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Real-life retrospective head-to-head comparison of the effect of bevacizumab, ranibizumab, and aflibercept on the visual acuity, intraocular pressure, central retinal thickness, total macular volume and retinal nerve fiber layer thickness, in the treatment of age-related macular degeneration

Inaugural-Dissertation zur Erlangung der Doktorwürde
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Widmung

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Abbreviations

AFB	Aflibercept
AMD	Age-related macular degeneration
AREDS	Age-Related Eye Disease Study
ARMS2	Age-Related Maculopathy Susceptibility 2
BCVA	Best corrected visual acuity
BMI	Body mass index
BrM	Bruch's membrane
BVZ	Bevacizumab
C-	Complement factor -
CFH	Complement factor H
CNV	Choroidal neovascularization
CRT	Central retinal thickness
CS	Complement system
CSF	central subfield thickness = CRT
Dpt	Diopters
ETDRS	Early treatment diabetic retinopathy study
FA	Fluorescein Angiography
GA	Geographic atrophy
IOP	Intraocular pressure
LogMAR	Logarithm of minimum angle of resolution
MAR	Minimum angle of resolution
n	Number of
NCT	Non-contact tonometer
OCT	Optical coherence tomography
ONH	Optic nerve head
PED	Pigment-epithelium detachment
PEDF	Pigment epithelium-derived factor
PRN	Pro re nata
RBZ	Ranibizumab
RNFLT	Retinal nerve fiber layer thickness
RPE	Retinal pigment epithelium
ROS	Reactive oxygen species
SD	Standard deviation
SD-OCT	Spectral domain-optical coherence tomography
TD-OCT	Time domain-optical coherence tomography
TnE	Treat and extend regimen
TMV	Total macular volume
VA	Visual Acuity
VEGF	Vascular endothelial growth factor
VEGFR	VEGF-receptor
WHO	World Health Organization

1. Zusammenfassung

Die altersbedingte Makuladegeneration (AMD), eine der Hauptursachen für Blindheit, weist viele Risikofaktoren auf, wobei das Alter der wichtigste ist. Abhängig von der Form der AMD wurden verschiedene Behandlungsansätze entwickelt. Einer dieser Ansätze ist die Verwendung von Anti-VEGF-Mitteln bei der Behandlung der exsudativen Form von AMD.

Dies ist eine retrospektive Studie mit dem Ziel, die Wirkung der drei verschiedenen Mittel Bevacizumab, Ranibizumab und Aflibercept, gegen den vaskulären endothelialen Wachstumsfaktor (Anti-VEGF) bei der Behandlung der exsudativen altersbedingten Makuladegeneration (AMD) im Bezug auf die Anzahl der Injektionen zu untersuchen und Head-to-Head zu vergleichen. Die untersuchten Messparameter sind: Netzhautnervenfaserschichtdicke, zentrale Netzhautdicke, Gesamtmakulavolumen, Sehschärfe und Augeninnendruck.

Die Stichprobe bestand aus 120 AMD-Patienten (80 Frauen, 40 Männer) mit der exsudativen Form, die mit mindestens 6 Injektionen entweder nach *pro re nata*- oder Treat and Extend Regimen behandelt wurden.

Die Ergebnisse zeigten, dass sich die Sehschärfe in den meisten Untergruppen leicht verbesserte, wobei einige Aflibercept-Untergruppen eine signifikante Verbesserung der Sehschärfe zeigten. Der Augeninnendruck zeigte nach der Behandlung mit den drei Anti-VEGF-Mitteln keine signifikante Veränderung. Es wurde eine signifikante Abnahme des Makulaödems festgestellt, die durch die signifikante Reduktion sowohl der CRT als auch der TMV deutlich wurde. Bevacizumab zeigte eine höhere Reduktion der CRT, gefolgt von Aflibercept und Ranibizumab, während Aflibercept die größte Reduktion des TMV zeigte, gefolgt von Bevacizumab und Ranibizumab. Die RNFLT änderte sich während der Behandlung nicht signifikant, mit Ausnahme einer Ranibizumab-Untergruppe, die nach der 3. Injektion eine signifikante Abnahme der RNFLT zeigte.

2. Summary

Age-related macular degeneration (AMD), a leading cause of blindness, has many risk factors with age being the most important one. Several treatment approaches have been developed, depending on the form of AMD. One of these approaches is the use of anti-VEGF agents in the treatment of the exudative form of AMD.

