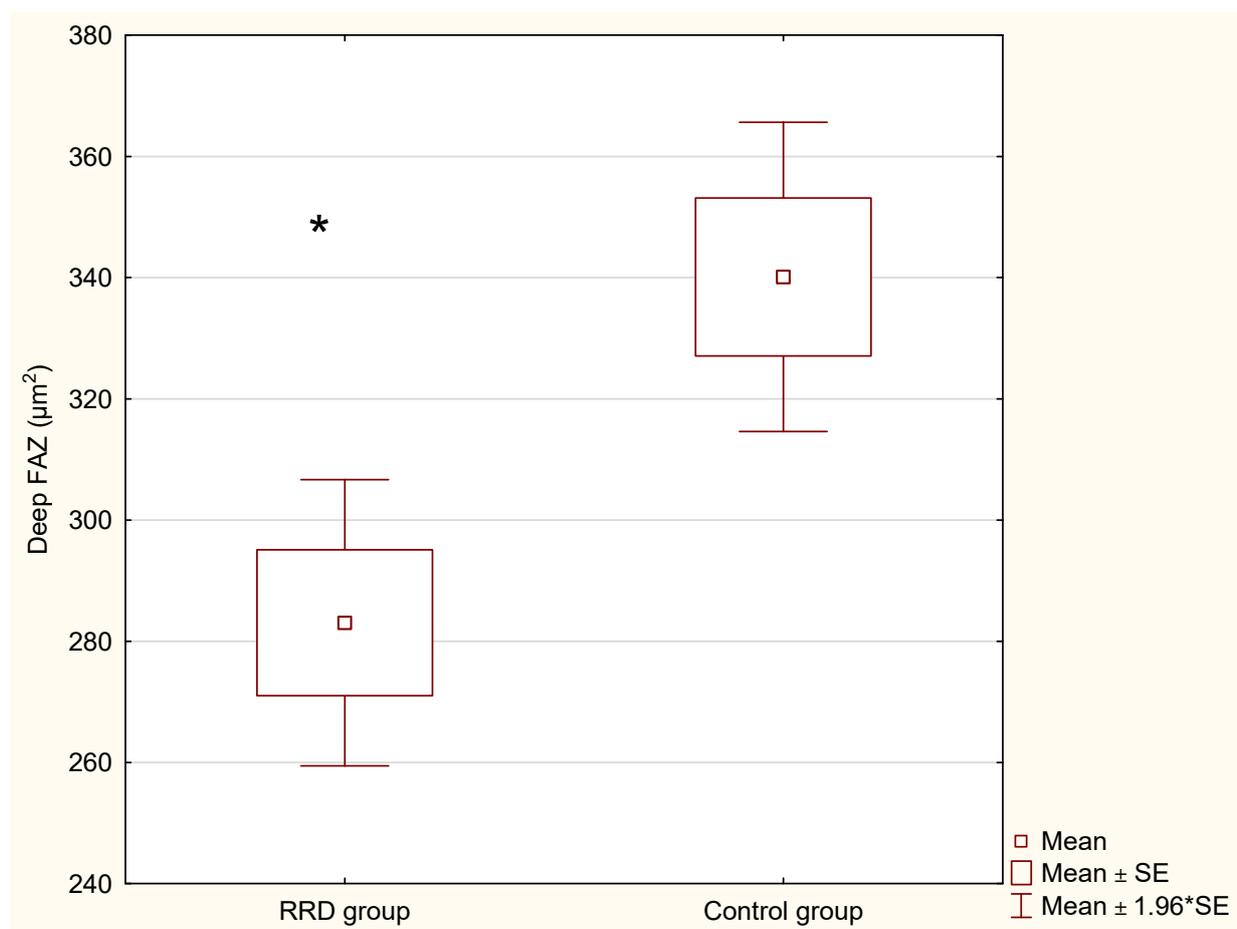
Figure 1. Changes in the deep vascular plexus of the FAZ in the RRD group and in the control group ($p < 0.05$). RRD—rhegmatogenous retinal detachment group; FAZ—fovea avascular zone; * statistically significant differences in comparison to control ($p < 0.05$).

The mean RNFL increased slightly in the post-treatment group only in the temporal quadrant, with the increase being statistically insignificant ($p = 0.104$).

The CMT in both groups exhibited similar values. On the other hand, in 78.95% of cases (90 eyes), the ELM was damaged, while in 50.00% of cases (57 eyes) it remained intact. Although the central macular thickness was normal, there was a visible diffuse edema. Furthermore, the RNFL thickness was below the norm in superior quadrants. The B-scan OCT shows a damaged ELM. The OCT-A scans of the same patient (upper row, left to right) show the en face image, superficial capillary plexus, deep capillary plexus, density map, outer retina and choriocapillaris. The vascular density map shows that the vessels in the temporal segments have lower density, which might result from the lower macular thickness in this region, as shown in the B-scan. Furthermore, the en face image shows the damage occurring to the ELM during PPV (Figure 2).

Changes in the tomography results between the groups are presented in Table 2 and Figure 1. Only differences in the deep FAZ between the RRD group and control group were found to be statistically significant ($p < 0.05$).
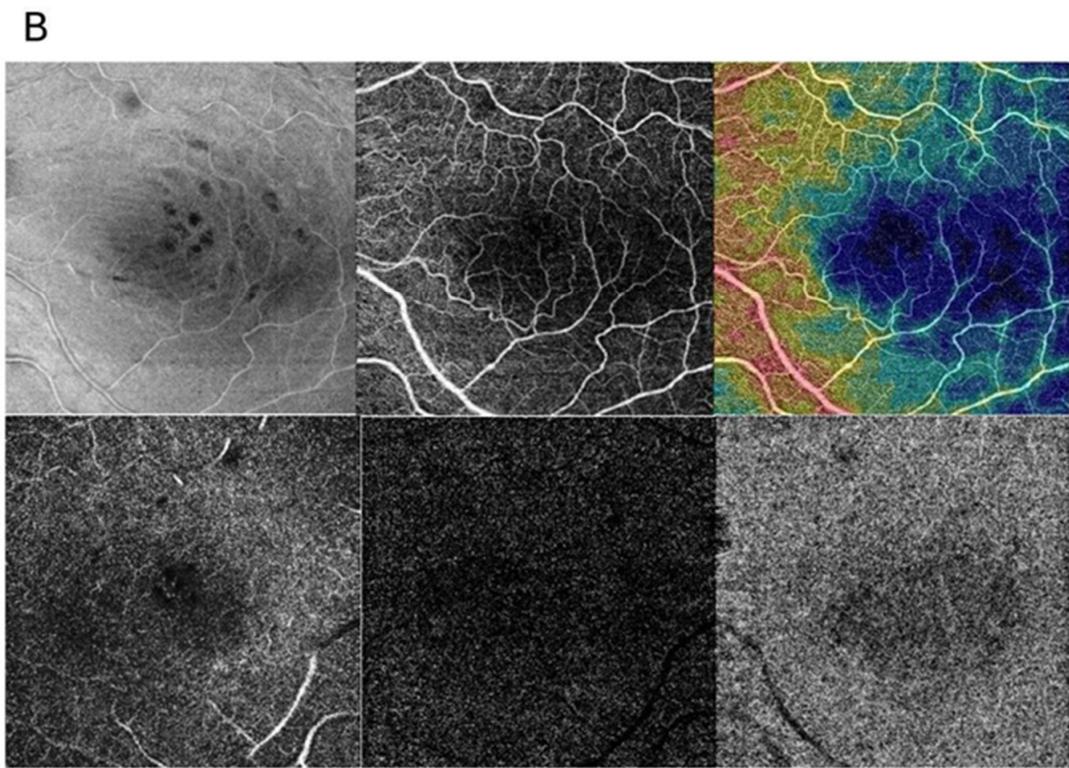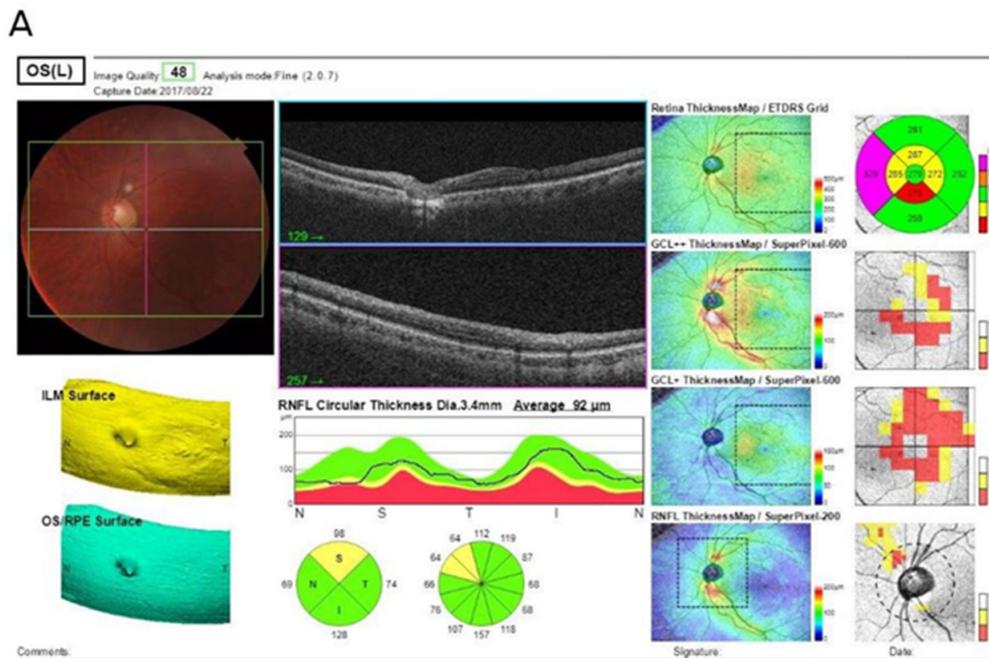
Figure 2. Comprehensive report of a patient who underwent PPV for RRD. (**A**) the OCT-A scans; (**B**) the vascular density map; PPV—pars plana vitrectomy; RRD—rhegmatogenous retinal detachment.

Table 2. Comparison of tomographic results between the groups.

Parameter	Group	Mean $\pm$ SD	$p < 0.05$
CMT (μm)	RRD	223.55 $\pm$ 11.25	$p = 0.91688$
	Control	225.29 $\pm$ 12.20	
RNFL	RRD	90.15 $\pm$ 4.04	$p = 0.19773$
	Control	82.44 $\pm$ 4.26	
Superficial FAZ (μm^2)	RRD	203.65 $\pm$ 31.69	$p = 0.28733$
	Control	215.30 $\pm$ 28.52	
Deep FAZ (μm^2)	RRD	284.79 $\pm$ 35.82	$p = 0.00924$
	Control	336.84 $\pm$ 32.23	
	RRD I	100.35 $\pm$ 6.56	$p = 0.39141$
	Control I	109.40 $\pm$ 8.12	
RNFL	RRD S	91.22$\pm$7.07	$p = 0.25636$
	Control S	104.20$\pm$8.76	
	RRD N	62.65$\pm$5.26	$p = 0.44899$
	Control N	69.07$\pm$6.52	
	RRD T	79.13$\pm$5.39	$p = 0.63863$
	Control T	75.07$\pm$6.68	

CMT—central macular thickness; RNFL—retinal nerve fiber layer; FAZ—foveal avascular zone; PPV—pars plana vitrectomy; ELM—external limiting membrane; SD—standard deviation.

4. Discussion

Experimental studies conducted on animal and human models demonstrate that apoptosis—the death of photoreceptor cells—occurs 1–3 days after retinal detachment [28–30].

Hagimura et al. [31] analyzed macular detachment in terms of macular elevation and changes that take place in an ablated retina. They determined that in the case of a slight elevation of the macula, the ablated retina was thicker, yet it did not exhibit intraretinal abnormalities. In the case of a considerable RRD with macular detachment, there were intraretinal separations of the neurosensory retina and wavy separation of external retina layers. They observed the occurrence of greater impairment of post-operative BCVA in eyes with a greater retinal plexus, despite a brief time of detachment, which suggests irreversible central retina damage [31]. This could explain why 1–2 days after macular detachment, visual acuity after surgical detachment of the retina was not higher than 20/50.

After analysis of our results, we did not determine any increase in CMT in comparison to the control group. We observed that an increased RNFL thickness occurred in the temporal quadrants, yet the increase was not statistically significant. In the remaining three quadrants, the RNFL thickness was slightly lower than in the control group. This result differs from that in currently published reports, which have indicated thinning changes in all four quadrants and, also, differences in the corresponding period in which they were observed (12–24 months) [32–35]. An increase in RNFL thickness in the temporal quadrants within 6 months after PPV with macular peeling due to ERM was observed [36], yet after this period, thinning occurred. This has been linked to the swelling of nerve fibers stemming from axonal transport disorder, leading to the apoptosis and atrophy of ganglion cells [37,38]. The maintenance of the increase in RNFL thickness in patients after PPV due to macula-off RRD may be linked to (1) intraretinal abnormalities related to macular detachment, (2) the presence of residual fluid related to RPE function disorder and (3) ILM peeling in the macular area.

ELM damage as observed in the OCT provides approximate information on the loss of nuclei of photoreceptor and Müller cells, leading to the loss of photoreceptor function, which results in reduced visual acuity in long-term observations [39]. Post-operative ELM integrity may constitute an important prognostic factor for restoration of the normal distribution of internal and external photoreceptor segments (IS/OS), being

linked to enhanced visual acuity [3]. Within our group, in the majority of tested patients, i.e., 90 persons (78.95%), the ELM integrity was disturbed; this BCVA was about 20/50 (0.624 ± 0.22).

In our study, we observed changes in the FAZ area between the RRD and control groups in the SCP (203.65 ± 31.69 μm^2 vs. 215.30 ± 35.82 μm^2; $p = 0.28733$; non-statistically significant) and the DCP (284.79 ± 35.82 μm^2 vs. 336.84 ± 32.23 μm^2; $p = 0.00924$).

This observation may also confirm the thesis that the shift and deformation of the pit may result in vascular changes [40], and macula-off retinal detachment may lead to changes being intensified in the DCP, caused by intraretinal separations of the neurosensory retina.

Another explanation for this vascular asymmetry may be the performance of ILM peeling, a procedure which, in the case of RRD, is recommended by many experienced vitreoretinal surgeons and is aimed at preventing the development of post-operative epiretinal membranes [1,41]. The ILM is a basement membrane for Müller cells, protecting retina neurons through neurotrophic factor release, which also helps neurons to thrive [41]. It has also been suggested that Müller cells play a role in regulation of the retinal blood flow and angiogenesis [41].

Our results are in line with the observations made by McKay et al. [18], who also reported lower SCP FAZ in the RRD group compared to the control. The opposite was true of the DCP FAZ, as in our study. However, the differences were not found to be statistically significant due to the relatively small size of the groups. At the same time, those authors indicated that the most likely cause of the poorer visual acuity in this group of patients was changes in vessel density (VD) in the DCP [18].

This was also confirmed through studies by Bonfiglio et al. [19], who also showed a lower VD in the RRD group after PPV with gas tamponade compared to the control group [19]. Additionally, Tsen et al. [T15.] showed a statistically significantly smaller area of both the SCP and the DCP in eyes with RRD compared to the control, which is consistent with our observations. In addition, it was noted that in the group of patients who underwent only PPV, they were characterized by higher post-operative vessel density and larger SCP and DCP area in the choroid compared to vitrectomized and scleral buckle eyes [15].

The observations in the study of Wang et al. are also interesting [14], in which macular perfusion changes were assessed in 14 patients (14 eyes) after 23-guage PPV with gas tamponade using OCT-A compared to fellow unaffected eyes. The superficial capillary plexus flow density, deep capillary plexus flow density and choriocapillary plexus flow density were characterized in the RRD group. It should be noted, however, that these investigators found that the FAZ area was significantly larger in the eyes with RRD after the 2-month post-operative period compared to the control eyes [14]. As a consequence, macular hypoxia continued. Furthermore, Woo et al. [20] noted that the superficial and deep FAZ area was significantly larger in the RRD group compared to the control group in a study of 34 patients (34 eyes). The authors suggested that the enlargement of the FAZ area in the RRD group compared to the control group may be due to ischemic damage to the retinal capillary plexus in the fovea [20].

These two observations contradict our results, where the superficial and deep FAZ area was greater in the control group than in the study group. It should be noted, however, that the size of the groups in our study was larger than in the abovementioned studies, which may explain the discrepancy. It should also be remembered that in the studies of Wang et al. [14] and Woo et al. [15], all patients enrolled in PPV underwent gas endotamponade. Meanwhile, in our study, patients received either gas endotamponade (80 patients) or silicone oil (34 patients), which may have had an impact on the obtained results. It should be remembered that the utilization of oil tamponade is not just associated with benefits for the patients but also with side effects. Raczyńska et al. [42] analyzed the influence of silicone tamponade on the ganglion cell complex, engaged in the transmission of visual information from the retina to the brain. They determined that in a group of 57 patients for whom PPV was conducted due to rhegmatogenous retinal detachment (RRD) where sili-

cone oil was utilized, a reduction in the ganglion cell complex was observed; additionally, a deterioration in vision was observed. They also indicated that determining the number of ganglion cells should be the gold standard of determining an improvement or deterioration in vision after eye surgery with silicone oil tamponade [42]. Furthermore, Inoue et al. [43] assessed the influence of perfluorocarbon liquid (PFCL) and silicone oil on human retinal pigment epithelium (RPE) cells and retinal ganglion cells (RCGs), except under in vitro conditions. They observed that PFCL and silicone oil decrease the vitality of RPE cells after 7 days of exposure. Moreover, they also determined that the damage caused by PFCL had a cytotoxic character, and that of silicone oil was mechanical [43], whereas in our research, we did not indicate a cytotoxic effect of oil endotamponade, which may be related to the fact that the silicone oil was removed after a maximum period of 1.5 months in all patients, among other factors. Of course, in light of the abovementioned research [42,43], it seems that a valuable supplement to the research would be the inclusion of a group of patients in whom oil endotamponade or gas would be removed at a later time.

Furthermore, Christou et al. [44] showed a lower VD in SCPs among 23 RRD patients who underwent PPV and applied gas endotamponade compared to the control. The fellow eye was used as a control. It is true that the SCP FAZ in the RRD group was greater than that in the control group, i.e., different than in our study, but the differences were not statistically significant ($p > 0.05$) [44].

Chatziralli et al. [45] assessed changes in the retinal microvasculature in 89 RRD patients who underwent a PPV and gas endotamponade procedure without internal limiting membrane peeling. As a control, they used eyes from healthy volunteers rather than the patient's healthy eye, as in our study. On the basis of the results from the OCT-A study, they found a statistically significant enlargement of the SCP FAZ and DCP FAZ in the study group after surgery compared to the control ($p < 0.05$). The differences in the scores obtained by Chatziralli et al. [45] and our group may result from the difference in the selection of control groups. Therefore, to address these discrepancies, one more control group should be included in future study [45].

In another study, Chatziralli et al. [46] showed that age, duration of RD, presence of proliferative vitreoretinopathy (PVR), central retinal thickness (CRT) and condition of the ellipsoid zone (EZ) and the ELM should be included among the independent factors affecting visual acuity after PPV surgery in patients with RRD [46].

Informing patients about the factors affecting BCVA will help to better visualize their prognosis after surgery, and this will be helpful in managing the patients. Furthermore, considering the conducted statistical analysis and its results, it would be appropriate to increase the size of the groups. Moreover, an interesting supplement to our research would be the inclusion of a control group comprising healthy volunteers.

Thus, performing ILM peeling in eyes after RRD may result in reduced flow in the capillaries of the internal retina. The possibility for a non-invasive retinal examination provides us with new information on the healing process of this highly important eye structure [46].

The factors that limit our work and results include the retrospective nature of the research presented in this paper. Next, we plan to conduct a prospective study that will include a control group comprising non-peeled eyes. In addition, at a later stage, vessel density analysis should be carried out, and the size of the groups should be increased. Nevertheless, the results obtained, and conclusions drawn from our study, are important, although further research is necessary.

Changes in the FAZ occurred in the study group and were more prevalent in the DCP. After surgical detachment of the retina, not only do anatomical changes take place but, most importantly, the action that is responsible for acquisition of a renewed neurosensory retina with retinal pigment epithelium (RPE) also occurs, resulting in enhanced visual acuity. This depends on numerous factors, the analysis of which has allowed for improved prognostic evaluation of individual cases of RRD in the last decade due to the presence of OCT. This might be related to changes in the neurosensory retina after macular peeling.

5. Conclusions

OCT-A is a promising method allowing for non-invasive visualization of the vascular network of the retina, which constitutes a very important component of the repair and nutritional processes in the course of different retinal diseases. A better understanding of the anatomical and hemodynamic changes taking place in the retina after macula-off RRD might be helpful to answer the question as to why the BCVA in these cases is "only" or "as much as" from 0.4 to 0.1.

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Review

Prevention of the Onset of Age-Related Macular Degeneration

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Abstract: Age-related macular degeneration (AMD) represents the leading cause of irreversible blindness in elderly people, mostly after the age of 65. The progressive deterioration of visual function in patients affected by AMD has a significant impact on quality of life and has also high social costs. The current therapeutic options are only partially able to slow down the natural course of the disease, without being capable of stopping its progression. Therefore, better understanding of the possibilities to prevent the onset of the disease is needed. In this regard, a central role is played by the identification of risk factors, which might participate to the development of the disease. Among these, the most researched are dietary risk factors, lifestyle, and light exposure. Many studies showed that a higher dietary intake of nutrients, such as lutein, zeaxanthin, beta carotene, omega-3 fatty acids and zinc, reduced the risk of early AMD. Regarding lifestyle habits, the association between smoking and AMD is currently accepted. Finally, retinal damage caused by ultraviolet rays and blue light is also worthy of attention. The scope of this review is to summarize the present knowledge focusing on the measures to adopt in order to prevent the onset of AMD.

Keywords: age-related macular degeneration; prevention; nutrients; lifestyle

1. Introduction

Age-related macular degeneration (AMD) represents nowadays the leading cause of irreversible blindness in elderly people, mostly after the age of 65 [1]. Recent studies predict that by 2040, the number of affected individuals worldwide will be circa 288 million [2]. In addition, the progressive increase in life expectancy of the world's population and, in particular, that of developing countries, will lead to a further increase in the elderly population [3]. By 2025, it has been estimated that the geriatric population will reach the amount of circa 1.2 billion people worldwide, 70% of whom residing in developing countries [4]. This process may contribute strongly to a steady increase in the number of people suffering from AMD, even greater than that assumed by previous studies.

AMD is a retinal disease that typically affects the macula, causing progressive loss of central vision [5]. Early-stage AMD is mainly characterized by clinical signs such as drusen and alterations of the retinal pigment epithelium. Late-stage AMD can assume two different forms: neovascular, also known as wet or exudative, or non-neovascular, also named as atrophic, dry, or non-exudative. AMD progression to late stages results in loss of central vision, leading to severe and persistent visual impairment and legal blindness, thus resulting in a significant impact on the quality of life and also hindering the functional independence of affected individuals [6].

Early AMD is clinically characterized by typical disturbs, such as mild central distortion and reduced reading capacity with decreased luminance. Difficulty to clearly recognize faces and central scotoma are also additional signs that can appear. Nevertheless, in this stage, affected people are mostly asymptomatic. Late AMD may progress to a sudden and rapid deterioration in case of a neovascular form, while a slow and gradual decline of central visual function is typical of the atrophic form. The main feature of neovascular AMD (nAMD) is the presence of choroidal neovascularization and its repercussions, such

as intra- and subretinal fluid, hemorrhages, pigment epithelial detachments, hard exudates and, finally, fibrotic tissue. Geographic atrophy is characterized by outer retinal thinning, and it has been evaluated that the progression rate corresponds to circa 2 mm^2/year on average [7]. It is important to point out that, according to the previous reports and classifications, what will be described regarding the prevention of AMD is relative to the "drusen-driven" AMD, without considering the spectrum of pachychoroid diseases.

Nowadays, although the use of intravitreal anti-vascular endothelial growth factor VEGFinjections has contributed considerably to improve the treatment of nAMD, no effective treatment has been yet found for both early AMD and geographic atrophy. The absence of a therapy that might at least stop the unavoidable progression of the clinical course determines a functional limitation in daily activities and, consequently, a worsening of the quality of life for the patients.

In the last years, many researchers have tried to define modifying risk factors, especially how to address retinal oxidative changes related to both onset and progression of the disease. In particular, positive behavioral modifications including smoking cessation, exercise, and healthy diet with the addition of nutritional supplements [8,9].

The attention of these studies has been mainly focused on the ability to intervene on the progression of early AMD towards more advanced stages of the disease and geographic atrophy, thereby modifying the aforementioned risk factors.

The aim of this review is to focus the attention on all the modifiable risk factors that might help to prevent, slow, or reduce the onset of AMD in healthy individuals.

2. Prevalence and Incidence of AMD

The most relevant information regarding the epidemiology of AMD has been provided by three large, population-based studies: the Blue Mountains Eye Study (BMES), Beaver Dam Eye Study (BDES), and Rotterdam Study (RS), in which data were collected and analyzed, mainly related to both incidence and prevalence of AMD in white populations [10].

The above-mentioned studies have found a prevalence of late AMD of 0.2% for people aged between 55 and 64 years, while an increased prevalence (13.1%) has been observed for people over 85 years [11]. Specifically, BMES demonstrated for early AMD a 15-year incidence of 22.7% and an incidence of 6.8% for late AMD, with an overall incidence greater in women rather than men [12]. At the same time, a meta-analysis from the European Eye Epidemiology Consortium including 14 population-based cohort studies evidenced a prevalence of 13.2% for early AMD and 3.0% for late AMD for people with more than 70 years [13].

Another large meta-analysis reported a higher prevalence of early AMD in European white people when compared with Asian population (11.2% and 6.8%). Moreover, in comparison to African people, there is a major prevalence of both early and late AMD for European population, while no differences have been found between African and Asian populations. On the contrary, the prevalence of nAMD has been estimated to be similar in all ethnic groups (0.46%) [2].

Regarding the prevalence of AMD among different populations, it is noteworthy to underline that both population-based and the Age-Related Disease Eye Study AREDS studies were performed before the concept of pachychoroid spectrum diseases had been widely recognized. Specifically, the difference between pachychoroid neovasculopathy and "drusen-driven" neovascular AMD has not been exhaustively analyzed. Pachychoroid spectrum diseases are much common in Asian compared to Western populations. Asians currently constitute 60% of the world's population and likely will contribute most greatly to the global prevalence of AMD by 2040. Before the introduction of the concept of pachychoroid spectrum diseases, pachychoroid neovasculopathy had often been diagnosed as AMD especially in Asian populations. This may partly explain why in the previous population-based studies, there was no difference in the prevalence of neovascular AMD in different ethnic groups even though early AMD is more common in Western than in Asian populations [14].

The different prevalence of non-exudative AMD among different ethnic groups may be partly explained by genetic differences. With regard to this, a genetic study has demonstrated a lower frequency of the C-risk allele of the Y402H polymorphisms in Japanese as compared with white populations [15].

3. AMD Impact on Quality of Life and Social Costs

AMD may reduce quality of life with a significant impact on the performance of normal daily activities. The burden of AMD in terms of health has been analyzed with the aim of the disability-adjusted life years (DALY), which represents an indicator that measures the loss of years of life due to AMD [16,17].

Reports exhibited increased levels of life stress and depression in patients affected by AMD compared to healthy subjects, mostly when results of treatment do not correspond to patients' expectations [18,19]. In addition, it has been demonstrated in patients affected by AMD a greater risk of functional disability, as showed by BMES, where a two times higher risk of negative effects on daily living activity was observed [20].

An interesting paper has evaluated the correlation between AMD and the risk to develop a form of cognitive impairment, including Alzheimer's disease, throughout the course of life. The researchers found an increased risk for AMD patients to suffer from cognitive impairment and Alzheimer's disease, mostly in case of atrophic AMD [21].

Finally, it has been estimated by a large meta-analysis that AMD is associated with a 20% increased risk of overall mortality and, specifically, a 46% increased risk for cardiovascular disease [22].

A deepened understanding of global patterns in health burden related to AMD is fundamental to implement focused strategies of prevention. In this regard, a recent study has estimated the social costs of blindness related to AMD in 2020 in the United States. Specifically, the authors analyzed excess costs that occur because of blindness, measuring the differences in total costs between blind and non-blind individuals. They found that the annual amount of excess costs for each blind individual is about USD 5000. Translated to the whole society, it means a total societal cost of circa USD 20 billion, a value that is estimating to triple by 2050 [23].

In the last 25 years, the health burden of AMD has continuously grown without pause worldwide. Despite the introduction of anti-VEGF therapy that has radically improved the clinical history of at least exudative AMD, the DALY due to AMD has not accordingly improved [16]. This process has been exhaustively analyzed by previous reports across countries, which estimated that the proportion of individuals affected by both visual impairment and blindness due to AMD raised by 81% and 36% between the years 1990 and 2010, respectively [24]. These observations demonstrate that the socio-economic burden related to AMD has not been relieved with the introduction of an effective therapy. For this reason, due to the challenge determined by the increase of both prevalence and burden of AMD, it may be necessary for the next years to arrange additional resources on a global scale to fight this phenomenon.

4. AREDS Studies: Interventions to Stop AMD Progression

A key role in the pathophysiology of AMD progression is played by oxidative stress. Cellular aging is mainly characterized by the production of oxygen free radicals, which eventually leads through various mechanisms to cell death. The retina has a proper defensive mechanism against oxidative processes, which consists of vitamins C and E, carotenoids, lutein, and zeaxanthin [8]. In addition, the presence of a major structural lipid, docosahexaenoic acid (DHA), at the level of cones, is involved in membrane permeability and acts against the formation of new vessels [25]. Based on these abovementioned findings, researchers have made attempts over the years to develop a therapy with nutritional supplements that can hinder an unstoppable process, such as AMD.

As early as the late 1990s, attempts have been made to evaluate the impact of adding nutrients to diet that could somehow slow or stop the progression of the disease toward

blindness [26]. In 1988, Newsome et al. [27] demonstrated that a diet with zinc supplementation might reduce the visual acuity loss in patients affected by AMD. In addition, other researchers assessed the efficacy of vitamin formulations containing zinc and antioxidants for AMD, finding contrasting results [28].

The most important reports relative to the nutritional supplementation therapy have been shown by the two AREDS studies [29,30]. The AREDS study assessed the effect of high-dose vitamins C and E (500 mg and 400 IU, respectively), β-carotene (15 mg), and zinc supplementation (80 mg) on AMD progression. The patients included in the study were divided into four groups based on disease's gravity, ranging from early to advanced AMD. The study demonstrated that AREDS supplementation reduced the risk of AMD progression as compared to placebo. However, there was no significant effect on the risk of visual acuity loss. It was also recommended that nutrition supplements should only be used by people who do not smoke, because subsequent analyses of other studies has found a greater incidence of cancer in smokers or recent ex-smokers who assumed β-carotene [31].

The AREDS-2 clinical trial [30] was conducted with the aim to ameliorate the efficacy of the AREDS formulation. For this reason, a new formulation with the addition of lutein and zeaxanthin, which has the maximum concentration at the fovea, plus DHA and EPA (docosahexaenoic and eicosapentaenoic acid) was evaluated in order to find a therapy for decreasing the risk of AMD progression. Moreover, the new study also assessed the effect of the new formulation after eliminating β-carotene and adjusting the dose of zinc to only 25 mg. Therefore, it has been proved that the risk of AMD progression was not more reduced as compared to the previous AREDS study, and, in addition, it was suggested that the first formulation should have included lutein and zeaxanthin, without β-carotene supplementation. Nowadays, the recommended formulation based on AREDS-2 results for dry AMD contains vitamin E (400 IU), vitamin C (500 mg), lutein (10 mg), zeaxanthin (2 mg), copper (2 mg), and zinc (80 mg, but also available with the 25 mg formulation) (Table 1).

Table 1. Daily nutritional supplementation based on the results of AREDS-2 study.

AREDS-2 Formulation	
Nutritional Supplement	**Recommended Daily Dose**
Vitamin E	400 IU
Vitamin C	500 mg
Lutein	10 mg
Zeaxanthin	2 mg
Copper	2 mg
Zinc	80 mg

5. Prevention of AMD Onset: Dietary Modification and Nutritional Supplementation; Smoking and Lifestyle Modifications; Role of Blue-Light, UV Radiation, and Intraocular Lenses

5.1. Dietary Modification and Nutritional Supplementation

Both AREDS-1 and -2 studies have shown to prevent AMD progression by nutritional supplementation. However, there are no data regarding the possibility to prevent the onset of AMD, either by modifying dietary habits or by adding nutritional supplements to the diet.

The Rotterdam study of 2005 [32] evaluated whether the intake of antioxidants with the diet was associated with a reduced risk to develop AMD. A questionnaire was used to estimate the dietary intake for a total of 5836 people at risk of AMD, but without signs of pathology in either eye. Specifically, at baseline, participants had to complete a checklist at home that queried foods and drinks they had consumed at least twice a month, dietary habits, use of nutritional supplements, and prescribed diets. Finally, these data were assembled and transformed to total energy intake per day with the computerized Dutch Food Composition Table [33]. The study found that a high consumption with the diet of

β-carotene, both vitamins C and E, and zinc was correlated with a decreased risk of AMD in elderly persons.

The formation of drusen is mainly influenced by oxidative protein modifications, as shown by previous studies [34]. Since the onset of drusen is the first clear visible sign of AMD, it is logical to argue that the action of antioxidant molecules exerts its maximum effectiveness just before the onset of the disease. Therefore, as suggested by the Rotterdam study, a diet rich in antioxidants might hinder the onset of AMD and it should be recommended mostly for those people with a strong family history [35]. Nutrient sources of vitamin E are whole grains, vegetable oil, eggs, and nuts. A higher intake of zinc can be achieved by consuming meat, poultry, fish, whole grains, and dairy products. β-carotene can be found in carrots, kale, and spinach. Citrus fruits and juices, green peppers, broccoli, and potatoes are the main suppliers of vitamin C (Table 2).

Table 2. Nutrients and their food sources capable of preventing the onset of AMD.

Nutrients and Food Sources for AMD Prevention	
Nutrients	**Food Sources**
Vitamin C	Citrus fruits and juices, green peppers, broccoli, potatoes
Vitamin E	Whole grains, vegetable oil, eggs, nuts
β-Carotene	Carrots, kale, spinach
Zinc	Meat, poultry, fish, whole grains, dairy products
Lutein and Zeaxanthin	Chicken egg yolk, leafy green vegetables
Omega-3 (DHA and EPA)	Fish and seafood, nuts and seeds, plant oils

Note: Dietary modifications have demonstrated to be effective only in studies considering Western populations. Thus, these modifications do not necessary apply to people of other ethnicities.

Recently, the EYE-RISK Consortium [36] investigated the relationship between the adherence to the Mediterranean diet (MeDi) and the incidence of advanced AMD in two European population-based prospective cohorts: the Rotterdam Study 1 (RS-1) [37] and the Antioxydants, Lipides Essentiels, Nutrition et Maladies Oculaires (Alienor) [38]. Specifically, compliance to MeDi was assessed with the aim of a specific a score based on consumption of vegetables, fruits, legumes, cereals, fish, meat, dairy products, and alcohol, and the monounsaturated-to-saturated fatty acids ratio. The authors concluded that a regular adherence to the MeDi was correlated with a 41% reduction of the risk to develop advanced AMD. These findings support the role of a diet rich in healthful nutrient-rich foods such as fruits, vegetables, legumes, and fish for preventing AMD onset. In addition, the study has suggested that also reducing unhealthful foods, such as red and processed meats and savory and salty industrialized products, might give a strong contribution to prevent the onset of AMD.

The rationale behind the choice to analyze a specific type of diet, such as the Mediterranean one, can be explained by the fact that a single nutrient or food approach is not able to reach synergistic effects of food and nutrients consumed in combination in the diet. High intake of plant foods and fish, lower intake of meat and dairy products, olive oil as the main source of fat, and a moderate consumption of wine represents the cornerstones of MeDi [39] (Table 3). The results of the EYE-RISK Consortium are also in accordance with those of previous cross-sectional studies. For example, the Carotenoids in Age-Related Eye Disease Study (CAREDS) study has demonstrated a decreased prevalence of early AMD in American women who have strongly adhered to the MeDi [40]. In addition, reports from the Coimbra study have shown a reduced prevalence of any type of AMD in participants who properly adhered to the MeDi [41]. Lastly, the European Eye Study has proven that high levels of MeDi score are associated with a lower prevalence of nAMD [42].

Table 3. Mediterranean diet: a guide to daily food choices.

Mediterranean Diet	
Food Types	**Frequency of Food Servings**
Whole grains, bread, beans, legumes, nuts, and seeds	Daily (35% of total daily calories)
Vegetables and fruits	Daily (30% of total daily calories)
Extra virgin olive oil	Daily (10% of total daily calories)
Fish and seafoods	Few times per week (20% of total daily calories)
Diary products, eggs, poultry, and yoghurt	
Meat, sweets	Small amounts (5% of total daily calories)

Note: Dietary modifications have demonstrated to be effective only in studies considering Western populations. Thus, these modifications do not necessary apply to people of other ethnicities.

The role of lutein and zeaxanthin has been exhaustively studied in the last years not only for their properties to slow down the progression of AMD, but also to assess their capability for preventing AMD onset [43]. Delcourt et al. [44] conducted a prospective study on a total of 640 individuals with age over 60 who were affected by different forms of AMD to ascertain whether there exists an association between dietary lutein and zeaxanthin intake and the manifestation of the disease. The authors found a strong inverse correlation between serum levels of lutein and zeaxanthin and the presence of AMD, suggesting a protective role played by the two xanthophylls. These results have been later confirmed by the study of Huang et al. [45], which revealed that participants with high levels of serum lutein and zeaxanthin had a 79% reduced risk to develop AMD in comparison to subjects with lower serum concentration. Furthermore, the prevention role carried out by the xanthophylls was assessed in The Blue Mountains Eye Study [46], where it has been demonstrated that persons who assume higher amounts of lutein and zeaxanthin show a reduced risk to develop drusen or AMD. Dietary sources of lutein and zeaxanthin are represented by chicken egg yolk and leafy green vegetables, whose main sources are kale, parsley, spinach, and broccoli (Table 3). Moreover, it is possible to find trace amounts of the two xantophylls in wheat and grain products, like corn, einkorn wheat, and durum wheat.

High food consumption of omega 3 long-chain polyunsaturated fatty acids with the diet, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), has been demonstrated to be linked with a reduced risk of AMD onset [47,48]. Omega 3 fatty acids allow the continuous renewal of RPE cells and, if absent, may contribute to degradation of photoreceptor structures and accumulation of drusen at the level of RPE [49]. Specifically, it has been demonstrated that high DHA levels of oily fish are correlated with less risk to develop choroidal new vessels [50]. In addition, the Nutritional AMD Treatment 2 (NAT2) Trial revealed that individuals with high blood levels of both EPA and DHA were significantly protected against AMD as compared to those with lower content of omega 3 [50,51]. However, a Cochrane meta-analysis asserted that there is no evidence to support that nutritional supplementation with omega 3 is able to prevent the onset of AMD [52]. Despite the discordant results that emerged in the scientific literature and the recent Cochrane meta-analysis, it might be reasonable for the physician to inform the patients about the positive effects of consuming fatty fish or omega 3 fatty acid supplements to prevent AMD onset.

Calcium regulation plays an important role in AMD onset, just as for other neurodegenerative diseases, including glaucoma, Alzheimer's, and Parkinson's disease [53]. The association between daily calcium intake and AMD onset has been extensively analyzed by Kaigi et al. in a cross-sectional study [54]. The authors found that individuals who consumed daily more than 800 mg of calcium showed a significantly higher probability to have AMD, although a dose relationship was not observed. It is noteworthy that the 800 mg/day cut off is lower than the daily intake of calcium for men and women in USA [55]. For these reasons, it should be necessary to conduct other studies in order to better evaluate the relation between high calcium consumption and AMD onset.

5.2. Smoking and Lifestyle Modifications

Cigarette smoking has been associated with the onset of AMD and represents a strong predictor of disease in two population-based longitudinal study [56]. A review by Thornton et al. [57] came to the conclusion that cigarette smoking represents a fundamental risk factor for the AMD onset, estimating with precision that the risk is circa 2- to 3-fold higher in smokers in comparison to nonsmokers. The EUREYE study [9] is a cross-sectional study that analyzed the relationship between cigarette smoking and both type and bilaterality of AMD in individuals aged 65 and older in European countries with the use of a single protocol for eye examinations and the evaluation of risk factors. The study reported an increased risk of neovascular AMD for smokers, and the attributable factor for AMD due to smoking was 27%. Interestingly, when compared with subjects affected by unilateral AMD, those with a bilateral form of AMD were more likely to be heavy smokers in the previous 25 years. Based on these findings, it is noteworthy to underline the possible risks to develop different forms of AMD associated with smoking and the benefit of quitting smoking. In particular, patients unilaterally affected by AMD who are current smokers should be informed about the possible risk to develop the disease in the other eye. A report of the Beaver Dam Eye Study [58] examined the association of pack-years smoked to incidence of AMD, showing that a greater number of pack-years smoked was associated with an increased risk of transforming from no AMD to minimal early AMD. The precise mechanism that determines the onset of AMD due to smoking has not yet been elucidated, but it seems that oxidative stress and alterations of both retinal and choroidal blood flow are involved [59].

Excessive or reduced sleep duration has been related with various negative health conditions, such as total mortality, cardiovascular diseases, diabetes, and arterial hypertension [60]. In this regard, Khurana et al. [61] studied the association between sleep patterns and AMD. A total of 1003 patients were surveyed for past sleep histories with the aim of a specific questionnaire and the patients' retina graded as no AMD, early AMD, neovascular AMD, and geographic atrophy. The authors concluded that longer sleep duration (more than 8 h) was correlated with a 7-fold higher risk of geographic atrophy. Nevertheless, it is also relevant to underline how it is only an association and not a cause–effect relationship. However, in order to take action to prevent the onset AMD it is important to consider the possibility that excessive sleep duration may contribute to the onset of AMD and, specifically, geographic atrophy. In addition, Perez-Canales et al. [62] investigated the association between self-reported sleep duration and neovascular AMD (nAMD). Four categories of sleep duration were identified: <6 h, 6–7 h, 7–8 h, and >8 h. The study demonstrated a higher prevalence of short sleep duration (<6 h) in subjects affected by nAMD as compared with age- and sex-matched controls. Moreover, the researchers observed a trend towards an increased risk to manifest nAMD in individuals with short and long sleep duration in comparison to those in the 7–8 h group, indicating a possible U-shaped relationship between sleep duration and nAMD.

Recent reports have recognized excess body weight and obesity as fundamental risk factors for cardiovascular disease [63]. Furthermore, overweight may determine different physical changes, such as a higher level of oxidative stress and inflammatory processes and an imbalance of blood lipids, all factors which are implicated in the pathophysiological mechanisms of AMD onset [64]. Previous studies proved that excess body fat can alter the transport and deposition of carotenoids from blood to macula, thus leading to a reduction of macular pigment levels at the fovea [65]. In the AREDS study, an increased risk of geographic atrophy was observed in individuals with higher BMI. Interestingly, Zhang et al. [66] evaluated the association between categories of body mass index (BMI) and AMD risk in different stages. Seven prospective cohort studies with 1613 cases identified among 31,151 subjects have been analyzed. The authors concluded that excess body weight and obesity are associated with a higher risk to develop AMD and, above all, it has been demonstrated that people affected by obesity have a major risk to manifest late AMD in comparison to subjects with a normal weight. Moreover, a potential linear

relationship between BMI and AMD risk was found, suggesting that maintaining normal body weight and preventing excessive weight gain might protect against the onset of AMD. Nevertheless, it has been demonstrated that the waist–hip ratio (WHR) and waist circumferences represent more reliable indicators of abdominal obesity compared to BMI. Further to this point, a decrease in WHR was found to be associated with reduced risk of AMD [67].