This is a retrospective study with the aim of investigating and comparing the effect of three different anti–vascular endothelial growth factor (anti-VEGF) agents, bevacizumab, ranibizumab and aflibercept in the treatment of exudative age-related macular degeneration (AMD). The studied factors are: retinal nerve fiber layer thickness, central retinal thickness, total macular volume, visual acuity, and intraocular pressure.

The study sample consisted of 120 AMD patients (80 females, 40 males), who were treated with anti-VEGF following either pro re nata or treat and extend regimens.
The results showed that the visual acuity slightly improved in most of the subgroups, with some aflibercept subgroups showing significant improvement in the visual acuity. The intraocular pressure showed no significant change after the treatment with the three anti-VEGF agents. A significant decrease in the macular edema, which was evident by the significant reduction in both CRT and TMV, was noticed. Bevacizumab showed higher reduction in CRT followed by aflibercept then ranibizumab, while aflibercept showed the greatest reduction in the TMV followed by bevacizumab then ranibizumab. The RNFLT did not change significantly during the treatment except for one ranibizumab subgroup which showed a significant decrease in RNFLT after the 3rd injection.

3. Age-related Macular Degeneration (AMD)

3.1 Introduction

Age-related macular degeneration (AMD) is considered one of the main causes of blindness worldwide, especially affecting the developed countries. It is an ophthalmological degenerative disease affecting the aging macula of the human retina, thus triggering progressive deterioration of patients' central vision, Fig.1 (Al-Zamil and Yassin, 2017). Early-stages of AMD are identified by several clinical signs, involving drusen formation and retinal pigment epithelium (RPE) abnormalities, while late-stages of AMD are classified into a neovascular (exudative or wet) or a non-neovascular (dry, atrophic, non-exudative) forms (Bhutto and Lutty, 2012; Damico *et al.*, 2012).

The majority of AMD patients are diagnosed with the dry form of AMD. The less prevalent wet form of AMD accounts normally for most cases of severe blindness, which has a foremost effect on patients' quality of life as well as their functional self-sufficiency (Little *et al.*, 2018). Due to an increasing prevalence of AMD, which is expected to double by 2050, a growing demand for new studies and novel therapies emerged. (Wright *et al.*, 2020). Taylor *et al.* (2016), mentioned that AMD negatively affects patients' visual perception, mobility, facial recognition, driving, reading, the use of electronic devices, and most importantly sufficient self-care. Patients suffering from AMD, especially those debilitated by the disease, were found to have a higher risk of developing depression.

Based on the World Health Organization (WHO) report on vision, AMD was found to be the third leading cause of vision-related impairment worldwide, directly after cataract and uncorrected refractive errors. The estimated number of people suffering from AMD will increase from 195.6 million in 2020 to 243.4 million people in 2030 (WHO, 2019). Although AMD affects Caucasians more, an increased prevalence of AMD in Asian countries has been noticed, which is mainly attributed to the change in diet, lifestyle westernization, in addition to demographic alteration (Velez-Montoya *et al.*, 2014). In the meta-analysis of Kawasaki *et al.*, (2010), the estimated prevalence of AMD among people aged between 40-79 years in four different Asian countries was 6.8% for early AMD and 0.56% for late-stage AMD. Jonas *et al.* , (2018), reported that the prevalence of early AMD is 1.4%, 0.20% for late AMD, and 0.10% for wet AMD among individuals aged between 55-85 years in China. In Saudi Arabia, AMD was found to account for 3.3% of blindness in those older than 50 years. (Hajar *et al.*, 2015).

A previous analysis by Smith *et al.*, (2001), of Caucasians living in Europe, the United States, and Australia, stated that the prevalence of AMD among individuals aged < 75 years is < 1%,

increasing to 4.6% among individuals aged 75–84 years and 13% among those older than 85 years. AMD was found to affect white European populations (12.3%) much more than those of Asian (7.4%) or African (7.5%) decent. Based on a global meta-analysis, a significant difference in AMD prevalence was not observed between Asians and Africans (Wong *et al.*, 2014). Consequently, AMD is the main cause of blindness in Europe with a 14% prevalence (Flaxman *et al.*, 2017). The prevalence of early-stage AMD in Germany is 11.9%, while the late-stage lies at around 0.2% (Brandl *et al.*, 2016). In Australia, Joachim *et al.*, (2015) reported that the 15-year incidence among individuals older than 40 years for early-stage AMD is 22.7% and 6.8% for late-stage disease. The estimated number of AMD patients in the United States in 2010 was 9.1 million, with a two-fold increase being expected in 2050 (Klein and Klein, 2009).