An increasing number of scientific reports evidence the positive influence of physical activity on morbidity and mortality, a better preservation of cognitive functions, and reducing biomarkers of aging [68]. Regarding the relationship between physical activity and AMD, studies demonstrated a protective role of physical activity towards AMD onset [69,70]. Nevertheless, current evidence is inconclusive because previous studies were conducted in nonhomogeneous populations with different systems of AMD classification and, in addition, the value of physical activity was not assessed uniformly. A recent systematic review and meta-analysis [71] investigated the association of physical activity and AMD among Caucasian population. The authors concluded that physical activity was associated with lower odds of both early and late AMD among Caucasians, suggesting that staying active throughout life might prevent the onset of AMD. The amount of activity considered as an active lifestyle was as little as three hours of moderate- to low-intensity physical activity per week, which reinforces the concept that also a small amount of physical activity may protect against the development AMD. It has been demonstrated that regular exercise might ameliorate both the activity of antioxidant enzymes and resistance to oxidative stress [72]. For these reasons, the increase in oxidative resistance resulting from moderate physical activity might lead to AMD prevention.

In conclusion, a recent review revealed that a healthy diet without food rich in sugar, fat, alcohol, refined starch, and oils, absence of smoking, and moderate physical activity are associated with a reduced risk of AMD [50]. Moreover, interestingly, the risk reduction was found greater when multiple lifestyles were considered together.

Public health interventions with the aim to adopt a correct lifestyle, including healthy diet, physical activity, and cessation of smoking, should be recommended strategies for AMD prevention (Table 4). These suggestions are crucial for people with genetic risk and also a family history of AMD.

Table 4. Lifestyle modifications suggested to reduce the risk of AMD onset.

Lifestyle and AMD Prevention	
Activity/Condition	**What to do to Prevent AMD Onset**
Smoking	Cessation
Sleep duration	7–8 h per night
Weight	Reducing obesity and overweight to normal Body Mass Index BMI values (18.5–24.9); reducing waist circumferences
Physical activity	At least three hours of moderate- to low-intensity physical activity per week

5.3. Role of Blue-Light, UV Radiation and Intraocular Lenses

The scientific literature has clearly shown that excessive exposure to light may damage surface tissues, such as the eye and the skin. For this reason the damage determined by sunlight exposure can also be involved in the onset of AMD [73]. In this regard, a fundamental role in the process of tissue damage is played by the so-called "blue light". The term blue light refers to a high-energy, short-wave visible light of 400–500 nm. It has been demonstrated that blue light is able to induce a photochemical damage of the retina because of the existence of photosensitizers in this specific wave-band [74]. Human healthy adults have developed special defense systems to protect eye structures from blue light damage. Indeed, visible light passes through the crystalline lens and macular pigments, which have the function to absorb blue light. The crystalline lens has the capability to

absorb higher amounts of both ultraviolet (UV) radiations and visible short-wave light, becoming yellow and accumulating oxidative damage with age, furnishing an increased protection from blue light [75]. In addition, the macular pigments, such as meso-zeaxanthin and lutein and zeaxanthin from dietary intake, represent natural intraocular filters that are able to absorb wavelengths between 400 and 520 nm, with an absorption peak at 460 nm [76]. High-energy UV radiation and blue light are mainly present in natural sunlight, while sources for UV radiation are represented by welding and UV lamps, and artificial sources for blue light include electronic devices and indoor lights [77].

The sun produces three different types of UV rays: UVA, UVB, and UVC. They have a wavelength, respectively, between 320 and 400 nm, 290 and 320 nm, and with a range of 200–290 nm [78]. UVB rays are those that can more likely cause damage to the retina and promote the onset of AMD. They target retinal pigment epithelium RPE cells with various mechanisms, such as direct DNA damage, oxidative stress, and activation of several pathways, among which are NLRP3, MAPK and JAK/STAT, which lead to inflammation, apoptosis, and cell death [79].

The introduction of blue light-filtering (BLF) intraocular lenses (IOLs) has allowed to have an intraocular lens able to filter short-wave light and to selectively decrease the transmission of UV radiation. It has been observed that IOLs not capable to absorb UV radiation might lead to significant retinal damage and, thus, facilitate AMD onset [80]. The addition of short-wave light filtering differentiates BLF-IOLs from other lenses because the presence of this filter makes them more structurally similar to the normal crystalline lens [81]. The rationale behind the use of BLF IOLs is that replacing the cataractous natural lens with an IOL without blue-light filter causes an unnatural condition, in which a high amount of blue light is transmitted inside the eye, producing the release of A2E, a short-wave photosensitizer that could damage the retina and RPE cells [82].

Regarding the possibility of preventing AMD onset, an interesting study has been published by Nagai et al. [83]. The authors examined the influence of BLF IOLs in the development of AMD, measuring changes in fundus autofluorescence after implantation of BLF (yellow-tinted) and UV-filtering (colorless) IOLs. They observed a significantly higher incidence of AMD in patients who received a UV-filtering IOL (11%) as compared with those where a BLF IOL was implanted (2%) 2 years after the implantation. These results show how it may be possible that the use of BLF IOLs could prevent the onset of AMD.

Despite the strong pathophysiological basis and studies that have demonstrated a favorable role of BLF IOLs in protecting against the onset of AMD, other data contradict these conclusions. For example, a recent analysis of systematic reviews about BLF IOLs for retinal protection has shown that benefits of using BLF IOLs are not currently supported by the best available research evidence, suggesting that surgeons keep in mind this limitation when adopting these devices in clinical practice [84]. In addition, recently, Achiron et al. [85] has assessed the effect of BLF IOLs on the prevention of nAMD after cataract surgery. In a large cohort study, including 11,397 eyes of 11,397 patients who received BLF and non-BLF IOLs, they demonstrated no apparent advantage exists of BLF IOLs over non-BLF IOLs in nAMD incidence.

As on the one hand it is possible to mitigate the phototoxic effect of blue light on the retina with the use of IOLs, it is at the same time possible to reduce the risk of retinal damage through the utilization of spectacle or contact lenses also in phakic eyes. In the last 10 years, there was a tremendous increase of compact fluorescent lamps and high-intensity light-emitting diodes (LEDs). Displays of both smartphones and tablets contain a large number of white-light LEDs, which appear white but have emissions at wavelengths corresponding to the peak of the blue light hazard function. This phenomenon may potentially lead to cumulative exposure to blue light and, consequently, to development of AMD. Standard spectacle lenses give protection against UV radiations and, with the addition of a yellow chromophore, it could be possible to decrease or eliminate blue light transmission. In order to ameliorate the retinal protection, antireflection interference coatings can be added to both anterior and posterior lens surfaces, attenuating a great part of the blue light [86]. A

review by Lawrenson et al. [87] has investigated the relative benefits and potential harms of blue-light-blocking (BB) spectacle lenses for macular health. Currently, no studies support the hypothesis that BB spectacle lenses might protect against the onset of AMD.

In conclusion, there are conflicting results in the scientific literature regarding the use of blue-light-blocking spectacles or intraocular lenses to counteract the development of AMD. Although the retinotoxic effects of blue light and UV radiations are known, more studies are needed to demonstrate with certainty the benefits of using such devices to prevent the onset of AMD.

6. Conclusions

AMD is the leading cause of irreversible blindness in elderly people and represents nowadays a high social and economic burden for health systems. Intravitreal medications for nAMD and dietary supplementation to slow down the progression of the dry form are the mainstay of AMD treatment. Despite the good results obtained, the impact of AMD on worldwide population remains huge in terms of visual outcomes and quality of life. Implementing preventive strategies could be an alternative way while waiting for new therapies capable to improve the clinical course of AMD even more than we are able to do today. Modification of dietary habits with a balanced supply of antioxidants, such as vitamins C and E, lutein, and zeaxanthin, zinc, β- carotene, and omega-3 fatty acids has demonstrated to be able to reduce the risk of AMD. Specifically, the Mediterranean Diet has shown to be effective to prevent the onset of AMD. In addition, lifestyle modifications, firstly smoking cessation, but also doing regularly physical activity, reducing excessive body weight and avoiding obesity, and sleeping circa 7–8 house per night, have shown to be effective in decreasing the possibility to develop AMD. Lastly, the role of intraocular lenses and eyeglasses regarding the AMD prevention is still controversial and needs to be further elucidated, although the use of blue-light-filters seems to protect the retina from photochemical damage.

In conclusion, a better lifestyle with balanced diet, no smoking, and regular physical activity might protect the eye from developing AMD. Therefore, it would be important for health systems to implement policies aimed to encourage a healthy lifestyle.

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Article

Meteorin Is a Novel Therapeutic Target for Wet Age-Related Macular Degeneration

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Abstract: The aim of this study was to evaluate the potential anti-angiogenic effect of MTRN (meteorin) in the laser-induced CNV rat model and explore its mechanisms of action. MTRN, thrompospondin-1, glial cell markers (GFAP, vimentin), and phalloidin were immuno-stained in non-human primate flat-mounted retinas and human retina cross sections. The effect of MTRN at different doses and time points was evaluated on laser-induced CNV at 14 days using in vivo fluorescein angiography and ex vivo quantification of CNV. A pan transcriptomic analysis of the retina and the RPE/choroid complex was used to explore MTRN effects mechanisms. In human retina, MTRN is enriched in the macula, expressed in and secreted by glial cells, and located in photoreceptor cells, including in nuclear bodies. Intravitreal MTRN administered preventively reduced CNV angiographic scores and CNV size in a dose-dependent manner. The highest dose, administered at day 7, also reduced CNV. MTRN, which is regulated by mineralocorticoid receptor modulators in the rat retina, regulates pathways associated with angiogenesis, oxidative stress, and neuroprotection. MTRN is a potential novel therapeutic candidate protein for wet AMD.

Keywords: retina; choroidal neovascularization; angiogenesis; meteorin; therapeutic innovation

1. Introduction

Age-Related Macular Degeneration (AMD) is the most common retinal disease, affecting 200 million individuals over 60 years old world-wide [1]. The "wet" form of AMD, also called neovascular or exudative AMD, represents approximately 50% of the cases, and is characterized by the growth of new abnormal blood vessels from the choroid into the retina, called choroidal neovascularization (CNV), causing macular edema, which is associated with rapid and severe vision loss. Anti-VEGF (Vascular Endothelial Growth Factor) intraocular injections stabilize vision in 80% of the cases at the cost of multiple injections [2,3]. However, after one year of optimal treatment regimens, only 40% of patients respond optimally to the treatment [4,5], since CNV continues to develop and edema persists.

The pathogenesis of AMD is multifactorial, but exact mechanisms leading to CNV and to RPE/choroid complex degeneration are incompletely understood. Identification and modulation of other molecular targets apart from VEGF is mandatory to improve the visual prognosis of patients with wet AMD.

Glucocorticoids (GCs) are amongst the most used drugs for macular edema of many origins, including diabetic retinopathy, vein occlusion, and inflammation [6]. However, in wet AMD, intraocular GCs have been inefficient to reduce CNV and the associated macular edema [5,7], suggesting that corticoid-induced regulatory mechanisms are altered in AMD. GCs bind to the glucocorticoid (GR) and the mineralocorticoid receptor (MR), both expressed in various retinal and choroidal cells including vascular endothelial cells and retinal pigment epithelium (RPE) [8,9]. In the retina and choroid, GR pathway activation is anti-inflammatory, anti-edematous [6], but MR pathway overactivation causes inflammation, oxidative stress, and choroidal pathology [8,10–12]. Using transgenic approaches, we previously showed that MR invalidation in vascular endothelial cells prevented laser-induced CNV and that pharmacologic MR antagonists exerted anti-inflammatory effects and anti-angiogenic effects [13]. In a small cohort of wet AMD patients, resistant to intensive anti-VEGF treatments, we showed that the oral MR antagonist (MRA) spironolactone potentialized the anti-edematous effects of anti-VEGF and that this effect was lost after spironolactone was stopped [13]. In the search for downstream MR antagonists molecular targets, we identified that MRA anti-angiogenic effects were mediated at least in part through the up-regulation of decorin (DCN) in the RPE/choroid complex [13]. In the rat retina transcriptome, meteorin was identified also as a MR molecular target [13].

Meteorin (METRN) is a newly identified secreted protein and a member of poorly characterized, evolutionarily conserved, two-members growth factor family. The other member of the family is meteorin-like or cometin. The receptor of MTRN has not yet been characterized. During brain development, MTRN is expressed in neural stem cells and radial glial cells and in immature neurons, where it induces axonal extension [14,15]. In the retina, it promotes glial Müller cells differentiation via activation of the Jak-STAT3 pathway [15]. The potential of MTRN as a neurotrophic factor was shown by the over-expression of MTRN in brain excitotoxic models. Lentiviral MTRN overexpression in the striatum following excitotoxic injury did not enhance neurogenesis but significantly increased the proportion of new cells with astroglial and oligodendroglial features [16]. Using a cell-based encapsulated technology, the in vivo slow delivery of MTRN, protected striatal neurons from quinolinic acid-induced excitotoxicity, reduced lesion size, and improved neuronal performance [17]. More recently, MTRN was shown to reduce hyperalgesia in a chronic nerve constriction injury rat model [18]. In photochemically-induced sciatic nerve injury and chronic constriction injury of the sciatic nerve in rats, MTRN reduced signs of pain and mechanical and thermal hypersensitivity, and these effects lasted after MTRN administration, suggesting a nerve effect rather than a simple pain relief mechanism [19].

MTRN also intervenes in the glio vascular crosstalk, regulating normal angiogenesis in the brain and in the neural retina [20]. Indeed, in vitro experiments showed that MTRN exerted anti-angiogenic activity via astrocyte-derived thrombospondin-1/-2 expression and secretion, suggesting a role in angiogenesis attenuation and vascular maturation by astrocytes [20].

In the retina, MTRN expressed in astrocyte endfeet that surround blood vessels could contribute to vascular maturation and maintenance, but its exact localization in the human adult retina and its role in neovascular diseases have not been studied. The aim of this study was to confirm that MTRN expression is regulated by the mineralocorticoid pathway in the retina, to localize MTRN expression in the human retina, and to evaluate its effect in the rat laser-induced CNV model, a validated model for human wet AMD.

2. Material and Method

2.1. Ethics for Animal Use

All experiments were performed in accordance with the European Communities Council Directive 86/609/EEC and French national regulations and approved by local ethical committees. Animals were kept in pathogen-free conditions with food, water, and litter, and housed in a 12-h/12-h light/dark cycle. Anesthesia was induced by intramuscular

ketamine 40 mg/kg and xylazine 4 mg/kg in rats and with topical oxybuprocain. Animals were sacrificed by carbon dioxide inhalation. Intravitreous injections (IVT) were performed using microfine (300 μL) syringes with 31G needles under topical anesthesia (tetracaine 1%, Aldrich, Lyon, France).

2.2. Regulation of MTRN Expression in Rat Retina by Aldosterone and/or Spironolactone, Which Regulate the Mineralocorticoid Pathway

Eight-week-old male Lewis rats were used in this experiment (ethical approval: #4488 Charles Darwin). Rat eyes received one single injection of 5 μL aldosterone 1 μM diluted in 0.9% saline, corresponding to a final concentration of 100 nM in the vitreous or 5 μL spironolactone corresponding to a final concentration of 10 μM in the vitreous. Control rat eyes were injected with 5 μL saline. Both eyes of 6 rats per group were injected.

At 24 h, rats were sacrificed by CO_2 inhalation and eyes were enucleated and dissected on ice to retrieve the neural retina and the RPE/choroid complex. Samples were snap-frozen in liquid nitrogen and stored at $-80\ °C$ until use. Total RNA was isolated from tissues using the RNeasy Mini Kit (Qiagen, Hilden, Germany) including DNase I treatment. First-strand complementary DNA was synthesized from the total mRNA using random primers (ThermoFisher Scientific, Saint Aubin, France) and SuperScript II reverse transcriptase (ThermoFisher Scientific). Transcript levels of MTRN were analyzed by quantitative PCR performed in CFX384 Touch Real-Time PCR Detection System with SYBR Green detection using the following primers. *Mtrn*: Forward: 5′ GTG ACT TTG TGA TCC ATG GG 3′, Reverse: 5′ TGG AAC AGT GGC AGT GTC TG 3′ and *Gapdh* Forward: 5′ GAC ATG CCG CCT GGA GAA AC 3′, Reverse: 5′ AGC CCA GGA TGC CCT TTA GT 3′. Delta CT threshold calculation was used for mRNA relative quantification results.

Statistical analysis was performed using the Kruskal–Wallis non-parametric test followed by a Dunn's multiple comparison test. $p < 0.05$ was considered significant.

2.3. Immunohistochemistry on Rat Retina

To localize the expression of MTRN on flat-mounted neural retina and RPE, two additional Lewis rats were used. For the retinal flat-mount, the eyes were dissected and retinas were flat-mounted, fixed 10 min in acetone at $-20\ °C$, blocked with fetal bovine serum 10% in PBS with Triton 0.1% for 30 min, and incubated with an anti-Meteorin antibody (Abcam rabbit antibody) and with an anti-GFAP coupled to CY3 in blocking solution overnight. Retinas were then rinsed and incubated with secondary antibody (Alexa Fluor conjugated 488 anti-rabbit, Invitrogen, Carlsbad, CA, USA). Flat mounts were mounted with fluorescent aqueous mounting medium (Dako Ltd., Ely, UK). For the RPE flat-mount, following the blocking step described above, RPE flat-mounts were incubated with primary antibody anti-MTRN at dilution 1:100 (Abcam rabbit antibody) in blocking solution overnight. After rinses, flat-mounts were incubated with anti-rabbit secondary antibody at 1:200 (Molecular Probes Alexa Fluor 488), and with Rhodamin Phalloidin (Life Technologies) at a 1:300 dilution for 1 h and with DAPI at a 1:5000 dilution for 5 min under constant agitation. Flat-mounts were finally mounted with fluorescent aqueous mounting medium (Dako Ltd., UK). Images were acquired with a confocal microscope (LSM 510 Carl Zeiss, Oberkochen, Germany).

2.4. Immunohistochemistry on Non-Human Primate and Human Samples

Two eyes from one eight-year-old *Sapio anubis* non-human primate were used. Two hours after enucleation, the eyes were sectioned and dissected in order to retrieve the neural retina, that was fixed in 4% paraformaldehyde for 24 h at $4\ °C$ and then rinsed in PBS.

Immunohistochemistry for MTRN, thrombospondin, and glutamine synthetase was performed in order to evaluate the distribution of MTRN in relation to glial Müller cells in the macula and fovea.

Human eyes were obtained from the oncology department of the University of Lausanne. The use of human subjects adhered to the tenets of the Declaration of Helsinki and was approved by the local Ethics Committee of the Swiss Department of Health on research

involving human subjects (CER-VD 340/15 and CER-VD 19/15) and patients signed an informed consent. Two retinas were obtained from patients with anterior uveal tumors but intact retinas. One eye from a 30-year-old woman was enucleated for an untreated ciliary body melanoma. One eye from a 54-year-old woman was enucleated due to a massive peripheral nasal melanoma. In both eyes, the macula was normal. The enucleated eyes were sectioned and the anterior part (including retina up to the equator) was used for classical pathologic examination. Posterior retinas of the eyes were used for immunohistochemistry on cryosections. Due to the enucleation procedure, fresh tissues were available for analysis.

Human samples were fixed in 4% paraformaldehyde for 24 h at 4 °C. They were then rinsed in PBS 1X and included in the optimal cutting temperature for cryosectioning. For fluorescence immunohistochemistry, 10 μm thick neuroretina sections were incubated overnight in primary antibodies at 4 °C listed in (Table 1). After washing with PBS supplemented with 10% fetal calf serum and 0.1% Triton X-100, sections were incubated 3 h with their corresponding secondary antibodies AlexaFluo®488 or AlexaFluo®594 or AlexaFluo®647 (Table 1). All antibodies were diluted in PBS supplemented with 10% fetal calf serum and 0.1% Triton X-100. Sections were counterstained with 4′, 6-diamino-2-phenylindol (DAPI). Images were observed and captured with a confocal microscope (LSM 710 software, ZEISS, Oberkochen, Germany).

Table 1. List of antibodies.

Antibodies	Species	Reference	Lab Provider	Dilution
Anti GFAP Antibody, Cy3 Conjugate	Mouse	MAB3402C3	Dako cytomation	1/200
Anti Meteorin	Rabbit	Ab12956	Abcam	1/100
Anti Glutamine synthetase clone GS-6	Mouse	MAB 302	Merck Millipore	1/300
Anti TSP1	Mouse	MA5-13398	Invitrogen	1/100
SUMO1	Mouse	Sc5308	Santa Cruz	1/100
PML	Mouse	PG-M3 sc-966	Santa Cruz	1/100
AlexaFluo®488 – AlexaFluo®594 – AlexaFluo®647		Ab12956	Invitrogen/Thermofischer	1/300
4′, 6′ –diamino-2-phenylindo DAPI			Sigma-Aldrich	1/5000

2.5. Laser-Induced CNV Rat Model

Eight-week-old male Long Evans rats from the Janvier Breeding Center (Le Genest-Saint-Isle, France) were used for CNV induction (local ethical committees' approval #2541-2015110210279792 v3). After anesthesia and dilation of the pupils, coverslips were positioned on the cornea as a contact glass. Six laser burns were performed 2 to 3 optic disc diameters away from the optic nerve on both eyes with an Argon laser (532 nm) mounted on a slit lamp (175 mW, 0.1 s and 50 μm), and the rupture of Bruch's membrane was assessed by the presence of a bubble.

2.6. Treatments

Treatment with MTRN was evaluated in the rat model of CNV. We used recombinant mouse MTRN protein (R&D Systems, Lille, France). After laser photocoagulation, two separate experiments were performed. In the first experiment, to test the preventive effect of MTRN, rats were injected with MTRN in the vitreous just after laser lesions were performed. They were divided into 4 treatment groups: (1) Intravitreous injection of PBS (5 μL), (2) intravitreous injection (IVT of 5 μL) of mouse recombinant MTRN (R&D System, Lille, France) at 50 g/mL in PBS, (3) IVT of 5 μL of MTRN at 200 ng/mL, (4) IVT of 5 μL of MTRN at 1 μg/mL. In a second experiment, to test the curative effect of MTRN, rats received the IVT of MTRN immediately or at seven days after laser burns were performed. They were divided into 4 groups: (1) IVT of PBS (5 μL), (2) IVT of 5 μL of MTRN at 1 μg/mL

at day 0, (3) IVT of 5 µL of MTRN at 6 µg/mL at day 0, (4) IVT of 5 µL of MTRN at day 7. In both experiments, 12 eyes per group were used. Finally, to decipher the transcriptional regulations of MTRN in the neural retina and the RPE/choroid complex, we performed a third experiment using IVT of MTRN at 1 µg/mL at the time of laser burn induction and sacrificed the animals at day 7. For this experiment, 4 rats per group were used.

2.7. Fluorescein Angiography (FA)

FA was performed 14 days after laser induction. After pupil dilatation, fluorescein (0.2 mL of 10% fluorescein in saline) was injected intravenously in the tail of rats. Early- and late-phase angiograms were recorded 1–3 and 5–7 min, respectively, after fluorescein injection. Simultaneously, infrared images were acquired to detect the site and effective presence of laser burn. For each laser-induced lesion, fluorescein leakage was graded qualitatively by evaluating the increase in size/intensity of dye between the early and late phases. Angiographic scores were established by 2 blinded observers according to the following criteria: grade 0, no hyperfluorescence; grade 1, slight hyperfluorescence with no increase in intensity nor in size; grade 2, hyperfluorescence increasing in intensity but not in size; grade 3, hyperfluorescence increasing both in intensity and size. Mean severity gradings were compared using the Kruskal–Wallis non-parametric test followed by a Dunn's multiple comparison test. $p < 0.05$ was considered significant. Results were also presented as the frequency of distribution of the severity grading scores as previously performed [13].

2.8. RPE/Choroid Flat-Mounts and CNV Quantifications

Two days after FA examination (time necessary for fluorescein elimination), eyes were enucleated, fixed in 4% PFA for 15 min at room temperature, and sectioned at the limbus; the cornea and lens were discarded. The retina was separated from the RPE/choroid complex. Eight radial incisions were made on the RPE/choroid, which was then flat-mounted and post-fixed with acetone for 15 min at −20 °C. After washing with 0.1% Triton × 100 in PBS, FITC-GSL I-Isolectin B4 (1:200, Vector, AbCys, Paris, France) was applied on two days at −4 °C. After washing with PBS, the RPE/choroid was flat-mounted and observed with a confocal microscope (Zeiss LSM710, Le Pecq, France). Images of the CNV were captured with a digital video camera coupled to a computer system. Horizontal optical sections (at 1 µm intervals) were obtained from the CNV surface. The deepest focal plane in which the surrounding choroidal vascular network connecting to the lesion could be identified was judged to be the floor of the CNV lesion. The area of CNV-related fluorescence on each horizontal section was measured using the ImageJ software. The summation of the entire fluorescent area on z-stack images from the top to the bottom of the CNV was used as an index for the CNV volume. On the same flat-mounts, MTRN immunohistochemistry was performed. Volume reduction was measured as compared to the vehicle-treated group and expressed as a percentage of reduction.

2.9. Statistics

Comparison between 2 groups was performed using Mann–Whitney U test. Comparison between multiple groups was analyzed using non-parametric Kruskal–Wallis followed by Dunn's test (GraphPad Prism 5 for Windows, GraphPad Software Inc., San Diego, CA, USA). p-values of 0.05 or less were considered significant.

2.10. RNA-Sequencing Data Analysis

RNA samples from either the neural retina or the RPE/choroid complex were sent for sequencing at the iGenSeq transcriptomic platform of the Brain and Spine Institute (ICM, Paris, France). RNA quality was checked by capillary electrophoresis (Agilent 2100 Bioanalyzer system) and RNA with integrity numbers (RIN) ranging from 7.8 to 8.2 was accepted for library generation. Quality of raw data has been evaluated with FastQC. Poor quality sequences have been trimmed and adaptors removed with Fastp software to retain

only good quality paired reads. Star v2.5.3a [21] has been used to align reads on reference genome rn6 using standard options. Between 30 and 38 million reads were mapped. Quantification of gene and isoform abundances has been done with rsem 1.2.28 [22], prior to normalization on library size with edgeR [23] bioconductor package. Low-expressed genes have been filtered-out. Reproducibility of replicates has been controlled with PCA representations. Finally, differential analysis has been conducted with the GLM framework likelihood ratio test from edgeR. Multiple hypothesis adjusted *p*-values were calculated with the Benjamini–Hochberg procedure to control FDR. Pheatmap Bioconductor package has been used to represent as a heatmap for the differentially expressed genes. GSEA function from clusterProfiler package has been used on HALLMARK geneset; on Gene Ontology; on Reactome gene sets; and on KEGG pathways, to get deregulated pathways from analysis, with a representation in dot plot. A genes/pathways network has been created with cytoscape, node color representing log fold change.

3. Results

3.1. MTRN Is Widely Distributed in the Rat Neural Retina and in the Retinal Pigment Epithelium and Its Expression Is Regulated by Mineralocorticoid Pathway

On the neural retina flat-mount from adult rats, MTRN is concentrated around the optic nerve head (Figure 1a, arrows), all over the ganglion cell layer (GCL), and around the vessels, which are surrounded by GFAP positive astrocytes (Figure 1b). MTRN is secreted and is still visible in the deep outer nuclear layer and at the outer limiting membrane (OLM) that constitutes the junctions between the glial Müller cells and the photoreceptor segments (Figure 1c). Higher magnification shows the presence of MTRN secreted by astrocytes around the vessels (Figure 1d). On the RPE flat-mount, RPE cells are well delineated by phalloidin staining and MTRN appears as dots in the cytoplasm, but also located in the nuclei of cells (Figure 1e). Using RT-PCR, we show that *Mtrn* is expressed in the rat neural retina and in the RPE/ choroid complex and that intravitreous aldosterone injection significantly reduces *Mtrn* expression in the neural retina at 24 h ($p < 0.001$) [11]. Conversely, spironolactone up-regulates *Mtrn* expression in the RPE ($p < 0.05$) as compared to aldosterone (Figure 1f). These results show that MTRN is found not only in the neural retina but also in the RPE/choroid and is a mineralocorticoid pathway transcriptional regulated target.
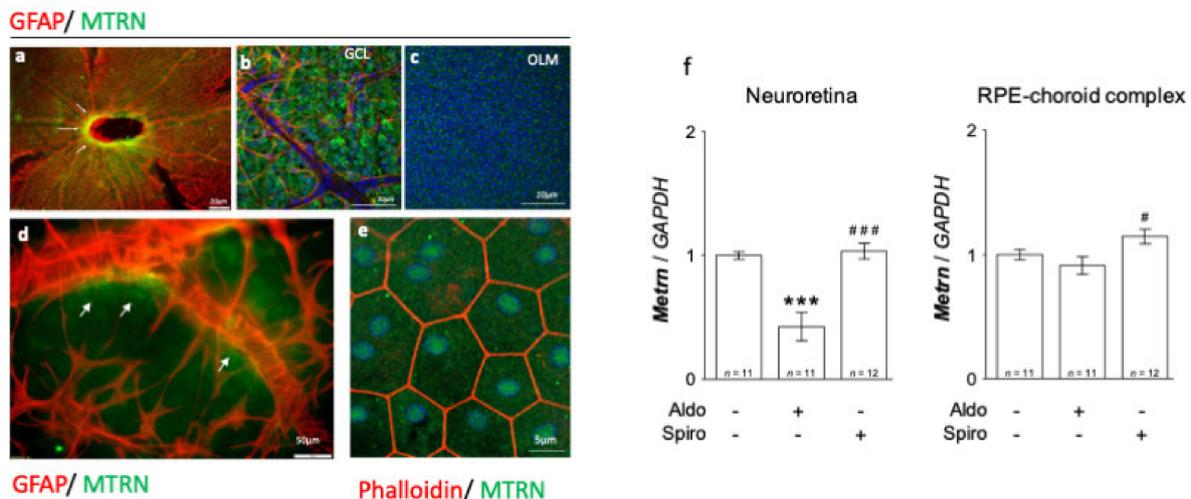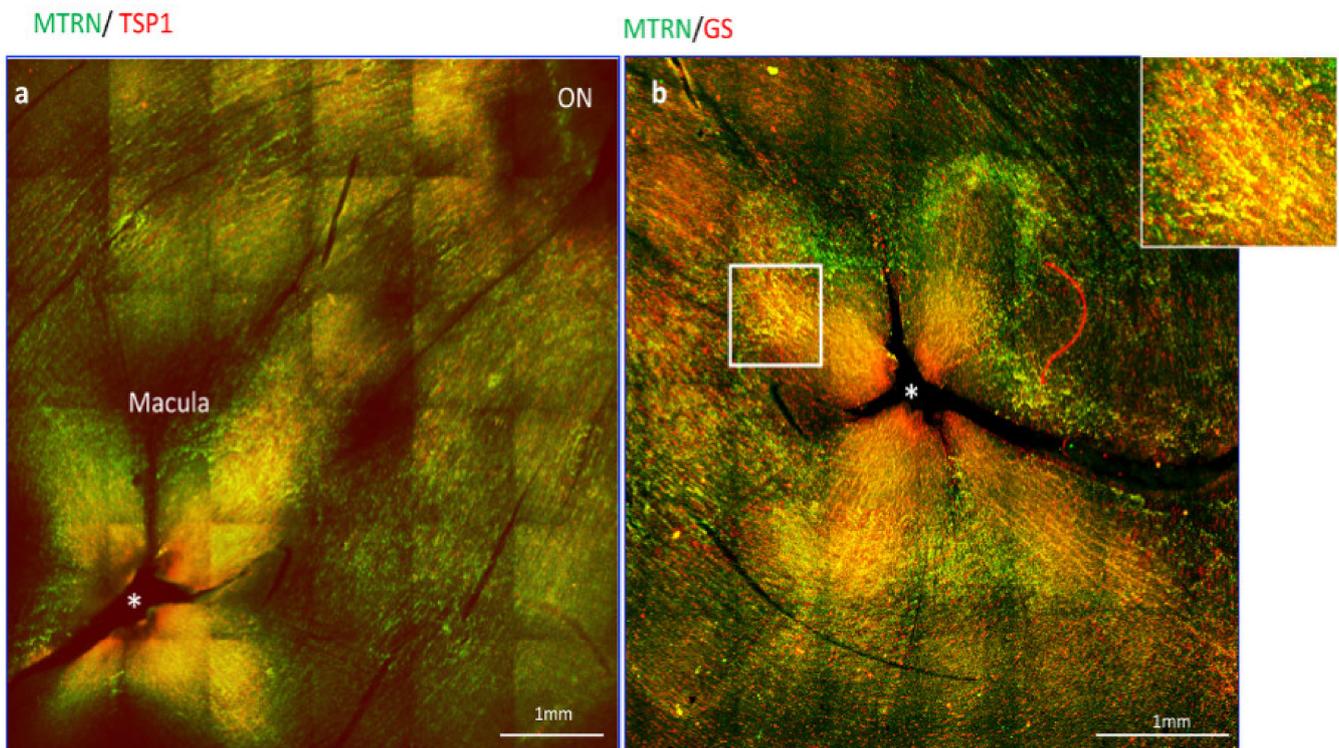

Figure 1. MTRN immunostaining on rat flat-mounted retina and RPE regulation in the retina and RPE/choroid complex at 24 h after MR agonist and antagonist intravitreous administration. MTRN immunostaining on the flat-mount retina (**a**–**d**). MTRN (green) is concentrated around the optic nerve head (**a**), also expressed in ganglion cell layer (GCL) (**b**), and at the outer limiting membrane (OLM). GFAP staining represents astrocytes located around vessels in the inner retina (**b**,**d**). MTRN

is secreted around vessels at the endfeet of astrocytes (**d**, arrows). In RPE cells delimited by phalloidin staining, MTRN is expressed in the cytoplasm but also in the nuclei (**e**). Quantitative analysis of MTRN expression in neuroretina and RPE-choroid complex 24 hrs after aldosterone (100 nM) or spironolactone (10 µM) in vivo exposure (**f**). Significant reduction of *Mtrn* expression is observed in neural retina at 24 h after aldosterone injection ($p < 0.001$). Spironolactone up-regulated *Mtrn* compare to aldosterone, but not compared to vehicle. There is significant up-regulation of *Mtrn* expression exclusively in RPE/choroid at 24 h after spironolactone injection # ($p < 0.05$ as compared to Aldo), *** ($p < 0.01$ as compared to untreated controls), ### ($p < 0.001$, as compared to Aldo treatment).

3.2. MTRN Is Concentrated in the Macula and Co-Localized with Thrombospondin in Non-Human Primate

Since thrombospondin expression and release from astrocytes was shown to be enhanced by MRTN, we analyze the distribution of MTRN and TSP1 on flat-mounted non-human primate retina flat-mounts. Interestingly, we observed that MTRN and TSP1 are highly concentrated around the optic nerve head (Figure 2a, ON), where astrocytes density is very high, but that both proteins are also highly concentrated and co-localize at the macula and around the fovea (Figure 2a,b, star), which is avascular and devoid of astrocytes. We thus co-labelled MTRN with glutamine synthetase (GS), which is a marker of glial Müller cells, to detect potential co-localization of MTRN with these cells at the fovea. Indeed, MTRN is highly expressed in Müller glia at the fovea and in the macula, where it co-localizes with TSP1. MTRN and TSP1 could contribute to the maintenance of the avascular zone.


Figure 2. MTRN localization in non-human primate retina flat-mount. Immunostaining on the flat-mount retina of MTRN (green), TSP1 (red), and GS (red). MTRN and TSP1 are highly concentrated around the optic nerve head (NO = Optic Nerve), and co-localize at the macula and around the fovea* (**a**). Colocalization of MTRN with GS expressed in Müller cells at the macula (**b** and inset).

3.3. MTRN Is Distributed in All Retinal Layers of the Human Neural Retina

Outside of the macula, MTRN is found in the nerve fiber layer (NFL), in and around astrocytes, in the nuclei of ganglion cells in the ganglion cell layer (GCL), in some of the nuclei of the outer nuclear layer (ONL), particularly in granules of the cone nuclei

(Figure 3a, inset, arrows), and in the inner segments of photoreceptors (IS). MTRN is present in astrocytes around vessels and diffuses at the surface of the retina (Figure 3b). In the posterior retina, MTRN is localized in astrocytes, in the deepest nuclei of the inner nuclear layer (Figure 3c,d), and in the extensions of glial Müller cells of the Henle fiber layer (Figure 3c, HFL, arrows). Co-labeling of MTRN with TSP1 shows co-localization of both proteins in the macula (Figure 4a), in both cytoplasm and nuclei of ganglion cells, and mostly in nuclei of inner and outer nuclear layers (Figure 4a). In addition, secreted MTRN is observed in all retinal layers. In the macula (Figure 4b–d), MTRN co-localize with TSP1 in all layers and is present along retinal glial Müller cells (Figure 4c). In the photoreceptors, MTRN and TSP1 co-localize in cone nuclei, within nuclear bodies (Figure 4d). MTRN signal is also very strong in the nerve fibers (Figure 4e, arrows). Note that in the outer nuclear layer (which represents the nuclei of photoreceptors), MTRN expression is more intense in the macula (Figure 4b) than outside of the macula (Figure 4e). Interestingly, we observed that in human retina, MTRN is abundant in the Bruch's membrane (BM) (Figure 4f), with a gradient towards the choroidal vessels, where TSP1 and MTRN co-localize in some nuclei of the endothelial cells.
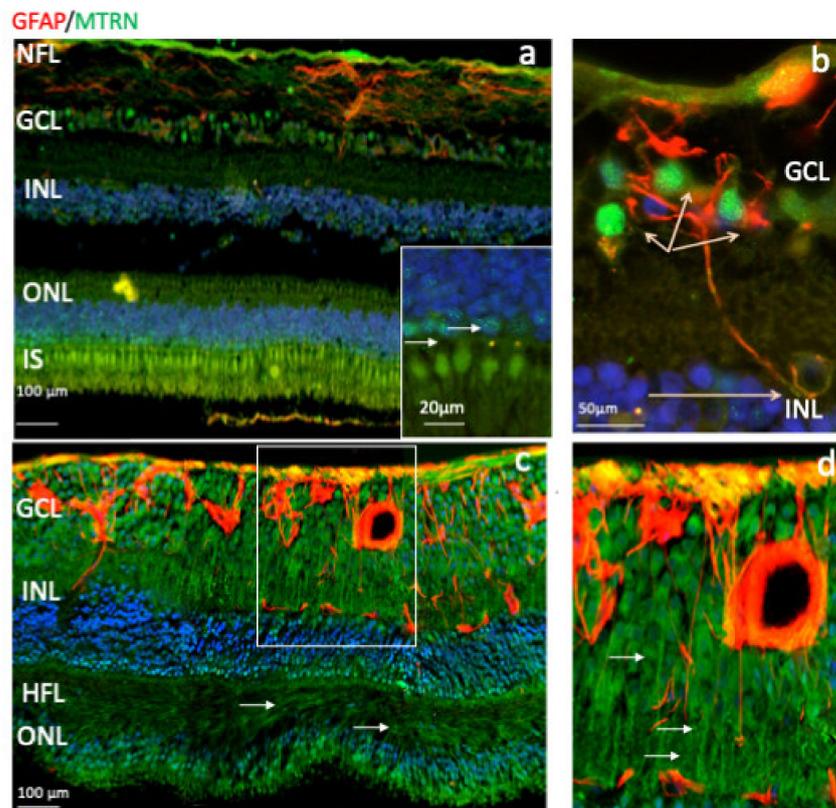
Figure 3. Human immunostaining of MTRN and GFAP on retinal cryosection. Immunostaining on human retinal cryosection of MTRN (green), GFAP (red), outside the macula (**a**,**b**), and in posterior retina (**c**,**d**). MTRN localized in the nerve fiber layer (NFL) (**a**), in Henle fiber layer (HFL) (**c**), in and around astrocytes, in the nuclei of ganglion cells layer (GCL), in some of the nuclei of the inner and outer nuclear layer (INL, ONL) (**a**,**c**), particularly in granules in the cone nuclei, and in the inner segments of photoreceptors (IS) (**a** and inset). MTRN is colocalized with GFAP in astrocytes and around the vessel (**b**,**d**).
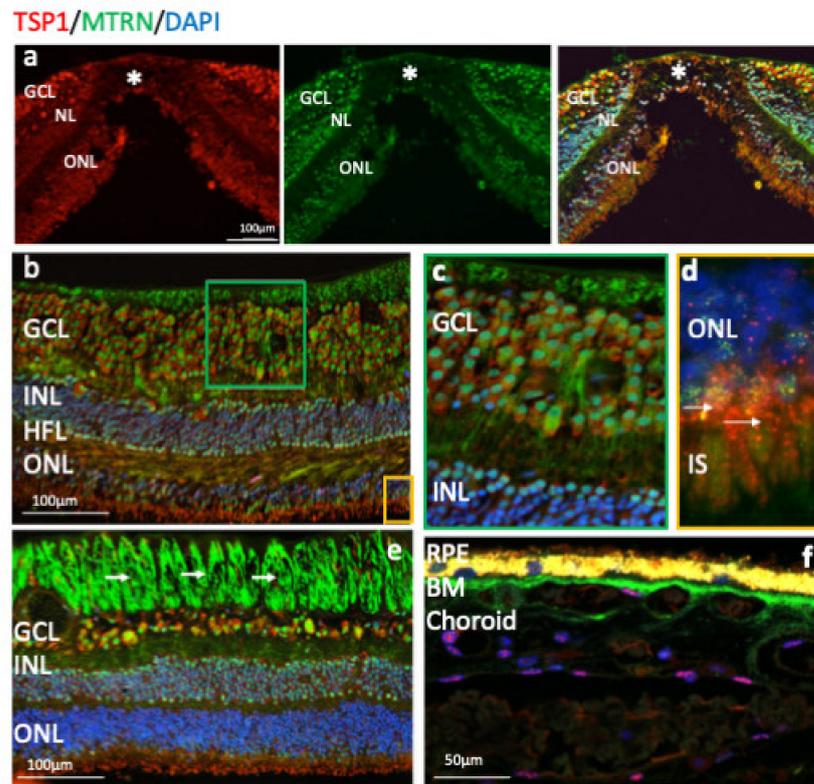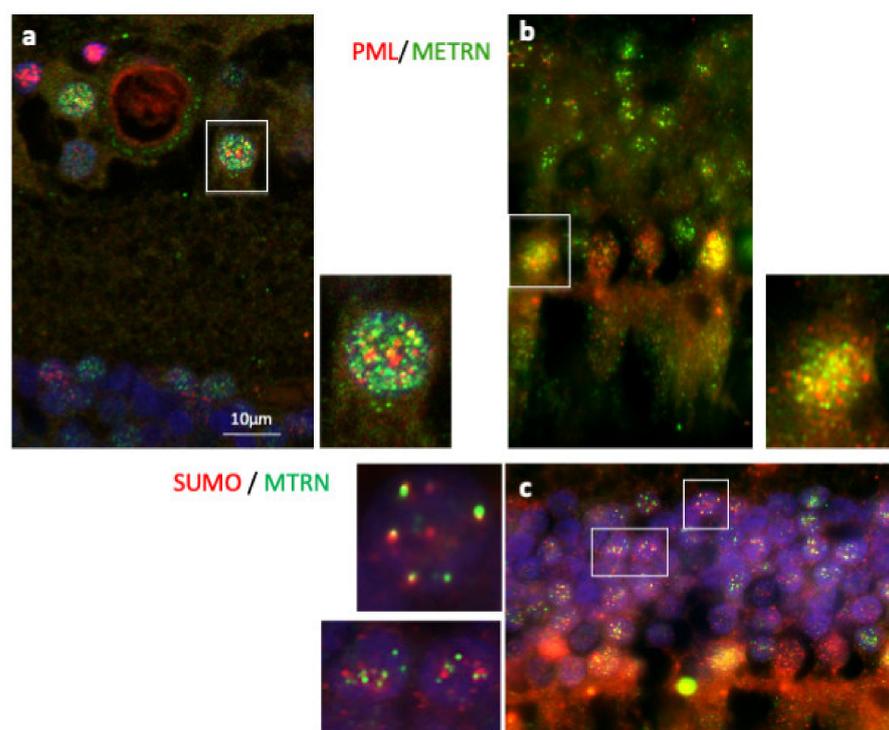
Figure 4. Human immunostaining of MTRN and TSP1 on retinal cryosection. Immunostaining on human retinal cryosection of MTRN (green), TSP1 (red), counter marked with DAPI (blue). MTRN (green) colocalized in all layers with TSP1, in macula (**a**) and is expressed in the cytoplasm and in nuclei of ganglion cells, in nuclei of inner and outer layers (INL, ONL) (**b**), in Müller fibers (**c**), and in the inner segment of photoreceptors (**d**). Close to the optic nerve, MTRN is strongly present in nerves fibers (**e**), in Bruch's membrane (BM) (**f**). MTRN is also found as a gradient starting from the BM towards the deeper choroidal vessels (**f**).

MTRN is highly expressed in the human retina and the RPE/choroid complex and is enriched in the macula. It co-localizes with TSP1 in nuclear bodies.

### 3.4. MTRN Is Secreted and Sequestered in PML Nuclear Bodies

In order to further characterize MTRN localization in nuclear bodies, we performed a co-staining of MTRN with the promyelocytic leukemia protein (PML), known to be a key regulator of nuclear bodies organization [24] and with SUMO, as SUMOylation is an essential post-translational modification involved in partner retention in PML nuclear bodies [24]. As shown in Figure 5, both in ganglion cells (Figure 5a and inset) and in photoreceptor cells (Figure 5b and inset), MTRN co-localizes, at least in part, with PML. In addition, MTRN co-localization with SUMO (Figure 5c), suggesting that part of MTRN could be SUMOylated, which could favor its sequestration in PML nuclear bodies.

Figure 5. Immunostaining of PML and SUMO on human retina cryosection. Immunostaining of MTRN (green), PML (red), and SUMO (red). MTRN co-localized with PNL in nuclear ganglion cells (**a** and inset), and in nuclear photoreceptor cells (**b** and inset), but also with SUMO in cell nuclei (**c** and inset).

3.5. MTRN Reduces Laser-Induced Choroidal Neovascularization Both in a Preventive and Curative Regimen

At preventive doses, MTRN reduced the angiographic leakage scores in a dose dependent manner with a significant reduction from 1 µg/mL (5 µL corresponding to a final concentration of 1 ng/µL in the vitreous), although a reduction of the 3 grading score frequency was already observed with the lower dose of 200 ng/mL (0.2 µg/mL final vitreous concentration). When administered at day 7, when CNV has already begin to form, MTRN was still efficient, although at a higher dose (6 ng/µL final vitreous concentration) (Figure 6). Reduction of CNV was further confirmed by quantification of neovascular membrane surface on flat-mounted choroids (Figure 7a), showing a 62% reduction in the preventive MTRN 1000 group and a 31% CNV reduction in the MTRN 200 group. When MTRN was administered at high dose (MTRN 6000 group), 80% of CNV reduction was observed, whether at a curative or preventive dose (Figure 7b). MTRN significantly reduced CNV when administered at the time of laser burn for the 200, 1000, and 6000 doses and when administered at the highest dose at day 7 ($p < 0.01$ for the 200 dose and <0.001 for other doses). At the level of the laser burn, on the neural retina, GFAP activation could be detected either in the superficial (Figure 7c, inset) or in the deep layers (Figure 7d inset), associated with TSP1 diffusion around the activated glial cells in eyes treated with PBS. Intense TSP1 staining was also observed at the level of CNV (Figure 7f). In the MTRN 1000 treated eye, high TSP1 signal could be observed at the endfeet of astrocytes on the vessel (Figure 7g,h) and despite the reduced size of CNV, TSP1 signal appears larger than the CNV (Figure 7i,j). Altogether, these results show that MTRN has an anti-angiogenic effect on CNV and that this effect could be mediated, at least in part, through TSP1.
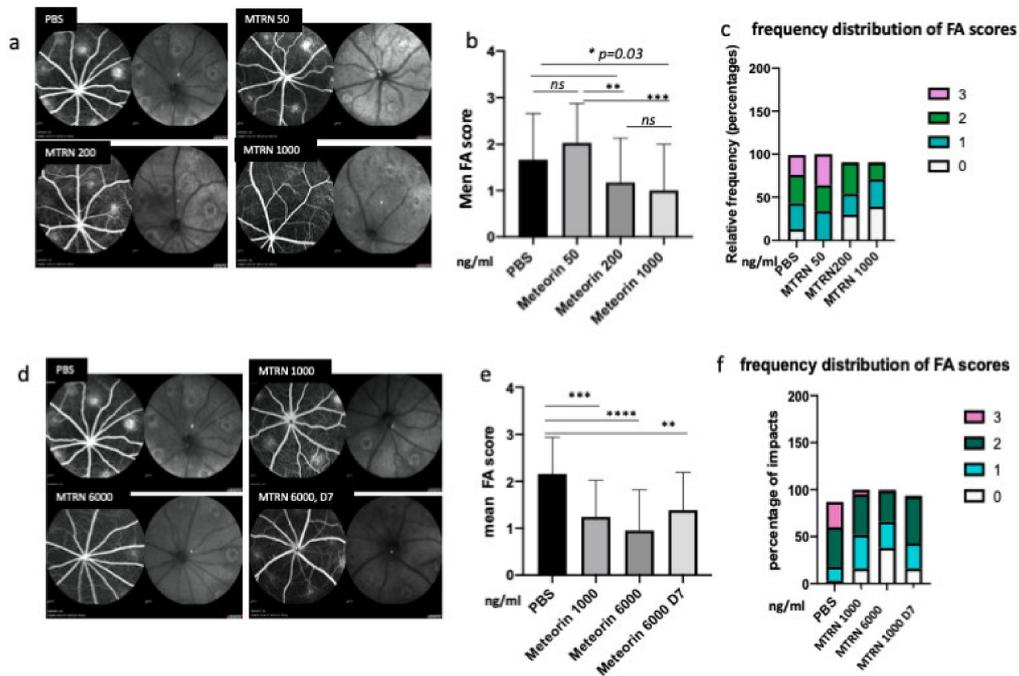
Figure 6. MTRN effect of CNV angiographic scores at day 14. Examples of fluorescein angiographic images at the left and infrared at the right of each eye fundus image with different tested doses (a, d). Mean grading scores and frequency of scores distribution in the preventive dose-response experiment (**b**,**c**) and in the preventive vs. curative dose experiment (**c**,**f**). MTRN: meteorin, 50 ng/mL, 200 ng/mL, 1000 ng/and 6000 mL (ng/mL), D7: treatment at day 7. Treatment. * ($p = 0.03$), ** ($p < 0.01$), *** ($p < 0.001$), **** ($p < 0.0001$).
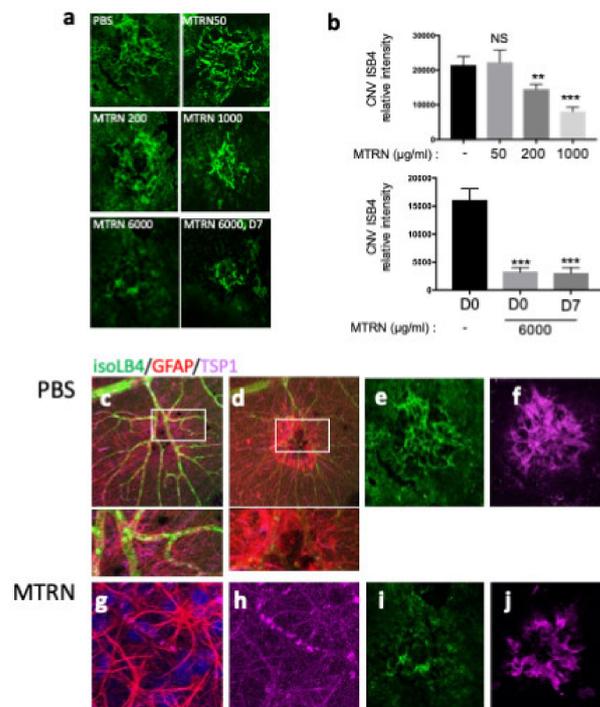

Figure 7. MTRN effect on CNV quantified by isolectin B4. Choroidal flat-mount laser impact images, for each treatment, stained with ß4-isolectin (green) (**a**). Quantification of neovascular volume (**b**). Immunostaining of isoLB4 (green), GFAP (red), and TSP1 (purple) on neuroretina treated by PBS (**c**,**d** and insets) and MTRN (**g**,**h** and insets), and on laser impacts of RPE-choroid complex treated by PBS (**e**,**f**) and MTRN (**i**,**j**). * ($p = 0.03$), ** ($p < 0.01$), *** ($p < 0.001$).

3.6. MTRN Regulates Genes and Pathways Involved in Angiogenesis Independent from VEGF in the RPE/Choroid and in the Retina

In the RPE/choroid complex at 7 days after laser induction and MTRN treatment, 42 genes were significantly up-regulated and 69 genes were significantly down-regulated (EdgeR, log2 FC > 0.5, *p* < 0.05). A full list of differentially regulated genes is available in Supplementary Table S1. The gene-scaled MA plot showed homogenous regulations of highly and poorly expressed genes (Figure 8a). GSEA, using the Hallmark gene sets, was identified as regulated in coagulation, TNFA via NFkB, IL6-Jak-STAT3 signaling, allograft rejection, fatty acid metabolism, IL2 STAT5 signaling, mitotic spindle, TGF beta signaling, epithelial mesenchymal transition, and oxidative phosphorylation (Figure 8b). In TGF beta signaling and IL6 Jak-STAT5 signaling pathways, genes encoding proteins were involved in barrier properties such as *Tjp1* and *Cdh1*, and genes encoding proteins involved in angiogenesis such as *Tgf beta*, *Il18*, and Thbs1, were up-regulated (Figure 8c). In the oxidative phosphorylation pathway, all genes were down-regulated (Figure 8c). GSEA, using the canonical pathway analysis, shows that MTRN regulates pathways in the extra cellular matrix, core matrisome, ECM glycoproteins, collagens, and secreted factors. Genes encoding decorin, biglycan, versican, aggregan, and the small leucin rich protein 2a were regulated by MTRN in these pathways. Reactome pathways analysis showed regulation of extracellular matrix, proteoglycan and glycosaminoglycan (Figure 9), VEGF and PDGF signaling, and cell surface interactions at the vascular wall. In the VEGF, Reactome pathway, genes encoding *Vegf*, or its receptors were not down-regulated (see Supplementary Table S1).
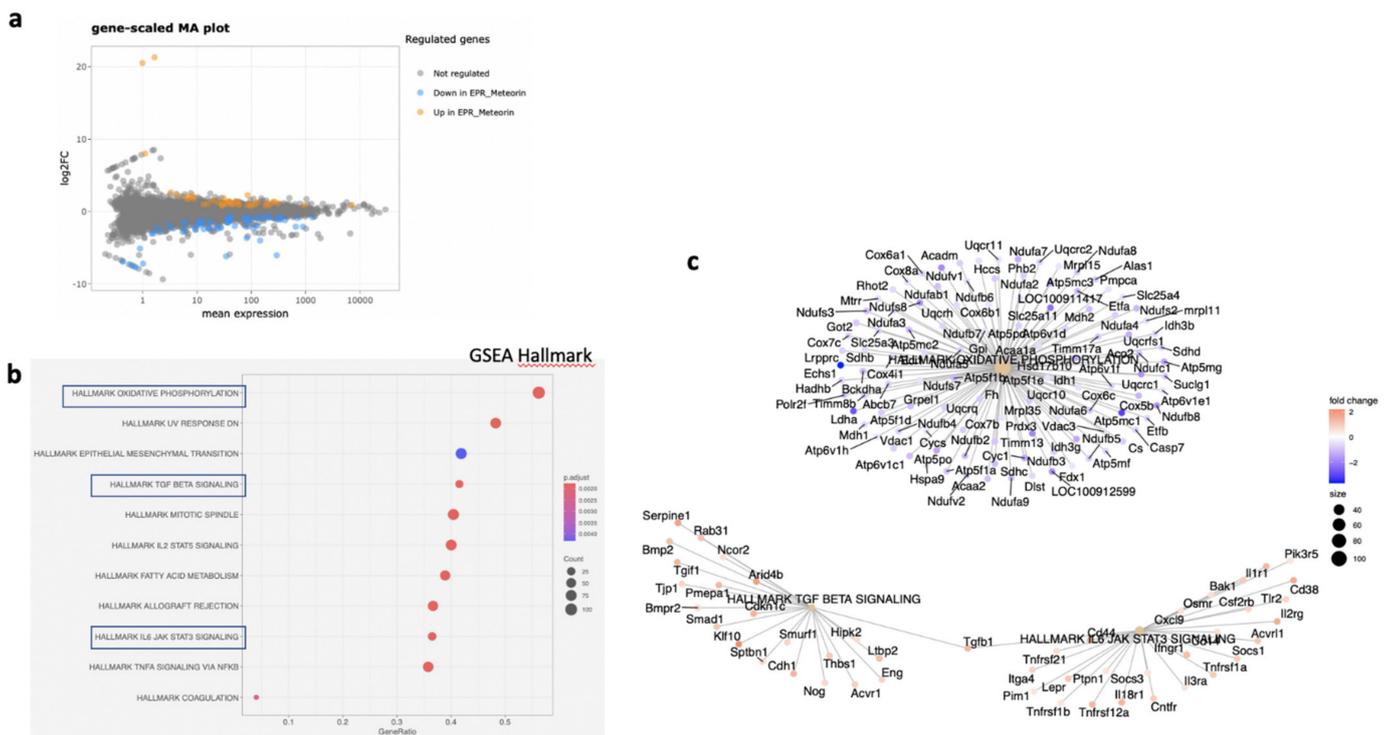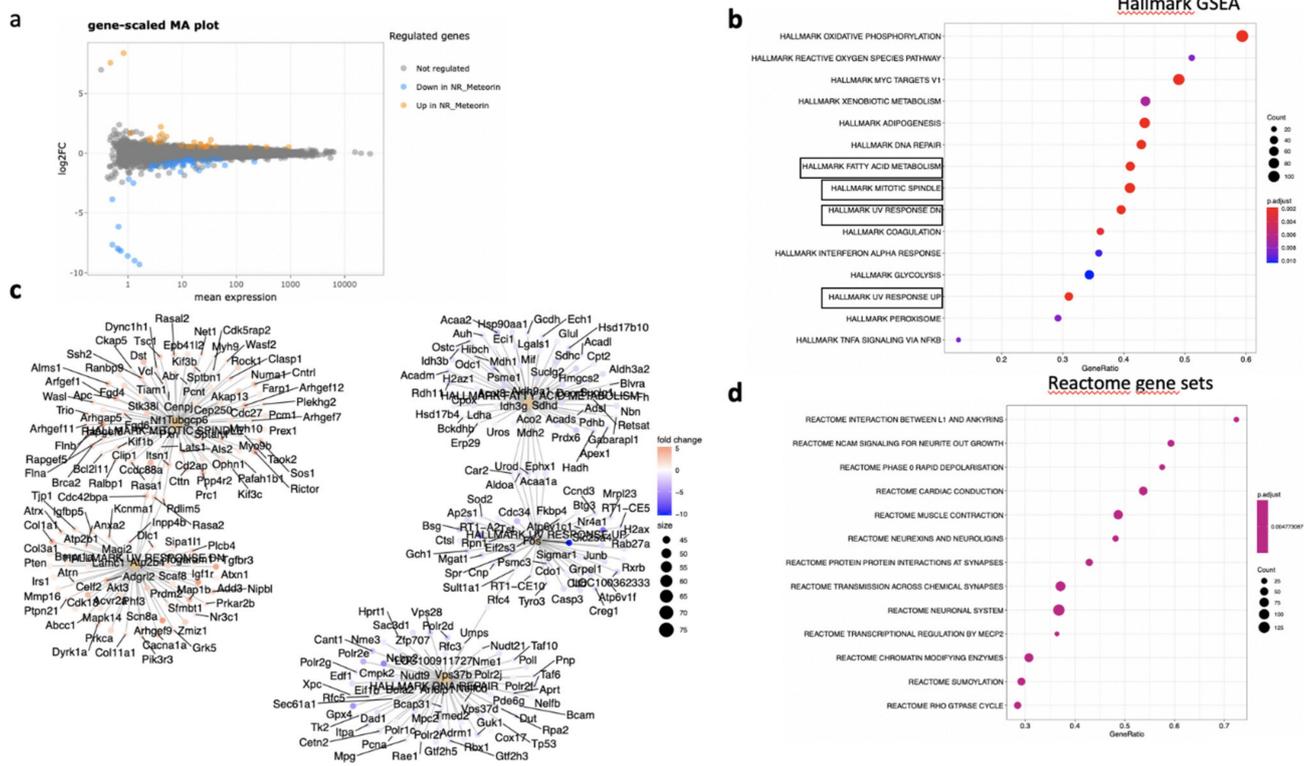

Figure 8. Transcriptomic analysis of the neural retina at day 7 after MTRN (1 µg/mL) was administered at the time of laser delivery. Gene-scaled MA plot. (EdgeR, log2 FC > 0.5, *p* < 0.05) (**a**), GSEA Hallmark. The dot plot depicts the gene ratios (number of core genes over the total number of genes in the set). The dots are colored by the adjusted *p*-value and their size is proportional with the size of the gene set (**b**), genes in the pathways in boxplots are detailed in the gene concept network, (**c**) gene pathway association using the Gene-Concept Network that provides information on the linkages of genes and pathways. Genes are colored by their FC and the size of the dots are proportional to the size of the gene set.
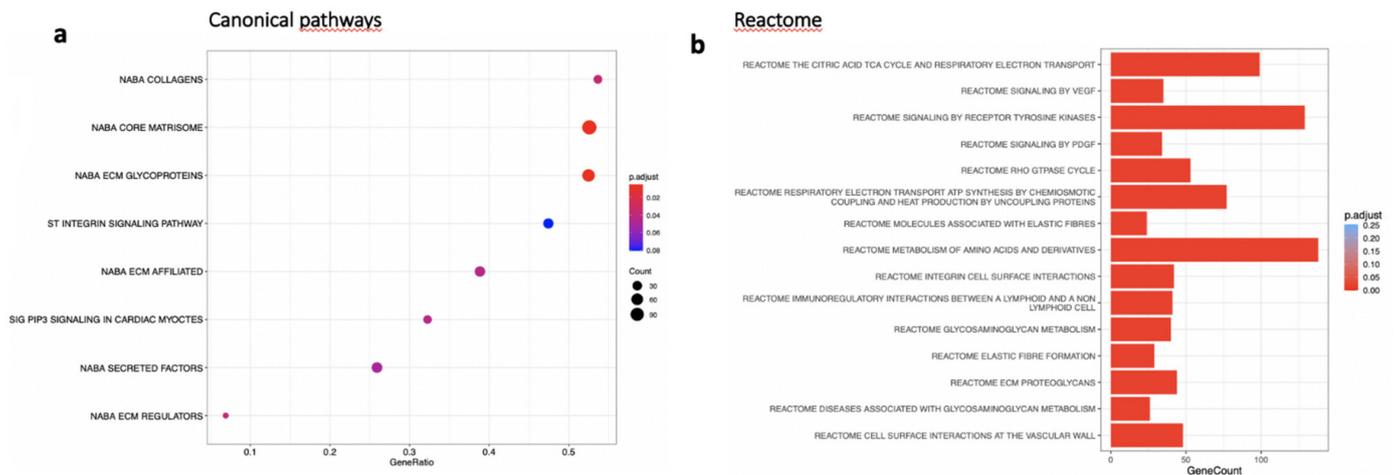
Figure 9. Transcriptomic analysis of the neural retina at day 7 after MTRN (1 µg/mL) was administered at the time of laser delivery. (**a**) Enriched term plot from canonical pathways gene sets from pathway databases. The dot plot depicts the gene ratios (number of core genes over the total number of genes in the set). The dots are colored by the adjusted *p*-value and their size is proportional with the size of the gene set (**b**), Reactome-enriched term plots from Reactome gene sets. The dot plot depicts the gene ratios (number of core genes over the total number of genes in the set). The dots are colored by the adjusted *p*-value and their size is proportional with the size of the gene set.

In the neural retina, 22 genes were significantly down- and 10 genes were up-regulated (DESeq2, log2FC > 1, *p* < 0.05). A full list of regulated genes is available in Supplementary Table S2. The gene-scaled MA plot showed homogenous regulations of highly and poorly expressed genes (Figure 8a). GSEA, using the Hallmark gene sets (Figure 10b), identified TNFA signaling via NFKB, several pathways involved in oxidative stress such as peroxisome (all genes are down-regulated), reactive oxygen species (all genes down-regulated), UV-response down (all genes up), UV-response up (all genes down-regulated), DNA repair (all genes down-regulated) (Figure 10c), indicating reduction of oxidative stress markers in MTRN-treated retina. Additional regulated pathways were coagulation (all genes down-regulated including *Mmp3, Timp1, Clu, Sparc, S100a1, Thbd*, and others), oxidative phosphorylation (all down-regulated), and mitotic spindle (all up-regulated including *Cdc42, Rock1*, and *2*). These regulated pathways indicate that MTRN reduces oxidative stress, activates microtubules formation and organization, and acts on fatty acid and mitochondria metabolism.

Reactome pathway analysis (Figure 10d) identified SUMOylation and several pathways involved in the neural system, neurite outgrowth, synapses, neuroligin, and neurexin, suggesting an effect on neural structural organization.

Figure 10. Transcriptomic analysis of the RPE-choroid complex at day 7 after MTRN (1 µg/mL) was administered at the time of laser delivery. Gene-scaled MA plot. (DESeq2, log2 FC > 1, *p* < 0.05) (**a**), GSEA Hallmark. The dot plot depicts the gene ratios (number of core genes over the total number of genes in the set). The dots are colored by the adjusted *p*-value and their size is proportional with the size of the gene set (**b**), genes in the pathways in boxplots are detailed in the gene concept network, (**c**) gene pathway association using the gene concept network that provides information on the linkages of genes and pathways. Genes are colored by their FC and the size of the dots are proportional to the size of the gene set. (**d**) Reactome-enriched term plots from Reactome gene sets. The dot plot depicts the gene ratios (number of core genes over the total number of genes in the set). The dots are colored by the adjusted *p*-value and their size is proportional with the size of the gene set.

4. Discussion

In the first part of this study, we confirmed that MTRN is a mineralocorticoid target gene. Indeed, in our previous study, we showed that antagonism of the mineralocorticoid receptor (MR) pathway had anti-angiogenic effect in the laser-induced CNV model in rodents and that this effect was unrelated to VEGF, but rather mediated by decorin [13] and by other proteins, up-regulated by MR, and detected in a transcriptomic study, including MTRN [11,13]. Aldosterone, a specific MR agonist, significantly down-regulated MTRN in the retina in vivo, whilst spironolactone rather increased its expression as compared to aldosterone in the RPE/choroid complex. These differences in agonist and antagonists effects could be related to the biodisponibility of spironolactone that has a very short half-life in the vitreous and strong hydrophobicity [25] and could thus be rapidly eliminated in the deep retinal layers.

Having identified MTRN as a potential anti-angiogenic effect of spironolactone, we then have studied its distribution in the adult retina. Immunolocalization of MTRN in the rodent and human retina has confirmed that in adults, MTRN is expressed and secreted by glial cells including astrocytes and glial Müller cells [20]. MTRN was shown to promote GFAP expression in astrocytes, favoring their differentiation via the Jak-STAT3 pathway [15]. Interestingly, our study shows that MTRN is enriched in the macula of non-human primates and in humans, where it is mostly expressed and secreted by glial

Müller cells, which have specific features and organization in the macula [26], but it is also expressed in cone photoreceptors and localized in nuclear bodies, including PML ones. In the macula, MTRN associates with thrombospondin-1, which is produced by glial cells and is a strong negative regulator of angiogenesis [27]; it could be hypothesized that MTRN could contribute to the maintenance of the avascular foveal zone. In addition, we clearly identified MTRN in RPE cells both in rats and in humans, where it is secreted towards the Bruch's membrane and the choroidal vessels and is also sequestered in nuclear bodies in RPE. The expression of MTRN in human RPE cells derived from iPSc was confirmed as well (manuscript under revision). However, MTRN was also found in ganglion cells and in nerve fibers and in some nuclei of the deeper inner nuclear layer. MTRN is thus ubiquitous in the human retina and choroid. The localization of MTRN in nuclear bodies was further shown by SUMOylation of the protein, which is a process that favors its retention in PML nuclear bodies [24,28]. Indeed, PML nuclear bodies can act as reservoirs for stress-response proteins [29,30], which can be very quickly released in case of stress such as hypoxia, known to be a strong enhancer of MTRN production [20]. In the neural retina, Reactome pathway analysis, SUMOylation was regulated by MTRN, confirming that this protein modification could regulate its activity.

In the laser-induced CNV model, intravitreous injection of MTRN was performed either preventively at increasing doses or curatively at 7 days after laser induction. The mouse recombinant MTRN has 87% of sequence homology with the rat and a molecular weight of 30KD, which is comparable to therapeutic proteins injected in humans to treat wet AMD with monthly injections [31], suggesting that a single injection should cover the duration of the experiment, which was 15 days. However, in order to reach efficient concentrations of therapeutic proteins in the deep retinal layers, and maintain these levels for several weeks, high initial concentrations are required. This is confirmed in our experiment, since an estimated initial concentration of 1 to 6 ng/mL was required to reduce significantly the angiographic leakage and size of the choroidal neovascular membrane. The highest MTRN dose tested (6 ng/mL in the vitreous) maintained an efficient anti-angiogenic effect both on leakage and CNV size when injected at 7 days, which is relevant in the prospect of clinical use of MTRN. We can ensure that MTRN activity was maintained for at least 7 days since transcriptional regulations in the neural retina and in the RPE/choroid complex could be observed at this time point.

The mechanisms of action of MTRN on CNV are complex and not related to VEGF expression regulation, since we did not observe any decrease in VEGF or its receptors in the transcriptomic study and using RT-PCR at day 3 and 7 (not shown). The transcriptomic analysis at seven days did not show a significant down-regulation of VEGF or its receptors nor an increased expression of thrombospondin-1, although GSEA Hallmark pathway analysis revealed an increase in *Thsp1* and *Thsp2* gene expression in RPE/choroid-treated rats. Accordingly, we observed strong thrombospondin release from astrocytes endfeet at the site of laser burn at 14 days and high thrombospondin expression in the CNV lesion despite its significantly reduced size. It can thus be hypothesized that MTRN act, at least in part, through secretion of thrombospondin, known to be reduced in eyes with wet AMD [32].

The transcriptomic analysis provides a broader view of the potential transcriptional mechanisms of action of MTRN. Although it gives a snapshot picture of expression levels at a single time point after treatment (day 7), it indicates major pathways regulated in RPE/choroid and in the retina treated with MTRN. As expected, the IL6-Jak-STAT3 pathway is regulated by MTRN, as already demonstrated in the brain and the retina [15,33].

Interestingly, MTRN regulates in RPE/choroid numbers of genes encoding proteins and glycoproteins involved in Bruch's membrane maintenance including decorin, biglycan, mimecan, and aggregan, amongst others, and is known to be de-regulated in AMD [34]. Decorin gene, up-regulated by MTRN, was shown to decrease hypoxia-induced Met, Rac1, HIF-1α, and VEGF expression in ARPE-19 cells in vitro [35], but to exert anti-angiogenic activity in the CNV model, without reducing VEGF [13]. MTRN also regulates metalloprotease expression and particularly down-regulates TIMP1, MMP10, and MMP3, involved in

AMD. MMP3, mostly located in the Bruch membrane [36], is up-regulated in RPE, submitted to oxidative stress [37], and in complement activation-induced injury [38]. In the TGF beta signaling pathway, the gene encoding thrombospodin-1 was up-regulated, indicating potential additional anti-angiogenic effect through thrombospondin-1 regulation [27]. Altogether, these results indicate that MTRN plays an important role in Bruch's membrane/RPE complex integrity and could regulate choroidal neovascularization through extracellular matrix and endothelial cells interactions.

In the neural retina, MTRN regulates several pathways that represent oxydative stress, mostly towards a reduction of oxydative stress, involving matrisome and mitochondria. Moreover, Reactome pathways show that MTRN interact with neurite outgrowth, synapses and the tubulin-associated proteins, which is in line with its effects on nerves both in pain and in excitotoxic injury models [16,17,19,33]. Whether MTRN could play additional neuroprotective and antifibrotic effects in AMD remains to be demonstrated.

Like in any study, there are limitations due to experimental potential biais. Here, we analyzed the localization of MTRN on human retina from individuals that have been enucletaed, which offers the unique opportunity to analyze fresh tissue. On the other hand, these eyes have anterior tumors that have indicated the enucleation. Thus, although the posterior retina did not show morphological changes and GFAP, which is a very sensitive stress marker in retinal Müller cells, was not activated, we cannot exclude potential pathological influence on the results. On the other hand, MTRN enrichment in the macula was not only observed in humans, but also in non-human primate macula, and we also found similar enrichment using differential transcriptomic analysis of macula versus periphery in monkeys (personnal unpublished data). Nevertheless, these results should be confirmed in non-pathologic human eyes from donors of different ages.

In conclusion, we report herein that MTRN is a ubiquitous protein that is not only secreted by glial cells in the human retina but also sequestered in nuclear bodies in photoreceptor and ganglion cells. It is a target gene of the MR antagonist, that through its anti-angiogenic effects, it could contribute to the effect observed in patients with wet AMD treated with spironolactone in addition to anti-VEGF [13].

Further studies should validate the regulatory target genes, identified by a single time point transcriptomic analysis, and evaluate potential synergic effects of MTRN with currently used anti-VEGF drugs.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Switching to Brolucizumab in Neovascular Age-Related Macular Degeneration Incompletely Responsive to Ranibizumab or Aflibercept: Real-Life 6 Month Outcomes

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Abstract: *Purpose*: The aim of this study was to evaluate the effect of switching treatment in eyes with neovascular age-related macular degeneration (nAMD) and treatment intervals of ≤6 weeks to brolucizumab. *Methods*: In this prospective series, eyes with persisting retinal fluid under aflibercept or ranibizumab every 4–6 weeks were switched to brolucizumab. Visual acuity (BCVA), reading acuity (RA), treatment intervals, central subfield thickness (CST), and the presence of intra- and subretinal fluid were recorded over 6 months. *Results*: Seven of 12 eyes completed the 6 month follow-up and received 4.4 ± 0.5 brolucizumab injections within 28.0 ± 2.8 weeks. Treatment intervals increased from 5.3 ± 0.9 weeks to 9.0 ± 2.8 weeks (95% confidence interval of extension (CI): 1.6 to 5.9). BCVA improved from 67.8 ± 7.2 to 72.2 ± 7.5 (95% CI: −0.3 to 9.1) ETDRS letters, RA improved from 0.48 ± 0.15 to 0.31 ± 0.17 LogRAD (95% CI: 0.03 to 0.25), and CST improved from 422.1 ± 97.3 to 353.6 ± 100.9 μm (95% CI: −19.9 to 157.1). Treatment was terminated early in five eyes (two intraocular inflammations with vascular occlusion without vision loss, one stroke, and two changes in the treatment plan). *Conclusions*: Improvement in visual performance and longer treatment intervals in our series over 6 months indicate the potential of brolucizumab to reduce the treatment burden in nAMD, while two instances of intraocular inflammation were encountered.

Keywords: brolucizumab; ranibizumab; aflibercept; anti-VEGF; neovascular age-related macular degeneration; visual acuity; reading acuity; disease activity; treatment change; treat and extend

1. Introduction

The prevalence of neovascular age-related macular degeneration (nAMD) is increasing, along with an increasing treatment burden for patients and healthcare systems [1]. Current regulatory approved anti-vascular endothelial growth factor (anti-VEGF) drugs include ranibizumab since 2006 (Lucentis®, Novartis AG, Switzerland), aflibercept since 2012 (Eylea®, Bayer Pharmaceutical AG, Germany), and brolucizumab since 2020 (Beovu®, Novartis AG, Switzerland). Despite these options, disease stability is not achieved in all eyes, with averages of 62.9% and 56.0%, respectively, after 12 and 24 months of treatment, whereas treatment intervals may be extended during the same periods to ≥12 weeks, with stability at 37.7% and 42.6%, respectively [2]. HAWK and HARRIER, two phase 3 trials of brolucizumab, reported a better morphological stability of central retinal subfield thickness with noninferior visual acuity, compared to aflibercept [3]. ALTAIR, a phase 4 trials of aflibercept, investigated a treatment interval extension to 16 weeks under a treat-and-extend (T&E) protocol. In this trial, 43.9% of eyes maintained an interval of 16 weeks after 96 weeks [4]. Obviously, an unmet medical need exists with regard to the portion of eyes achieving disease stability and safely reaching treatment intervals of 3 months or more. Currently, the available limited evidence indicates an increasing potential to achieve disease stability and allow a treatment interval extension to ≥12 weeks from ranibizumab

over aflibercept to brolucizumab [2]. The hope that brolucizumab might more effectively achieve disease stability in the subset of eyes which cannot be extended to 8 or more weeks under their current anti-VEGF therapy led to switching to brolucizumab with the aim of reducing the treatment burden in many cases upon its approval. Recent studies showed a short-term reduction of foveal thickness 4–8 weeks after switching to brolucizumab in eyes with active nAMD despite the consequent anti-VEGF treatment every 4–8 weeks, indicating its superior efficacy regarding anatomic criteria [5–7]. Adding to this short-term experience, the aim of this study was to evaluate the functional and anatomic outcomes after switch to brolucizumab over 6 months in eyes with active nAMD under treatment intervals of ≤6 weeks with other anti-VEGF agents.

2. Materials and Methods

This is an ongoing prospective open-label single-center (Berner Augenklinik am Lindenhofspital) cohort study, analyzing the effect of a switch to brolucizumab in eyes with nAMD insufficiently responsive to ranibizumab or aflibercept. Primary endpoints are the change in morphologic markers of disease activity measured by central subfield thickness (CST) and the presence of intra- and subretinal fluid, as well as single optotype distance and text reading visual acuity. Secondary outcomes include changes in injection intervals, annual number of injections, and qualitative changes in activity signs of macular neovascularization (MNV) using optical coherence tomography angiography (OCTA). The study is designed for 2 years. Here, we report the preliminary 6 month data.

The study followed the standards of the Declarations of Helsinki, and local ethical board approval was granted (Kantonale Ethikkommission Bern, Switzerland, reference number 2020-00412). Patients from February to June 2020 were offered a switch to brolucizumab with the following inclusion criteria: treatment for nAMD for at least 1 year and functionally relevant persisting intra- and/or subretinal fluid despite treatment intervals of 6 weeks or less. Exclusion criteria were denial of informed consent, macular scarring preventing a change in visual function, and other causes of intra- or subretinal fluid. Treatment-naïve patients were not included in this study. Treatment was switched after the confirmation of MNV activity by dye leakage in fluorescein angiography (FA) and persisting fluid in optical coherence tomography (OCT). Initial therapy was initiated with three monthly loading injections using ranibizumab or aflibercept at the discretion of the treating physician, thereafter following a treat and extend (T&E) regimen with 2 weeks increments according to morphological signs of activity in OCT. Upon switching, treatment with brolucizumab was initiated within maximally 6 weeks of the last injection, followed by a second injection after 1 month, thereafter again following a treat-and-extend protocol (T&E) according to morphological response in OCT. Treatment intervals were extended by 2 weeks in the case of resolved intra- and resolved or stable subretinal fluid over three consecutive injection intervals. At every visit, best corrected distance visual acuity (BCVA) was measured on a Snellen decimal scale and converted to Early Treatment of Diabetic Retinopathy Study (ETDRS) letters equivalent, where a Snellen decimal BCVA of 1.0 was defined as 85 ETDRS letters for the statistical analysis; reading acuity (RA) was measured on a standardized text, which is comparable to Radner and Birkhäuser charts, with logarithmically reduced font size, ranging from 0.1 to 1.6 Snellen decimal equivalent (https://szb.abacuscity.ch/de/A~51.952/Nahsehprobe-D-Erw.-R%C3%BCckseite%3A-ETDRS-_-LCS, accessed on 1 June 2021). Results were converted to the negative logarithm of decimal reading acuity (logReading Acuity Determination = LogRAD) for statistical analysis. OCT angiography (OCTA) was also carried out (Heidelberg Spectralis OCT 2 with 880 nm wavelength, axial resolution 3.9 μm and lateral resolution 5.7 μm, Heidelberg Engineering, Heidelberg, Germany). Lastly, a thorough ophthalmologic examination including dilated fundoscopy was performed to exclude signs of inflammation. Central subfield thickness was defined as the average thickness between the internal limiting membrane (ILM) and Bruch's basal membrane (BM) within the central 1 mm of the fovea. Centering to the fovea and BM segmentation were manually controlled and adjusted if needed for fixation or segmentation

misalignments. The 6 month data were analyzed using SPSS (software package V.23 (IBM Inc., Armonk, New York, NY, USA)). Changes of BCVA in ETDRS letters, RA in LogRAD, CST, injection intervals, and number of injections are reported with their respective 95% confidence intervals (CIs). Given the small sample sizes, we decided not to apply statistical analyses for this descriptive dataset.

3. Results

Twelve eyes of 12 patients were switched to brolucizumab according to the above-mentioned criteria between February and May 2020 in our center. The complete baseline characteristics and principal measurements are listed in Table 1.

Table 1. Baseline characteristics of patients and reason for dropouts. Legend: #IVT, total number of IVTs applied in total (months since baseline and number of injections) and within the last 6 months (number); m, male; f, female; R, right; L, left.

|				**#IVT Pretreatment**			**Treatment Discontinuation**
				Demographic and Disease Characteristics			
Measure	**Age**	**Sex**	**Laterality**	**Total**		**6 months**	
Unit	**Years**	**m/f**	**R/L**	**Months**	*n*	*n*	
Included	77	m	R	61	51	4	no
	84	f	R	27	19	4	no
	65.1	f	L	75	42	5	no
	77.2	f	L	113	91	6	no
	85.1	f	R	34	24	4	no
	84.6	m	R	53	30	5	no
	76.1	m	L	54	46	5	no
Excluded	76.3	m	R	13	11	5	14 weeks from baseline: transitory ischemic attack, 3 weeks after third brolucizumab injection. Not related to treatment. Discontinuation for safety precautions.
	84.4	f	R	27	22	6	4 weeks after baseline: anterior and intermediate uveitis without vasculitis, 4 weeks after first brolucizumab injection.
	78.1	m	L	72	41	5	10 weeks after baseline: panuveitis and retinal occlusive vasculitis 6 weeks after second brolucizumab injection.
	87.1	f	L	13	14	6	4 weeks after baseline: progressive pigment epithelial detachment; switched back to aflibercept upon decision of the treating physician.
	88.8	f	R	48	10	5	21 weeks after baseline: adjunctive treatment with intravitreal dexamethasone for persistent intravitreal fluid, 5 weeks after the fifth brolucizumab injection (see text).

Seven eyes reached a follow-up of 6 months (28.0, SD ± 2.8 weeks), in which they received 4.4 ± 0.5 brolucizumab injections compared to 4.7 ± 0.8 during the 26 weeks before baseline. The mean treatment interval was extended from 5.3 ± 0.9 weeks before switch to brolucizumab 9.0 ± 2.8 weeks thereafter (Figure 1, 95% CI of extension: 1.6 to 5.9 weeks).

Central subfield thickness regressed from 422.1 ± 97.3 µm to 353.6 ± 100.9 µm (95% CI: −19.9 to 157.1). A complete resolution of the previously persisting intraretinal and/or subretinal fluid was found in two out of seven eyes (29%). Intraretinal fluid was present in four (57%) and subretinal fluid in three eyes (43%) at baseline, and intraretinal fluid was present but reduced in two (29%, example in Figure 2) and subretinal fluid remained present in three eyes (43%), to a lesser extent in two eyes, and increased in one eye after 6 months. Distance BCVA changed from 67.8 ± 7.2 at baseline to 72.2 ± 7.5 ETDRS equivalents after 6 months (Figure 3a, 95% CI: −0.3 to 9.1). Reading acuity improved from

0.48 ± 0.15 at baseline to 0.31 ± 0.17 LogRAD after 6 months (Figure 3b, 95% CI: 0.03 to 0.25), which represents a two-line increase in RA from approximately 0.32 to 0.5 Snellen decimal. Table A1 (Appendix A) shows all measurements per patient at baseline and at the 6 month follow-up visit.
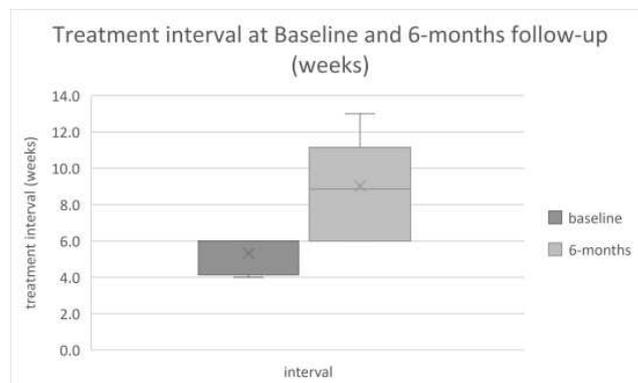
Figure 1. The last treatment interval at baseline before switch to brolucizumab was 5.30 ± 0.93 weeks with persistent disease activity before switch to brolucizumab and 9.0 ± 2.8 weeks at 6 months (95% CI of extension: 1.6 to 5.9, *n* = 7).
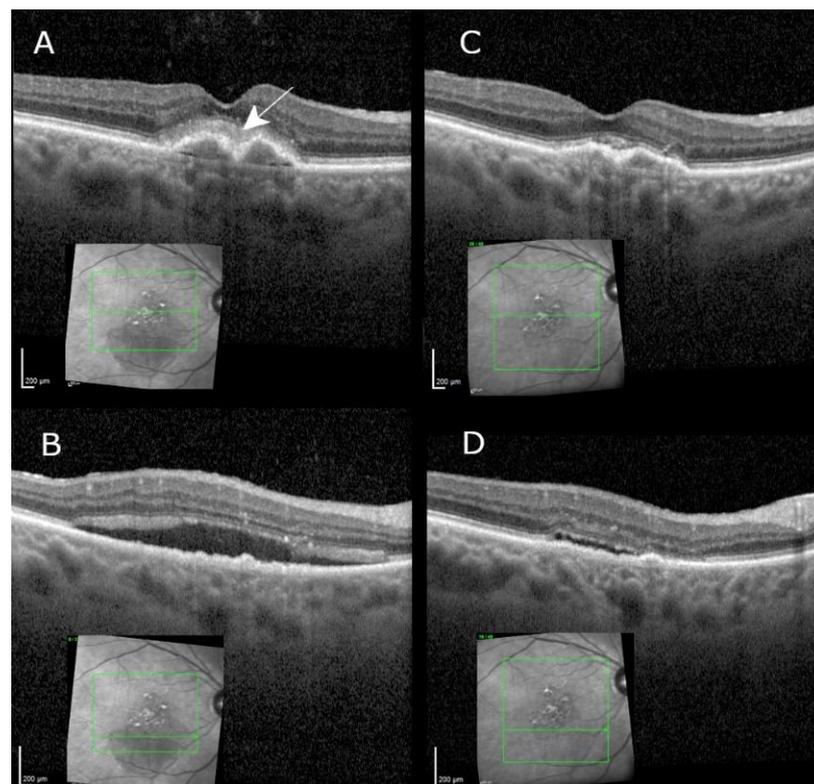
Figure 2. Optical coherence tomography (OCT) images of an 85 year old patient through the fovea (upper row, (**A**,**C**)) and the inferior parafoveal macular area (lower row (**B**,**D**)) at switch, 6 weeks after the last anti-VEGF injection (left row (**A**,**B**)), at the 6 month measurement visit 29 weeks from baseline, and 11 weeks after the fourth Brolucizumab injection. The figure shows resolved foveal subretinal hyperreflective material (arrows) and massively regressed subretinal fluid inferior to the fovea, despite a longer treatment interval. Meanwhile, distance visual acuity improved from 60 to 75 ETDRS letters, and reading acuity improved from 0.25 to 0.32 Snellen decimal (0.6 to 0.5 logRAD = logReading Acuity Determination).