On the brighter side, Colijn *et al.*, (2017) noted a decrease in the prevalence, especially that of advanced, vision-threatening AMD in Europe. This was attributed to the adoption of a healthier lifestyle, as well as the increased use of the vision-preserving anti-vascular endothelial growth factor (anti-VEGF) agents as a treatment modality for the exudative AMD form. These agents have an effect of more than 90% in stabilizing or even improving vision after a treatment period of almost two years (Wong *et al.*, 2014). Anti-VEGF agents have the same pharmacological targets, but since their structures differ, various mechanism of actions and different pharmacokinetics arise, thus affecting their risk to benefit ratio (Platania *et al.*, 2015; Andrés-Guerrero *et al.*, 2017). Since the pathogenesis of early AMD remains unclear, prevention of AMD or the development of treatment approaches for the early stages of the disease, is unfortunately still not possible. Moreover, patients with the late geographic atrophy form still have no available therapy to help preserve their vision (Whitmore *et al.*, 2015).

New advances in diagnostic imaging techniques, genetic studies, and empirical findings in several studies, provided a new and better understanding of AMD natural history (Holz *et al.*, 2017; de Oliveira Dias *et al.*, 2018). Despite of all the above-mentioned advancements and the breakthrough findings of important cellular events in terms of AMD pathogenesis and progression (Toomey *et al.*, 2018; Fisher and Ferrington, 2018), further data is still needed to better understand, and eventually, better apply, effective therapies for the late stages of AMD. This is particularly true for the dry form, for which still no treatment exists, as well as the wet form, in order to reduce the frequency of anti-VEGF injections needed, or help in the development of more potent treatment forms.

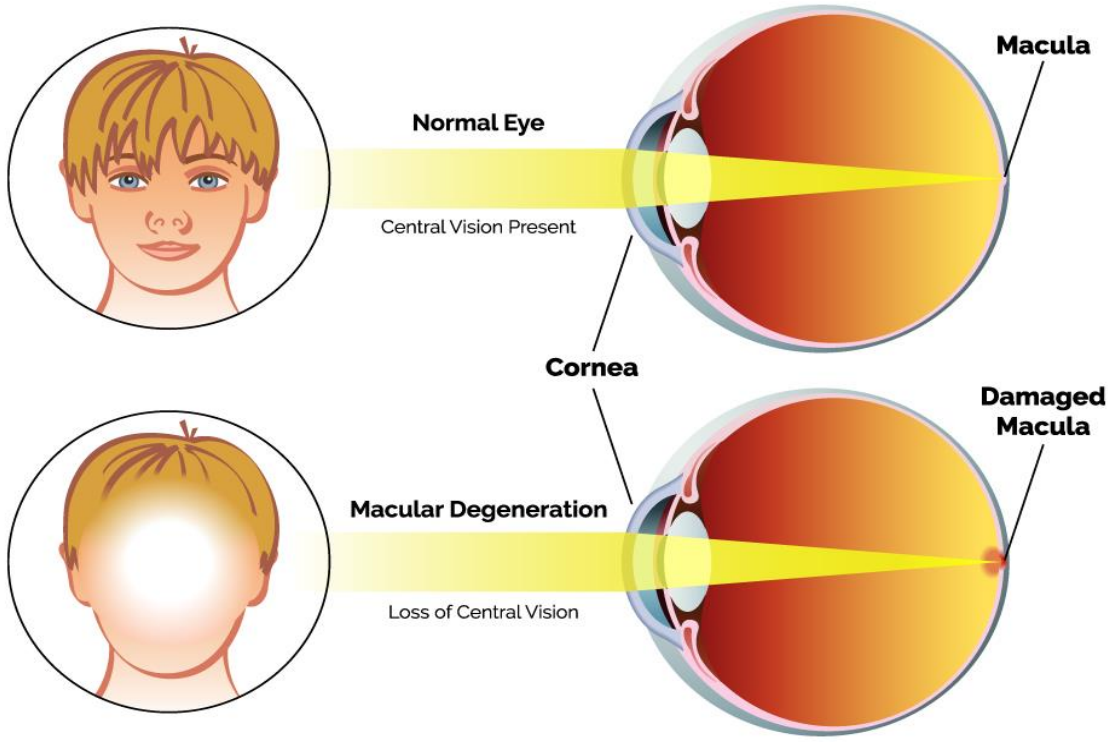


Fig.1: Loss of central vision in AMD (American Academy of Opthalmology, 2016)