(a)

(b)
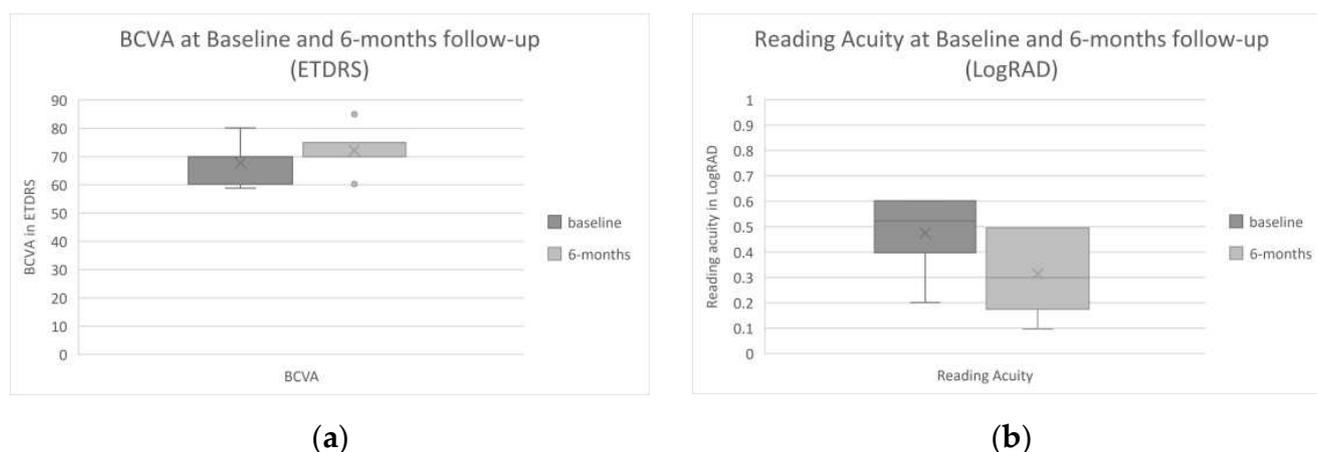
Figure 3. Box-and-whisker graph indicating changes in best corrected visual acuity (BCVA, 1A) and reading acuity (RA, 1B) from baseline to the 6 month follow-up exam. BCVA (**a**) was 67.8 ± 7.2 at baseline and 72.2 ± 7.5 ETDRS equivalents after 6 months (95% CI: −0.3 to 9.1, *n* = 7). Reading acuity (**b**) improved from 0.48 ± 0.15 (approximately 0.32 Snellen decimal) at baseline to 0.31 ± 0.17 (approximately 0.5 Snellen decimal) LogRAD after 6 months (95% CI: 0.03 to 0.25, *n* = 7), indicating a possible two-line increase in reading acuity.

Five patients were excluded from the analysis because treatment had to be discontinued prior to reaching the prescheduled study end after 6 months. Two patients developed intraocular inflammation with extramacular vascular occlusion after one and two injections, respectively, needing systemic corticosteroid treatment and the discontinuation of brolucizumab therapy, followed by a switch back to the previous anti-VEGF agents. The two cases are described in detail in the next section. One patient suffered a transitory ischemic attack. Since he had a previous history of stroke, this was probably not attributed to brolucizumab, but the therapy of the affected poorer eye was discontinued as a precautionary measure, and later resumed with the previous anti-VEGF agent. One patient with massive intraretinal fluid subjectively experienced further visual deterioration despite better visual acuity after one brolucizumab injection and requested to be switched back to the previous anti-VEGF therapy. In one patient, therapy was supplemented with intravitreal dexamethasone to reduce persistent injection burden by the attending physician, according to reported experience [8]. In the analysis of the excluded subset, no statistically significant change in BCVA or CST after 6 months compared to baseline was observed (Table A1, Appendix A).

Case Reports: Two Patients with Intraocular Inflammation after Brolucizumab

Case 1: 86 year old woman with a history of myocardial infarction. After 22 anti-VEGF injections within 27 months, treatment was switched to brolucizumab. Four weeks after switching to brolucizumab and escaping the patient's attention, a two-line reduction in best corrected visual acuity (BCVA) from 20/32 to 20/50, confluent keratic precipitates (Figure 4A), and moderate anterior and intermediate uveitis (2+ anterior chamber and vitreous cells, vitreous haze 2+) were observed. Wide-field fluorescein angiography showed arterial branch occlusions in the inferior vessel arcades (Figure 4B) without vascular leakage. Three-hourly treatment with topical prednisolone and systemic prednisolone 1 mg per kg of body weight (mg/kg of body weight per day for 1 week was tapered off thereafter while continuing acetylsalicylate and clopidogrel. After 1 week, precipitates disappeared. Ocular inflammation improved within 2 weeks, while BCVA decreased to 20/63 before recovering to 20/40 after 1 month. Intraretinal fluid was reduced for 2 months before increasing to the baseline-level 12 weeks after brolucizumab injection, at which time treatment with aflibercept was resumed.
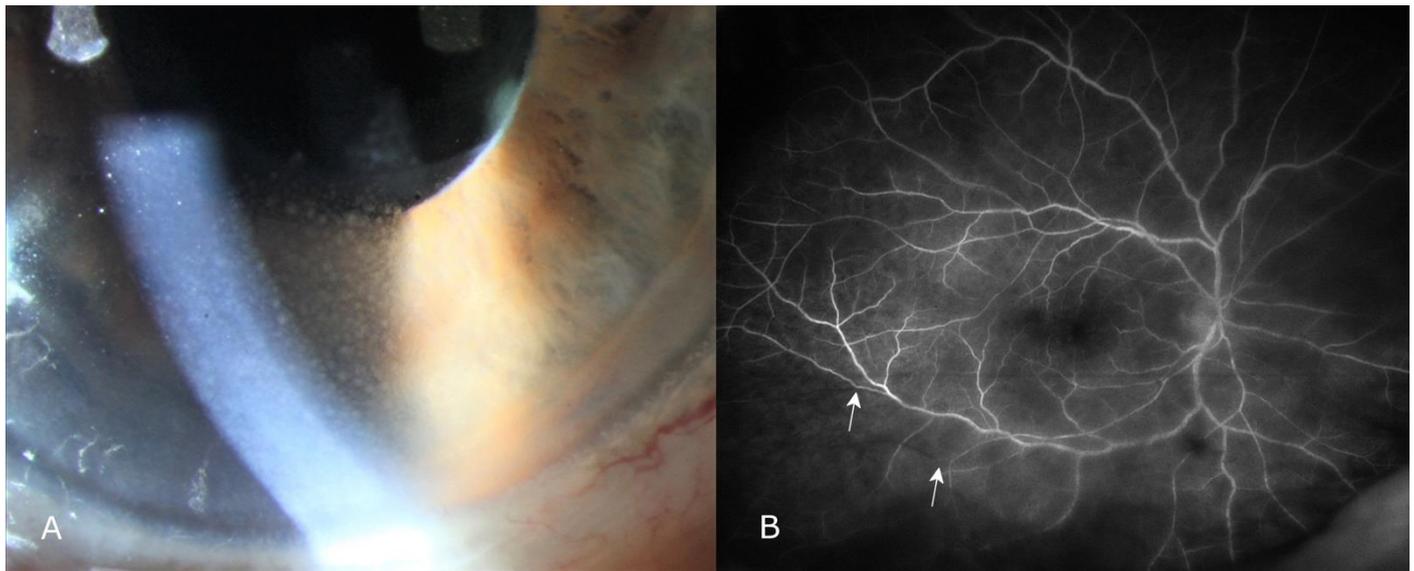
Figure 4. Case 1, an 86 year old woman with anterior uveitis and confluent mid-sized keratic precipitates (**A**): slit-lamp photograph and intermediate uveitis with mid-peripheral arterial occlusion of two arterial branches (arrows, (**B**): wide-field fluorescein angiography) 4 weeks after the first intravitreal brolucizumab injection. Intraretinal fluid slightly regressed for 12 weeks, and anterior uveitis and keratic precipitates completely regressed within 2 weeks of systemic and topic prednisolone. Visual acuity regained close to baseline values after temporary deterioration.

Case 2, 84 year old male with a history of myocardial infarction, stroke, and coronary and valvular surgery, under hemodialysis for renal failure after glomerulonephritis. After a total of 41 intravitreal injections within 72 months and significant retinal fluid despite a treatment interval of 4–5 weeks in his better eye, treatment was switched to brolucizumab. Four weeks after the second brolucizumab injection, the patient reported floaters, whereas BCVA improved from 20/40 to 20/32 and IRF resolved. We found a significant panuveitis with preretinal infiltrates, but no vascular sheathing. Widefield angiography revealed segment arterial leakage and extramacular branch arterial occlusions (Figure 5A–D). Intravenous methylprednisolone (40 mg) was given on two consecutive days, followed by oral prednisolone for 3 days (at 1 mg/kg bw then tapered off over 1 month), accompanied by acetylsalicylate. After 1 month, BCVA remained 20/32 in the absence of IRF with persisting vitreal infiltration. Further follow-up showed stable BCVA, recurrence of IRF, and retrograde staining of the occluded vessels 10 weeks after the last brolucizumab injection.
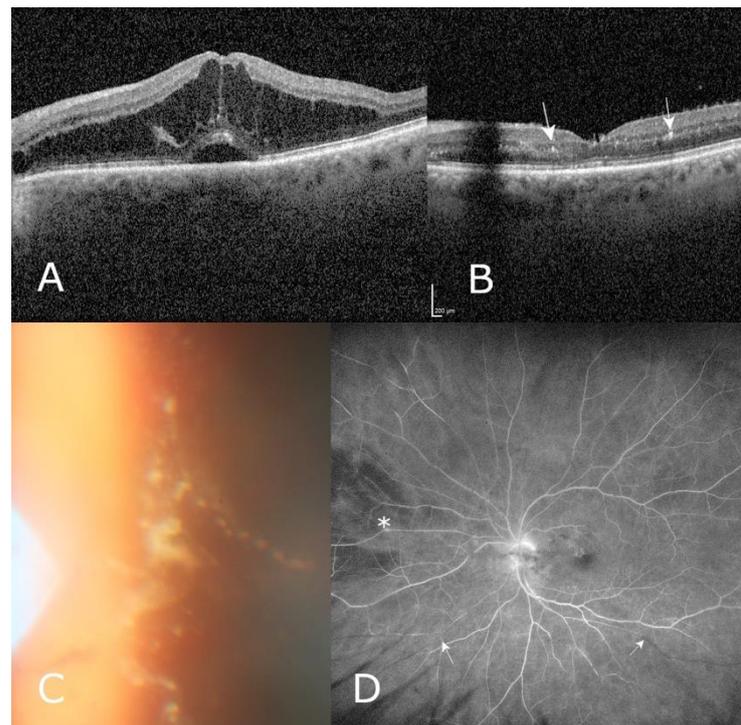
Figure 5. Case 2, an 84 year old male with persistent significant intraretinal fluid in optical coherence tomography (**A**) despite monthly treatment with aflibercept for age-related macular degeneration. Six weeks after the second four-weekly brolucizumab injection, intraretinal fluid completely regressed (**B**). The patient reported floaters without visual deterioration, and posterior uveitis with typical preretinal infiltrates (arrows indicate intraretinal hyperreflective spots in (**B**)) was observed (**B**,**C**). Wide-field fluorescein angiography (**D**) revealed vasculitis (asterisk) and branch retinal artery occlusion (arrows). Four weeks later, fluorescein angiography (not shown) revealed a probably retrograde reperfusion of previously occluded arterial branches.

4. Discussion

Our data indicate a benefit of switching to brolucizumab with an increased reading visual acuity and increased treatment interval in eyes that were insufficiently responding to previous anti-VEGF agents in intervals of ≤6 weeks following a T&E regimen for nAMD. This confirms previous reports of a reduction in CST within the first 2 months after the switch to brolucizumab [5–7], and better morphological outcomes and a functional noninferiority compared to aflibercept over 2 years in two phase 3 studies [3]. Moreover, in accordance with previous studies, distance BCVA did not change significantly after switching from other anti-VEGF agents to brolucizumab, which is well in line with previous experiences of switching from ranibizumab to aflibercept [8–15]. In contrast to these publications, a sustained reduction in CST after 6 months was not recorded in our cohort, which may be linked to insufficient power. Nevertheless, we found a sustained improvement of reading acuity after 6 months. Along with a high satisfaction of our successfully switched patients due to a perceived improvement in visual performance, this provides further support that single-letter distance visual acuity is not representative of daily visual tasks [12,16–18].

A correlation of retinal thickness in OCT with reading abilities has been demonstrated in nAMD [19], but reading tests are not widely adapted in clinical practice and research, and testing is not standardized [20]. While ETDRS reading scores are standard for functional outcomes in large randomized studies, vision-related quality of life correlates more with contrast sensitivity and binocular reading speed, but ETDRS letter score does not correlate with binocular reading speed [16]. Reading speed and comprehension are substantially reduced in AMD [21], and reading disabilities have a significant impact on quality of

life in AMD [22]. Furthermore, microperimetry revealed correlations of reading speed with fixation stability, foveal absolute scotoma, reading acuity, and sociodemographic characteristics [23]. Similar findings are known from postoperative outcomes after the peeling of epiretinal membranes, where an increase in reading visual acuity and critical print-size was shown in patients despite unchanged BCVA [24]. Taken together, visual acuity measured with single optotypes seems unable to represent pathological changes in AMD or relevant parameters for the quality of life of affected patients. Reading requires the recognition of closely spaced letters or signs, which requires a larger parafoveal area of the retina than simply the resolution between two points. The discrepancy of impaired reading with maintained visual acuity is explained in diseases such as nAMD by the many small microlesions of the sensory cells in the center of the macula. To address this problem and achieve a more patient-centered therapy, different visual assessments have been compared [20]. Our data support the inclusion of reading acuity in the routine assessments of patients with nAMD as it has a greater informative value with regard to vision-related quality of life, as well as pathophysiology.

An obvious limitation of our study is the small sample size and the large dropout rate, together with a relatively short follow-up period. With respect to this, we decided to report only 95% confidence intervals without formally stating significance. The high dropout rate is explained by a surprisingly high incidence of treatment complications in our cohort with two cases of relevant intraocular inflammation and retinal vascular occlusion after one and two brolucizumab injections. HAWK and HARRIER already reported relatively high rates of intraocular inflammation (IOI) in eyes treated with brolucizumab but a similar rate of severe vision loss compared to the controls treated with aflibercept [3]. The HAWK and HARRIER reported rates for Aflibercept were, however, also higher than observed in a review of 10 phase 3 trials for aflibercept [25]. After the regulatory approval of brolucizumab, a case series of intraocular inflammation with retinal vascular occlusion was reported, some of them with significant vision loss [26–28]. As a result of these reports, treatment preferences are based on expert opinions, and the impact of treatment choice on visual outcome in real life has remained narrative [29,30]. The pathophysiological basis and risk factors for this IOI signal are currently a topic of intensive research within the community and the manufacturer. A post hoc analysis of the study data by an expert committee showed that most IOI with severe visual loss happened within the first 3–6 months of treatment [31]. After analysis of first interpretable data of the MERLIN trial, on 28 May 2021, Novartis® advised against the use of brolucizumab with intervals shorter than 6 weeks after up to three monthly loading injections, and terminated ongoing studies that allowed this possibility [32]. Therefore, efficacy and possible therapeutic improvement must be balanced against a potential risk, while our cohort showed a disproportionate incidence of such responses. This said, both of our patients recovered above baseline after treatment with topical and systemic corticosteroids, before treatment was resumed with the previous anti-VEGF agents.

In summary, in this small cohort, we observed an improved reading acuity and longer treatment intervals 6 months after switch to brolucizumab in patients with persistent nAMD activity despite intensive anti-VEGF treatment. Larger sample sizes are required to estimate the strength of this effect, which will prospectively be addressed in the currently recruiting Falcon study (ClinicalTrials.gov Identifier: NCT04679935).

5. Conclusions

Based on the preliminary data of our cohort, brolucizumab led to an increased reading acuity and longer treatment intervals in patients with high treatment demand for other anti-VEGF drugs, while distance BCVA measured with standard ETDRS or Snellen charts and CST remained unchanged. It, thus, seems that brolucizumab has a place at least as a second-line anti-VEGF agent in patients with high treatment demand. The increase in reading acuity seems to go along with an improved control of disease activity after switch to brolucizumab and may well contribute to an improved vision-related quality

of life. Given the risk of intraocular inflammation and vascular occlusion, careful patient selection and education remain essential for early detection and successful treatment of possible complications. We strongly advocate the use of standardized reading charts for the assessment of functional evolution in nAMD under treatment, appearing to be a better physiological and psychological representation of the functional impact of disease activity with regard to vison-related quality of life.

Appendix A

Table A1. Measurements before and 6 months after switch to brolucizumab. **Legend:** BCVA: best corrected visual acuity for distance and single optotypes; CST: central subfield thickness; ETDRS: Early Treatment of Diabetic Retinopathy Study letters; Inj: total number of Injections applied during the observation period; interval: interval from last injection; IRF: intraretinal fluid present in B-Scan optical coherence tomography imaging; IVT: intravitreal treatment; logRAD: log reading acuity determination = reading acuity equivalent of logMAR; Obs: observation time from baseline to the 6 month measurements in weeks (varies due to individual treat and extend intervals); RA: reading acuity; SRF: subretinal fluid present in B-Scan optical coherence tomography imaging; y: yes/present; n: no/not present; #IVT: number of IVTs applied within the specified time; 6 mths: last 6 months before switch; mos: total treatment duration of the disease before switch in months.

		Baseline Measurements								Six Months Measurements						
Measure	Interval	BCVA	RA		CST	IRF	SRF	Obs	Inj	Interval	BCVA	RA		CST	IRF	SRF
Unit	Weeks	ETDRS Score	Snellen	Log-RAD	μm	y/n	y/n	Weeks	#IVT	Weeks	ETDRS	Snellen	logRAD	μm	y/n	y/n
Included	5	70	0.3	0.523	499	y	n	28	5	6	75	0.63	0.201	541	y	n
	6	80	0.63	0.201	367	n	y	33	4	11	85	0.8	0.097	263	n	n
	4	70	0.4	0.398	368	n	y	28	5	6	70	0.63	0.201	305	n	y
	4	70	0.4	0.398	316	y	n	26	4	9	70	0.4	0.398	279	y	n
	6	60	0.25	0.602	341	n	y	29	4	11	75	0.32	0.495	335	n	y
	6	65	0.25	0.602	570	y	n	28	5	7	70	n/a	n/a	311	n	n
	6	59	0.25	0.602	494	y	n	24	4	13	60	0.32	0.495	441	n	y
Excluded	4	50	0.13	0.886	485	y	y	26	n/a	n/a	39	0.13	0.886	523	y	y
	4	75	0.63	0.201	323	y	y	24	n/a	n/a	75	0.5	0.301	313	y	n
	5	70	0.32	0.495	708	y	y	25	n/a	n/a	75	0.63	0.201	314	y	n
	4	41	0.13	0.886	933	n	y	22	n/a	n/a	20	n/a	n/a	1250	n	y
	5	45	n/a	n/a	417	y	n	30	n/a	n/a	50	n/a	n/a	n/a	n/a	n/a

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The Spectrum of Central Choriocapillaris Abnormalities on Swept-Source Optical Coherence Tomography Angiography in the Fellow Eye of Unilateral Exudative Age-Related Macular Degeneration Patients: From Flow Deficits to Subclinical Non-Exudative Neovascularization

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Abstract: We evaluated the spectrum of choriocapillaris (CC) abnormalities in the fellow eyes of unilateral exudative age-related macular degeneration (AMD) patients using swept-source optical coherence tomography angiography (SS-OCTA). Fellow eyes of unilateral exudative AMD patients were prospectively included between May 2018 and October 2018. Patients underwent a multimodal imaging including a SS-OCTA. Demographics and clinical findings were analyzed. The estimated prevalence of macular neovascularization (MNV) was computed. Number and size of flow deficits (FDs) and percentage of flow deficits (FD%) were computed on the compensated CC flow images with the Fiji software. We included 97 eyes of 97 patients (mean age was 80 ± 7.66 years, 39 males, 58 females). The prevalence of MNV in the studied eyes was 8.25% (8/97 eyes). In the 89 non-neovascular eyes, FD% averaged $45.84\% \pm 11.63\%$, with a corresponding total area of FDs of 4.19 ± 1.12 mm^2. There was a higher prevalence of drusenoid pigment epithelial detachment in eyes with subclinical neovascularization ($p = 0.021$). Fellow eyes with unilateral exudative AMD encompassed a series of CC abnormalities, from FDs of the aging CC to subclinical non-exudative MNV.

Keywords: AMD; oct-angiography; fellow eye; choriocapillaris; quiescent macular neovascularization

1. Introduction

Age-related macular degeneration (AMD) is a leading cause of vision loss in developed countries [1]. Among the hallmarks of AMD, drusen and pigment epithelial changes play a major part. Recently, Yu et al. have shown on the Age-Related Eye Disease Study Research Group 2 (AREDS2) eyes that, by 5 years, 67% of eyes with drusenoid pigment epithelial detachments (PED) progress to late a AMD, such as geographic atrophy (GA) or macular neovascularization (MNV) emanating from the choroid (type 1 and 2) or from the deep retinal layer (type 3) [2]. An increasing body of literature shows that besides inflammation or genetic predisposition, dysfunction of the complex comprised of retinal pigment epithelium (RPE)-Bruch's membrane-choriocapillaris (CC) is mainly involved [3–5] in AMD pathogenesis and progression.

Choriocapillaris is a key source in the nutrition and oxygenation of the RPE. Bhutto and Lutty have shown that in atrophic AMD, the death and dysfunction of photoreceptors

and CC appear to be secondary to the RPE loss, while in exudative AMD, the loss of choroidal vasculature may be the initial insult to the complex [6]. Loss of CC generates hypoxia in the RPE and consequently angiogenic substances are produced, abutting in the growth of neovascularization. Thus, the loss of CC might also be a stimulus for drusen formation because the disposal system for retinal debris and exocytosed material from RPE would be limited. Ultimately, the photoreceptors die of lack of nutrients, leakage of serum components from the neovascularization, and scar formation [6].

With the advent of optical coherence tomography angiography (OCTA), a depth-resolved non-invasive imaging technique, a detailed study of the CC in vivo has been possible [7,8]. Zheng et al. recently showed that, in normal aging eyes, the percentage of CC flow deficits (FDs) increases with age across the central 5 mm of the macula [9]. In intermediate AMD eyes, Borrelli et al. demonstrated an increased CC flow impairment, which co-localized to the area of CC beneath and immediately surrounding drusen [10]. Moreover, previous histological studies by Green and Sarks have described the presence of neovascularization that was not accompanied by exudation in postmortem eyes [11,12]. Querques et al. described this type of neovascularization in intermediate AMD eyes, coining the term of "quiescent" MNV by means of multimodal imaging accompanied by a functional characterization [13]. The detection of such quiescent neovascularization was possible by means of both indocyanine green angiography (ICGA) and spectral domain optical coherence tomography (SD-OCT), the latter revealing, at the site of quiescent MNV, an irregular, slightly elevated RPE with its major axis in the horizontal plane. Nonetheless, in recent years, OCTA has proven to be highly useful in detecting such quiescent neovascularization [14,15]. Therefore, given that subclinical, non-exudative MNV may precede the onset of exudation [16], our aim was to evaluate the spectrum of CC abnormalities in the fellow eyes of patients with unilateral exudative AMD using OCTA.

2. Materials and Methods

Consecutive patients with unilateral exudative AMD were enrolled in this prospective cross-sectional study at the Department of Ophthalmology at Créteil Hospital between May and October 2018. The institutional review boards of the University Paris-Est Créteil approved the study. The study was conducted in accordance with the tenets of the 1964 Declaration of Helsinki.

Fellow eyes of unilateral exudative AMD patients were included. Therefore, the fellow eye was considered as the study eye. Exclusion criteria consisted of active MNV, prior anti-vascular endothelial growth factor (anti-VEGF) treatment in the study eye, GA, and other confounding retinal disease, such as adult-onset foveomacular vitelliform dystrophy, high myopia, and diabetic retinopathy. Patients with a signal strength index <8 were also excluded.

Study eyes were classified as early AMD if drusen was <125 μm in diameter without any other abnormalities [17] present and as intermediate AMD if drusen was >125 μm with or without pigmentary abnormalities [17] present. Presence of small drusen (<63 μm), large drusen (>125 μm), drusenoid pigment epithelial detachment (PED) (>350 μm) and reticular pseudodrusen (RPD) were reported.

Subclinical non-exudative macular neovascularization (MNV) was defined as MNV without intraretinal/subretinal exudation on OCT but well detectable on fluorescein angiography (FA) and ICGA [13–15].

2.1. Imaging

All patients underwent a complete ophthalmological evaluation, including multi-modal imaging: infrared imaging (IR), blue fundus autofluorescence (FAF), (Spectralis; Heidelberg Engineering, Heidelberg, Germany), spectral domain optical coherence tomography (Spectralis; Heidelberg Engineering, Heidelberg, Germany), and swept-source OCTA (Plex Elite, Carl Zeiss Meditec, Inc., Dublin, CA). All patients had had at least one FA

and ICGA (Spectralis; Heidelberg Engineering, Heidelberg, Germany) previous to study inclusion.

Swept-source OCTA had an A-scan rate of 100,000 A-scans per second and a light source of 1050 nm (1000–1100 nm full bandwidth). The axial resolution was 6.3 μm and transverse resolution was 20 μm. We obtained 3×3 mm^2 OCTA images centered on the fovea.

Grading was performed by expert readers on 3×3 mm^2 SS-OCTA and corresponding structural OCT images, as well as on SD-OCT images and FAF. SS-OCTA and structural SS-OCT images were independently analyzed by two expert readers (SF and AMB) to confirm the presence/absence of drusen, subclinical non-exudative macular neovascularization, or macular atrophy. In case of disagreement, a third reader (AM) adjudicated discordances. SD-OCT images were analyzed by two expert readers (AKK and VC) to confirm presence and type of drusen and to measure central macular thickness (CMT) and choroidal thickness (CT). In case of disagreement, a third reader (ES) adjudicated discordances. Presence/absence of atrophy was assessed on FAF images (CJ, AM). CMT was recorded from the retinal thickness ETDRS grid generated by the Spectralis software (Version 1.10.4.0, Heidelberg Engineering, Heidelberg, Germany). CT was determined using a scan passing through the central fovea and defined as the distance between the retinal pigment epithelium–Bruch's membrane complex and the sclerochoroidal interface [18]. Calipers provided by the OCT software were used for this measurement.

2.2. Image Processing

En face CC structure and en face CC flow images were extracted from the OCTA device. Manual segmentation was used to segment the CC slab as previously described [10] (10 μm thick segments, starting 31 μm posterior to the RPE fit reference). Segmentation errors, if present, were manually adjusted by one reader (AM) in order to correct segmentation. Structural and vascular images were then processed.

Images were then imported into the Fiji Software (National Institute of Mental Health, Bethesda, MD, USA). In order to compensate the shadowing generated by drusen onto the CC, we used a previously described method of compensation for the signal attenuation under drusen [10,19] (Figure 1).

En face CC flow images were compensated with en face CC structures as follows: To an en face CC structure image, an inverse transformation was applied using the Fiji "Invert" function. Gaussian blur filter was applied on the en face CC structure image for smoothing. Multiplication between the en face CC flow image and the processed en face CC structure image was performed using "Image Calculator". Thus, we obtained a compensated en face CC image. Binarization of the compensated en face CC flow image was performed using the Phansalkar threshold (radius: 15 pixels) in order to obtain a quantitative analysis of the flow deficits as described in recent literature [10]. The percentage of flow deficits (FD%), number and size of flow deficits (FDs), the total FDs area were obtained using the "Analyze Particles" module (size: 0–infinity, circularity: 0–1) [10].

The number of FDs was the number of contiguous black pixel areas representing the flow deficits. The size of FDs was the mean area, in μm^2, of contiguous black pixel area. The total FDs area represents the area, in mm^2, of all the FDs. The FD% was computed as the total FDs area on the total image (black and white pixels) area.
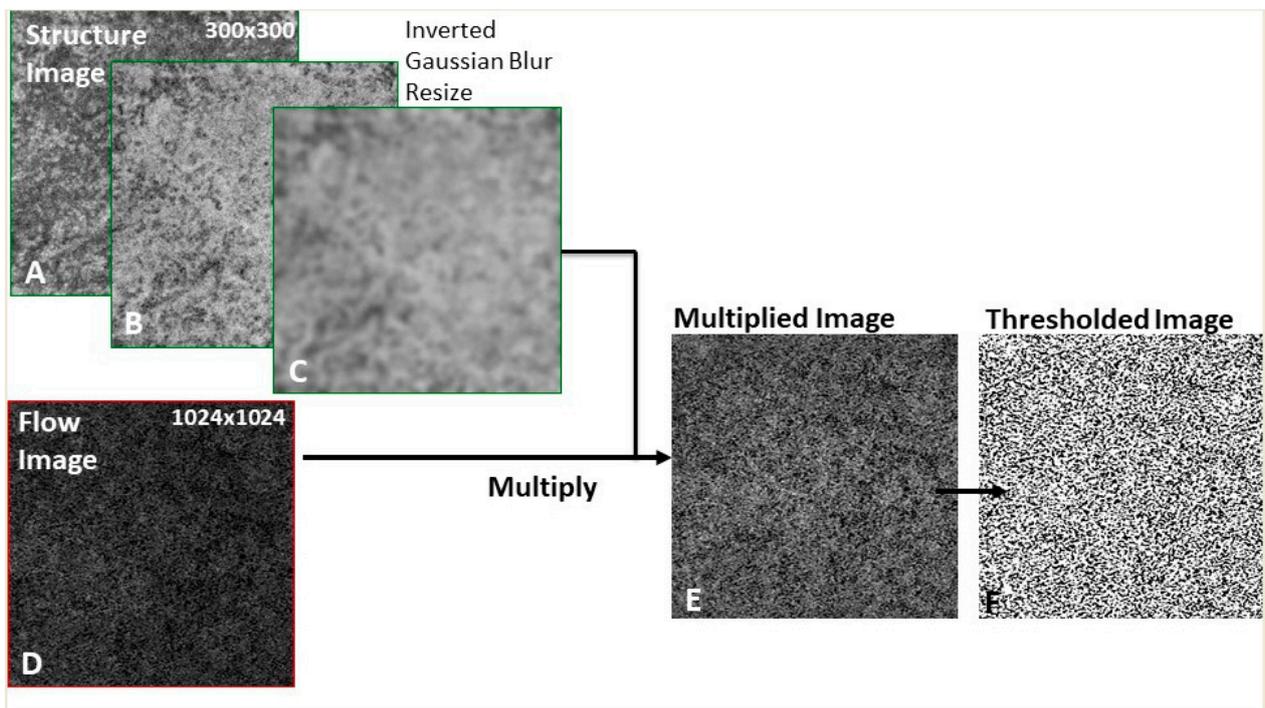
Figure 1. Representation of the algorithm used to investigate the choriocapillaris (CC). To the en face CC structure image (**A**) an inverse transformation was applied using the Fiji "Invert" function (**B**). Smoothing was obtained using the Gaussian blur filter (**C**). Multiplication between the en face CC flow image (**D**) and the processed en face CC structure image (**C**) was performed using "Image Calculator". A compensated en face CC image was obtained (**E**). Binarization of the compensated en face CC flow image was performed using the Phansalkar threshold (radius: 15 pixels) in order to obtain a quantitative analysis of the flow deficits as described in recent literature (**F**).

2.3. Statistical Analysis

Qualitative variables were expressed in percentages and quantitative variables were expressed by their mean with standard deviation. Comparisons of qualitative variables were performed using the Fischer exact test. Comparisons of quantitative variables were performed using the Mann–Whitney test. We tested and compared different radii within the compensation algorithm [20]: radius of 4 pixels, corresponding to 26.37 microns, radius of 8 pixels (49.80 microns), 10 pixels (61.52 microns), and 15 pixels (90.82 microns). The coefficient regression values were calculated using the linear regression method. Multivariate analyses were performed to search factors associated with FD%, size and number of FDs expressed as log10. They took into account the following parameters: age, best corrected visual acuity (BCVA, in logMAR), CMT, CT (in log10), small drusen, large drusen, drusenoid PED, RPD, and atrophy that were associated in univariate analysis ($p < 0.20$) and/or clinical significance. A relation between the FD size and FD number was assessed by using the logarithm of the FD size and FD number values and by carrying out a linear regression [7]. $p < 0.05$ was retained as significant. Analyses were performed with STATA, version 13.0. (Texas, USA).

3. Results

3.1. Patient Demographics and Clinical Characteristics

We included 97 eyes of 97 patients (mean age was 80 ± 7.66 years, 39 males, 58 females). In our series, unilateral exudative AMD consisted of type 1 MNV in 61/97 eyes, type 2 MNV in 15/97 eyes, type 3 MNV in 8/97 eyes and mixed type 1 and 2 in 13/97 eyes.

The fellow eye was considered as the study eye. In our series, 62/97 study eyes presented with small drusen, 69/97 with large drusen, 44/97 with drusenoid PED, 54/97 with reticular pseudodrusen (RPD), while 19/97 presented with macular atrophy on FAF

imaging and SD-OCT. In all the eyes, macular atrophy was present outside the 10 central degrees analyzed.

On the overall cohort, mean CMT was 238.49 µm ± 67.49 µm and mean CT was 210.51 µm ± 83.21 µm.

3.2. OCTA Spectrum of Choriocapillaris Abnormalities

Mean PlexElite Image Quality was 8.99 (±0.74).

3.2.1. Neovascularization: Subclinical Non-Exudative MNV

Table 1 summarizes the characteristics of the neovascular study eyes (*n* = 8).

Table 1. Demographics, clinical findings, and multimodal imaging features of patients (*n* = 8) with subclinical non-exudative macular neovascularization on the study eye.

| Patient	Gender	Age	BCVA (logMAR)	BCVA (Snellen Equivalent)	Small Drusen	Large Drusen	Drusenoid PED	Atrophy	Reticular Pseudo-drusen	CMT (µm)	CT (µm)
1	Female	72	0.1	20/25	1	1	1	0	1	252	257
2	Female	78	0.0	20/20	0	0	1	0	0	211	139
3	Male	80	0.2	20/32	0	0	1	0	0	338	136
4	Male	82	0.1	20/25	0	0	0	0	0	249	289
5	Female	91	0.3	20/40	1	1	1	0	1	264	243
6	Female	72	0.1	20/25	1	1	1	0	1	244	382
7	Female	89	0.2	20/32	0	0	1	0	1	239	268
8	Female	88	0.0	20/20	0	1	1	0	0	204	102

BCVA—best-corrected visual acuity; PED—pigment epithelial detachment; CMT—central macular thickness; CT—choroidal thickness; 0—absence; 1—presence.

Of the 97 study eyes, OCTA detected the presence of neovascularization in 8 (Figures 2 and 3), thus the estimated prevalence was 8.25%. Table 2 shows the comparison between the non-neovascular and the neovascular study eyes. None of the 8 neovascular eyes presented with macular atrophy. However, 3/8 of these presented with small drusen, 4/8 with large drusen, 7/8 with drusenoid PED, and 4/8 with RPD. Mean CMT in these eyes was 250.13 µm (±40.95) and mean CT was 227 µm (±94.38).

Table 2. Comparison between the non-neovascular (*n* = 89) and the neovascular (*n* = 8) study eyes.

	Non-Neovascular Study Eyes (*n* = 89)	Neovascular † Study Eyes (*n* = 8)	*p*-Value
Mean Age (years)	79 (±7.70)	82 (±7.41)	0.41 *
BCVA (logMAR)	0.18 (±0.46)	0.12 (±0.10)	0.21 *
Small drusen (*n*,%)	59 (66.29%)	3 (37.5%)	0.13 **
Large drusen (*n*,%)	65 (73.03%)	4 (50%)	0.22 **
Drusenoid PED (*n*,%)	37 (41.57%)	7 (87.50%)	0.021 **
Reticular pseudodrusen (*n*,%)	50 (56.18%)	4 (50%)	1.0 **
Atrophy (*n*,%)	19 (21.35%)	0 (0%)	0.34 **
CMT (µm)	231.95 (±43.74)	250.13 (±40.95)	0.20 *
CT (µm)	209.09 (±83.04)	227 (±94.38)	0.63 *

†—subclinical non-exudative macular neovascularization; *—Mann–Whitney Test; **—Fischer's exact test; BCVA—best-corrected visual acuity; PED—pigment epithelial detachment; CMT—central macular thickness; CT—choroidal thickness.
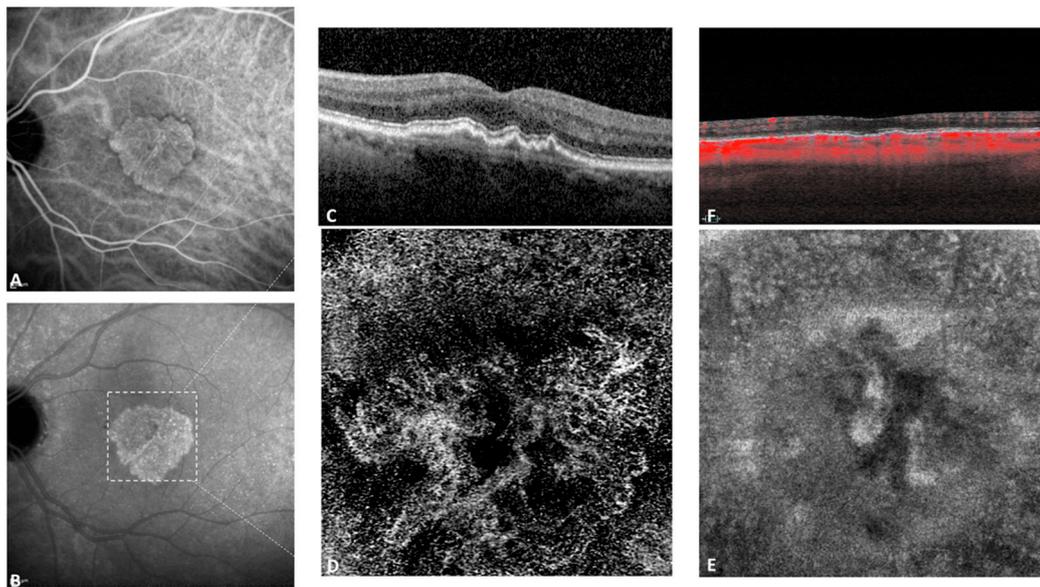
Figure 2. Multimodal imaging of an 80 years old patient with subclinical macular non-exudative neovascularization on the study eye. Intermediate (**A**) and late (**B**) indocyanine green angiography (ICGA) frames, revealing a hyperfluorescent plaque in the late frame. Spectral domain optical coherence tomography (SD-OCT) (**C**) showed a shallow pigment epithelial detachment (PED). The outer retina to choriocapillaris (ORCC) segmentation of the 3 × 3 mm² OCTA (**D**) revealed the presence of a high flow neovascular network. (**E**). Corresponding structural en face image at the choriocapillaris and (**F**) Corresponding B-scan with the choriocapillaris segmentation.
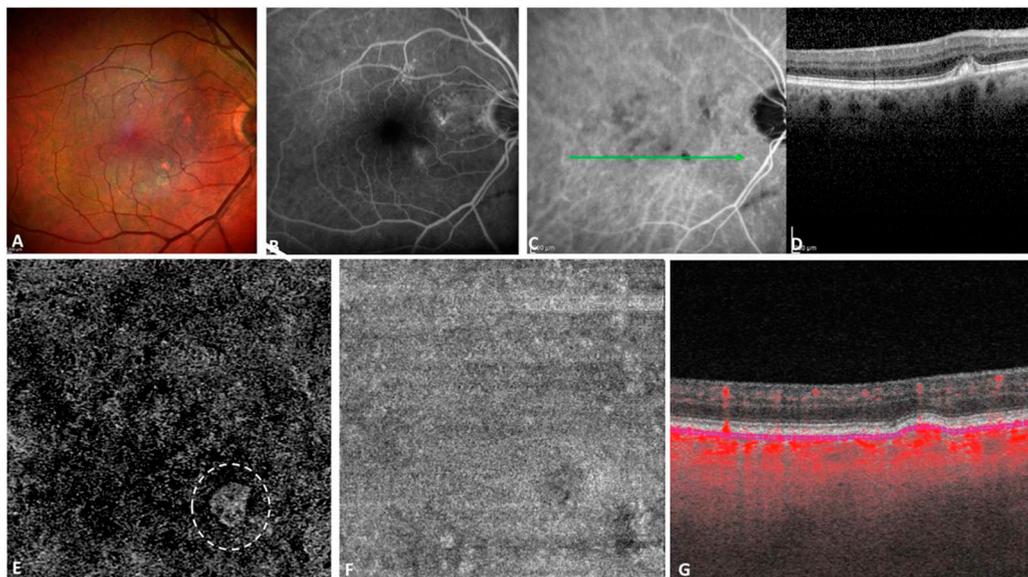
Figure 3. Multimodal imaging of an 86 years old patient with a small subclinical macular non-exudative neovascularization on the study eye. Multicolor imaging revealing soft drusen (**A**) that appeared hyperfluorescent on fluorescein angiography (FA) (**B**) and hypofluorescent on the late indocyanine green angiography (ICGA) frame. (**D**) Spectral domain optical coherence tomography (SD-OCT) revealed a small hyperreflective drusen, corresponding to a hypofluorescent area on ICGA (**C**). The manual 10 μm thick segmentation located 31 μm posterior to the RPE fit reference with projection artifact removal (**E**), revealed the presence of a small high flow neovascular network (dashed white circle) with the en face structural slab (**F**). The corresponding B-scan with flow overlay (**G**) confirmed the presence of flow within the drusen.

There were no statistically significant differences between the non-neovascular (*n* = 89) and the neovascular (*n* = 8) eyes in terms of quantitative and qualitative variables except for the presence of drusenoid PED (*p* = 0.021) (Table 2).