3.2 Genetics and Pathobiology of AMD

The pathophysiological mechanisms behind the development of AMD were until recently poorly understood. Based upon the data from over-thirty years ongoing studies, reliable evidence verifies that the AMD pathogenesis is a multi-factorial and complex interplay between genetic, functional, metabolic and environmental-related factors (Kijlstra and Berendschot, 2015).

A considerable amount of studies has clearly shown that the family history plays a vital role in the acquisition of AMD. Kumaramanickavel, (2016) studied the effect of genetic factors and its link to AMD. The results revealed that, although some genes are related to the disease, their effect was not always significant to the acquisition or the progression of AMD. These studies provided further evidence that AMD is a complex multifactorial disease, which involves several molecular and cellular mechanisms, as well as numerous other risk factors. Recognized molecular mechanisms commonly attributed to the pathophysiology of AMD include the following: oxidative stress, dysregulated antioxidant processes, impaired lipid metabolism, angiogenesis, and inflammation-mediating mechanisms (van Leeuwen *et al.*, 2018; Kauppinen *et al.*, 2016; Jarrett and Boulton, 2012).

AMD affects mainly the central section of the retina, known as the macula, which is responsible for central vision (Mitchell et al., 2018). Histopathological examination of eyes affected with AMD provided evidence, that this degenerative disease is characterized by local destruction of the macular region of the retina. Macular related changes typically found in AMD include the loss of the retinal pigment epithelium (RPE), photoreceptors and choriocapillaris, as well as the accumulation of lipids and protein deposits below the RPE or Bruch's membrane (BrM), scarring, and the development of choroidal neovascularization (CNV) (Bhutto and Lutty, 2012; Damico et al., 2012). Furthermore, inflammatory responses in the form of the involvement of microglia, macrophages as well as the activation of the complement system, are also associated with AMD (Natoli *et al.*, 2017).

What until now is known about AMD is largely based upon empirical studies that reported RPE as the main site of injury (Mettu *et al.*, 2012). The RPE plays a key role in balancing retinal homeostasis, which is achieved by regulating nutrients and metabolites transportation, light absorption, phagocytosis of photoreceptor outer segments after shedding, as well as retinal visual pigment recycling for continuous phototransduction. Consequently, impairment of the RPE will result in retinal degeneration (Saito *et al.*, 2013). Several molecular events are commonly associated with RPE deterioration in AMD patients. These include the age-related lipofuscin accumulation that results from poor phagocytosis of photoreceptor outer segments (Celkova, Doyle and Campbell, 2015). The built up of Lipofuscin beneath the RPE leads to an increase in the oxidative damage caused by an increase in the amount of free radicals generated, in addition to the further inhibition of the phagocytic degradation of damaged biomolecules. (Olchawa *et al.*, 2017).

Besides RPE pathological injury, extracellular deposits, such as basal lamina deposits and drusen, are usually present in AMD. BrM associated drusen and basal deposits are the key factors in AMD development (Curcio, 2018). The usual thinning of the choriocapillaris in retinas with AMD leads to a decrease in the removal of extracellular material, thus to an increase in the formation of drusens (Biesemeier *et al.*, 2014). This is usually proceeded by thickening of the Bruch's membrane collagenous layers, degeneration of elastin and collagen fibers, as well as calcification of the BrM. (Leuschen *et al.*, 2013). The subretinal deposits usually consist of protein or lipids, which are either formed by inadequate RPE metabolism and poor RPE cell degradation, which increases the amount of debris, or by the chronic inflammation resulting from complement system activation at the site of debris (Buschini *et al.*, 2011).

Drusen are classified into soft, with a size > 65 µm, that are highly associated with AMD progression, and hard, with a size <65 µm, which are commonly associated with the normal

aging process of the human retina (Lim *et al.*, 2012). BrM is a semipermeable membrane responsible for the nutrients and metabolites transportation between the choriocapillaris and the outer retina. Hence, the accumulation of deposits in BrM limits the diffusion of molecules, resulting in RPE and photoreceptors damage (Booij *et al.*, 2010). Moreover, the accumulated lipid deposits can easily become oxidized, thus promoting oxidative stress (Kinnunen *et al.*, 2012). Pathological changes in the RPE play a major role in the early damage associated with AMD, while the pathophysiology differs in the late stages (Bird, Phillips and Hageman, 2014).

3.3 Complement and Immune-related Pathways

Growing evidence supports an association between drusen formation, the inflammatory cascade and AMD progression. Although this correlation has been ambiguous and even unnoticed in the past few years, immune-mediated and inflammatory pathways have appeared to be the hallmarks related to the pathogenesis of AMD. The complement system (CS) is normally triggered by bacterial infections and is a vital protective measure against it. Nevertheless, there is growing evidence that this system likewise protects the body's metabolic tissues, such as the RPE, against reactive oxygen species (ROS) (Gemenetzi and Lotery, 2016). Dysregulation of the CS, due to genetic-related mutations, polymorphisms, or any other cause, can result in illnesses similar to AMD. The CS functions primarily by marking pathogens, including bacteria, through the binding of an indicator protein. This leads to the readily recognition of the pathogen by the immune system and it’s elimination from the body through the process of phagocytosis, which is accomplished by macrophages and other dendritic cells (Lambert *et al.*, 2016a).

The CS has three different pathways: the classical, lectin, and the alternative pathway. These pathways usually initiate the formation of a (C3 or C5) convertase enzyme, which triggers the pro-inflammatory molecules and the assembly of the membrane attack complex (N. S. Merle *et al.*, 2015). Complement factors such as (C3a, C3b) attract the leukocytes, which in turn also stimulates the expression of vascular endothelial growth factor (VEGF), hence resulting in the formation of choroidal neovascularization (CNV) (Chen and Xu, 2015). Complement factor H (CFH), of all other complement factors, is responsible for nearly 50% of the significant attributable risk, especially the genetic risk, to develop AMD. This makes it a very likely new drug target to diminish the incidence of AMD (Parsons *et al.*, 2019). An increased risk of AMD is linked to a dysregulated complement factor H (CFH) activity due to gene polymorphism, in which the complement system pathway is activated, with a consequent subretinal inflammatory response within the drusen (Johnson *et al.*, 2011).

In contrast to CFH, complement factor B may have a protective effect against the progression of AMD, since any related mutation or polymorphism would limit the creation of drusen (Thakkinstian *et al.*, 2012). On the other hand, complement factor D may enhance the development of AMD (Stanton *et al.*, 2011). More studies are still needed to further understand this association; interestingly, inhibitors of the complement factor D are currently in the developmental phases (Abdel-Magid, 2014). C5, C3 as well as C2 complement factors are known to be proinflammatory, thus leading to the deposition of new drusen (Hageman *et al.*, 2001). Polymorphisms in C2 and C3 may intensify the risk of developing AMD (Qian-Qian *et al.*, 2015; Sergejeva *et al.*, 2016). C3 polymorphisms account for 22% of the populations' determinable AMD risk (Yates *et al.*, 2007).

Similarly, any mitochondrial DNA gene deletions or mutations will result in a decreased cellular mitochondrial number and size, provoking oxidative stress and increasing the risk of AMD (Farrar *et al.*, 2013). Although it remains unclear what exact part the (ARMS2) gene may play in mitochondrial function and complement system activity (Smailhodzic *et al.*, 2012), it has been shown that a single copy of ARMS2 -related alleles is responsible for 53% of late-stage AMD population derivable risk (Klein *et al.*, 2013). ARMS2 gene polymorphism, together with the risk alleles of CFH, result in a considerable increase in the likelihood of AMD development (Wyatt *et al.*, 2013). Moreover, the population derivable risk for AMD increases to 76% if ARMS2, CFH, in addition to C3 risk variants, are combined (Spencer *et al.*, 2008).

3.4 Angiogenesis in Age-Related Macular Degeneration

Angiogenesis is defined as the formation of new blood vessels from pre-existing vessels through the splitting or sprouting process (Carmeliet and Jain, 2011). Angiogenesis has a key role in human development, repair, and reproduction; while it’s disruption can lead to destructive disorders (Salajegheh, 2016).