3.2.2. Choriocapillaris Flow Deficits in Non-Neovascular Fellow Eyes

Concerning the CC abnormalities of non-neovascular fellow eyes (*n* = 89), FD% averaged 45.84% (±11.63). Figure 4 illustrates the CC FD in a non-neovascular fellow eye. The mean number of FDs was 1965 (±1063) and the mean size of FDs was 5076.49 μm² (±9984.51). Figure 5 illustrates the log–log graph between FD size and FD number with an intercept of +2267 and a slope of −0.0604, showing an inverse linear relation. The mean area of FDs was 4.19 (±1.12) mm². Table 3 summarizes the mean values of CC abnormalities.
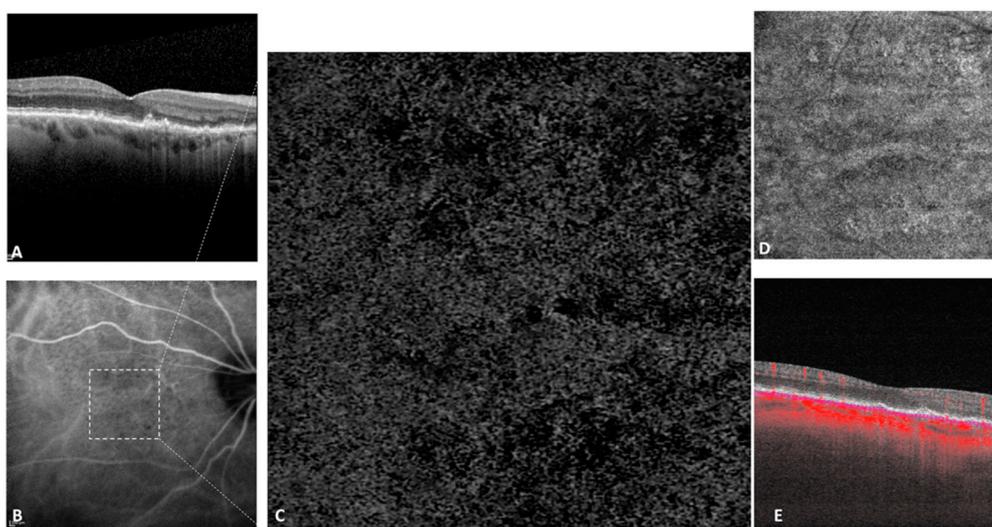
Figure 4. Choriocapillaris abnormalities of an eye with intermediate AMD. Enhanced depth imaging spectral domain optical coherence tomography (EDI-SD-OCT) visualized numerous drusen (**A**) which appeared hypofluorescent on indocyanine green angiography (ICGA) (**B**). On the manual choriocapillaris segmentation of the 3 × 3 mm² OCTA (**C**), multiple flow deficits were noticed in the CC flow image, corresponding in part to signal attenuation generated by drusen. Panel (**D**) corresponds to the structural en face CC slab, while panel (**E**) represents the corresponding B-scan with flow overlay.

Table 3. Choriocapillaris abnormalities in the non-neovascular group (*n* = 89) using radius 15 pixels.

	Mean (SD)
Percentage of flow deficits (%)	45.84 (±11.63)
Number of flow deficits	1964.74 (±1063.19)
Size of flow deficits (μm²)	5076.49 μm² (±9984.51)
Area of flow deficits (mm²)	4.19 (±1.12)
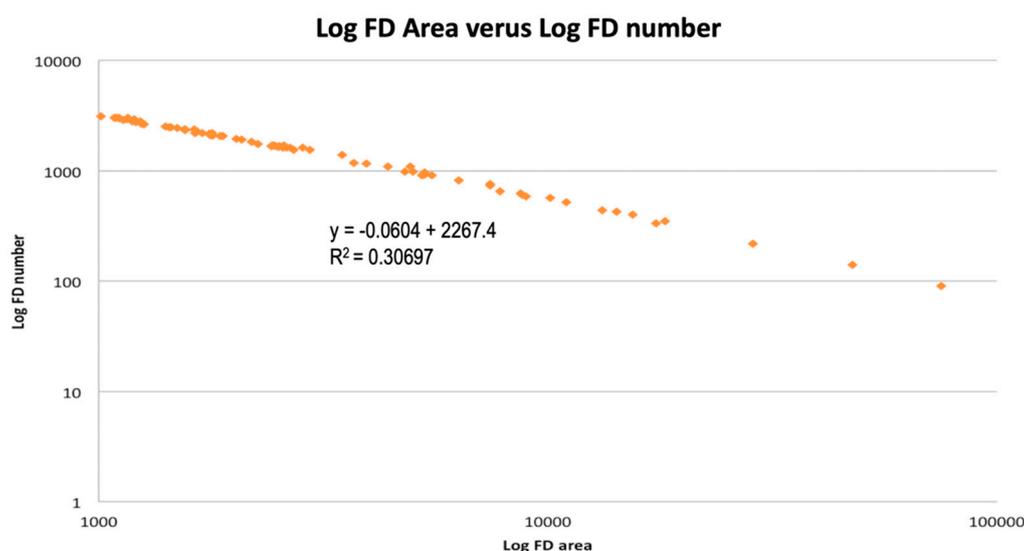
Figure 5. Log–log graph expressed in log10 between the size and number of flow deficits. When the log flow deficits were plotted against the log of the size of the flow deficits, a linear relationship was observed, with an intercept of +2267.4 and a slope of −0.0604.

In the univariate analysis, FD% in the non-neovascular fellow eyes was not statistically associated with CMT ($p = 0.59$), small drusen ($p = 0.55$), nor drusenoid PED ($p = 0.09$), but it was statistically associated with CT ($p = 0.003$), large drusen ($p = 0.03$), atrophy ($p = 0.02$), and RPD ($p = 0.007$). These associations were not found in the multivariate analysis, where FD% was associated in a statistically significant manner with age (linear regression, coefficient 0.62, $p = 0.02$) and the presence of small drusen (linear regression, coefficient 0.13, $p = 0.017$).

The size of FDs was not correlated with variables such as CMT ($p = 0.64$), small drusen ($p = 0.49$), large drusen ($p = 0.07$), and drusenoid PED ($p = 0.13$) in the univariate analysis, where it was associated with atrophy ($p = 0.029$) and RPD ($p = 0.02$). These associations were not found in the multivariate analysis. However, age (linear regression, coefficient 2.69, $p = 0.019$) and choroidal thickness (linear regression, coefficient −0.68, $p = 0.005$) were associated, in the multivariate analysis, with the size of FDs.

The number of FDs was not correlated with variables such as CMT ($p = 0.59$), small drusen ($p = 0.76$), large drusen ($p = 0.19$), nor drusenoid PED ($p = 0.21$) in the univariate analysis. However, the number of FDs was correlated with findings such as atrophy ($p = 0.03$) and RPD ($p = 0.048$) on multimodal imaging. These correlations were not found in the multivariate analysis, where the number of FDs was associated with age (coefficient −2.25, $p = 0.007$) and CT (coefficient 0.46, $p = 0.008$). Table 4 summarizes these findings.

3.2.3. Sensitivity Analysis

We tested the compensation algorithm for different radii values—radius of 4 pixels, corresponding to 26.37 microns, radius of 8 pixels (49.80 microns), 10 pixels (61.52 microns) and 15 pixels (90.82 microns) (Figure 6)—and the results are summarized in Table 5. According to the different radii, FD% ranged from 41.74 to 45.84%, mean number of FD from 3381.08 to 1964.74, and mean size of FD from 4198.77 to 5548.82 μm^2.

Table 4. Linear regression on percentage of flow deficits (FD), size of FD, number of FD in non-neovascular study eyes.

Linear Regression on	Variable	Univariate Analysis			Multivariate Analysis		
		Coef. [†]	Std. Err.	*p* Value	Coef. [†]	Std. Err.	*p* Value
Percentage of flow deficits *	Age *	0.77	0.26	0.004	0.62	0.26	0.02
	CMT *	−0.04	0.07	0.59			
	CT *	−0.16	0.05	0.003			
	Small drusen	0.03	0.05	0.55	−0.13	0.05	0.017
	Large drusen	0.12	0.06	0.03			
	Drusenoid PED	0.09	0.05	0.09			
	Atrophy	0.14	0.06	0.02			
	RPD	0.14	0.05	0.007			
Size of flow deficits *	Age *	3.44	1.13	0.003	2.69	1.13	0.019
	CMT *	−0.15	0.32	0.64			
	CT *	−0.81	0.23	0.001	−0.68	0.23	0.005
	Small drusen	0.16	0.24	0.49			
	Large drusen	0.47	0.25	0.07			
	Drusenoid PED	0.34	0.23	0.13			
	Atrophy	0.61	0.27	0.029			
	RPD	0.53	0.22	0.02			
Number of flow deficits *	Age *	−2.74	0.80	0.001	−2.25	0.80	0.007
	CMT *	0.12	0.23	0.59			
	CT *	0.57	0.17	0.001	0.46	0.17	0.008
	Small drusen	−0.05	0.17	0.76			
	Large drusen	−0.24	0.18	0.19			
	Drusenoid PED	−0.21	0.16	0.21			
	Atrophy	−0.43	0.19	0.03			
	RPD	−0.33	0.16	0.048			

*—expressed in log10; [†]- regression coefficient; CMT—central macular thickness; CT—choroidal thickness; PED—pigment epithelial detachment; RPD—reticular pseudodrusen.
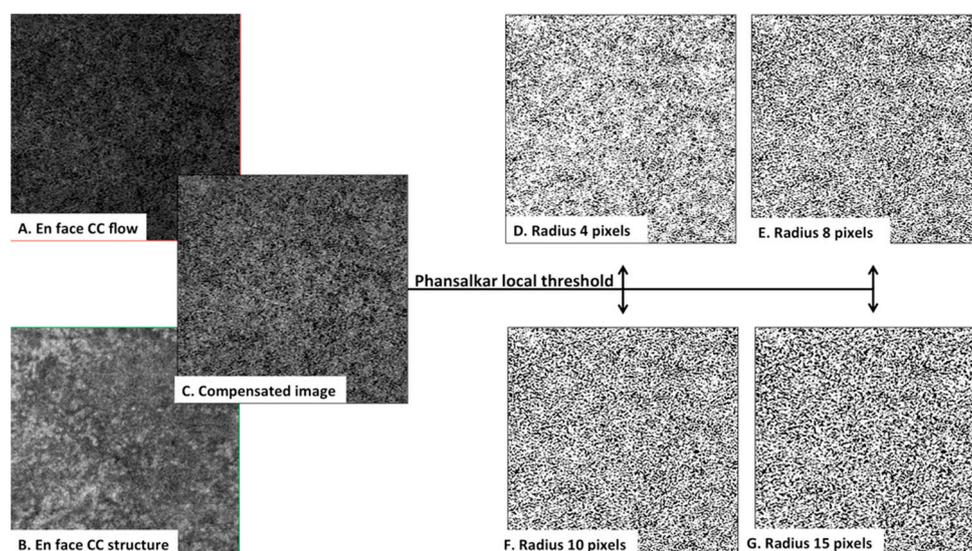
Figure 6. Phansalkar's local thresholding method with window radii of 4, 8, 10, and 15 pixels on a 3 × 3 mm² swept source optical coherence tomography angiography (SS-OCTA) scan from an intermediate AMD eye. Flow choriocapillaris (CC) SS-OCTA image (**A**) and the en face CC structure slab (**B**) generated the compensated image (**C**). Panels (**D**) to (**G**) show the effect of choosing different window radii ((**D**) radius 4 pixels, (**E**) radius 8 pixels, (**F**) radius 10 pixels, (**G**) radius 15 pixels)in Phansalkar's local thresholding method for 3 × 3 mm SS-OCTA images.

Table 5. Choriocapillaris abnormalities in the non-neovascular group according to radii (*n* = 89).

	Mean (SD) Radius 4	Mean (SD) Radius 8	Mean (SD) Radius 10	Mean (SD) Radius 15 Pixel
Percentage of flow deficits (%)	41.74 (±15.26)	44.81 (±12.43)	45.52 (±12.01)	45.84 (±11.63)
Number of flow deficits	3381.08 (±1735.01)	2405.87 (±1347.19)	2151.26 (±1218.96)	1964.74 (±1063.19)
Size of flow deficits (μm^2)	4198.77 (±14,929.13)	5253.17 (±13,861.42)	5548.82 (±13,471.32)	5076.49 (±9984.51)

4. Discussion

In our study, we showed that in the fellow eye of exudative AMD patients, there is a wide spectrum of macular choriocapillaris abnormalities, ranging from subclinical non-exudative neovascularization to an increase in CC FD%.

Measurable changes of the flow within the CC were found with age and hypertension, and significant alterations in the flow pattern were demonstrated in eyes with RPD and in eyes with late AMD in the fellow eye [3,7,9,10,21–24].

Zheng et al. recently showed that in normal eyes, there was an increasing number and percentage of CC FDs with ageing [9]. Thus, the mean FD% in normal eyes within the age category corresponding to our cohort (80–89 years) was 17.0% ± 3.3% on the 2.5 mm circle on the 3 × 3 mm² slab, while in our study, the mean percentage of CC FD% in our non-neovascular, mostly intermediate AMD (iAMD) group (*n* = 89) was 45.84% (±11.63) on the whole image.

Furthermore, Sacconi et al. performed CC perfusion density (PD) in healthy subjects using SS-OCTA and a similar image processing method [21]. The authors found a significant negative association between perfusion of CC and age in healthy subjects. In 12 eyes of 70–80 years old healthy patients, a mean PD of 71.791% ± 4.113% on the 3 mm annulus around the fovea was reported, accounting for a CC FD% of 28.209%. Nevertheless, there is a significant difference between these results of PD and/or FD% and those of Zheng et al. [9]. These differences may be explained by the fact that small variations in image processing (thresholding, used radius, slab selection) can greatly influence the results [25,26]. Hence, these considerations should be accounted for the discrepancies between our study and previous studies. Moreover, Chu et al. recently demonstrated that the local window radius should be optimized before using the Phansalkar's thresholding method [20]. Therefore, in the present study, radius 15 was chosen for the main analysis, in accordance with recent literature. However, a supplementary sensitivity analysis with different radii values (4, 8, and 10) was also performed (Table 5 and Figure 6), showing up to 4.10% difference for FD%. As too small radii may not include enough pixels and larger radii generate larger FD% values, a consensus on the parameters of choriocapillaris analysis is needed. Slight differences in manual segmentation and image processing methods lead to an important variation in the CC quantitative variables. In the present study, we tried to overcome this variability by using the same image processing method as other SS-OCTA studies [10] and testing different radii.

Borrelli et al. quantitatively analyzed the CC signal voids' topographical distribution in single images of iAMD eyes [10], showing consistent results with those found among our (non-neovascular) iAMD eyes. In an analysis comparing the entire CC area in both control eyes and the drusen-free region in iAMD, the multiple regression analysis showed that only age was associated with the CC variables. In our study, we performed a linear regression on FD%, size of FD, and number of FD showing, in the multivariate analysis, a statistically significant association with age for each of the CC variables studied. Moreover, FD size and FD number demonstrated an inverse linear relationship on the log–log plot in Figure 5, which is consistent with recent literature [7]. Additionally, in the multivariate

analysis, small drusen was also associated with FD%, while CT was associated with the size and number of FD (Table 4).

Nassisi et al. recently showed that there is a correlation between the CC flow impairment around the atrophic lesions and the yearly growth rate of GA, with more CC flow impairment meaning a faster growth of atrophy [27]. In our study, the presence of atrophy was associated, in the univariate analysis, with FD%, FD number and size, suggesting that the increase of FD% may indeed correlate with atrophy development.

In our study, subclinical non-exudative neovascularization was present in 8 out of 97 eyes, suggesting an estimated prevalence of neovascular complications of 8.25% in the fellow eye of exudative AMD. Interestingly, Treister et al. showed a slightly higher prevalence of subclinical neovascularization (14.7%) in the 34 fellow eyes of unilateral late AMD patients, analyzed by SD-OCTA [28].

On one hand, de Oliveira Dias et al. have recently shown, by means of SS-OCTA, a prevalence of 14.4% of subclinical non-exudative neovascularization among the 160 eyes included in their cohort [29]. Recently, Yang et al. [16] found, also by means of SS-OCTA, that 13.2% of their cohort (non-exudative AMD, either iAMD or late AMD) had subclinical MNV at first imaging.

The use of a small scanning area, as well as the lack of subfield analysis, are among the limitations of our study, together with the small sample size and the absence of a control group. The absence of a control group that would have underwent the same image processing method is the reason why we cannot conclude, despite the comparison with other studies, that the observed changes in the CC were due to iAMD rather than to other factors. Moreover, the CC slab in eyes with reticular pseudodrusen and decreased choroidal thickness might generate the displacement of the slab from the CC to the anterior choroid. It remains difficult to select a cutoff of choroidal thickness from which this slab should be adapted. The manual segmentation was manually corrected if necessary. Nonetheless, choosing a 3×3 mm^2 scanning area allowed us to use the highest resolution scanning area in order to assess CC FDs.

One of the strengths of the present study was the use of the SS technology, which allowed for a better detection of MNV and a more reliable assessment of CC nonperfusion.

In conclusion, fellow eyes of unilateral exudative AMD encompass a series of CC abnormalities, from FDs of the ageing CC to subclinical non-exudative MNV.

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Review

Management of Cataract in Patients with Age-Related Macular Degeneration

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Abstract: Cataract and age-related macular degeneration (AMD) are two of the most common eye diseases of aging. This review addresses the pre-operative, intra-operative, and post-operative considerations in managing cataract in patients with age-related macular degeneration. Surgery for visually significant cataracts in patients with AMD can substantially improve the quality of life and reduce the risk of falls. Pre-operative optical coherence tomography is now recommended where possible to identify pre-existing macula disease. Careful counselling of patients is required before cataract surgery, especially with respect to the expected visual outcome, intraocular lens choice and potential risks of surgery. Real-world data has suggested 6 months of intravitreal anti-VEGF therapy for neovascular AMD before cataract surgery is compatible with optimum long-term visual outcomes. Patients receiving intravitreal therapy for neovascular AMD should be advised of the slightly higher risk of intraoperative complications and the surgeon should be prepared to manage these during the operation. During cataract surgery, unnecessary light exposure should be avoided to reduce phototoxicity. Careful planning of intravitreal therapy for neovascular AMD just before cataract surgery allows the eye greater recovery time in the post-operative period before further planned intravitreal therapy.

Keywords: cataract surgery; age-related macular degeneration; clinical trials; real-world evidence

1. Introduction

Cataract and age-related macular degeneration (AMD) are two of the most common causes of visual impairment globally, with the incidence set to increase in upcoming decades with an ageing population [1]. A recent study in patients over 50 years of age identified that 20% of 411 eyes listed for cataract surgery had some evidence of AMD on optical coherence tomography (OCT) imaging [2]. This review provides guidance on the pre-operative, intra-operative, and post-operative considerations in managing cataracts in patients with age-related macular degeneration (Table 1). Evidence is derived from available clinical trials, real-world evidence and expert opinion.

2. Pre-Operative Considerations

2.1. Counselling Regarding Visual Acuity and Quality of Life Outcomes

The Age-Related Eye Disease Studies (AREDS1 and AREDS2), identified a mean improvement of +4 and +11 logMAR letters in those with intermediate AMD undergoing cataract surgery [3,4]. Additionally, for patients with driving level vision (better than 20/40 Snellen), final visual acuity (VA) outcomes were similar to control patients.

In neovascular AMD, a post-hoc analysis of the MARINA (Minimally Classic/Occult Trial of the anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD) and ANCHOR (Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in AMD) clinical trials demonstrated that cataract surgery was beneficial for eyes with an average improvement of >10 logMAR letters [5].

Although cataract surgery may not reliably improve VA in fovea-involving geographic atrophy, it may improve other critical aspects of visual function such as contrast sensitivity, peripheral vision, glare, and color vision as well as help with quality of life indices [6–8].

A recent study identified the risk of falls decreased by 54% (incidence rate ratio (IRR) = 0.46, 95% CI = 0.22–0.97, p = 0.04) after first eye cataract surgery only, compared with the period before the first eye surgery [9]. The risk of falls decreased by 73% (IRR = 0.27, 95% CI = 0.11–0.63, p = 0.002) after the second eye cataract surgery, compared with the period before the first eye surgery. Improved binocular VA (IRR = 5.49, 95% CI = 1.19–25.28, p = 0.03) and contrast sensitivity (IRR = 0.26, 95% CI = 0.070–0.94, p = 0.04) were associated with a decrease in the number of falls.

2.2. Does Cataract Surgery Cause AMD to Progress?

There has been much debate and conflicting evidence as to whether cataract surgery causes AMD progression [10]. A Cochrane review of two small randomised controlled trials recommended that physicians must make recommendations to their patients with AMD regarding cataract surgery based on experience and clinical judgment until large controlled trials are conducted and their findings published [11–13]. It concluded that although cataract surgery provides improvement in vision in eyes with AMD compared with no surgery at six months, it is unclear whether the timing of surgery has an effect on longer-term visual outcomes. The Cochrane review also stated that "*ethical considerations preclude withholding surgery, or delaying it for several years, if it may be a potentially beneficial treatment*" [11]. Another systematic review that included a variety of study designs concluded the link between cataract surgery and AMD remained equivocal due to limited available evidence [14].

2.3. Screening for Macula Disease with Optical Coherence Tomography

A number of studies have identified the benefit in screening eyes with macula OCT imaging prior to cataract surgery [2,15,16]. In one study, all patients underwent routine spectral-domain OCT scanning prior to cataract surgery [2]. The scans were reviewed by a retinal specialist for macular pathology and compared to pre-operative biomicroscopic fundus examination findings. Overall, the management of 107 (26.0%) out of 411 patients was modified due to macular spectral-domain OCT findings, which were either missed (22.8%) or underestimated (3.2%) by the fundus biomicroscopy examination. Changes in pre-operative patient management included altering patient consultation regarding presbyopia correction options (73 eyes, 17.8%) and referral to a retina specialist (34 eyes, 8.3%). Routine macular spectral-domain OCT scans for cataract surgery candidates helped to identify macular pathologies that might be missed or underestimated by standard fundus biomicroscopy examination.

2.4. How Long Does Neovascular AMD Need to Be Treated with Intravitreal Therapy before Considering Cataract Surgery?

The Fight Retinal Blindness (FRB!) registry investigated the timing of cataract surgery in patients with neovascular AMD [17]. Patients who had cataract surgery within 6 months of initiating anti-VEGF therapy were more likely to lose vision. It is possible that only after the first 6 months after commencing anti-VEGF therapy the lesions were sufficiently controlled. The average time from the first injection to cataract surgery was 14 months in the post hoc analysis of the ANCHOR and MARINA clinical trials [5]. A preoperative exudation-free period may be an important parameter when considering cataract surgery in patients with neovascular AMD, although the exact minimum period of inactivation has not yet been determined [18]. Cataract surgery within 6 months of starting treatment for neovascular AMD should be avoided if possible.

2.5. Predicting Visual Acuity Outcomes

Predicting the extent of VA improvement in patients with concurrent cataract and AMD is challenging. The FRB! registry identified that the angiographic subtype of neovas-

cular AMD did not impact the visual outcomes of cataract surgery [17]. Prognostic factors in patients with AMD undergoing cataract surgery have recently been investigated in a retrospective study [19]. Better pre-operative VA predicted smaller VA gains ($p < 0.007$). Longer duration of AMD in intermediate AMD, ellipsoid zone disruption in neovascular AMD, and lower central subfield thickness in geographic atrophy were associated with poorer VA outcomes ($p < 0.05$). Further studies are required to quantify the impact of these biomarkers on visual outcomes to allow for more accurate counselling of patients pre-operatively.

2.6. Discussion of Intraocular Lens Choices with Patients

There are a number of specific considerations of intraocular lens (IOL) choice in patients with AMD undergoing cataract surgery:

2.6.1. Avoiding Multifocal Intraocular Lenses

Multifocal IOLs aim to address presbyopia by splitting light rays to different focal points to reduce spectacle dependence [20]. They are classified as refractive, diffractive, or a combination of these [20]. Bifocal and now trifocal IOL options are available. Multifocal IOLs are however associated with reduced contrast sensitivity and more higher order aberrations than monofocal IOLs [20]. Multifocal IOLs are relatively contraindicated in patients with AMD [21].

There is a suggestion that extended depth of focus (EDoF) IOLs may have less of a deleterious effect on contrast sensitivity and cause less higher order aberrations than trifocal IOLs [22]. However, the quality of vision would still be expected to be worse than a monofocal IOL [23]. Evidence that EDoF IOLs are well tolerated in patients in AMD would be required before advocating them.

2.6.2. Toric Intraocular Lenses

Toric IOLs do not compromise contrast sensitivity and can be used in patients with AMD if there is significant astigmatism [24]. Toric IOLs do need to be dialed to a specific alignment. Therefore, a back-up non-toric IOL should be available in case of zonular weakness.

2.6.3. Aspheric Intraocular Lenses

Aspheric IOLs negate the positive spherical aberrations of the cornea. Clinical trials have reported improved contrast sensitivity, glare sensitivity and coma compared with conventional IOLs [25–27]. This could be beneficial in patients with AMD although at this stage further evidence in this patient population is required to support this hypothesis [28].

2.6.4. Blue-Blocking Intraocular Lenses

Ultraviolet filtering IOLs that block harmful UV-C energy outside the visible spectrum reaching the macula have been available for over 30 years [29]. Most commercially available intraocular lenses contain this filter as a default and there is widespread acceptance of the benefit in the medical community.

The story with blue light filtering IOLs is less clear cut [30]. Blue light–blocking IOLs are designed to filter short-wavelength light in the visible spectrum in addition to ultraviolet light and mimic the natural crystalline lens. An in vitro study identified that an ultraviolet- and blue light-absorbing intraocular lens demonstrated significantly better protection against light-induced oxidative stress, senescence, and structural retinal pigment epithelial damage than the ultraviolet-absorbing intraocular lens [31]. A small retrospective study reported that geographic atrophy progression in the UV-blocking IOL group to be significantly greater than the combined UV and blue-blocking IOL group after one year [32].

Conversely, blue light has a role in helping scotopic vision and suppresses melatonin, helping regulate the circadian rhythm [33,34]. Concerns about impaired night vision, disturbed sleep, and impacts on quality of vision have been raised although not definitively proven.

A registry-based cohort study with data from the Swedish National Cataract Register and the Swedish Macula Register from 2010 to 2017 compared eyes with and without preoperative AMD that had undergone cataract surgery and subsequently treated for neovascular AMD to eyes not treated within the study period [35]. All first-eye surgeries registered from 2010 to 2017 and matching eyes found in the Swedish Macula Register that had undergone treatment for neovascular AMD >1 year after the cataract procedure were included. A blue-blocking IOL did not statistically significantly decrease the likelihood of subsequent neovascular AMD treatment in eyes with pre-operative AMD (53% vs. 57%, $p = 0.11$). The authors concluded that if the use of a blue-blocking IOL offers any protection from undergoing neovascular AMD treatment after cataract surgery, such an effect must be very small.

2.6.5. Intraocular Lenses Providing Magnification or Prismatic Effect

Patients with AMD can benefit from additional magnification, especially for reading tasks. Handheld magnifiers can be difficult to use especially if other comorbidities such as significant arthritis of the upper limbs is present. Some of this is mitigated by improved accessibility options on modern tablets and phones. Magnifying IOLs have been developed. The most common approach to magnification, used in implantable miniature telescope (IMT) lenses, the IOL-VIP System, and iolAMD is a Galilean type telescope, where two optical elements with high positive and negative power should be used in combination with the cornea [36]. Another approach to magnification is the Lipshitz macular implant, based on a Cassegrain configuration, which uses mirrors instead of lenses [36]. A description of all the available magnifying intraocular lens devices is outside the scope of this review but is well covered elsewhere [10,36,37]. There is an inherent compromise between improved magnification and loss of field of view. Some magnifying IOLs such as the Scharioth Macula Lens attempt to address this by having high magnification in the central optic only. Generally, magnifying IOLs are more of an option in advanced AMD. Long-term safety data is required, especially for devices that occupy significant volume in eyes with shallow anterior chambers.

Prism-based intraocular lenses do not necessarily provide magnification but instead displace the retinal image from the damaged central macula to a more peripheral healthier area. The Fresnel prism approach has a potential problem of diffraction and scattered light at the edges of each Fresnel zone, which might be a source of significant glare [36,38].

3. Intra-Operative Considerations

There are factors for the surgeon to consider during cataract surgery in patients with AMD.

3.1. Timing of Intravitreal Anti-VEGF Therapy and Cataract Surgery

Most modern day phacoemulsifaction cataract surgery is performed without sutures, aiding the speed of visual recovery. It is preferable to allow time for these self-sealing wounds to heal before performing further intravitreal therapy. Therefore, clinicians aim to deliver an intravitreal anti-VEGF injection for neovascular AMD in the weeks preceeding cataract surgery. In the ANCHOR and MARINA clinical trials, intravitreal ranibizuamb was delivered in the month prior to cataract surgery with good outcomes [5].

There have also been studies reporting good outcomes when delivering intravitreal anti-VEGF at the time of cataract surgery to reduce the risk of activating the choroidal neovascular (CNV) lesion [39–41]. However, the FRB! registry did not observe a relationship between intravitreal anti-VEGF for neovascular AMD in the two weeks before the cataract surgery and VA outcomes [17]. This is in contrast to diabetic macular oedema, where the FRB! registry identified benefit of intravitreal therapy even if at the time of cataract surgery [42].

If intravitreal anti-VEGF therapy is anticipated in the first week after cataract surgery, it may be prudent to place a suture to secure the main wound.

3.2. Managing Posterior Capsular Rupture or Zonular Dialysis during Cataract Surgery

Eyes receiving intravitreal therapy for neovascular AMD appear to be at increased risk of intra-operative complications during cataract surgery. Possible explanations include inadvertent crystalline lens capsule trauma and zonular trauma either directly or from local scleral deformation at the time of intravitreal injections [43]. Identification of cases at higher risk assists the operative planning and allows patients to be better informed about potential surgical risks.

Posterior capsular rupture (PCR) is a complication of cataract surgery that can be associated with significantly worse visual outcomes [44]. The Royal College of Ophthalmologists of England National Ophthalmology Database study of cataract surgery for cases between 2010 and 2018 reported a mean PCR rate of just less than 1% [45]. The hypothesis that previous intravitreal therapy is a predictor of increased risk of PCR during cataract surgery was tested on a large registry of eyes undergoing cataract surgery from 20 UK Hospital Trusts between 2004 and 2014 [46]. Data were available on 65,836 cataract operations, of which 1935 eyes had received previous intravitreal injections (2.9%). Of these injections, 80% were intravitreal anti-VEGF therapy for neovascular AMD. Univariate regression identified advanced cataract, patient age, junior cataract surgeon grade, and the number of previous intravitreal injections were associated with increased risk of PCR. Analysis considering intravitreal injections as a categorical variable identified 10 or more previous injections that were associated with a 2.6 times greater likelihood of PCR ($p = 0.003$) after adjusting for other significant independent predictors. Three independent studies have supported this observation, one from the USA, and two others from the UK [47–49]. Much like we see with COVID-19 vaccine development, rare adverse events can be identified outside of a clinical trial setting in real-world settings [50].

Cataract surgeons may have to manage zonular dialysis in patients who have received previous intravitreal anti-VEGF therapy. This seems biologically plausible given the proximity of zonules to the site of intravitreal injections, although at this stage there is no supportive evidence. A large retrospective study identified improved short and medium-term visual outcomes when a capsule tension ring (CTR) was used for zonule dialysis [28]. The records of 22,312 consecutive eyes undergoing cataract surgery were reviewed. The incidence of zonular dialysis was 0.50% (111 eyes). A CTR was inserted in 46 eyes. Using a multivariate linear regression model, better initial pre-operative VA ($p = 0.019$), the use of a CTR ($p = 0.014$), and the absence of vitreous loss during surgery ($p = 0.008$) were associated with improved early postoperative VA. Better medium-term postoperative VA was significantly associated with the use of a CTR during surgery ($p = 0.004$).

3.3. Reducing Unnecessary Light Exposure

Phototoxicity to the retina has been described with solar retinopathy or laser-light induced retinopathy [51,52]. The macula is already not healthy in AMD and hence may be more vulnerable to phototoxic damage [53]. Prolonged surgery and bright light settings during cataract surgery are potential risk factors for phototoxic macula damage [53]. A balance has to be struck between reducing light exposure and adequate visualization to perform the surgery safely. The visually significant cataract should reduce the extent of light exposure early in the cataract operation. Once the new intraocular lens is inserted, UV light should be blocked. The main time of risk would therefore be when the eye is aphakic and the surgeon can take extra precautions to reduce light exposure during this period of the cataract surgery.

3.4. Femtosecond Laser-Assisted Cataract Surgery

A small retrospective study compared femtosecond laser and conventional cataract surgery for patients with neovascular AMD undergoing cataract surgery [54]. Overall, the postoperative course between neovascular AMD after femtosecond laser and conventional cataract surgery was equal. During the early follow-up, however, femtosecond laser-treated eyes had less subclinical macular oedema on optical coherence tomography

imaging, suggesting that the role of femtosecond laser-assisted cataract surgery in eyes with macular vulnerability is an area for further research. It may be that the shorter phacoemulsification time reported in femtosecond laser-assisted cataract surgery leads to less cystoid macular oedema.

4. Post-Operative Considerations

4.1. Increased Risk of Acute and Delayed Endophthalmitis

Over 200,000 Medicare beneficiaries in the USA underwent cataract surgery from 1 January 2009 to 31 December 2013. By using a 5% sample of Medicare claims data, the risks of adverse outcomes in beneficiaries with a history of intravitreal injections relative to those without were calculated using the Cox proportional hazard model [55]. Prior injections were associated with increased risk of both acute (HR, 2.29; 95% CI, 1.001–5.22) and delayed-onset endophthalmitis (HR, 3.65; 95% CI, 1.65–8.05). The authors recommended increased post-operative vigilance in patients with a history of intravitreal injections undergoing cataract surgery.

4.2. Intravitreal Therapy for Patients with Neovascular AMD after Cataract Surgery

The FRB! registry investigated whether there was a change in the frequency of intravitreal anti-VEGF injections required for neovascular AMD in the 12 months after cataract surgery compared with the 12 months prior [17]. Disease activity grading and intravitreal injection numbers were similar in both periods for patients having cataract surgery, whereas both slightly decreased in the control group in the latter 12 months, suggesting that cataract surgery modestly increased the level of activity of the CNV lesion. Studies without a control group suggested cataract surgery did not influence CNV activity [56,57].

4.3. Consider Additional Lighting, Magnification and Reading Aids

After cataract surgery, the benefit of additional lighting or magnification in patients with AMD should be considered. Patients may find low vision aids more beneficial once they have clear media after cataract surgery. Local patient support groups can offer routes to access these. Modern portable electronic devices have desirable properties such as the flexible use of magnification.

Glasses are usually updated one month after cataract surgery. There was no good evidence from a Cochrane Review of low vision aids to support the use of filters or prism spectacles in patients with low vision [58].

Table 1. Summary of factors to consider when carrying out cataract surgery in patients with age-related macular degeneration.

	Recommendation/Consideration	Level of Evidence
Pre-operative	There are quality of life benefits in carrying out surgery for visually significant cataract in patients with all stages of AMD	Level 2
	Screening for macula disease with pre-operative optical coherence tomography is recommended	Level 2
	Avoid multifocal intraocular lenses in patients with macular disease	Level 3
	Cataract surgery within 6 months of starting treatment for neovascular AMD should be avoided if possible.	Level 2
	Intravitreal anti-VEGF therapy for neovascular AMD in the month before cataract surgery is compatible with good long-term visual outcomes.	Level 2

Table 1. *Cont.*

	Recommendation/Consideration	Level of Evidence
Intra-operative	Slightly increased risk of posterior capsule rupture in eyes that have received intravitreal therapy	Level 2
	Reduce unnecessary light exposure during cataract surgery	Level 3
Post-operative	Slightly increased risk of acute or delayed endophthalmitis in patients undergoing cataract surgery who have had previous intravitreal injections.	Level 2
	The frequency of intravitreal anti-VEGF therapy for eyes with neovascular AMD is likely to be similar in the 12 months before and after cataract surgery	Level 2
	Offer access to additional lighting, magnification and reading aids	Level 2

Evidence is graded on three levels [59]: Level 1: evidence based on results of randomised controlled trials, power calculations, or other recognised means to determine the statistical validity of the conclusion. There is a lack of high-quality Level 1 evidence in the field. Level 2: evidence based on results of case studies, case series, or other non-randomised prospective or retrospective analysis of patient data. Level 3: evidence based on expert opinion, consensus opinion, or current recognised standard of care criteria where no formalcase series analysis was available.

5. Conclusions

Real-world evidence has complemented the limited available clinical trial data to provide useful insights into how to optimize outcomes in patients with AMD undergoing cataract surgery. Further studies are required to provide more accurate prediction of the visual potential of eyes undergoing cataract surgery, to understand whether blue-blocking intraocular lenses offer long-term visual benefits, to investigate the long-term safety of magnifying intraocular lenses, and to identify optimal surgical techniques. It may be that some of these questions can be answered by clinical trials whereas others will be better addressed by large registry studies.

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Brolucizumab for Choroidal Neovascular Membrane with Pigment Epithelial Tear and Subretinal Fluid

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Abstract: The aim of this study was to determine the utility of brolucizumab in the management of choroidal neovessels (CNV) with a retinal pigment epithelial (RPE) tear and subretinal fluid. We used a case series of patients with CNV who developed an RPE tear either spontaneously or following an intravitreal injection. All patients received intravitreal brolucizumab as primary or switch therapy. Appropriate data were collected. Follow-up was one year. The paired t-test was used to determine the significance of the results. The primary outcome measure was the change in best corrected visual acuity (BCVA). Secondary outcome measures were the change in subretinal fluid and complications, if any. A total of five patients were included in the analysis. The age range was $67-74$ years and baseline BCVA was from 20/80 to 20/100. On average, all patients showed improvement in BCVA ($p = 0.012$) and also showed a significant anatomical improvement ($p = 0.03$). None of the patients had any complications, and all patients responded to additional anti-VEGF injections. In conclusion, all patients showed significant visual and anatomical improvement with brolucizumab; no complications were noted. All patients, including those who received switch, demonstrated a favorable anatomical and visual response to intravitreal brolucizumab without safety concerns.

Keywords: age-related macular degeneration; anti-vascular endothelial growth factor; brolucizumab; epithelial tear; exudation; optical coherence tomography

1. Introduction

Retinal pigment epithelial (RPE) tears can occur in certain ocular conditions spontaneously or with intervention [1–4]. The visual prognosis depends largely, but not entirely, on the precise location of the scrolled-up RPE margin [1,3]. If subfoveal, it can lead to permanent visual dysfunction. Histopathological evidence shows that choroidal neovessels (CNV) may involve an area much wider than what is apparent clinically, and CNV activity can continue even if there is a tear [4]. The size of the pigmented epithelial detachment (PED) and a high number of intravitreal therapies are known risk factors of this complication [5]. In the event of an RPE tear, the subretinal fluid (SRF) enters the subretinal space, and intravitreal injections of anti-vascular endothelial growth factor (VEGF) agents are generally prescribed to aid its resolution [1]. Currently, intravitreal anti-VEGF therapy is considered the best recourse in such a situation [1]. However, limited literature works are available on the management of patients with CNV who develop RPE tears and have SRF.

The HAWK and HARRIER studies established the non-inferiority of the new molecule brolucizumab vis-a-vis aflibercept, with some analyses suggesting a superior anatomic

outcome [6]. Brolucizumab is a single-chain antibody fragment (scFv), which is the smallest functional unit of an antibody. The small size of the molecule allows the delivery of a higher molar dose compared with larger molecules such as ranibizumab, aflibercept and bevacizumab. Brolucizumab was developed by grafting regions of an anti-VEGF antibody to a human scFv scaffold.

Nearly 50% of enrolled patients could receive 12 weekly injections, considerably reducing the treatment burden. However, concerns about safety with special reference to intraocular inflammation and vasculitis dampened the initial enthusiasm for the drug [7]. As data continue to evolve, the risk of serious adverse events is currently pegged at 4.6% [8,9].

This small case series looks at patients with CNV and RPE tears who received intravitreal brolucizumab as primary or switch therapy.

2. Materials and Methods

This was a retrospective, observational study conducted at the Alphavision Augenarzt Praxis, Bremerhaven, Germany. All patients with a diagnosis of RPE tear treated by brolucizumab were included. Treatment-naive eyes, as well as patients already treated with intravitreal injections, were included. We recorded the best corrected visual acuity (BCVA), and details of the anterior and posterior segment exam as well as the systemic examination and special investigations such as fluorescein angiography (and indocyanine green angiography where necessary), autofluorescence imaging (both using the Zeiss Visucam 450, Zeiss Meditec, Dublin, CA, USA) and optical coherence tomography raster scans using a spectral domain OCT (Cirrus, Zeiss Meditec, Dublin, CA, USA). This study complied with the tenets of the Declaration of Helsinki and was approved by an international review board (Ethics Committee of the French Society of Ophthalmology, IRB 00008855 Société Française d'Ophtalmologie IRB#1). Patients gave their informed consent to participate in the study.

All patients were given at least three intravitreal anti-VEGF injections each a month apart and monitored for BCVA, fluid status and RPE tears. In the case of switch therapy, patients were considered for change of anti-VEGF agents after at least 3 consecutive monthly anti-VEGF agents. Standard guidelines as elaborated upon in the HAWK and HARRIER trials were followed with respect to intravitreal brolucizumab therapy. Intravitreal injections were administered using a standardized aseptic technique.

Follow-ups were scheduled on post-intravitreal injection days 1 and 7 and then monthly for one year.

The primary outcome measure was the change in BCVA with treatment. The secondary outcome measures were the reduction in SRF and the complications, if any. Statistical analysis was performed using the paired t-test. Statistical significance was set at $p < 0.05$.

3. Results

We included five patients (five eyes) with CNV who developed an RPE tear either spontaneously (three patients) or during the course of intravitreal therapy (two patients). There were three males. All patients were free of systemic disease. The demographic characteristics and the baseline and final BCVA values are listed in Table 1.

Three patients had a mixed (type 1 + type 2) CNV; the other two had type 1 CNV as noted on fluorescein angiography. All patients showed significant visual and anatomical improvement until the end of the follow-up period. All patients had received at least five injections of brolucizumab until the last follow-up. All patients had a grade 3 tear.

The age range was 67−74 years and baseline BCVA was from 20/200 to 20/100. On average, all patients showed improvement in BCVA ($p = 0.012$) and also showed significant anatomical improvement ($p = 0.03$). None of the patients developed any complication nor did any patient require a switch of anti-VEGF therapy.

A total of three patients were treatment-naïve and two patients were previously treated with intravitreal injections of aflibercept. They were switched to aflibercept due to

persistent fluid in the retina. These two patients showed BCVA improvement as well as retinal fluid regression.

Table 1. Important characteristics of patients with pigment epithelial tears who received brolucizumab.