Progression into the late-stage of AMD, particularly the wet form, is related to the formation of new choroidal blood vessels within the central retina, signifying the loss of imposed controls on angiogenesis (Ng *et al.*, 2017). The wet form of AMD, distinguished by CNV, is considered the chief cause of blindness related to AMD among the elderly (Rudnicka *et al.*, 2015). The process of new capillaries formation encompasses a cascade of actions. It starts with the degradation of the basement membrane of the pre-existing blood vessel by the proteolytic activity of the plasminogen activator system and matrix metalloproteinases (Roma-Lavisse *et al.*, 2015). This is followed by endothelial cells proliferation, along with chemotactic migration into the extracellular matrix, lumen formation, and endothelium maturation (Cooley and Bikfalvi, 2018).

Recent evidence suggests that VEGF is the main proangiogenic factor predisposing to CNV (Parmeggiani *et al.*, 2010). VEGF is synthesized under normal physiological status by the RPE, and controlled by pigment epithelium-derived factor (PEDF), an antiangiogenic regulator that is also produced by the RPE, in order to maintain the homeostasis of the retina (Tong and Yao, 2006). In abnormal conditions such as ischemia, inflammation, or hypoxia, neovascularization is promoted through an increased expression of VEGF (Grossniklaus, Kang and Berglin, 2010). VEGF usually binds to three receptors, with different affinities as well as different actions. Binding to the VEGFR-2 receptor promotes angiogenesis and vascular permeability (Ferrara, Gerber, and LeCouter, 2003). The VEGFR-1 acts as a competitive receptor of VEGF, thus negatively regulating the activation of VEGFR-2 (Melincovici *et al.*, 2018). VEGFR-3 receptor induces embryo angiogenesis, gliomas and colon carcinomas (Smith *et al.*, 2010). The significance of the VEGF role is based upon clinical data, which revealed that the VEGF levels are significantly elevated in AMD patients with CNV in comparison to unaffected individuals (Velez-Montoya *et al.*, 2010). This role is further supported by the suppression of neovascularization, along with vascular permeability, after the application of the anti-VEGF therapy, namely aflibercept (AFB), bevacizumab (BVZ), and ranibizumab (RBZ), hence preserving vision in AMD patients (Schmidt-Erfurth, Chong, *et al.*, 2014).

3.5 Classification of Age-Related Macular Degeneration

According to AREDS, AMD has three progression stages; initial (A) with small hyperpigmentation areas and < 20 drusen of middle-size, intermediate (B) with one or several larger drusen or a small geographical atrophy not involving the center of the macula, in addition to advanced (C and D). The advanced stages are further divided in to dry, with a large geographical atrophy involving the central macula, or exudative AMD, associated with neovascularization (Kassoff *et al.*, 2001). Clinically, late-stage AMD is generally classified into two types (Fig 2), degenerative (dry) that accounts for 80% of cases, and neovascular (wet). Geographic atrophy (GA) is the late form of advanced non-neovascular AMD (dry), accounting for a 35% prevalence among late-stages cases, as well as for 20% of AMD associated irreversible blindness (Klein *et al.*, 2007). Dry AMD can also slowly progresses into the neovascular form for unknown causes.