Patient Age(y)/Sex	Baseline VA (Snellen)	Therapy Status (*n*: Number of Injections)	PED Height (μm) Prior to Tear	Central Macular Thickness (μm) Baseline	SRF after RPE Tear (μm)	Number of Brolucizumab Injections *	Central Macular Thickness (μm) 1 Year	VA at 1 Year
74/M (Figure 1)	20/160	Switch (4 AFB)	225	328	289	2	275	20/100
69/M	20/200	Treatment-naive	279	389	296	4	268	20/50
67/F	20/120	Treatment-naive	342	367	272	2	282	20/80
72/M	20/100	Treatment-naive	328	402	301	2	259	20/50
68/F	20/100	Switch (5 AFB)	303	331	277	3	272	20/70

AFB—aflibercept, F—female, M—male, PED—pigment epithelial detachment, SRF—subretinal fluid, RPE—retinal pigment epithelium, VA—visual acuity, y—year. * Indicates number of injections required to achieve complete resolution of fluid in all compartments. All patients received therapy as per HAWK and HARRIER protocols.
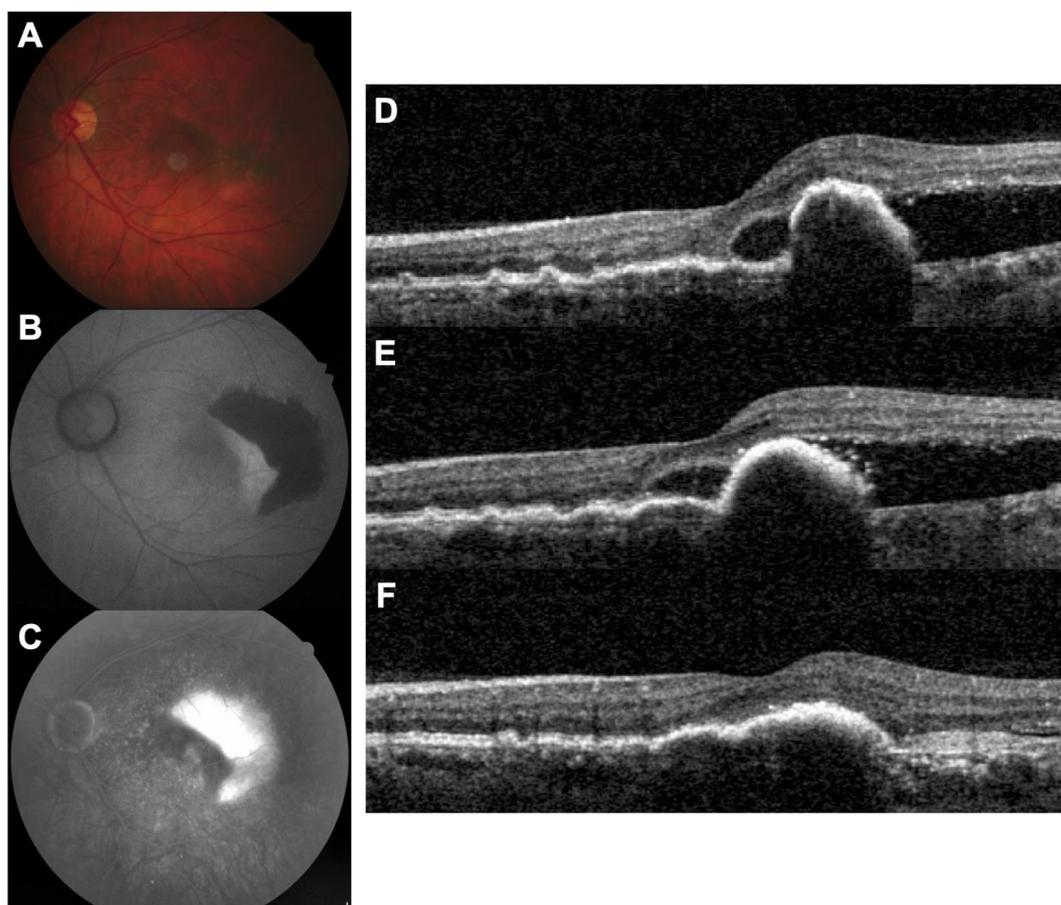
Figure 1. RPE tear complicating a type 1 CNV of a 74-year-old man (case 1). (**A**). Fundus photography showing RPE hyperpigmentation in the foveal area; (**B**). fundus autofluorescence demonstrating a large area of hypoautofluorescence in the temporal macula; (**C**). fluorescein angiography showing a window effect in front of the RPE tear; (**D**). baseline OCT showing a double layer sign under the fovea and a large PED with an absence of RPE in the temporal macula (i.e., RPE tear). SRF is present. (**E**). OCT after 4 monthly intravitreal injections of aflibercept, showing persistent SRF; (**F**). OCT after 2 intravitreal injections of brolucizumab showing a favorable response.

4. Discussion

This small case series shows good anatomical and functional results with intravitreal brolucizumab in patients with CNV and RPE tear with active leak. A total of three patients were treatment-naïve and two patients received switch therapy from aflibercept to brolucizumab. Both patients who received switch therapy showed further improvement in vision from baseline (at the time of switch). No side effects or complications, either as a consequence of the disease process or the administered therapy, were noted until the end of the follow-up period. None of the patients had a recurrence until the end of the follow-up period.

RPE tears [1–4] tend to eventually have a poor prognosis because of subretinal fibrosis, especially if the tear folds and encroaches upon the foveal center. In the event, it is necessary to ensure adequate therapy and achieve complete resolution of the associated fluid, thereby minimizing the detrimental effect of the condition on the retinal tissue.

The role of brolucizumab in the treatment of the exudative form of age-related macular degeneration (AMD) has been established through landmark Phase III trials. The smaller size of the brolucizumab molecule means a larger concentration of the drug can be delivered to the posterior segment of the eye via intraocular injection. This probably accounts for the improved efficacy and durability. On the other hand, as is true for any biological agent, it may account for a higher incidence of hypersensitivity such as a reaction.

There have been reports of adverse events with brolucizumab [7]. Notwithstanding, the drug is a useful alternative to currently available anti-VEGF agents for exudative AMD and, indeed, can eventually be the drug of choice given its potency, especially to reduce the treatment burden. More data will be available for perusal as the drug steadily penetrates global markets.

With just five cases to report, we have limited evidence on the efficacy and safety of brolucizumab in this particular subgroup of patients with exudative AMD, although none of the patients developed any complications. Further trials will be necessary to provide conclusive evidence. We suggest that patients with CNV who develop an RPE tear may benefit from initiation of brolucizumab therapy. Additionally, switch to brolucizumab is a valid option in these patients if the disease process seems unresponsive to primary therapy with other anti-VEGF agents, such as aflibercept (Figure 1).

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Visual Outcome after Intravitreal Anti-VEGF Therapy for Macular Neovascularisation Secondary to Sorsby's Fundus Dystrophy: A Systematic Review

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Abstract: The aim of this paper is to summarise our own and to review published experience regarding the long-term outcome of intravitreal treatment for macular neovascularisation (MNV) secondary to Sorsby's fundus dystrophy (SFD). A systematic literature search using the MeSH terms [Sorsby] and [anti-vascular endothelial growth factor (VEGF)] was conducted in NCBI/PubMed, Cochrane Central Register of Controlled Trials (CENTRAL), ScienceDirect, Google Scholar and ClinicalTrials.gov to identify publications reporting anti-VEGF treatment outcomes in SFD. Treatment outcomes were extracted for this meta-analysis from 14 publications and an own patient reporting a total of 31 cases with a mean follow-up (FU) of 54 months. Both eyes were affected in ten (32.3%) instances. Heterogenous reporting limited the comparability of the outcomes. All papers in common, however, reported satisfied to excellent responses to anti-VEGF therapy if patients were diagnosed and treated immediately after onset of symptoms. Of 20 eyes, for which visual acuity was reported before and after treatment, five worsened and seven improved by more than 1 line, whereas eight eyes maintained their function by end of the follow up, and 11 eyes (55%) maintained a driving vision (Snellen VA $\geq$ 0.5). Of six eyes with a VA < 0.5, VA improved in one to VA $\geq$ 0.5, whereas of 14 eyes with an initial VA $\geq$ 0.5, this dropped to <0.5 despite therapy. In MNV secondary to SFD, the delay between first symptoms and access to anti-VEGF treatment determines subretinal scar formation and thereby, functional prognosis. If treated early, this is generally favourable under regular controls and a consequent anti-VEGF treatment of MNV activity.

Keywords: Sorsby's fundus dystrophy; Sorsby; hereditary retinal dystrophy; choroidal neovascularisation; macular neovascularization; anti-VEGF treatment; long-term FU; treatment outcome

1. Introduction

Sorsby's fundus dystrophy (SFD) is a rare, autosomal dominant inherited retinal disease with complete penetrance affecting both genders similarly, typically becoming symptomatic after the second decade of life, with an average onset in the 4th to 5th decade of life, leading to severe bilateral vision loss and blindness if left untreated [1,2]. The pathophysiological mechanisms underlying the disease have yet to be identified while it is known to be caused by mutations in the gene encoding tissue inhibitor of metalloproteinases-3 (TIMP3) [3]. TIMP3 regulates remodeling of the extracellular matrix by inhibiting metalloproteases (MMPs) and competes with VEGF in binding to its receptor VEGFR2, thereby inhibiting angiogenesis [4–6]. It is expressed by retinal pigment epithelium (RPE) cells and is an element of Bruch's membrane in healthy individuals. Altered structure and aggregation of the protein can lead to characteristic accumulations in Bruch's membrane in SFD patients, resulting in Drusen-like deposits and thickening of the membrane [7,8].

What remains to be discovered is whether the accumulation of TIMP3 directly leads to disruption of Bruch's membrane, or indirectly, by the failure to inhibit MMP activity and VEGF-driven angiogenesis, resulting in the development of choroidal neovascularisation (CNV) or, due to the underlying pathophysiology more appropriately synonymously used, macular neovascularisation (MNV) [9,10].

SFD is characterised by the loss of central vision due to the development of a classical MNV (Figure 1a,b), and in the clinical course central geographic atrophy (Figure 2) [11]. Classical MNV was found to be a significant risk factor for a poor long-term prognosis in response to foveal scar formation in aged related macular degeneration [12]. Early symptoms in SFD include metamorphopsia, reduced colour vision, difficulties with dark adaptation and nyctalopia [2,13]. The typical clinical presentation of affected patients also includes drusen, reticular pseudodrusen and peripheral pseudodrusen. The hallmark of the angiogenic switch to macular neovascularisation is subretinal haemorrhage and exudation, whereas disciform macular scarring and central pigment epithelium atrophy represent the late stages (Figure 3) [13–15]. Progressive peripheral chorioretinal atrophy (Figure 4) and loss of ambulatory vision may be seen [11,13].

(a)

(b)

(c)

(d)
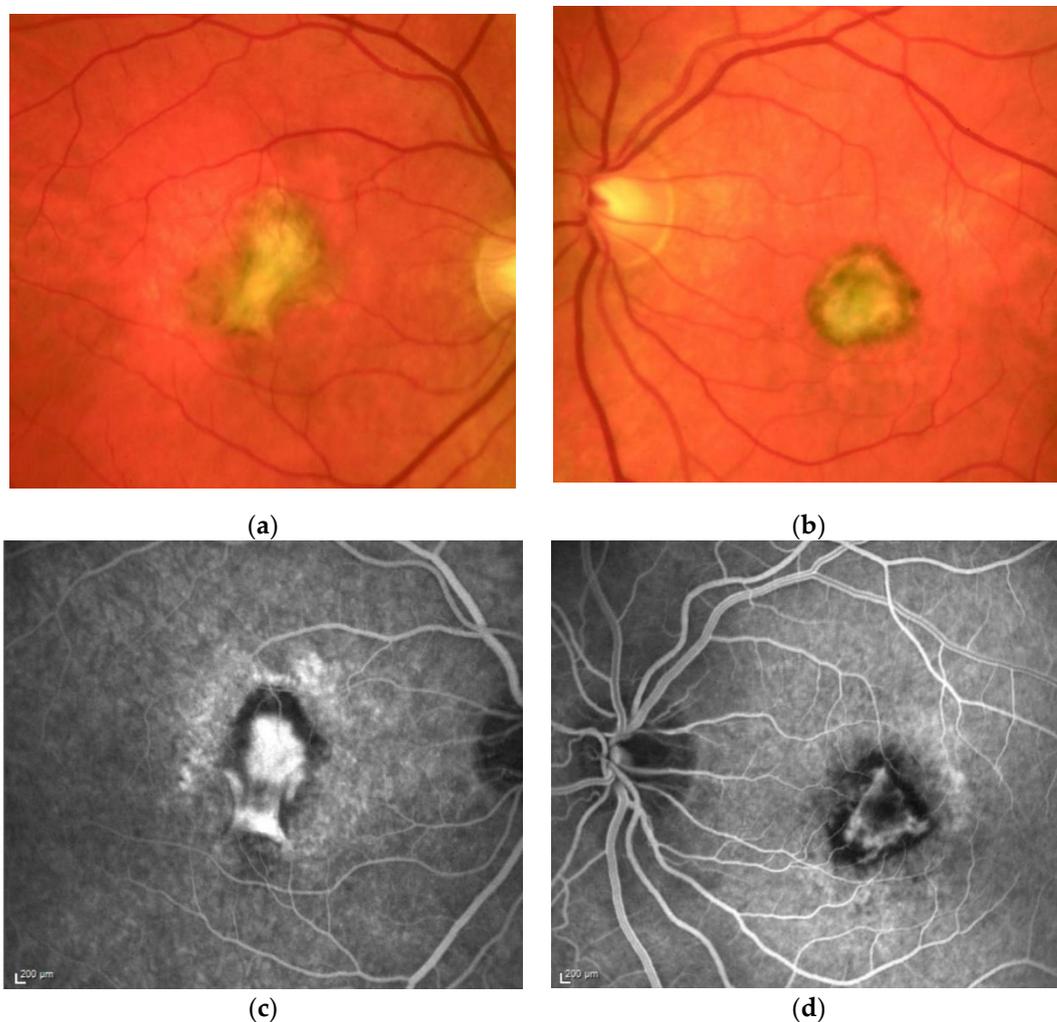
Figure 1. M, 35 years, M. Sorsby. Clinical image of both eyes with a significant submacular fibrovascular lesion after three courses of photodynamic therapy in the right (**a**) and four in the left eye (**b**), prior to the start of intravitreal therapy. (second panel). Same patient, fluorescein angiography (R middle (**c**), L early arteriovenous phase (**d**)) confirming a low-active predominantly classic macular neovascularisation.

(**a**)   (**b**)
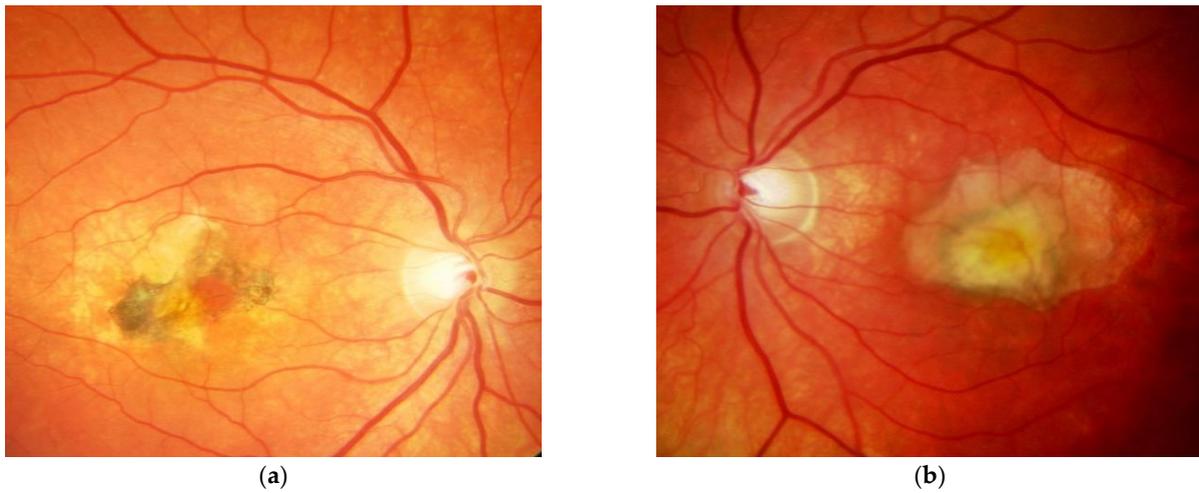
Figure 2. Same patient, 5 years later. First reactivation of macular neovascularisation evidenced by vision loss and a small macular hemorrhage as well as newly present intraretinal fluid in OCT in the right eye (**a**) and macular pigment atrophy in both eyes (**a**,**b**).

(**a**)   (**b**)
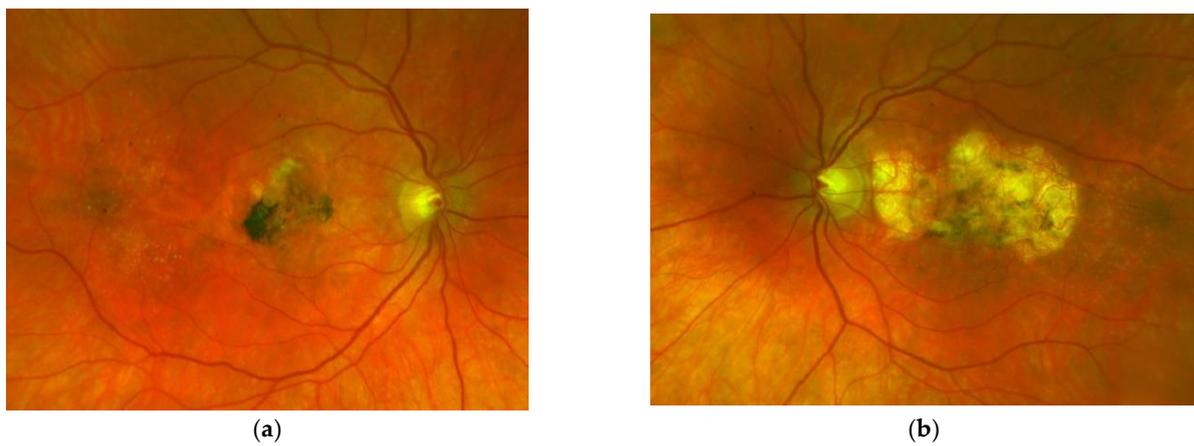
Figure 3. Same patient, 2016, 10 years after the start of intravitreal therapy; no lesion activity after 22 intravitreal Ranibizumab injections in the right eye (**a**) and a remarkable progressive macular atrophy despite a stable scar in his left eye (**b**).

(**a**)   (**b**)

Figure 4. *Cont.*

(**c**)

(**d**)
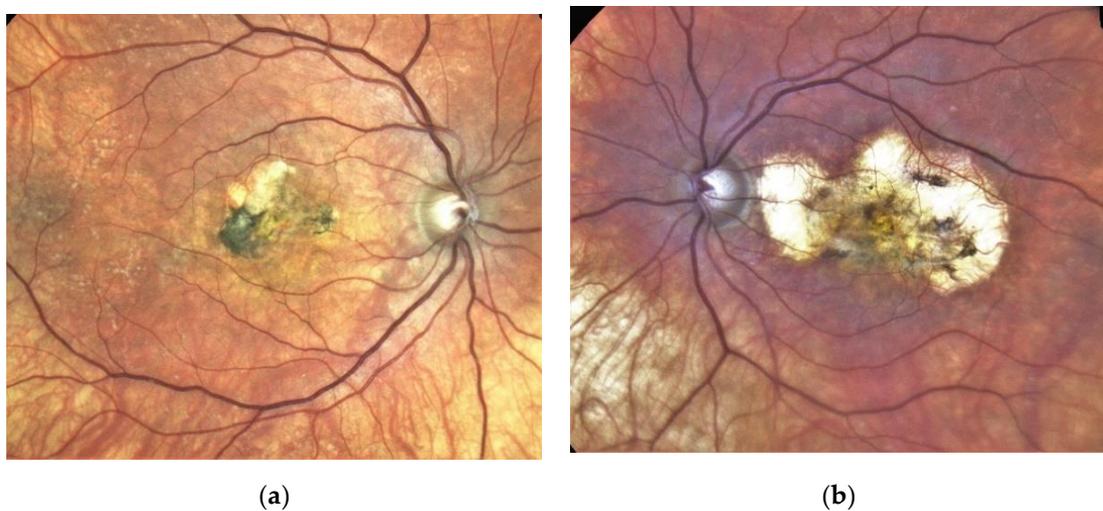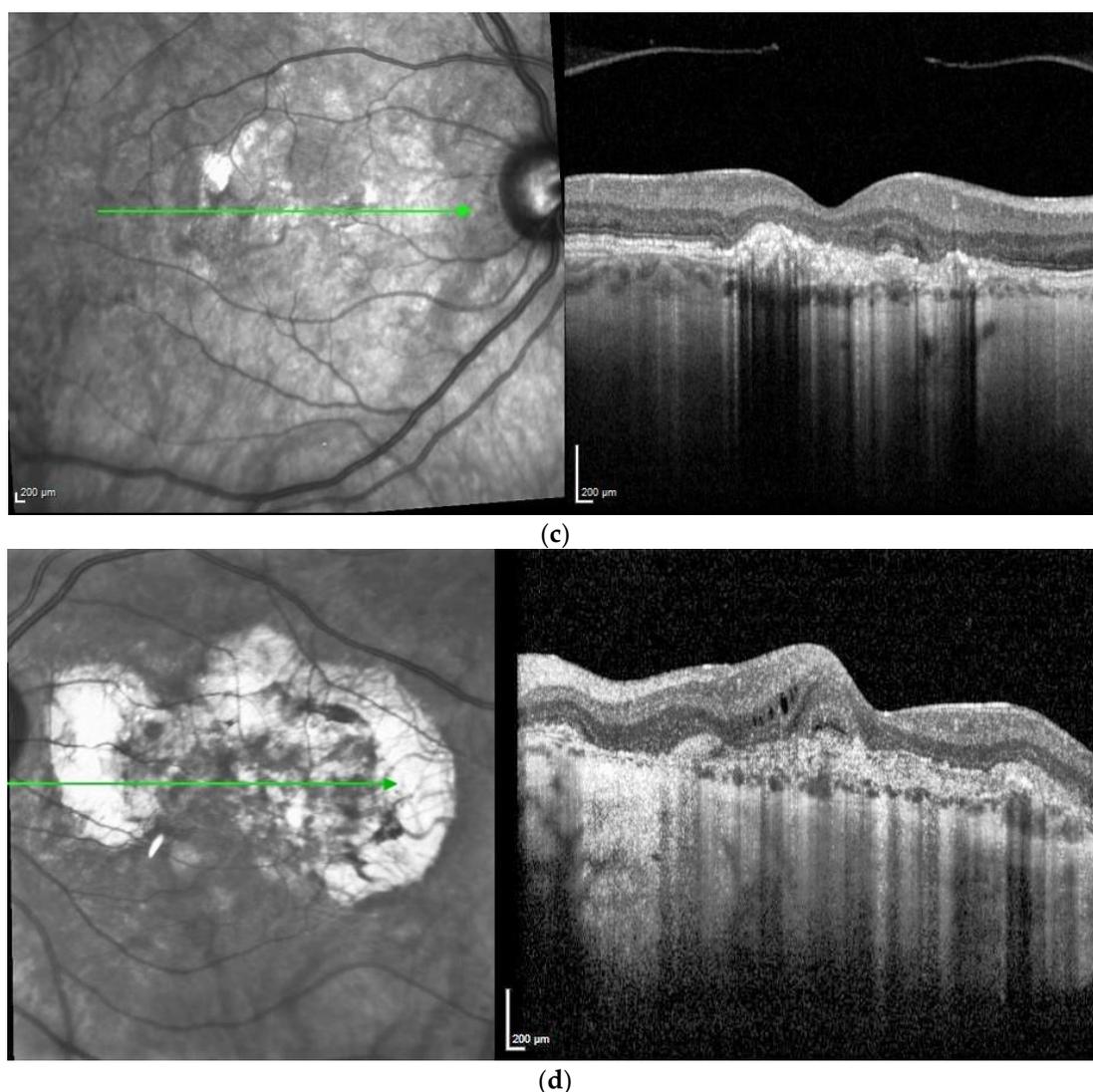
Figure 4. Same patient, 2021, meanwhile 51 years old. Eighteen years after diagnosis and 15 years after the start of intravitreal therapy visual acuity was maintained at Snellen 1.0 (20/20) in his right (**a**) and 0.16 (32/200) in his left eye (**b**), though reading and contrast-enhancing optical aids are required for near visual performance; no lesion activity after 22 intravitreal Ranibizumab injections in the right eye and a widely unchanged macular situation. Progressive macular scarring in both eyes. Upper panel: Clinical pictures of R + L eye (**a,b**), second panel, redfree picture and OCT of the right eye (**c**), bottom same, left eye (**d**). The arrows in redfree frames on the left side in Figure 4c,d indicate the location of the line scans on the right side. Note the progression of severity and extension of RPE changes, Drusen formation and choroidal sclerosis during the observation period. With consequent clinical controls and Ranibizumab treatment immediately upon first signs of lesion reactivation, his quality of life is perceived as excellent, he can follow his daily professional and private activities without relevant restrictions.

The differential diagnosis in this relatively young population is mostly straight forward with a positive family history and includes other inherited macular dystrophies, presenting an age-related macular degeneration (AMD)-like morphology and secondary MNV pathologies, though these but rarely present bilateral [16]. As there is no causal therapy available, current symptomatic treatment has focused on the management of hemeralopia and neovascular complications. Vitamin A has been used, to some extent, to improve night blindness [3]. While lower doses lack efficacy, high doses increase the risk of hepatotoxicity [17]. The formation of MNV is the main cause of severe visual impairment. Thermal laser photocoagulation of MNV has failed to improve vision, but was found to induced frequent recurrences [11,18]. In the early 2000s, verteporfin became

available and photodynamic therapy (PDT) was used to treat subfoveal MNV alone or combined with intravitreal corticosteroids. The effect of PDT on MNV activity was limited and not predictable [11,17,19–24]. Five years later, access to intravitreal anti-VEGF therapy provided a new treatment option for different types of MNV, including SFD. Until the advent of anti-VEGF drugs, SFD had a poor prognosis and eventually led to bilateral loss of central vision [11,14]; however, more than a decade later, several reports demonstrated promising long-term results preserving a meaningful VA. This compelled us to review the literature regarding long-term visual outcomes in patients with SFD since the advent of anti-VEGF treatment. We also added the experience of our own patient, who has been treated for the past 18 years in our clinic and retained a VA of 20/20 in his better eye.

2. Materials and Methods

A systematic literature search was conducted on 15 February 2021 of the NCBI/PubMed Cochrane Central Register of Controlled Trials (CENTRAL), ScienceDirect, Google Scholar and ClinicalTrials.gov databases using the key and MeSH terms [Sorsby] AND [anti-VEGF OR bevacizumab OR ranibizumab OR aflibercept OR photodynamic] and according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. To ascertain maximal exhaustiveness, cross-checking was performed in the reference lists of all papers, including meta-analyses and systematic reviews, to further identify cases meeting the diagnosis and treatment requirements, but not appearing under the above-mentioned MeSH terms and key words. Only articles and conference abstracts providing sufficient information to allow the assessment of the evolution of visual function with anti-VEGF treatment over a minimal FU of at least 3 months and written in English, German or French were included.

2.1. Eligibility Criteria

Criteria applied for studies to be considered eligible for this meta-analysis were:

- Report of single or multiple patient case or cohort study including patients diagnosed with Sorsby's Fundus Dystrophy published or treated until February 2021;
- Additional or pre-treatment with corticosteroids or photodynamic therapy was accepted;
- Reporting of evolution of visual function.

2.2. Information Retrieved from the Included Publications

The following parameters were retrieved: authors, publication date, title of the publication, gender of patient(s), age at onset of disease and at treatment initiation, time since diagnosis, family history, treatment history, laterality of affected eyes, evolution of VA under therapy, time gap between symptomatic vision loss and treatment initiation, FU duration after first anti-VEGF injection, total number of injections, additional treatment, and, if provided, genetic mutations. The same was applied to both eyes of our own patient.

Whenever necessary and to contain a uniform data format, we converted VA scores into Snellen decimal VA. For the analysis, data for each affected eye were recorded separately (one line in the table). For maximal completeness of the data sets, data from eyes represented in several citations were composed, if the supplemental articles added additional information on this study.

2.3. Assessment of Risk of Bias

Since this systematic review summarises case studies, we decided to integrate raw data instead of effect sizes from those reports with no underlying study design that could be biased. Following, a specific assessment of bias is not applicable. Some selection bias based on the orphan disease diagnosis may indeed be present, since the target population is very narrowly outlined. Our demographic data nevertheless show that we have a range of age in the predicted window (32 to 57 years) as well as a comparably balanced gender ratio (60.9% male). Based thereon, we assume that selection bias might not be a relevant problem.

3. Results

The systematic literature search generated a total of 907 records (PRISMA search flow, Figure 5). After exclusion of duplicates and the first screening of titles and abstracts, 21 full-text articles remained. After full-text reading, 14 publications reporting on 30 cases were included in the final analysis. All cases were independently coded by two raters. Interrater reliability was calculated in order to show agreement between the two raters. Cohen's kappa [25] yielded 92%, indicating a high interrater agreement. Differences in data extraction were resolved by discussion. These data were completed by results of an own case under long-term treatment for SFD.
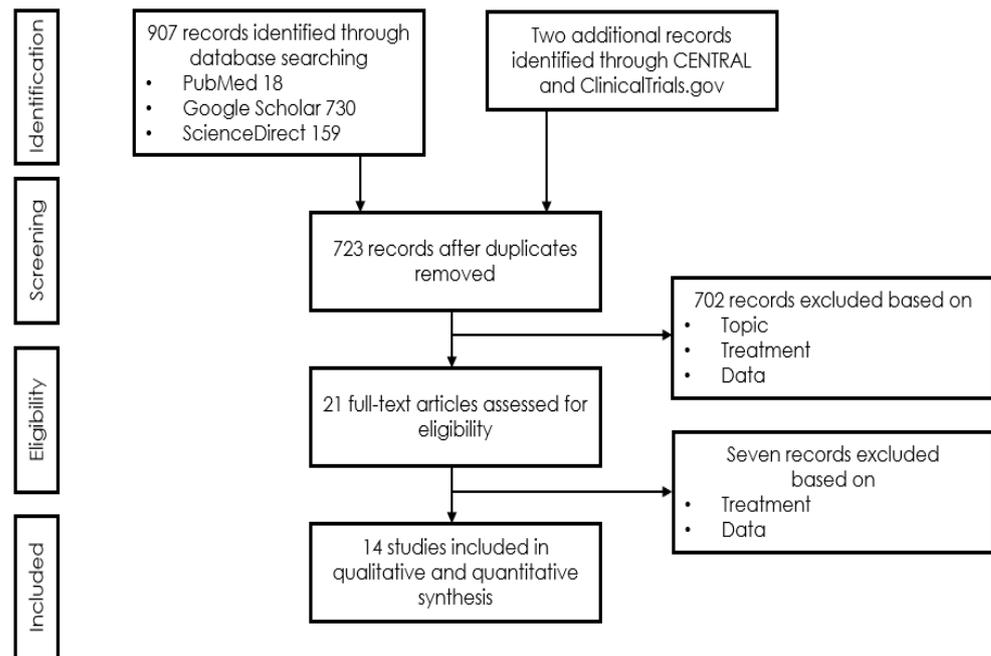
Figure 5. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) search flow.

Meta-Analysis

The overall FU time was 54 months. Eighteen of the thirty-one patients were case reports (Table 1). These 18 cases (six female, ten male, two unknown) referred to 27 affected eyes. Mean age at onset of MNV was 40 years. Mean VA at onset of MNV was 0.63 and last reported was 0.55 in all 18 cases. Considering only cases with both onset and last reported VA, it sums up to 0.63 and 0.62, respectively. Mean FU time was 52.8 months. VA was reported for all 27 eyes at the end of FU and for 20 of these eyes before and after treatment. Beyond all 27 eyes, 67% maintained a Snellen VA of 0.2 or better, and 51% maintained a value $\geq$0.5. Beyond the 20 eyes with VA known before and after treatment, five (28%) lost >one line, three (17%) $\geq$three lines, whereas seven eyes (39%) remained stable ($\pm$one line), six (33%) gained >one line, and beyond these, three (17%) gained three or more lines. When comparing patients with immediate (13 eyes) and delayed treatment (five eyes), we found that immediate treatment led to an increase of 0.16 of VA, whereas delayed treatment led to a decrease of 0.38 of VA by the end of observation. It must be considered, however, that VA at onset was better for the delayed treatment group (1.12) compared to the immediate treatment group (0.46).

Table 1. Treatment outcomes of case and cohort studies with Sorsby's fundus dystrophy, part I.

Patient	First Author	Year of Publication	Gender	NR of Eyes	Age at Onset	Family History Positive	Prior Treatment	VA before Onset	Eye
1	Sivaprasad	2008	m	1	nr	yes	PDT	nr	nr
2	Gemenetzi	2011	f	2	34	yes	no	nr	r
2	Gemenetzi	2011	f		37	yes	PDT	1.00	l
3	Gemenetzi	2011	f	1	44	yes	no	1.00	r
4	Gray	2012	f	1	38	yes	no	nr	l
5	Balaskas	2013	m	1	41	nr	no	1.25	r
6	Copete-Piqueras	2013	m	2	32	nr	no	nr	r
6	Copete-Piqueras	2013	m		32	nr	no	nr	l
7	Fung	2013	m	1	44	yes	no	1.00	r
8	Kapoor	2013	m	2	57	yes	no	1.25	r
8	Kapoor	2013	m		57	yes	no	1.00	l
9	Gliem	2015	nr	1	54	yes	no	1.00	l
10	Gliem	2015	nr	1	56	yes	no	1.00	r
11	Gliem	2015	m	1	45	yes	no	1.00	r
12	Keller	2015	m	2	32	yes	nr	nr	r
12	Keller	2015	m		32	yes	PDT	nr	l
13	Keller	2015	m	2	28	yes	no	nr	r
13	Keller	2015	m		28	yes	no	nr	l
14	Mohla	2016	f	1	52	nr	no	0.63	r
15	Menassa	2017	m	2	44	yes	no	1.60	r
15	Menassa	2017	m		38	yes	no	nr	l
16	Tsokolas	2020	f	2	34	yes	no	nr	r
16	Tsokolas	2020	f		37	yes	PDT	nr	l
17	Tsokolas	2020	f	2	36	yes	no	1.25	r
17	Tsokolas	2020	f		38	yes	no	1.00	l
18	Own patient	2004	m	2	33	yes	no	nr	r
18	Own patient	2004	m		33	yes	no	nr	l
19–23 *	Kaye	2017	nr	5	nr	nr	nr	nr	nr
24–31 *	Sanz	2013	62.5% m	9	45.3 (6.9)	nr	nr	nr	nr
Mean			62.5% m	41	41.2	48.4%	9.8%	1.08	

200

Table 1. *Cont.*

Patient	First Author	VA at Onset	Treatment Delay (Months)	Last VA	Follow-Up after Onset of Anti-VEGF Treatment (Months)	Total Number of Intravitreal Injections	Drug	Mutation
1	Sivaprasad	0.50	2	0.50	6	2	2 Bev	Ser181Cys
2	Gemenetzi	0.10	0	0.16	33	6	6 Bev	p.S204C
2	Gemenetzi	1.60	0.75	1.25	5	3	3 Bev	p.S204C
3	Gemenetzi	0.10	0	1.00	3	1	1 Bev	p.S204C
4	Gray	1.00	1.5	1.00	13	3	3 Bev	Ser181Cys
5	Balaskas	0.16	nr	0.40	27	14	14 Ran	c.610A4T (p.Ser204Cys)
6	Copete-Piqueras	0.63	0	1.00	6	1	1 Ran	mutations in Exon 5 of gene 22.12.3
6	Copete-Piqueras	0.80	0	1.00	6	1	1 Ran	mutations in Exon 5 of gene 22.12.3
7	Fung	0.63	0	0.80	48	6	6 Bev, PDT	Tyr159Cys
8	Kapoor	0.50	0	0.10	55	8	8 Bev, several Bev-Dex	normal coding sequence (codons 124–188 of the mature protein)
8	Kapoor	0.63	0	0.40	77	31	8 Bev, min. 18 Bev-Dex, 5 Ran, PDT	normal coding sequence (codons 124–188 of the mature protein)
9	Gliem	0.80	0	1.00	12	1	1 Bev	c.530A > G (p.Tyr200Cys)
10	Gliem	0.63	0	1.00	8	nr	multiple Bev	c.530A > G (p.Tyr200Cys)
11	Gliem	nr	0	1.00	nr	35	35 Bev	c.545A > G(p.Tyr182Cys)
12	Keller	nr	nr	0.70	60	nr	several Ran and Bev	nr
12	Keller	nr	nr	0.03	60	3	PDT, 3 Ran	nr
13	Keller	nr	nr	0.10	48	nr	Multiple Ran	nr
13	Keller	1.00	nr	0.20	48	nr	Multiple Ran	nr
14	Mohla	0.10	0	0.32	7	2	2 Bev	p.Arg204Cys
15	Menassa	1.25	0.3	0.80	6	5	5 Ran	c.610A > T
15	Menassa	nr	nr	0.10	nr	6	6 Ran	c.610A > T
16	Tsokolas	0.10	0	0.08	144	5	5 Bev	Ser204Cys
16	Tsokolas	1.25	1	0.16	108	79	79 Bev	Ser204Cys
17	Tsokolas	nr	4	0.06	72	24	24 Bev	Ser204Cys
17	Tsokolas	nr	0	0.50	60	42	42 Bev	Ser204Cys
18	Own patient		0	1.0	192	24	3 PDT, Tri, 24 Ran	mutation in the TIMP3 gene
18	Own patient		0	0.16	192	9	4 PDT, multiple Tri, 9 Ran	mutation in the TIMP3 gene
19–23 *	Kaye	0.8 (0.8)	nr	0.2 (0.4)	Min. 60	16	Bev	mutation in tissue inhibitor of metalloproteinases-3 (TIMP3)
24–31 *	Sanz	0.25 (0.2)	nr	nr	nr	9.11 (6.01)	Bev, Ran	p.Ser204Cys
Mean		0.56	0.45	0.49	54	12.78		

Abbreviations: nr, not reported; VA, Snellen visual acuity; FU, follow-up; f, female; m, male; r, right eye; l, left eye; nr, not reported; Bev, bevacizumab; Dex, dexamethasone; Ran, ranibizumab; PDT, photodynamic therapy; Tri, triamcinolone. * Cohort studies: values are reported as mean (standard deviation).

An additional 13 cases participated in two cohort studies [26,27] (Table 1), of which five were male and three were female (five unknown). The mean age was 45 years, and mean FU was 60 months. Five patients in the first series [26] experienced remarkable protection against severe vision loss over 24 months with anti-VEGF treatment (22.2% of the treated eyes suffered significant vision loss compared to 100% of the eyes in the control group). The second series [27] included nine eyes of eight patients that experienced VA gain with anti-VEGF treatment that was maintained over five years. However, the authors observed a linear decrease in VA of 0.1 logMAR units per year until scar formation.

4. Discussion

Secondary MNV is the landmark for the breakdown of VA in SFD. With the introduction of intravitreal anti-VEGF therapy, this previously rapidly blinding disease [11] has, for the first time, found an unprecedented treatment that may preserve useful central vison over many years if initiated early, with 51% of eyes maintaining a reading and driving vision ($\geq$0.5) and 67% of vision allowing reading with reading aids ($\geq$0.2) [11,16,17,19,21,22,28–33]. Even under consequent treatment of neovascular activity, the underlying, so far only partially understood heredo-degenerative pathology may progress and result in central geographic atrophy and/or progressive night blindness, for both of which there is currently no treatment available. Fortunately, such progression has not been observed in our patient over the past 18 years (Figures 2–4).

Central vision may be maintained as long as a central fibrovascular scar has not developed. In neovascular age related macular degeneration ani-VEGF therapy was found to delay scar formation [12]. A significant number of the published patients (Table 1) retained their central vision at least partially over four to seven years after occurrence of MNV, if anti-VEGF agents were administered shortly after occurrence of MNV. Sanz et al. estimated that the risk of significant visual loss may be reduced by 96% over 24 months, based on their case series of eight eyes if MNV was treated early with anti-VEGF drugs. In their series, 22.2% of treated eyes suffered a significant vision loss, compared to 100% of the eyes in the historical control group [26]. Kaye et al. reported a stabilization of VA with anti-VEGF treatment for MNV in five patients during the five-year observation period. They found, however, a linear annual decrease in VA of 0.1 logMAR units, with macular scar formation as the causative factor [27].

Before the availability of anti-VEGF drugs, treatment aimed at preserving some vision with a series of PDT and parabulbar or intravitreal triamcinolone that may stabilise small lesions as in the right, but not so in the left eye of our patient. The functional success of PDT, however, is unavoidably linked to a significant subretinal fibrovascular scar formation, which in the long term is accompanied by severe vision loss. Fortunately, disease remained quiet in the right eye of our patient over seven years. By then, anti-VEGF treatment had become available. This has allowed to maintain a full vision with a total of 24 intravitreal ranibizumab injections on a PRN basis over meanwhile ten years. Given the long periods of inactive MNV, the treatment burden remained supportable for this patient under a PRN regimen. Long times of inactivity of MNV are not unique, why a treatment following a treat-and-extend strategy in this generally relatively young population cannot generally be recommended.

Though our study is inherently limited by the paucity of retrospectively reported cases, this did not question the tremendous effect of early anti-VEGF therapy. The length of FU period in our and previously published cases proved the long-term efficacy of anti-VEGF treatment for MNV in SFD. Affected patients deserve to be correspondingly educated that there is a good chance to retain useful central vision, and to understand the importance of immediately consulting an ophthalmologist in case of visual irregularities, ideally before severe VA loss is encountered.

Though there exists, in conclusion, no cure for this heredo-degenerative disease, anti-VEGF treatment has dramatically changed the prognosis for patients with Sorsby's fundus dystrophy. The visual function may be preserved in the vast majority for a significant

period of the patients' lives. More than half of the patients will maintain a driving and reading vision if macular neovascularisation is diagnosed and treated early.

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Abstract: Age-related macular degeneration (AMD) is one of the most common causes of vision loss. Advanced forms of AMD are seen in primarily two types—neovascular AMD (nAMD) with the presence of choroid neovascularization and non-neovascular AMD (nnAMD) with geographic atrophy. Neovascular AMD is characterized by choroidal neovascularization (CNV), which leads to a cascade of complications, including exudation, leakage, and ultimately fibrosis with photoreceptor loss. Inhibition of VEGF represents the current standard of care. However, there is a tremendous gap between the outcomes in randomized clinical trials and real-world settings. New agents for nAMD might offer the potential to improve treatment outcomes and reduce treatment of frequent intravitreal injections. We summarize all the newer molecules, their pivotal clinical trial results, and their unique mechanisms of action; these include longer-acting agents, combination strategies, sustained release, and genetic therapies.

Keywords: emerging treatment; neovascular age-related macular degeneration (nAMD); Vascular Endothelial Growth Factor (VEGF)

1. Introduction

Age-related macular degeneration (AMD) is a leading cause of degenerative vision loss in elder individuals [1–3]. Due to an aging population, the global prevalence of AMD is projected to rise from 170 to 288 million by the year 2040 [4]. AMD may be classified as early, intermediate, and advanced types based on severity [5,6]. In early AMD, multiple small- and medium-sized drusen lipids deposit under the retina, or there are mild pigmentation abnormalities of the retinal pigment epithelium (RPE) in at least one eye. Intermediate AMD is characterized by at least one large druse, retinal pigment abnormalities, or geographic atrophy of the RPE that does not involve the center of the fovea. Lastly, advanced AMD is vision threatening and is seen in primarily two types— neovascular AMD (nAMD) and non-neovascular AMD (nnAMD) with geographic atrophy.

"Wet" or neovascular AMD (nAMD) is defined by choroidal neovascularization (CNV) that causes bleeding, fluid accumulation, and fibrosis of the macula [7]. While CNV only affects 10–15% of patients diagnosed with AMD, it accounts for 90% of severe vision loss caused by AMD [8,9]. Macular photocoagulation has been historically used to limit damage from choroidal lesions [10]. However, the past 15 years have experienced a paradigm shift in the treatment of nAMD. Intravitreal antivascular epithelial growth factor (VEGF) agents—bevacizumab, ranibizumab, and aflibercept—now represent the standard of care. Additionally, a fourth intravitreal drug, brolucizumab, was approved by the FDA in the last quarter of 2019. A large body of evidence from randomized clinical trials has helped to guide clinicians to use these agents with great success.

However, despite their proven efficacy, anti-VEGF agents still face issues. Firstly, there is a high treatment burden due to their short duration of action. Patients may require monthly injections over many years of treatment. Furthermore, long-term administration of

intravitreal anti-VEGF injections is not ideal. Studies reflect worse visual acuity outcomes in the real world than those achieved in clinical trials [11,12]. This may be explained by challenges with compliance to regular injections, resulting in a large share of real-world patients being undertreated. Additionally, anatomic features, such as the development of fibrosis, may affect this outcome. In the CATT study, 25% of patients on aggressive anti-VEGF therapy developed some degree of fibrosis at 2 years [13]. Furthermore, there was an increased risk of developing retinal scarring and geographic atrophy in nAMD patients 2 to 5 years after initiating treatment [14]. Complications such as vitreous and subconjunctival hemorrhage, fluid accumulation under the fovea, increased intraocular pressure, endophthalmitis, and ocular inflammation have also been described [15–18].

There is clearly an unmet need for more durable and longer-acting treatment against nAMD. Several promising agents are in development, which improve upon current anti-VEGF therapy, exploit novel pathways, use innovative delivery systems, or offer combination therapy. This review aims to summarize these emerging therapies, their mechanisms of action, and their pivotal clinical trial results (Table 1).

Table 1. Currently available and experimental treatments for neovascular age-related macular degeneration (nAMD).

Drug	Mechanism of Action	Company	Relevant Studies	Phase of Study
Faricimab	Angiopoetin-2 and VEGF-A antibody	Genentech	TENAYA, LUCERNE, AVONELLE-X	3
Port Delivery System (PDS) with Ranibizumab	Surgically implanted reservoir with anti-VEGF	Genentech/Roche	ARCHWAY	3
Abicipar Pegol	Anti-VEGF DARPin	Allergan	CEDAR, SEQUOIA	3
Brolucizumab	Single-chain anti-VEGF antibody fragment	Novartis	HAWK, HARRIER	3
KSI-301	Antibody biopolymer conjugate	Kodiak Sciences	DAZZLE	1b
Conbercept	Recombinant VEGF receptor antibody	Chengdu Kanghong Biotech Company	PANDA-1, PANDA-2	3
OPT-302	VEGF-C and VEGF-D blockade	Molecular Partners	ShORe, COAST	3
GB-102	Depot formulation of sunitinib malate	Graybug Vision	ADAGIO, ALTISSIMO	1/2a, 2b
RGX-314	Gene therapy	REGENXBIO	ATMOSPHERE, AAVIATE	2b/3
ADVM-022	Gene therapy	Advernum Biotechnologies	OPTIC	1

2. Methods of Literature Search

The literature search was conducted by searching PubMed and Google Scholar, along with sources cited from companies' websites. The latter allowed us to locate findings that were presented at recent conferences. Only articles in the English language were included. The search was conducted up to the end of April 2021.

3. Pathophysiological Aspects of Current and Future Therapy

The pathophysiology of AMD is multifactorial and complex. In addition to strong age dependence, there are a variety of metabolic, functional, genetic, and environmental factors at play [19–26]. At least four key processes contribute to disease: lipofuscinogenesis, drusogenesis, neovascularization, and local inflammation [27].

With aging, several metabolites accumulate within the retina, leading to elevated levels of the age-related pigment, lipofuscin [27]. Lipofuscin is the product of incomplete metabolism of external segments of photoreceptors by phagolysosomes. Elevated concentrations of this pigment have been associated with cell damage and oxidative stress.

These toxic effects impair the RPE, which is responsible for the maintenance of photoreceptor cells and is involved in the recycling of visual pigments and daily phagocytosis of constantly shed photoreceptor outer segments. Additionally, A2E (breakdown product of lipofuscin) has been found to activate the complement system, further contributing to pathogenesis [21].

Another component of AMD pathogenesis is the development of lipid deposits called drusen, which may be "soft" or "hard" depending on size and shape. Drusen are composed of similar protein components to the plaques found in Alzheimer's disease [20]. Soft drusen appear as large, pale-colored, dome-shaped elevations that can resemble localized serous RPE detachments. In contrast, large drusen are usually a sign of diffuse thickening of Bruch's membrane with basal linear deposit. Studies suggest that local inflammation and activation of the complement cascade actively contribute to drusogenesis, photoreceptor degeneration, and Bruch's membrane disruption.

Lastly, choroidal neovascularization (CNV) characterizes pathology for which nAMD gets its "wet" name. CNV leads to uncontrolled growth of leaky blood vessels under the macula in a variety of exudative eye conditions, such as AMD and diabetic retinopathy. This process may be mediated, in part, by local inflammation and immune reactions [21]. Neutrophils, macrophages, mast cells, and activated microglia can release an array of proangiogenic factors, including VEGF [28]. The VEGF family of proteins regulate vascular permeability in the retina and are the target of current therapy.

Among these proteins, VEGF-A is the principal driver of CNV, binding to the extracellular ligand-binding domains of two tyrosine kinase receptors (VEGFR-1 and VEGFR-2). This cascade leads to the activation of genes for angiogenesis and vascular permeability. However, aside from VEGF-A, there has been recent therapeutic interest in targeting VEGF-C and VEGF-D. These isoforms have been shown to be increased in response to inhibition of VEGF-A [29–32]. Selective blockade of VEGF-A may trigger compensatory upregulation of other members of the VEGF family. Therefore, avenues to suppress this mechanism of resistance are under active exploration.

Additionally, angiogenesis receives a contribution from alternative pathways, such as the Ang-Tie 2 axis. In this pathway, angiopoietin-1 and angiopoietin-2 are key cytokines that interact with transmembrane receptor tyrosine kinase (Tie-2) [33]. In healthy states, Tie-2 is bound by angiopoietin-1, which is a protective factor, promoting vascular stability, pericyte recruitment, and the inhibition of vascular permeability factors [34]. However, in angiogenic states, the competitive inhibitor angiopoietin-2 is upregulated, displacing Ang-1, and causing endothelial destabilization, inflammation, and breakdown of the blood–retina barrier [35]. Combination therapies that target these non-VEGF angiogenic factors may provide additional benefit over current standard of care. However, this is yet to be determined. The agents discussed in this review are organized according to the mechanism of action in Figure 1.
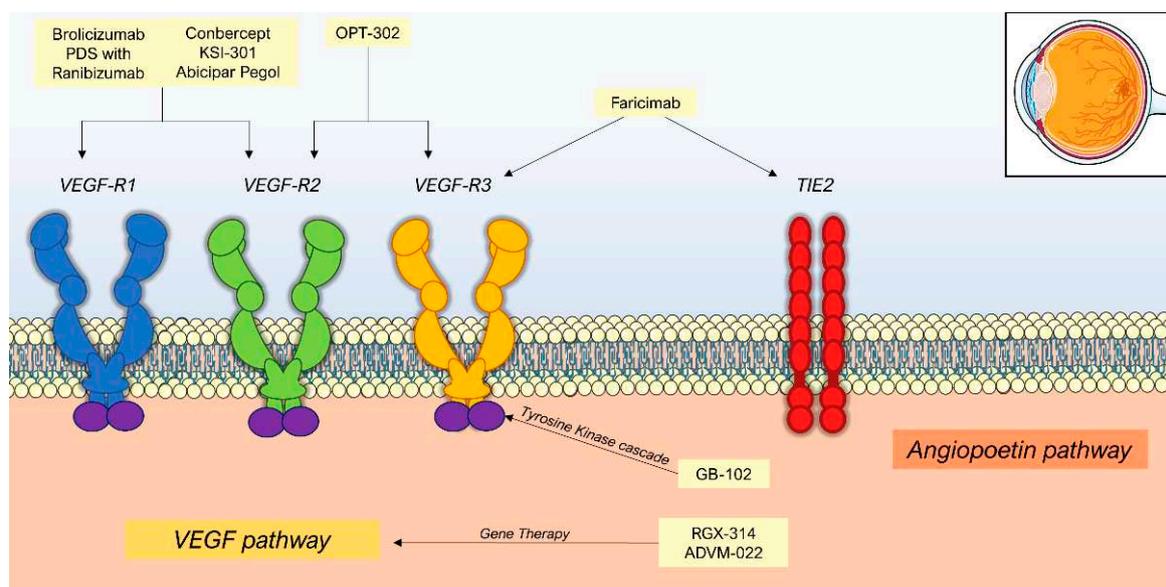
Figure 1. Mechanisms of action of Neovascular age-related macular degeneration therapies in development.

4. Emerging Neovascular AMD Therapies

4.1. Faricimab

The current first-line therapy for nAMD exclusively targets VEGF, creating an unmet need for anti-VEGF subresponsive patients. Faricimab aims to fill this void by exploiting the alternative Ang-2/Tie-2 pathway [33]. Faricimab is the first bispecific monoclonal antibody designed for intraocular use [34]. With two arms, the antibody independently binds and neutralizes both VEGF-A and angiopoetin-2 (Ang-2), which together synergistically promote vascular stability.

TENAYA and LUCERNE were Phase 3, multicenter, randomized studies to evaluate the efficacy and safety of faricimab in patients with nAMD [36,37]. Patients were randomized to receive faricimab 6 mg up to every 16 weeks or aflibercept 2 mg every 8 weeks after three initial loading doses. With the faricimab arm, they had four initial loading doses with disease activity assessments at Weeks 20 and 24 based on best-corrected visual acuity (BCVA) and central subfield thickness (CST) criteria, along with the investigator's evaluation. At Week 20, if there was active disease, those patients continued on at every 8 weeks. At Week 24, if there was active disease, those patients continued on for every 12 weeks. If there was no disease activity, those patients maintained every 16-week interval with faricimab. The primary endpoint was change in BCVA from baseline averaged over three visits, Weeks 40, 44, and 48. Results showed meaningful and comparable reductions in CST from baseline through Week 48 with faricimab up to every 16 weeks and aflibercept every 8 weeks. The median number of injections for the faricimab arm was six vs. eight in aflibercept. The initial BCVA gains were sustained, with a majority of patients in the faricimab arm up to every 16 weeks. Faricimab showed excellent durability with 45% of patients on every 16-week and almost 80% of patients on every 12-week dosing by Week 48. Concerning safety, the rates of intraocular inflammation in these studies were low. Intraocular inflammation was reported on average in 2% and 1.2% of patients for faricimab and aflibercept, respectively. There were no cases of vasculitis.

TENAYA and LUCERNE are 2-year studies; the long-term extension study, AVONELLE-X, will generate 4-year long-term data. These data on faricimab offer hope of exploiting additional mechanisms beyond VEGF. With much still being elucidated about the role of angiopoietin in vessel stabilization, it will be interesting to study if faricimab provides additional benefits from having a unique mechanism of action.

4.2. Port Delivery System (PDS)

The success of traditional anti-VEGF therapies raised the question of whether a longer-acting ranibizumab could be delivered via an implantable reservoir. Thus, the Port Delivery System (PDS) was developed, allowing for the continuous release of ranibizumab into the vitreous via passive diffusion [38]. PDS is intended to reduce the frequency of intravitreal injections and potentially allow patients with nAMD to go several months before needing a refill of the implant. The device is a self-sealing eye implant that requires surgical implantation and can be refilled in the office via injection through the conjunctiva. Currently, the PDS holds 20 μL of a customized formulation of ranibizumab (100 mg/mL). This dosage was found to be the most effective dose from the Phase 2 LADDER trial in wet AMD, looking at visual and anatomic success [39].

Results of the Phase 3 ARCHWAY trial showed that PDS at every 24 weeks was noninferior and equivalent to monthly ranibizumab at its primary endpoint at Week 40 [40]. As expected, there was a transient postsurgical drop in vision in the PDS arm that recovered by Week 40. By Week 72, patients in the PDS arm had two refill exchanges at Weeks 24 and 48, with vision and anatomic outcomes comparable with monthly ranibizumab. There was equivalent vision and controlled retinal thickness, and PDS patients required five times fewer treatments over a mean duration of 78 weeks.

The idea of a surgically implanted VEGF depot is intriguing but does carry potential risks. VEGF has been found to be a key neurotrophic factor involved in the maintenance of retinal vasculature [41]. Potent, long-term inhibition may be disruptive to the health of neurovascular cells. There is evidence of increased risk of geographic atrophy in patients treated monthly as opposed to patients treated pro re nata [42,43]. With any of the extended durability anti-VEGF treatments in development, including PDS, little is known about the adverse events associated with the prolonged antagonism of VEGF. Additional considerations include risks from the surgical procedure itself and the possibility of endophthalmitis or vitreous hemorrhage. These risks are still being evaluated by the FDA for consideration for use. Nonetheless, the promising results from ARCHWAY are an excellent step towards increased longevity of nAMD therapy.

4.3. Abicipar Pegol

New to the world of protein therapeutics, designed ankyrin repeat protein (DARPin) molecules are small, single-domain proteins that can selectively bind to a target protein with high affinity and specificity [44,45]. These molecules are highly stable, providing advantages over currently available antibodies or antibody fragments. At present, abicipar pegol is a DARPin developed for use against nAMD [46]. Abicipar binds all isoforms of VEGF-A with excellent tissue penetration. Furthermore, it has a longer intraocular half-life compared with ranibizumab (>13 days vs. 7.2 days) [47].

Following encouraging results from the Phase 2 REACH study [48], two identical global Phase 3 studies were conducted (CEDAR and SEQUOIA) [49]. Participants with nAMD were divided into three arms: three monthly abicipar 2 mg injections followed by an injection every 8 weeks, two monthly abicipar 2 mg injections followed by an injection after 8 weeks and every 12 weeks thereafter, and monthly ranibizumab injections. Results showed mean change in BCVA during Year 2 was similar when compared to Year 1 across all treatment arms. Precisely 93% of patients in the 8-week abicipar group, 90% of patients in the 12-week abicipar group, and 94% of patients in the 4-week ranibizumab group achieved stable vision. Only four intravitreal injections of abicipar were required to maintain the outcomes, as compared to monthly intravitreal ranibizumab injections. Overall, abicipar demonstrated non-inferiority compared with ranibizumab, meeting its primary endpoint.

However, roughly 15% of abicipar-treated eyes experienced intraocular inflammation (IOI). In efforts to reduce this adverse effect, the manufacturing process has since been modified. The MAPLE study, a 28-week safety evaluation, was performed to determine the rates of adverse events in 128 patients after the manufacturing process was changed. The

data showed a reduced intraocular inflammation rate of 8.9%, and only 1.6% of these cases were deemed moderately severe or severe [50]. The improvement in the rate of adverse effects is because reformulation is a step in the right direction; however, additional research is required to validate the efficacy and extended duration of abicipar.

4.4. Brolucizumab

At a size of ~26 kDa, the humanized single-chain antibody fragment brolucizumab may provide enhanced tissue penetration, clearance, and drug delivery characteristics compared to more traditional anti-VEGF agents [51]. By comparison, ranibizumab and aflibercept have molecular weights of 48 and 115 kDa, respectively [52]. The molar dose of brolucizumab is 11.2 to 13.3 times higher than that of aflibercept, permitting greater drug concentrations and therefore longer duration.

The safety and efficacy of brolucizumab were compared to aflibercept in two Phase 3 trials, HAWK and HARRIER [53]. The primary endpoint in both studies was noninferiority to aflibercept in mean change in BCVA from baseline to Week 48. In HAWK, patients were randomized to intravitreal brolucizumab 3 mg, brolucizumab 6 mg, or aflibercept 2 mg. HARRIER randomized patients to brolucizumab 6 mg or aflibercept 2 mg. Brolucizumab was noninferior to aflibercept in the primary outcomes in both studies. In the superiority analysis of HAWK at Week 16, the incidence of disease activity was significantly lower with brolucizumab 6 mg compared with aflibercept (24.0% vs. 34.5%). Intraretinal fluid/SRF was present in fewer brolucizumab-treated eyes versus aflibercept-treated eyes at Week 16 in both trials. Rates of ocular and nonocular AEs were similar with brolucizumab and aflibercept.

Despite the efficacy of brolucizumab for nAMD, and its superior pharmacokinetics, many retina specialists are concerned about the risk of occlusive vasculitis and blindness with the drug. The rate of uveitis was 2.2% with brolucizumab 6 mg and 0.3% with aflibercept in HAWK, and <1% with both drugs in HARRIER. The incidence of iritis was 2.2% with brolucizumab 6 mg and 0% with aflibercept in HAWK, and <1% with both drugs in HARRIER. While the FDA has recently given approval towards brolucizumab use, it is unclear whether these adverse events will outweigh the potential benefits.

4.5. KSI-301

Mechanically, KS-301 resembles the classic anti-VEGF agents; however, it is based on a 950 kDa antibody biopolymer conjugate (ABC) platform that is engineered specifically for increased durability [54]. Preclinical pharmacokinetic studies have demonstrated KSI-301's extended ocular half-life of 10–12 days. In a Phase 1b study, patients received three loading doses at Weeks 0, 4, and 8. There was a durability assessment from Weeks 12 to 72 with an extension study from weeks 76 to 148. The efficacy of KSI-301 was determined by change from baseline to Week 52 in mean BCVA and optical coherence tomography (OCT) thickness. There was an observed mean 5.7-letter improvement to 69.7 ETDRS eye chart letters (~20/40 Snellen) at Year 1 [55]. Additionally, thickness had decreased by 105 microns. Patients received three loading doses, followed by an average of two individualized doses thereafter, resulting in a total of five mean injections in Year 1.

There was an excellent safety profile for KSI-301. Most adverse effects were assessed as mild and consistent with the profile of intravitreal (IVT) anti-VEGF injections. To date, 43 serious AEs (SAEs) have been reported in 24 subjects; however, none were drug related. Additionally, three ocular SAEs in the study eye not drug related were all resolved. Only two AEs of IOI (2/710, 0.28%) were noted, both traced to 1+ vitreous cell with complete resolution.

4.6. Conbercept

Conbercept is a 141 kDa engineered fusion protein that, like aflibercept, acts as receptor decoy against VEGF [56]. However, conbercept has higher binding affinity and contains an additional fourth binding domain of VEGFR2. This design is hypothesized

to provide increased stability of the receptor-ligand complex and extend the half-life of conbercept [56–59]. Two global Phase 3 trials for nAMD were initiated: PANDA-1 and PANDA-2. Each trial is evaluating 1140 patients randomized to conbercept, 0.5 or 1 mg, or aflibercept 2 mg, with primary efficacy analysis at 36 weeks. In PANDA-1, patients received three loading doses through Week 8, then continued with dosing every 8 weeks through Week 92. In PANDA-2, dosing was pro re nata after Week 40, with the 0.5 mg conbercept and aflibercept groups on the same regimen as PANDA-1 up until that point, after which the conbercept 1 mg arm shifted to 12-week dosing after 8 weeks and moved onto PRN at Week 40. The PANDA trials recently reached a milestone by completing 36-week primary endpoint visits of enrolled patients in December 2020.

4.7. OPT-302

With traditional VEGF blockade, current therapies target VEGF-A, which is considered to be the most pathologic isoform. However, in this process, other VEGF isoforms are upregulated [29–32]. OPT-302 is a novel "trap" molecule that binds and neutralizes the activity of VEGF-C/-D, blocking their activation of receptors VEGFR2 and VEGFR-3 [60]. There is hope that combining OPT-302 with currently available anti-VEGF-A may address mechanisms of resistance associated with existing therapies. Two concurrent global Phase 3 trials known as Study of OPT-302 in combination with Ranibizumab (ShORe) and Combination OPT-302 with Aflibercept Study (COAST) have begun [61]. These trials build upon the successful Phase 2b nAMD clinical trial while additionally evaluating the administration of OPT-302 in combination with ranibizumab and aflibercept over a longer treatment period and in a greater number of patients. ShORe and COAST will enroll approximately 990 treatment-naive patients each and assess the efficacy and safety of intravitreal 2.0 mg OPT-302 in combination with 0.5 mg ranibizumab or 2.0 mg aflibercept, compared to ranibizumab or aflibercept monotherapy, respectively. The primary endpoint of both studies is the mean change in BCVA from baseline to Week 52 for OPT-302 combination therapy compared to anti-VEGF-A monotherapy.

4.8. GB-102

Like PDS, GB-102 is another sustained-release anti-VEGF delivery system [62]. However, GB-102 is formulated as an intravitreal formulation of sunitinib malate-containing, biodegradable microparticles. The controlled microparticle release is intended for biannual injection to maintain comparable visual acuity and central subfield thickness outcomes. The ADAGIO Phase 1/2a study consisted of patients with nAMD who received four escalating dose cohorts of eight patients, each receiving a single dose of either 0.25, 0.5, 1, or 2 mg of GB-102 [63]. Precisely 88% of the patients at 3 months and 68% of the patients at 6 months were maintained on a single dose of GB-102. Positive outcomes were observed for up to 8 months. CST was significantly reduced at all months compared with pretreatment. However, one concern that emerged was the nonaggregation of the drug once in the vitreous cavity, resulting in particle dispersion. Nine of thirty-two subjects experienced related symptoms, including eye pain, photophobia, and blurriness [64]. A new manufacturing process was developed to eliminate the microparticle dispersion and incomplete aggregation, which was used for future trials.

Phase 2b ALTISSIMO was initiated to further evaluate GB-102 for CNV lesions in previously treated nAMD patients [65]. The study consists of three cohorts: 1 mg of GB-102, 2 mg of GB-102, or 2 mg of aflibercept at baseline. The GB-102 cohorts will then receive their same initial dose every 6 months, whereas the latter control group will continue to receive aflibercept 2 mg every 2 months. The primary outcome is the proportion of treated subjects remaining rescue free through Month 10. While the efficacy of GB-102 is still being determined, it provides an immediate bridge towards longer-lasting therapies further down the pipeline. GB-102 has by far the longest time needed between treatments, and the development of GB-103, which aims for once-a-year dosing, has already begun

4.9. RGX-314

Gene therapy has shown promise for the treatment of inherited retinal diseases, and recently there has been a push for gene therapy solutions for nAMD. RGX-314 is a vector designed to bind and neutralize VEGF in a manner similar to ranibizumab [66]. RGX-314 utilizes adeno-associated virus serotype 8 (AAV8) as its vector, with research suggesting that AAV vectors provide long-term transgene expression [67]. The gene therapy vector is preferentially taken up by retinal cells, leading to high levels of production of the monoclonal antibody fragment. The company is advancing two separate routes of ocular administration of RGX-314: a one-time subretinal administration during vitrectomy; and in-office suprachoroidal delivery. The hope is that the long-standing and stable production of the anti-VEGF therapeutic protein could reduce the need for frequent intravitreal injections.

ATMOSPHERE is the first of two planned pivotal trials for the evaluation of subretinal delivery of RGX-314 in patients who have received prior treatment for nAMD [68,69]. Patients underwent vitrectomy and were delivered subretinal RGX-314 across five dose cohorts (3×10^9 genome copies (GC)/eye, 1×10^{10} GC/eye, 6×10^{10} GC/eye, 1.6×10^{11} GC/eye, 2.5×10^{11} GC/eye). RGX-314 continued to be generally well tolerated across all cohorts, with 20 serious adverse events (SAEs) reported in 13 patients, including 1 possibly drug-related SAE of a significant decrease in vision in Cohort 5. The most common nonserious adverse events in the eye were generally assessed as mild (87%). These included postoperative conjunctival hemorrhage (69% of patients), postoperative inflammation (36% of patients), eye irritation (17% of patients), eye pain (17% of patients), and postoperative visual acuity reduction (17% of patients). In 67% of patients across all cohorts, and in 83% of patients in Cohorts 3 through 5, retinal pigmentary changes were observed on imaging, the majority of which were in the peripheral inferior retina. Retinal hemorrhage was observed in 26% of patients and is an anticipated event in patients with severe wet AMD. There have been no reports of clinically determined immune responses, drug-related ocular inflammation, or postsurgical inflammation beyond what is expected following routine vitrectomy. In the two higher dose cohorts (four and five), patients at 1.5 years after treatment demonstrated stable visual acuity with a mean BCVA change of +1 letters and −1 letters from baseline, respectively, as well as decreased CRT, with a mean change of −46 and −93 μm, respectively. In Cohort 4, 4 out of 12 (33%) patients have received no anti-VEGF injections after 6 months following RGX-314 administration and demonstrated a mean BCVA change from baseline of +2 letters at 1.5 years. Eight out of eleven (73%) patients have received no anti-VEGF injections after 6 months following RGX-314 administration and demonstrated a mean BCVA change from baseline of −2 letters at 1.5 years. These data show a meaningful reduction in anti-VEGF treatment burden in both Cohorts 4 and 5. With the positive results of ATMOSPHERE, and the pending results of suprachoroidal RGX-314 delivery in AAVIATE, there is much promise for gene therapy in nAMD treatment.

4.10. ADVM-022

ADVM-022 is another gene therapy that aims to provide sustained anti-VEGF expression from the retina. In the OPTIC trial, the primary objective was to assess the safety and tolerability of a single IVT injection of ADVM-022. Secondary objectives were to evaluate BCVA and anatomy using spectral-domain OCT (SD-OCT), and to assess the need for rescue therapy [69]. All patients received aflibercept injection 1 to 2 weeks prior to dosing of ADVM-022. There was a 24-week safety and efficacy assessment. Again, the same was done at Week 52 with a follow-up at 104 weeks. Patients received oral steroid prophylaxis in Cohorts 1 and 2 and steroid eyedrop prophylaxis in Cohorts 3 and 4.

Overall, ADVM-022 continues to be well tolerated with a favorable safety profile at both high and low doses. It showed robust and sustained efficacy in both high and low doses. There was excellent durability out to 92 weeks from a single IVT injection with 0 supplemental injections in Cohort 1. There was robust aqueous anti-VEGF protein expression observed at 18 months in Cohort 1. This study showed a substantial reduction

in annualized injection frequency following ADVM-022. Most patients did not require any supplemental injection in OPTIC. Patients completing 2 years in OPTIC are being enrolled into an extension trial to be followed for up to 5 years. Two global Phase 3 (PIVOTAL-a and PIVOTAL-b) trials are targeted to initiate in the fourth quarter of 2021.

Gene therapy differs from other extended durability therapies as no hardware is implanted in the eye. This may circumvent potential complications such as conjunctival erosion. Moreover, there is tremendous value in the potential role of gene therapy in preventing chronic exudative eye conditions such as nAMD, as many researchers believe that early intervention is valuable in limiting the progression of nAMD. While one or two intravitreal injections are generally tolerable, ongoing treatment with no definite cessation for patients who are asymptomatic can often be untenable for them. So, there is much excitement about the possibility of a one-time treatment with sustained intraocular VEGF suppression that could slow the course of nAMD. However, a potential disadvantage to gene therapy is the inability to turn it off. The consequences of long-term VEGF blockade are still being elucidated.

5. Conclusions

While anti-VEGF agents have revolutionized our treatment of nAMD, the field continues to evolve in the hope of providing better options for our patients. As discussed, numerous novel molecular targets may allow us to improve upon the clinical outcomes achieved by the VEGF blockade. Beyond VEGF, there are several trials underway investigating alternative factors in retinal and choroidal angiogenesis, such as PDGF, FGF, and EGF. Furthermore, research towards longer-acting pharmaceuticals might yield good results with fewer treatments, helping to improve compliance, possibly allowing us to treat more patients.

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Real-World Performance of a Self-Operated Home Monitoring System for Early Detection of Neovascular Age-Related Macular Degeneration

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Abstract: The real-world performance of a home telemonitoring strategy (ForeseeHome AMD Monitoring System®, Notal Vision, Inc.,Manassas VA, USA) was evaluated and compared to the device arm of the AREDS2-HOME study among patients with intermediate AMD (iAMD) who converted to neovascular AMD (nAMD). All patients with confirmed conversion to nAMD who used the home monitoring system from 10/2009 through 9/2018 were identified by Notal Vision Diagnostic Clinic's medical records. Selected outcome variables were evaluated, including visual acuity (VA) at baseline and at conversion, and change in visual acuity (VA) from baseline to time of conversion. In total, 8991 patients performed 3,200,999 tests at a frequency of 5.6 ± 3.2 times/week. The 306 eyes that converted from iAMD to nAMD over the study period (a 2.7% annual rate) were included in the analyses. There was a median (interquartile range) change of -3.0 $(0.0-(-10.0))$ letters among converted eyes, 81% [95% confidence interval (72–88%)] maintained a VA $\geq 20/40$ at the time of conversion, while 69% of the conversion detections were triggered by system alerts. The real-world performance of an at-home testing strategy was similar to that reported for the device arm of the AREDS2-HOME study. The home telemonitoring system can markedly increase early detection of conversion to nAMD.

Keywords: ForeseeHome AMD Monitoring System®; neovascular age-related macular degeneration; AREDS2-HOME study

1. Introduction

Eyes with early and intermediate age-related macular degeneration (AMD) typically retain good central vision and overall vision [1]. Any subsequent vision loss is usually attributed to advanced AMD, specifically, geographic atrophy or neovascular AMD (nAMD). Both the Comparison of AMD Treatments Trial (CATT) [2,3] and a real-world analysis that employed the Intelligent Research in Sight (IRIS) registry [4] confirmed that baseline visual

acuity (VA) at the time of conversion from AMD to nAMD is a strong predictor of long-term visual outcomes under treatment with intravitreal anti-vascular endothelial growth factor (anti-VEGF) therapies. Significant visual improvement with these treatments, as shown in the landmark registration studies [5–7], is often not replicated in real-world outcome studies. Given that baseline vision is a strong determinant of long-term visual outcome and that long-term gains in vision are often unsustainable, early detection of nAMD can be pivotal to achieving favorable therapeutic results [8–11]. A recent cost-utility analysis reported that early treatment was 138–149% more cost-effective than late treatment, and those authors concluded that early treatment is critical for obtaining optimal vision and cost-effectiveness, as is long-term follow-up and adherence to treatment [12].

Despite the obvious benefit of detecting conversion to nAMD while vision is still good, many reports reveal that only 13 to 35% of patients are diagnosed and begin intravitreal anti-VEGF treatment when their VA is 20/40 or better [4,13–15]. One analysis of IRIS registry data on 13,859 subjects found that the mean VA at conversion to nAMD was 20/74 [4]. In another and larger IRIS database analysis, the mean VA of more than 140,000 newly converted eyes was 20/83 [14,15]. Similar findings of poor VA at conversion have been widely reported in other studies in which VA at the time of conversion ranged from 20/80 to 20/142 [16–22].

The incidence of conversion from dry to nAMD [13,14,23–25] suggests that frequent monitoring of eyes with intermediate AMD would be optimal. However, bringing a large, older population into clinics for monitoring visual and anatomic changes can be difficult for both the patient and the caregivers, particularly given current safety concerns related to the COVID-19 pandemic. One possible solution to address these difficulties is at-home self–monitoring. Patients and physicians have long used the Amsler grid as a home monitoring strategy, but it has limited efficacy due to factors, such as problems in filling in the information, lack of fixation, and poor compliance [26,27]. No well-designed clinical trial has shown that the Amsler grid is effective in detecting nAMD in patients with good VA. More effective types of home monitoring technologies and services may well have increasing relevance and desirability in a COVID-19 environment and beyond, as people may prefer to "stay at home" to limit their potential exposure to the virus [28].

The AREDS2-HOME study [29,30] evaluated one home self-monitoring strategy that included the ForeseeHome AMD monitoring system (Notal Vision, Inc., Manassas, VA, USA). The system uses preferential hyperacuity perimetry to detect minute differences in the relative spatial localization of two or more objects and was previously described [1]. In brief, during the test of each eye, the patient is responding to fast stimuli in random locations in the visual field of the central 14°. These flashing signals include artificial distortions with varying amplitudes. The marking of the patient in the location of the presented distortion, in a different location, or the absence of a response are collected, transmitted to the secured cloud location, and are analyzed by the system's artificial intelligence algorithm. Upon identification of a statistically significant change in the testing results compared to a baseline period, a change alert is communicated to the prescribing physician through a remote diagnostic clinic that provides the monitoring service. The study randomized subjects to either a device arm that included the home monitoring system in addition to standard care or to a control arm that included standard care alone. The study findings showed that a significantly higher percentage of patients were able to maintain VA of 20/40 or better after using the strategy that included home monitoring to help identify conversion in early stages.

The objective of this study was to evaluate the performance in real-world implementation of the strategy that includes the use of this at-home monitoring system in combination with standard care, i.e., the strategy implemented in the device arm of the HOME study. The system allows the patients to proactively monitor their visual status between doctor visits while at the same time establishing a safety net that is intended to prevent significant loss of vision during the conversion to nAMD. This evaluation focused on the most relevant parameters of vision preservation. We hypothesized that since the real-world implementation uses the same device, hyperacuity test, remote diagnostic clinic infrastructure, and

compliance reminder services all integrated into a monitoring program, then the efficacy of the system would be similar to the efficacy reported by the participants randomized to the device arm of the AREDS2-HOME study.

2. Patients and Methods

This is a retrospective review of data on all patients with available information on a confirmed conversion from intermediate AMD to nAMD during their participation in an at-home monitoring program any time during the period from October 2009 through September 2018. The inclusion criteria for enrollment into the program was diagnosis of intermediate dry AMD and best corrected visual acuity of 20/60 or better in any eye that was prescribed. Participation was defined as having a device at home and the availability of a valid baseline. Patients were identified from the medical records of the Notal Vision Diagnostic Clinic (Notal Vision Inc.; Manassas, VA USA), an independent diagnostic testing facility and medical provider of the home monitoring program. Patients were referred to the program by an eyecare professional. Upon referral, the NVDC contacted the patient over the telephone, provided explanations about the nature of the disease and the purposes of the device, and shipped the device to the patient's home. Following remote training on device operation, the patients established a baseline of testing results. They were instructed and encouraged to self-test daily. The NVDC monitored their compliance to these instructions and reminded patients to self-test when necessary. Artificial intelligence compared the patient's baseline and most recent sequence of testing results to a normative database and transmitted the information to the NVDC. If there had been a statistically significant change from baseline in recent self-test scores (an alert), a notification was sent to the NVDC. After the alert was reviewed by the NVDC, it was relayed to the referring physician's office staff who contacted the patient to schedule a clinical evaluation. Other complementary triggers for an in-office visit were routine and symptoms visits. All these visit-triggering modalities jointly comprise the home monitoring strategy under evaluation.

This study was granted an exemption of oversight by an independent institutional review board (IntegReview, Austin, TX, USA) on the basis of Code of Federal Regulations Title 45, Part 46.104. It does not contain any human participants or use of animals.

2.1. Outcome Variables

The datasets of eyes with reported conversion during the study period included demographics, overall duration of participation in the monitoring program (as reported in monitoring years as the sum of periods from the first test to the reported conversion of all the study eyes), the number of tests performed during the study period and the mean weekly frequency of testing, the annual observed conversion rates, and the dates of identification of conversion to nAMD confirmed by the treating physicians. They also included the VA at baseline and confirmed conversion (when available) as measured at the physician's office, and the modality that triggered the in-office visit in which the diagnosis was confirmed following a system alert (as opposed to detection of conversion during routine or symptom-driven visits).

For the purposes of this study, baseline was defined as the point in time when the patient was first prescribed the at-home monitoring system. The testing baseline used by the system in the ongoing monitoring may have been established few days or weeks later due to the delay from enrollment at the clinic to device delivery, setup, and first usage. Outcome measures included the time from baseline to the reported conversion, the proportion of conversions to nAMD detected following an alert by the at-home monitoring system vs. conversions reported as having been identified during an office visit, the VA at baseline and at the time of conversion, the numerical change in VA from baseline to conversion, the percentage of eyes with a VA $\geq$ 20/40 at time of conversion, and the percentage of eyes with a VA $\geq$ 20/40 at baseline that maintained a VA $\geq$ 20/40 at the time of conversion to nAMD. Conversion was confirmed based on the evaluation of nAMD by a retina specialist.

2.2. Statistical Analyses

Statistical analyses were primarily limited to descriptive statistics with comparison between sub-cohorts when applicable. VA was reported as Snellen equivalent and converted to Early Treatment Diabetic Retinopathy Study (ETDRS) letters for comparison when applicable and reported in letter changes or Snellen [31].

3. Results

During the study period, a total of 8991 patients were enrolled in the program and tested a total of 13,930 eyes, representing an average of 1.55 eyes per patient. They performed 3,200,999 tests during 11,525 years of monitoring. A total of 306 eyes were reported to have converted from intermediate AMD to nAMD during the study period representing a conversion rate of 2.7% per year, and they were included in the current analyses. The mean ($\pm$standard deviation, SD) weekly frequency of testing per eye and per patient were 3.7 ± 1.9 and 5.6 ± 3.2, respectively (Figure 1). The patients' mean age was 75 ± 7.1 years, and 199 (65%) were females (Table 1). Of the 306 eyes with confirmed disease progression, 211 (69%) were identified following the at-home system alert, and the remaining 95 (31%) were identified during a routine or a symptom-driven visit. The duration from time of baseline to conversion to nAMD by the alerting modality is shown in Table 1. The median 5.7-month difference in the time from baseline to conversion between the modalities was not significant ($p = 0.082$), although it did show a trend towards shorter duration for the system alerts.
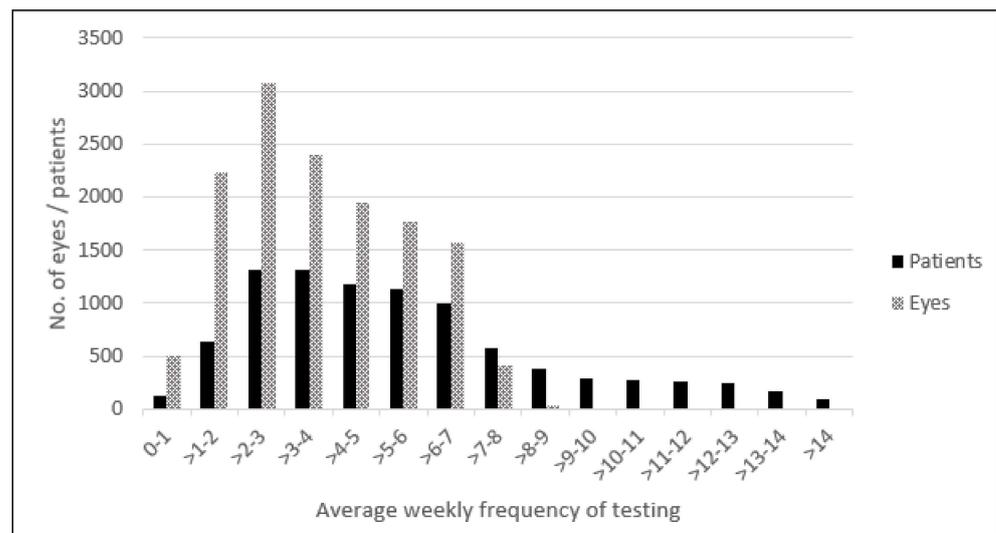
Figure 1. Distribution of the average weekly frequency of testing per eye and per patient.

Table 2 provides details about the VA for the entire cohort and by detection modality. The data included the VA at baseline, at conversion to nAMD, and the change in VA from baseline. Since this report is based on real-world, retrospectively collected data, not all VA values from both time points were available. There was a statistically significant difference in visual acuity between baseline and at the time of conversion ($p = 0.00014$). The mean (SD) and median (interquartile range, IQR) change in VA from baseline to conversion for the 121 eyes with data at both time points was -5.4 (10.0) and -3.0 [0.0–(-10.0)], respectively. For the 95 eyes with VA data that was triggered by system alert, the change in VA from baseline to conversion was a median (IQR) of -2 [0.0–(-10.0)] letters. In comparison, the change in VA from baseline to conversion in the 26 eyes where the conversion was detected during routine or symptom-driven office visits was a median (IQR) of -4.5 [0.0–(-16.5)] letters. The difference of 2.5 letters in median change of VA was not statistically significant ($p = 0.19$).

Table 1. Demographics and time to conversion from AMD to nAMD of patients in the at-home monitoring program by modality of detection.

Modality of Detection, *n* (%) of Conversions	All Conversions: 306 (100%)	Conversion Detected Following an Alert: 211 (69%)	Conversion Detected during Routine or Symptom-driven Visit to Physician 95 (31%)
Mean age in years (SD)	75 (7.1)	76 (6.9)	73 (7.3)
Female sex, *n*	199 (65%)	139 (66%)	60 (63%)
Mean (SD) time from initiation of the at-home device use to conversion, months	16.8 (16.3)	15.8 (15.4)	19.2 (18.0)
Median (IQR) time from initiation of the at-home device use to conversion, months	11.4 (4.3–23.5)	10.5 (3.9–22.3)	16.2 (5.4–24.2)

AMD: age-related macular degeneration; nAMD: neovascular age-related macular degeneration; SD: standard deviation; IQR: interquartile range.

Table 2. Visual acuity outcomes by detection modality.

VA Outcomes	Baseline VA	VA at Conversion to nAMD	VA at Conversion with Known Baseline VA	VA Change from Baseline to Conversion
No. of eyes	121	193	121	
Mean VA [SD] letters	77.7 (7.4)	72.4 (12.0)	72.3 (12.7)	−5.46 (10.0)
Mean VA Snellen	20/32	20/40	20/40	
Median VA [IQR] letters	79.0 (74.0–84.0)	75.0 (68.0–81.0)	74.0 (66.0–81.0)	−3.0 (0.0–(−10.0))
Median VA Snellen	20/25	20/32	20/32	
Eyes with nAMD detection triggered by a system alert with known VA				
No. of eyes	95	151	95	
Mean VA [SD] letters	77.6 (7.1)	72.7 (11.2)	73.1 (11.3)	−4.5 (8.6)
Mean VA, Snellen	20/32	20/40	20/40	
Median VA [IQR] letters	78.0 (74.0–83.0)	75.0 (69.0–81.0)	74.0 (68.5–81.0)	−2.0 (0.0–(−10.0))
Median VA Snellen	20/32	20/32	20/32	
Eyes with nAMD detection during a routine or symptom-driven visit to physician with known VA				
No. of eyes	26	42	26	
Mean VA [SD] letters	78.0 [8.7]	71.3 [14.7]	69.4 [16.8]	−8.6 [13.9]
Mean VA Snellen	20/32	20/40	20/40	
Median VA [IQR] letters	81.0 [71.75–85.75]	76.0 [62.25–81.0]	74.0 [56.25–82.5]	−4.5 [0.0–(−16.5]
Median VA Snellen	20/25	20/32	20/32	

VA; visual acuity; AMD: age-related macular degeneration; nAMD: neovascular age-related macular degeneration; SD: standard deviation; IQR: interquartile range.

Of the 193 eyes with information about the VA at conversion to nAMD, the VA of 144 eyes (75%; 95% confidence interval, CI [68–81%]) was equal or better than 20/40. Of the 109 eyes with a baseline VA ≥ 20/40, 88 eyes (81%, 95% CI [72–88%]) maintained a VA ≥ 20/40 at the time of conversion and initiation of treatment. Detection was triggered

by a system alert in 86 eyes with a baseline VA ≥ 20/40 that had information about VA at the time of conversion, and 71 of those eyes (83%, 95% CI [73–90%]) maintained a VA ≥ 20/40. Seventeen of the 23 eyes with a baseline VA ≥ 20/40 74% (95% CI [52–90%]) in which conversion was detected during a routine or symptom-driven visit maintained a VA ≥ 20/40. There was no significant difference between the alerting modalities and the proportion of eyes with a VA ≥ 20/40 (p = 0.38).

4. Discussion

This study reports on the real-world performance of a monitoring strategy that included an at-home self-operated monitoring system in conjunction with office visits (both routine and symptom-driven) for early detection of nAMD. This same strategy was evaluated in a prospective multicenter study that found patients in the home monitoring arm lost significantly less VA from baseline to the time of conversion than those being followed by standard care alone [29].

Our current study included a larger group of eyes (n = 306) that converted to nAMD compared to the AREDS2-HOME study (n = 82). Our reported conversion rate was 2.7% per monitoring year, which is typical for an intermediate AMD population [32,33], and provides an indication that a significant portion of conversions within this cohort was captured during the study. The mean (SD) weekly frequency of testing per eye was 3.7 ± 1.9, which is consistent with 3.44 ± 1.86 tests per week reported in a recent publication on real-world use of the ForeseeHome device [34]. The patients in our study tested themselves 5.6 ± 3.2 times per week, consistent with the frequency of 5.9 tests per week as calculated from a recent real-world report, [34] and more than the frequency of 4.4 ± 1.7 tests per week reported in the AREDS2-HOME study. A comparison of the distribution of detection between the triggering modalities revealed a similar rate of "first to trigger" by the home monitoring system was reported in both studies: it was 69% in the current study compared with 64% in the AREDS2-HOME study in a comparable per protocol (PP1) population of patients who were using the device at the time of nAMD detection, regardless of frequency of use [29]. Our findings suggest that the inclusion of the home monitoring program in an overall monitoring strategy allows the at-home system to detect the majority of disease conversions, thereby providing an effective safety net for patient vision. The median (IQR) change in VA for the entire cohort was −3.0 [0.0–(−10.0)] letters. In comparison, the median (IQR) change in VA for the device arm of the HOME study was very similar, at −3.0 [−1.0–(−10.0)] letters [29].

Our study demonstrated that 81% [95% CI (72–88%)] of eyes with a VA ≥ 20/40 at baseline retained that vision at the time of conversion, which was lower than the 91% reported for a comparable group in the device arm of the HOME study, but higher than the 62% reported in the control arm of the HOME study [29]. The current report also showed a much higher percentage of eyes with a VA of ≥20/40 (75%) at the time of conversion to nAMD when all eyes were considered, other real-world studies of newly diagnosed nAMD reported a percentage of eyes with a VA of 20/40 or better at the time of conversion that ranged from 13.1 to 34.3%, [14,16,35,36] notably including the IRIS registry dataset (n = 55,930 eyes), in which 34.3% of eyes had a VA ≥ 20/40 at the time of diagnosis [14]. Caution is recommended when interpreting these comparative data, however, since the population that elects to use a home monitoring technology does not necessarily represent the general population included in the IRIS database. Our current study showed that for this population, which is similar to the PP1 population in the AREDS2-HOME study, there was real-world efficacy in identifying patients with a better vision at the time of conversion through the use of an at-home monitoring system, a finding comparable to the results of the AREDS2-HOME study.

Overall, there was no significant difference between the alerting modalities in terms of the VA in eyes with good vision (>20/40) at the time of conversion in this population. One possible explanation for the different outcomes of the current and IRIS studies may be that the HOME study baseline VA was 83 letters on the ETDRS chart while it was 79 letters

in the current study, shifting the distribution of VAs below the threshold of 20/40. Another possible explanation is that the group of patients who converted to nAMD in this paper who elected to use a home-monitoring technology were more sensitive to small subjective changes in vision, than the IRIS population.

The main limitation of this study is that it is a retrospective database analysis. The VA was not available at baseline or at conversion for all patients, and some of the VA values were reported over the telephone, which may have introduced some bias. This review only included patients who converted to nAMD after establishing a baseline on the ForeseeHome monitoring system. In trying to evaluate the sensitivity of the findings to possible methodological limitations resulting from the retrospective, real-world study design, we considered a worst-case scenario in which an unbalanced, larger number of events were triggered by routine visits or symptoms that were not identified and therefore not included in the report, and we found the expected VA loss to be 4.5 letters. Another limitation is that we did not collect information about the outcomes of alerts issued by the home monitoring system that did not result in an immediate identification of conversion to nAMD. These may include alerts that led to conversions to nAMD that were identified after some delay, alerts that led to the diagnosis of non-nAMD pathologies, and those that wound up being false alerts. In some instances, the patient re-established a baseline and resumed testing. However, those limitations were mitigated by study strengths, which include the length of review (9 years) and the very large number of tests and monitoring years. Also, the same system was used in both the real-world and the AREDS2-HOME study, with the same support and reminders as reflected in the high weekly frequency of use. This allowed us to avoid some of the typical difference between studies and real-world evidence. Other strengths were the uniquely large number of newly diagnosed nAMD events that were detected early after a period of close follow-up with the ForeseeHome system, which differs from how a typical cohort of treatment-naïve nAMD eyes are traditionally diagnosed, treated, and reported during an office visit.

Our conclusions differ from those of another recently published real-world data set, [36] primarily because the reports evaluated two different populations of real-world patients. The current study evaluated a patient population that had a device with a valid baseline up to and including the time of conversion. Yu et al. [34] evaluated all patients who were prescribed the device, and they cite a large number of patients who did not or could not establish or re-establish a baseline, as well as a considerable number of patients who discontinued use of the device.

In conclusion, our current study suggests that the consistent long-term use of an at-home monitoring system may provide a significant benefit to patients as a means of increasing early detection of nAMD with good vision, a known strong predictor for long-term preservation of vision [13,14]. AMD has been long established as a common condition in the elderly population [37]. In today's COVID-19 pandemic environment and beyond, the ability to provide quality care to patients at high risk of converting to nAMD while limiting their potential exposure to COVID-19 bestows additional value. This study provides evidence to demonstrate that an at-home monitoring system can achieve those goals. It may drive physicians, patients, and their families to request access, gain benefit from the expected performance, and drive a large-scale improvement in the quality of life of this growing population.

Informed Consent Statement: Patient consent was waived due to the separation between the source data and case report forms in this retrospective study.

Data Availability Statement: The datasets generated and analyzed during the current study are not publicly available since the data could be used to derive business intelligence information about the company that owns the clinical data and considers them confidential.

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Article

Five-Year Outcome of Aflibercept Monotherapy for Exudative Age-Related Macular Degeneration with Good Baseline Visual Acuity

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Abstract: We investigated the long-term visual and anatomical outcomes of aflibercept monotherapy for exudative age-related macular degeneration (AMD) with good baseline best-corrected visual acuity (BCVA). A medical chart review was performed for 40 consecutive patients with baseline decimal BCVA $\geq$ 0.6 secondary to exudative AMD. Three monthly injections were administrated, and thereafter additional injection was performed if needed over 5 years. In total, 13 eyes with neovascular AMD (nAMD) and 27 eyes with polypoidal choroidal vasculopathy (PCV) were enrolled. In both groups, the mean BCVA significantly improved at the 12-month visit ($p < 0.05$). However, the significant improvement in BCVA disappeared at the 24-month visit, and the final mean BCVA was equivalent to that at baseline ($p = 0.17$ in the nAMD group and $p = 0.15$ in the PCV group). The median number of injections required after the loading dose was 15.0 during the 5-year follow-up (nAMD:15.0 vs. PCV:15). During the study period, 37 (92.5%) eyes required retreatment(s). Cox regression analysis demonstrated that the protective allele of *ARMS2* A69S was associated with a retreatment-free period from the initial injection ($p = 0.041$, repeated forward selection method). As-needed aflibercept monotherapy is a preferable treatment option for exudative AMD with good initial visual acuity regardless of nAMD or PCV during the 5-year study period.

Keywords: aflibercept monotherapy; polypoidal choroidal vasculopathy; neovascular age-related macular degeneration; good baseline visual acuity

1. Introduction

Age-related macular degeneration (AMD), one of the leading causes of legal blindness in advanced countries with older-aged populations [1], is a chronic inflammatory disease with a varied etiology [2]. The intravitreal injection of vascular endothelial growth factor (VEGF) inhibitors has greatly changed the treatment of exudative AMD. It was first reported that a monthly administration of ranibizumab improved the best corrected visual acuity (BCVA) in eyes with exudative AMD in the ANCHOR/MARINA [3,4]. However, a subsequent study, PIER, demonstrated that the quarterly administration of ranibizumab after three-monthly loading injections failed to improve BCVA in eyes with exudative AMD [5]. On the other hand, the PRONTO study demonstrated that monthly monitoring and as-needed reinjection after three monthly loading ranibizumab injections is an effective treatment option for improving BCVA [6]. Currently, anti-VEGF therapy has become the first-line treatment for exudative AMD worldwide; however, repeated injections after the loading phase are required for most eyes [7]. Therefore, the management of recurrence is essential for patients with AMD to maintain good vision for their lifetime.

Aflibercept is a fusion protein that has a stronger binding affinity to VEGF-A in comparison with ranibizumab and bevacizumab. The VIEW1/2 study demonstrated that

bimonthly aflibercept monotherapy after three monthly loading injections is comparable to monthly ranibizumab monotherapy for visual improvement [8]. Many studies have reported favorable outcomes of intravitreal aflibercept injections (IAI) for exudative AMD with various treatment protocols, including fixed interval dosing, as-needed regimen, and treat-and-extend (TAE) regimens [8–11]. However, the study period was ≤ 24 months in most studies. In large-scale randomized studies, the inclusion baseline best-corrected visual acuity (BCVA) is $\leq 20/40$ [3,4]; therefore, there have been few reports investigating the long-term visual outcome in patients secondary to exudative AMD with good baseline visual acuity.

In the present study, we investigated the 5-year visual and anatomic outcomes for patients secondary to exudative AMD with good BCVA ≥ 0.6, who were initially administrated three monthly aflibercept monotherapy followed by as-needed injection.

2. Methods

2.1. Participants

A retrospective medical chart review was performed in consecutive treatment-naïve eyes secondary to neovascular AMD (nAMD) or polypoidal choroidal vasculopathy (PCV) with baseline BCVA ≥ 0.6 in the decimal scale, receiving 3 monthly intravitreal aflibercept injections (IAIs) at the University of Yamanashi Hospital between January 2013 and July 2015. The present study was approved by the Institutional Review Board of the University of Yamanashi and was conducted as per the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients.

At baseline, all patients underwent comprehensive ophthalmic examinations, including the measurement of BCVA using Landolt chart, intraocular pressure, slit-lamp biomicroscopy with +78-diopter (D) lens, color fundus photography, fluorescein angiography (FA), indocyanine green angiography (ICGA) using a confocal laser scanning system (HRA-2; Heidelberg Engineering, Dossenheim, Germany), and spectral domain optical coherence tomography (SD-OCT) examination (Spectralis version 5.4 HRA + OCT).

We included patients secondary to exudative AMD, including PCV and nAMD. Eyes with nAMD show type 1 or type 2 neovascularization on SD-OCT and the absence of polypoidal lesions on ICGA. PCV shows a solitary or cluster of polypoidal lesions with or without branching vascular networks on ICGA [12]. Lesion size was defined as the greatest linear dimension (GLD). The GLD, which was defined as the fundus lesion covering the dye leak, pigment epithelial detachment, subretinal hemorrhage, and choroidal neovascularization, if present, was determined using the FA image. The central retinal thickness (CRT) was defined as the vertical distance between the inner border of the retinal pigment epithelium and the inner limiting membrane at the center of the macula in the SD-OCT image. The subfoveal choroidal thickness (SCT) was measured as the vertical distance between the outer border of the retinal pigment epithelium and the chorioscleral border, using the enhanced depth imaging mode equipped with HRA2 Spectralis ver 5.4.

The inclusion criteria were as follows: (1) eyes with treatment-naïve exudative AMD, including nAMD and PCV; (2) baseline decimal BCVA ≥ 0.6 in the Landolt chart; (3) receiving 3 monthly IAI (2.0 mg/0.05 mL); and (4) a follow-up period of 60 months after the initial IAI. The exclusion criteria were (1) previous treatment history for exudative AMD, including intravitreal injection of ranibizumab or photodynamic therapy; (2) eyes that had undergone vitrectomy; (3) eyes with retinal angiomatous proliferation; and (4) other macular abnormalities including myopic choroidal neovascularization (CNV), angioid streaks, and other secondary CNV. If both eyes were eligible, the second eye was included in this study.

2.2. Follow Up

All patients received IAIs (2.0 mg/0.05 mL) every 3 months, followed by monthly follow-up requiring SD-OCT examination, as well as routine ophthalmic examination. If residual or recurrent exudation was observed on SD-OCT, retreatment with a single

IAI was performed. All patients underwent monthly follow-up. When the eyes had not experienced lesion reactivation for more than 12 months, the follow-up interval was extended for a maximum of 2 months until recurrent exudation was observed. If massive subfoveal hemorrhage, dense vitreous hemorrhage, or cataract progression was observed, appropriate surgical procedures, including pars plana vitrectomy and cataract surgery, were performed. The patients who did not complete the 5-year follow-up period were excluded from this study.

2.3. Genotyping

We genotyped two major single nucleotide polymorphisms associated with AMD, namely rs800292 in the *CFH* gene, and rs10490924 in the *ARMS2* gene. Genomic DNA was purified using a PUREGENE DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) from the peripheral blood of the participants collected at the time of baseline FA/ICGA. Genotyping of the two genes was conducted using TaqMan genotyping assays with a 7300/7500 real-time polymerase chain reaction system (Applied Biosystems, Foster City, CA, USA) following the manufacturer's recommendations as previously described [13]. In detail, TaqMan genotyping assays contain sequence-specific primers to amplify the polymorphic sequence of the target genes, and two minor groove binders to stabilize the samples. Purified wet genomic DNA was mixed with a TaqMan genotyping assay and dispensed onto a reaction plate, and the genotyping with a real-time PCR system was performed. The allelic discriminatin plot was collected and analyzed by three researchers (W.K., Y.S., and S.Y.), and recorded on an anonymous basis.

2.4. Statistical Analysis

Statistical analyses were performed using the SPSS for Windows (SPSS, Tokyo, Japan). BCVA measured in the decimal scale with a Landolt chart was converted into a logarithm of the minimal angle resolution (log MAR). The differences between continuous and categorical variables were tested using the Mann–Whitney U test, the Kruskal–Wallis test, or the chi-square test. The paired *t*-test was used to determine the significance of the difference between values before and after treatments. Multivariate logistic regression analysis was performed to investigate the baseline risk factors for retreatment due to residual or recurrent exudation. Cox regression survival analysis was conducted to estimate the relative risk for retreatment. *p*-values less than 0.05 were considered statistically significant.

3. Results

3.1. Change in Log MAR BCVA

A total of 40 eyes of 40 patients were included in the study. The mean age was 71.8 ± 8.0, and 31 patients were male (77.5%). Table 1 shows the baseline characteristics of the patients. There were 13 patients with nAMD (nAMD group) and 27 with PCV (PCV group). There was no significant difference between the two groups except for the baseline BCVAand the risk allele frequency of *ARMS2* and *CFH*. The patients in the PCV group had better baseline BCVA (Mann–Whitney U test) and lower risk allele frequency for *ARMS2* A69S (rs10490924) and *CFH* I62V (rs800292, Chi-square test, Table 1). However, after adjustment for multiple testing by applying the Benjamini Hochberg method, these three variables were also insignificant.
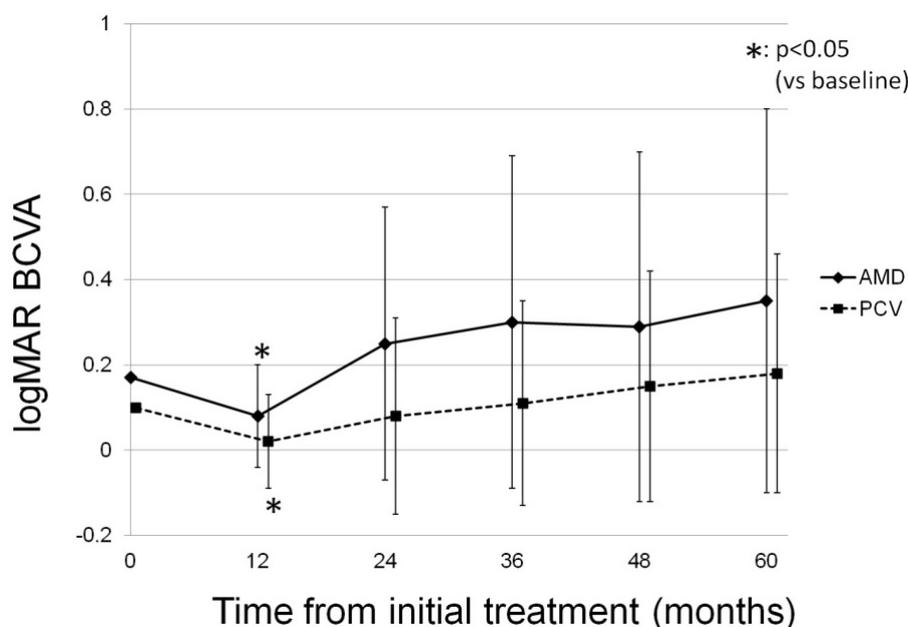
The change in mean BCVA of the patients in each group during the follow-up period is presented in Figure 1. Although the mean BCVA in each group significantly improved at 12 months from the baseline ($p < 0.05$), the significance disappeared at 24 months, and the final mean BCVA was equivalent to that at baseline ($p = 0.17$ in the nAMD group and $p = 0.15$ in the PCV group). To evaluate the reason for the decrease in BCVA improvement after month 12, we reviewed the SD-OCT images of the patients with a final BCVA deterioration of 0.3 logMAR or worse. The result revealed that there were 7(17.5%) patients with severe BCVA deterioration due to various macular or other ocular pathologies, including macular atrophy in two eyes, not achieving dry macula in two eyes, subfoveal hemorrhage

from PCV in one eye, cataract in one eye, and macular edema secondary to central retinal vein occlusion in one eye.

**Table 1.** Baseline characteristics of the subject.

	All Subjects $n = 40$	nAMD $n = 13$	PCV $n = 27$	*p*-Value (nAMD vs. PCV)
Age	71.8 ± 8.0	74.0 ± 9.3	70.8 ± 7.2	0.38
Gender male	31(77.5%)	11(84.6%)	20(74.1%)	0.45
Baseline BCVA	0.12 ± 0.09	0.17 ± 0.07	0.10 ± 0.09	0.02
Baseline CRT	377 ± 155	448 ± 231	343 ± 88	0.28
Baseline SCT	226 ± 98	185 ± 56	246 ± 108	0.10
Baseline GLD	2767 ± 1385	3049 ± 1554	2631 ± 1306	0.41
ARMS2 A69S (rs10490924)				
GG:TG:TT (T allele frequency)	12:10:18 (57.5%)	1:4:8 (76.9%)	11:6:10 (48.1%)	0.015
CFH I62V (rs800292)				
AA:GA:GG (G allele frequency)	3:15:22 (73.8%)	0:3:10 (88.5%)	3:12:12 (66.7%)	0.038

nAMD: neovascular age-related macular degeneration, PCV: polypoidal choroidal vasculopathy.

Figure 1. The change in mean best-corrected visual acuity of the participants in each group during the follow-up period. In the neovascular age-related macular degeneration group, the mean BCVA was 0.17 ± 0.07 at baseline, and 0.08 ± 0.12, 0.25 ± 0.32, 0.30 ± 0.39, 0.29 ± 0.41, and 0.35 ± 0.45 at 12 months, 24 months, 36 months, 48 months, and 60 months, respectively (*p* = 0.03, 0.39, 0.25, 0.31, 0.17, respectively). In the polypoidal choroidal vasculopathy group, the mean BCVA was 0.10 ± 0.09 at baseline, and 0.02 ± 0.11, 0.08 ± 0.23, 0.11 ± 0.24, 0.15 ± 0.27, and 0.18 ± 0.28 at 12 months, 24 months, 36 months, 48 months, and 60 months, respectively (*p* = 1.5×10^{-3}, 0.82, 0.80, 0.24, 0.15, respectively).

Figure 2 presents a representative case with severe vision. The patient required 32 IAIs after initial three monthly injections during a 5-year follow-up period. Though her initial BCVA was 0.8 on the decimal scale, macular atrophy had developed since month 48, resulting in a deteriorated BCVA of 0.1 at month 60.
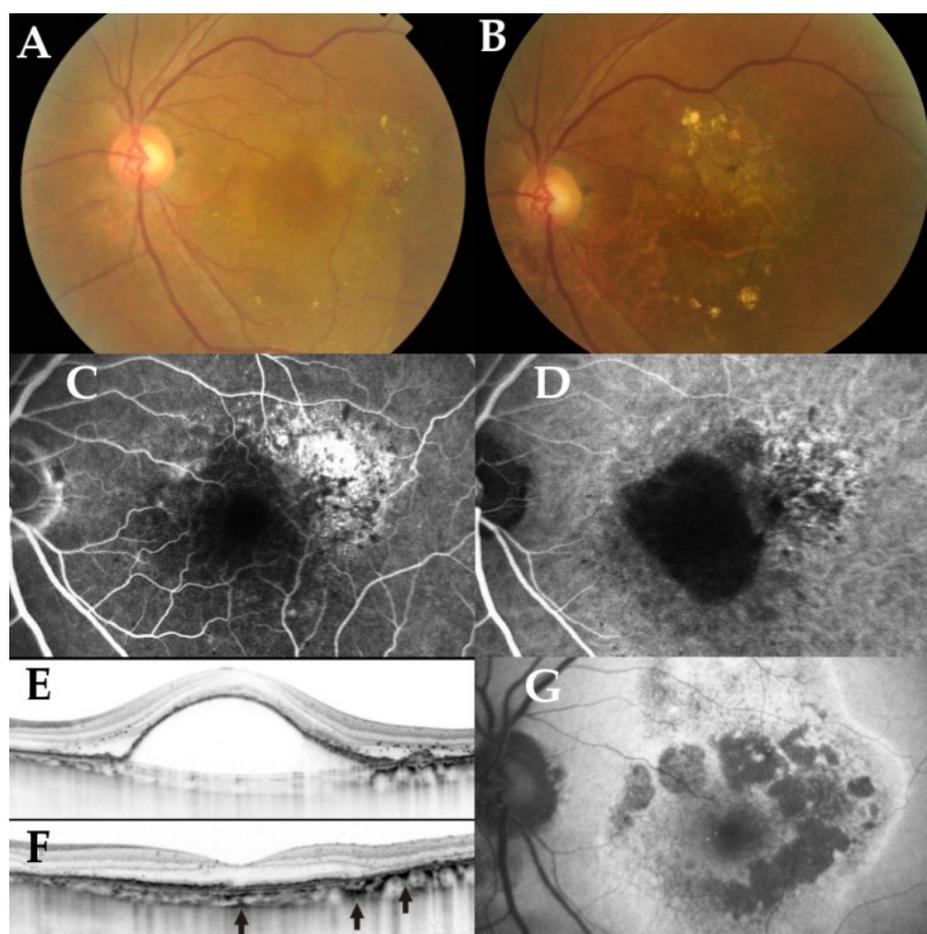
Figure 2. A representative case of a 69-year-old female with type 1 neovascularization in the left eye. (**A**) Color fundus photography in the left eye at baseline reveals serous retinal detachment (SRD) and pigmental epithelial detachment (PED) in the macula. (**B**) Color fundus photography in the left eye at 60 months from baseline reveals absorption of SRD and retinal pigment epithelium (RPE) atrophy around the fovea. (**C**) Fluorescein angiography (FA) of the left eye at baseline demonstrates window defect at the temporal of the fovea. (**D**) Indocyanine green angiography demonstrates PED including fovea as a hypofluorescent lesion. (**E**) Spectral domain optical coherence tomography (SD-OCT) of fovea in the left eye at baseline reveals large PED with double-layer sign and SRD. (**F**) SD-OCT of fovea in the left eye at 60 months from baseline reveals complete resolution of PED and SRD; however, choroidal hypertransmission due to subfoveal RPE atrophy is apparent (black arrows). (**G**) Fundus autofluorescense of the left eye at 60 months from baseline reveals RPE atrophy as hypoautofluorescent areas around the fovea.

To investigate the factors associated with BCVA change at 60 months, we conducted a multivariate regression analysis as a multiple comparison (Table 2). The results showed that female sex was associated with good BCVA improvement at 60 months. During the follow-up period, four patients underwent cataract surgery. However, the history of cataract surgery was not associated with BCVA improvement at 60 months (Table 2). None of the eyes required vitrectomy.

3.2. Change in OCT Parameters

During the follow-up period, the mean CRT of all participants significantly decreased from 376.7 ± 155.1 μm at baseline to 237.1 ± 62.7 μm at 60 months ($p < 0.05$). A significant reduction in CRT was also observed in both the nAMD and PCV groups ($p < 0.05$). Figure 3 illustrates the change in mean CRT in each group.

Table 2. Multivariate linear regression analysis of factors associated with mean BCVA change at 60 months.

Variables	β-Coefficient	*p*-Value
Age	0.01	0.25
Male gender	−0.36	0.023
Baseline log MAR BCVA	−0.23	0.76
Greatest linear dimension	$−2.8 \times 10^{-6}$	0.95
Central retinal thickness	4.1×10^{-4}	0.41
Subfoveal choroidal thickness	$−4.5 \times 10^{-4}$	0.50
Subtype (nAMD = 0, PCV = 1)	0.02	0.91
ARMS2 A69S T allele	2.0×10^{-3}	0.98
CFH I62V G allele	0.14	0.16
Cataract surgery	0.046	0.82
Total number of IAIs	5.6×10^{-4}	0.90

ARMS2: age-related maculopathy susceptibility; BCVA: best-corrected visual acuity; *CFH*: complement factor H; IAI: intravitreal aflibercept injection; logMAR: logarithm of the minimal angle resolution.
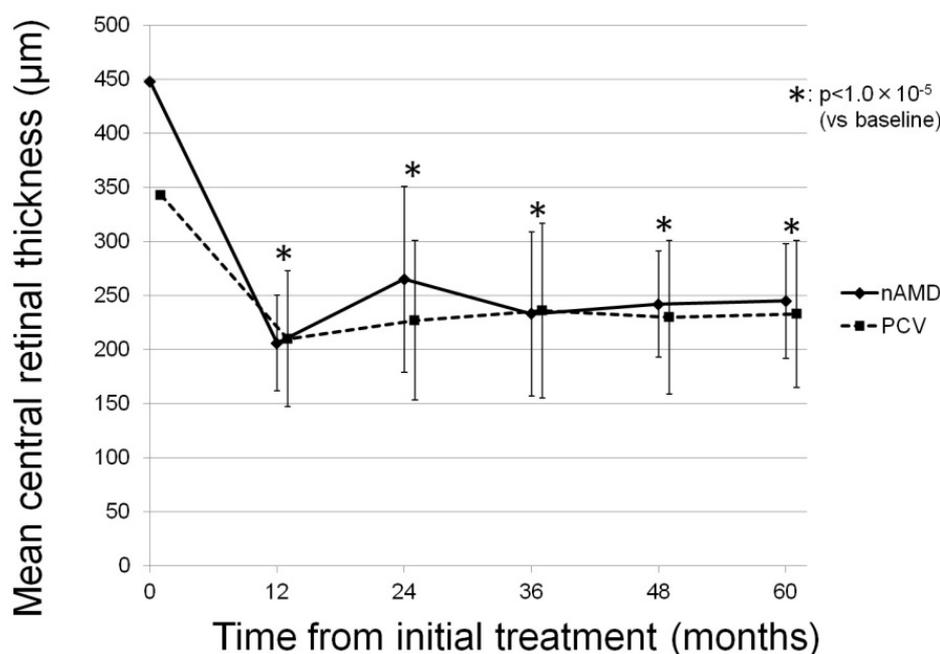
Figure 3. The change in mean central retinal thickness in each group during the follow-up period. Mean central retinal thickness (CRT) shows significant decreases from 448 ± 231 μm at baseline to 206 ± 44 μm at 12 months, 265 ± 86 μm at 24 months, 233 ± 76 μm at 36 months, 242 ± 49 μm at 48 months, and 245 ± 53 μm at 60 months ($p = 2.5 \times 10^{-3}$, 9.1×10^{-3}, 2.4×10^{-3}, 6.9×10^{-3}, 3.1×10^{-3}, respectively) in the neovascular age-related macular degeneration group, and mean CRT shows a significant decrease from 343 ± 88 μm at baseline to 210 ± 63 μm at 12 months, 227 ± 74 μm at 24 months, 236 ± 81 μm at 36 months, 230 ± 71 μm at 48 months, and 233 ± 68 μm at 60 months ($p = 1.0 \times 10^{-5}$, 1.0×10^{-5}, 4.0×10^{-4}, 1.0×10^{-5}, 1.0×10^{-5}, respectively) in the polypoidal choroidal vasculopathy group.

Similar to CRT change, the mean SCT of all participants significantly decreased from 226.0 ± 97.8 μm at baseline to 198.8 ± 80.0 μm at 60 months ($p < 0.05$). In the nAMD group, the mean SCT significantly decreased at 12 months from baseline ($p < 0.05$); however, the final mean SCT reduction was not significant at 60 months ($p = 0.37$). In contrast, in the PCV group, a significant decrease in mean SCT was maintained throughout the follow-up period ($p < 0.05$). Figure 4 shows the changes in the mean SCT in each group.
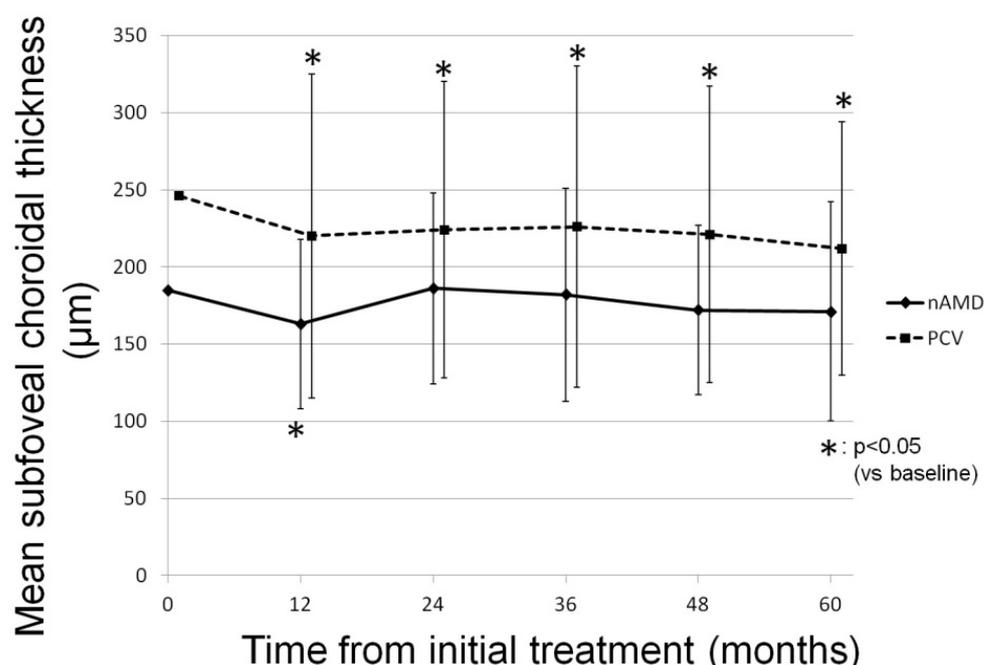
Figure 4. The change in mean subfoveal choroidal thickness in each group during the follow-up period. In the neovascular age-related macular degeneration group, mean subfoveal choroidal thickness (SCT) was 185 ± 56 μm at baseline, and 163 ± 55 μm, 186 ± 62 μm, 182 ± 69 μm, 172 ± 55 μm, and 171 ± 71 μm at 12 months, 24 months, 36 months, 48 months, and 60 months, respectively ($p = 0.013$, 0.95, 0.82, 0.28, and 0.37, respectively). In the polypoidal choroidal vasculopathy group, mean SCT showed a significant decrease from 246 ± 108 μm at baseline to 220 ± 105 μm at 12 months, 224 ± 96 μm at 24 months, 226 ± 104 μm at 36 months, 221 ± 96 μm at 48 months, and 212 ± 82 μm at 60 months ($p = 6.0 \times 10^{-5}$, 2.9×10^{-4}, 3.3×10^{-3}, 1.8×10^{-3}, 1.9×10^{-3}, respectively).

3.3. Retreatment

Among the 40 eyes, 37 (92.5%) needed IAI(s) as retreatment after the loading dose during the 60-month follow-up period. Figure 5 shows the distribution of patients according to the number of total injections required after the loading dose. Table 3 shows the mean number of total injections required after the loading dose and the distribution of mean number of injections year-by-year. There is no significant difference in the mean number of IAIs between the two groups (21.2 ± 14.2 in the neovascular AMD group and 17.1 ± 13.9 in the PCV group, $p = 0.36$). We conducted a multivariate linear regression analysis to investigate the factors associated with the number of total injections required after the loading dose as a multiple comparison. As shown in Table 4, the risk allele of *ARMS2* A69S (rs10490924) was the only factor associated with the number of total injections.

To investigate the factors associated with retreatment, a Cox regression survival analysis was conducted. The results showed that the protective allele (G allele) of *ARMS2* A69S (rs10490924) was the only factor associated with a retreatment-free period from the initial injection (β-coefficient = 0.42, $p = 0.041$, repeated forward selection method). Other variables including age, sex, baseline BCVA, baseline CRT, baseline SCT, or allele of *CFH* I62V (rs800292) were not associated with a retreatment-free period. Figure 6 shows the Kaplan–Meier curve for a retreatment-free proportion from the initial injection depending on disease subtypes, *ARMS2* A69S, and *CFH* I62V genotypes.
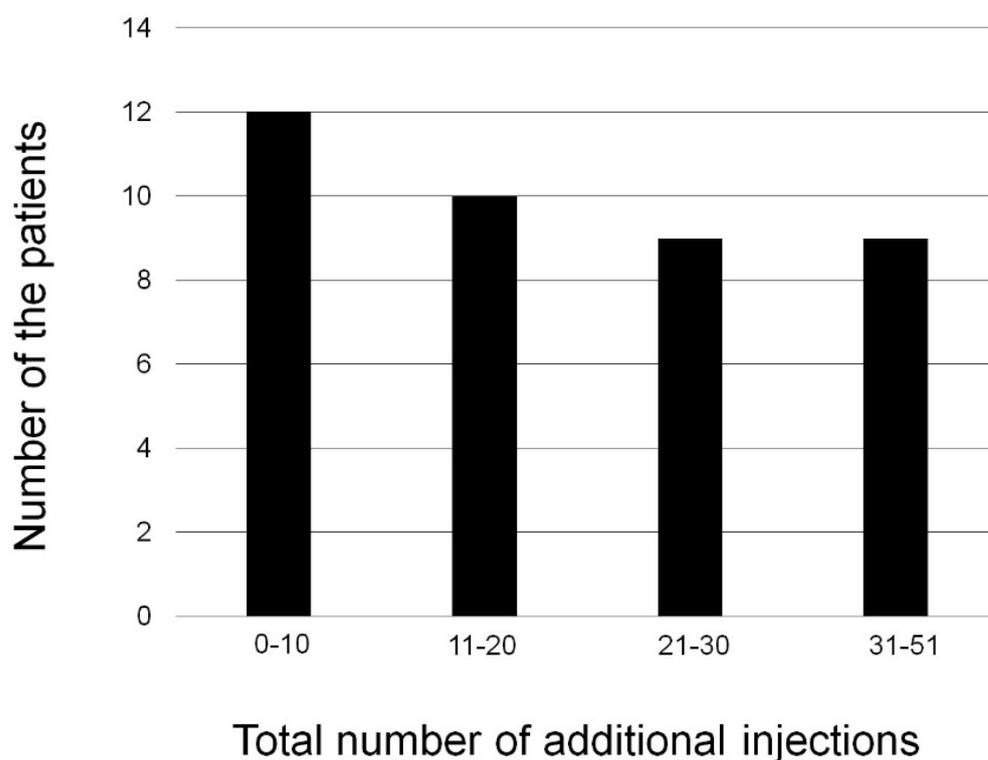
Figure 5. The distribution of patients according to the number of total injections required after the loading dose. In total, 12 (30%) patients were administered 0–10 injections, 10 (25%) patients 11–20 injections, 9 (22.5%) patients 21–30 injections, and 9 (22.5%) patients were administered 35–51 injections during the follow-up period. The median and mean numbers of intravitreal aflibercept injections required after the loading dose in all patients are 15.0 and 18.4 ± 14.0.

Table 3. Mean number of total injections required after the loading dose and the distribution of the mean number of injections year-by-year.

Total (0–60 Months)	1st Year (0–12 Months)	2nd Year (13–24 Months)	3rd Year (25–36 Months)	4th Year (37–48 Months)	5th Year (49–60 Months)
18.4 ± 14.0	2.4 ± 2.5	3.8 ± 3.1	3.7 ± 3.4	3.8 ± 3.4	4.4 ± 3.9

Table 4. Multivariate linear regression analysis of factors associated with number of total injections.

Variables	β-Coefficient	*p*-Value
Age	0.44	0.23
Male gender	−5.61	0.36
Baseline log MAR BCVA	39.3	0.18
Greatest linear dimension	-2.0×10^{-3}	0.32
Central retinal thickness	0.023	0.22
Subfoveal choroidal thickness	−0.029	0.27
Subtype (nAMD = 0, PCV = 1)	7.09	0.27
ARMS2 A69S T allele	6.63	0.025
CFH I62V G allele	0.87	0.83

ARMS2: age-related maculopathy susceptibility; BCVA: best-corrected visual acuity; *CFH*: complement factor H; logMAR: logarithm of the minimal angle resolution.
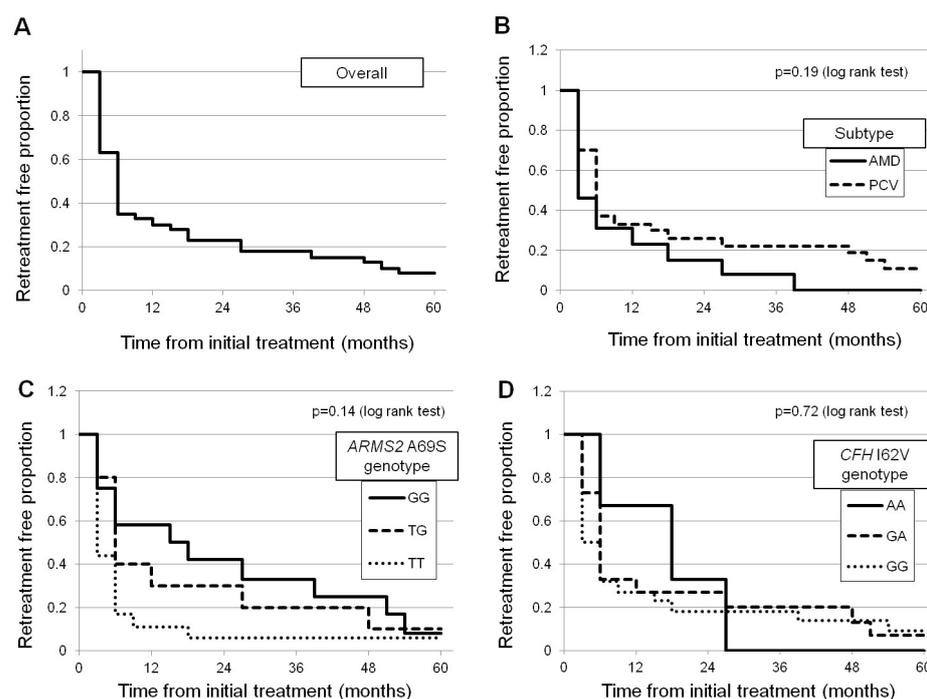
Figure 6. Kaplan–Meier curve regarding retreatment-free proportion from the initial injection. (**A**) The mean retreatment-free period after the initial intravitreal aflibercept injections (IAI) is 16.1 ± 18.7 months, and the retreatment-free proportion at 60 months is 7.5% in all patients. (**B**) The mean retreatment-free period is 11.2 ± 11.5 months and 18.5 ± 21.1 months in the nAMD and PCV groups, respectively. The retreatment-free proportion at 60 months is 0% and 11% in the nAMD group and the PCV group, respectively (*p* = 0.19, log-rank test). (**C**) Mean retreatment-free period is 24.6 ± 18.8 months, 18.8 ± 20.1 months, and 9.0 ± 13.2 months in patients with GG, TG, and TT genotypes of *ARMS2* A69S, respectively. The retreatment-free proportion at 60 months is 8%, 10%, and 6% in patients with GG, TG, and TT genotypes of *ARMS2* A69S, respectively (*p* = 0.14, log-rank test). (**D**) The mean retreatment-free period is 17.7 ± 10.7 months, 17.4 ± 19.6 months, and 15.0 ± 19.5 months in patients with AA, AG, and GG genotype of CFH I62V, respectively. The retreatment-free proportion at 60 months is 0%, 7%, and 9% in patients with AA, AG, and GG genotypes of CFH I62V, respectively (*p* = 0.72, log-rank test).

4. Discussion

To the best of our knowledge, this is the first study investigating the long-term treatment outcome of as-needed IAIs for exudative AMD in patients with relatively good initial visual acuity. Our study was a retrospective cohort study; therefore, we consider it as valuable as real-world data.

In the literature, there have been several studies evaluating the long-term efficacy and safety of anti-VEGF agents, including aflibercept, for exudative AMD. Khanani et al. reported on the five-year outcome of an intravitreal anti-VEGF agent treatment using a TAE regimen for nAMD in eyes with baseline vision 20/60 or better. They described that more than 75% of the patients could maintain their baseline VA, and the mean number of injections during years 1–5 was 7.9, 5.9, 5.6, 5.9, and 6.0, respectively [14]. Javidi et al. reported the treatment outcome of a similar intravitreal drug therapy for nAMD using a TAE regimen for up to 7 years [15]. The results were also preferable; the mean BCVA was maintained with a mean number of injections of 7.6 during the first three years, and 5.9 during years 4–7. The difference between our study and these studies is that we selected IAI only for the intravitreal treatment with an as-needed regimen, whereas they had chosen not only aflibercept but also ranibizumab or bevacizumab using the TAE regimen. As a result, the patients in our study achieved similar visual outcomes with relatively fewer additional injections than those in their studies.

Maguire et al. reported the long-term treatment outcomes of anti-VEGF agents, including bevacizumab and ranibizumab, for treating nAMD in an extended comparison of AMD treatments trial study [16]. They reported that the mean change in visual acuity at 5 years from baseline was −3 letters. In the present study, the mean BCVA changed from 0.12 ± 0.09 at baseline to 0.23 ± 0.35 at 60 months, and the BCVA change was equivalent to −6 letters on the early treatment diabetic retinopathy study chart. Given that the initial visual acuity in the present study was equal to or better than the decimal BCVA of 0.6, we consider that the as-needed aflibercept therapy was an effective treatment option for exudative AMD, even in patients with good initial BCVA. However, in the present study, a significant improvement in mean BCVA was apparent at 12 months in both the nAMD and PCV groups; thereafter, the mean BCVA returned to the baseline values and was maintained throughout the follow-up period. We consider that the discrepancy between the insignificant BCVA improvement and the significant reduction in CRT or SCT at month 60 was due to the patients with macular or other pathologies, which were independent of CRT of SCT. In the present study, 7 of 40 patients showed BCVA deterioration of 0.3 logMAR or worse due to these conditions. Even if these patients with severe BCVA deterioration were excluded from the analysis, it did not reach a statistical significance between baseline BCVA and BCVA at 60 months.

During a 60-month follow-up period, 37 (92.5%) out of 40 eyes needed a minimum of one retreatment. Cox regression analysis revealed that the protective allele of *ARMS2* A69S was associated with a retreatment-free period from the initial injection. In a previous study, we investigated the short-term prognostic factors associated with retreatment among patients who received an initial 3-month IAI for exudative AMD [17]. Cox regression analysis revealed that the risk allele of the *ARMS2* A69S gene and older age were associated with a shorter retreatment and reactivation time from the initial injection. In the present study, the positive association between the protective allele of *ARMS2* A69S and the retreatment-free period from initial injection was also confirmed even in patients with good baseline BCVA. Recently, we reported that the protective allele of *ARMS2* A69S was associated with a retreatment-free period in patients with PCV who received the combination therapy involving photodynamic therapy (PDT) and intravitreal injection of anti-VEGF agents [18,19]. The differences between the present study and the previous study are the better initial mean BCVA, including the patients with nAMD, and excluding PDT as a treatment modality in the present study. Consequently, there are several differences in the results between the studies. The proportion of the patients who had not required any retreatment was greater in the previous study than in the present study (27.9% vs. 7.5%), and the total number of retreatments during a 5-year follow-up period in the previous study was relatively smaller than that in the present study (7.51 ± 7.25 injections and 0.51 ± 1.01 combination therapies vs. 18.4 ± 14.0 injections). These results might indicate the good potential of PDT in terms of disease stability against PCV. On another front, the preferable results in the present study might shed light on the choice of treatment modality for PCV patients with initial BCVA ≥ 0.8 in the decimal scale, which had not been included in the previous studies. Moreover, from the previous and present results, it might be inferred that the risk allele of *ARMS2* A69S might be associated with lesion reactivation after treatment, regardless of the treatment modality.

There are several limitations to the present study. The first limitation is the retrospective nature of the analysis. The second limitation is the relatively small sample size. The third limitation is the wide fluctuations in the data. The standard deviations of mean BCVA, CRT, and SCT presented in Figures 1–3 were high, and the significant P values presented in the tables were slightly below the threshold of $p < 0.05$. These limitations might lead to some uncertainties about the interpretation of the findings. Further prospective analysis of the long-term visual and anatomical outcomes of aflibercept for exudative AMD with a large sample size is necessary.

In conclusion, IAI is a preferable treatment option for exudative AMD for prolonged disease stabilization, even in patients with good initial visual acuity. The risk allele of the *ARMS2* A69S gene is associated with earlier retreatment after the initial three monthly IAIs.

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