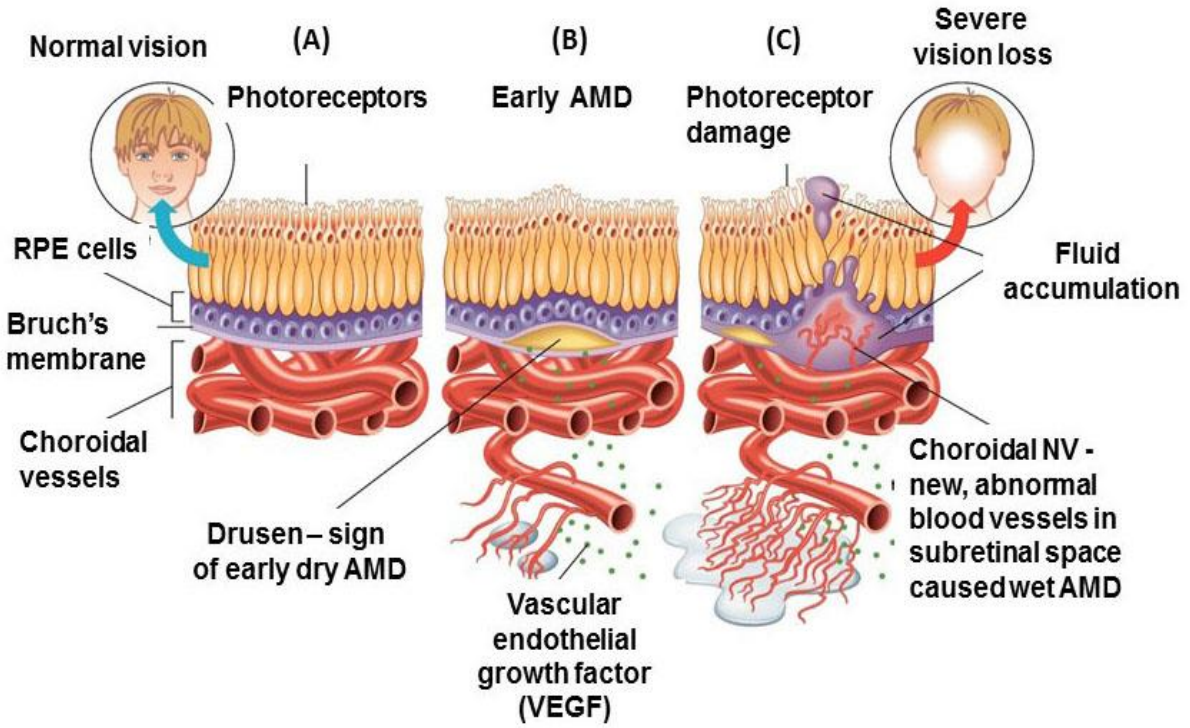


Fig.2 Stages of AMD (A) normal retina with RPE layer. (B) Drusen presence between the RPE and Bruch's membrane in dry AMD and induction of VEGF expression; (C) Abnormal blood vessels formation in response to VEGF in the choroid (CNV), decreased Bruch's membrane and RPE layer integrity, resulting in subretinal leakage and fluid accumulation, thus causing visual impairment in the late stage of AMD. Adopted from (Salimiaghdam *et al.*, 2020).

In dry AMD, areas of the retina with complete RPE atrophy were found to occur earlier than the loss of the choriocapillaris in nearby areas, hereby indicating that the RPE is the main site of injury. On the other hand, the loss of choriocapillaris occurred earlier in wet AMD or choroidal neovascularization (CNV), indicating that the choriocapillaris are the primary injury site, which induces hypoxia in the nearby RPE and upregulates the expression of vascular endothelial growth factor (VEGF), therefore promoting choroidal neovascularization (Mcleod *et al.*, 2009).

The CNV associated with wet AMD results in a subretinal outflow of fluids, blood, lipids, as well as fibrous scar formation (Arya *et al.*, 2018). It is often differentiated into three broad types; occult CNV, localized in the sub-RPE area, classic CNV, found in the sub-retinal area, and the third type is described as intraretinal angiomatose proliferation (Farecki *et al.*, 2017). Several imaging techniques were developed to facilitate the diagnosis and to monitor the response to the applied therapy in AMD patients. These techniques include mainly Optical Coherence Tomography (OCT) and Fluorescein Angiography (FA), with the proper method selection being based on the patients' clinical condition (Freund, Toth and Zarbin, 2019).

AMD severity could be evaluated based on the better-seeing eye Snellen visual acuity (VA) test, and thus categorized (20/20–20/40) as mild, (20/50–20/100) as moderate, (20/200) or worse as severe, and finally (20/800) or worse as very severe (Tsou and Bressler, 2017). In the study (Wong *et al.*, 2008), best-corrected visual acuity (BCVA) is given as a logarithm of the minimum angle of resolution (logMAR). LogMAR is the $\log_{10}$ of the MAR (Minimum angle of resolution), and MAR is defined as the angle in minutes of arc, stroking one fifth of the of a Snellen optotype subtended at the eye (Oduntan & Mashige, 2009). If wet AMD remains untreated, the visual acuity elapses with a logMAR increase of 0.4 after one year and 0.6 after three years in nearly 41% of all neovascular AMD patients.

3.6 Risk Factors

Identification of the following risk factors has a potential effect on predicting the progression of AMD:

3.6.1 Age

Age is considered the most powerful demographic non-modifiable AMD predictor (Lambert *et al.*, 2016b). Aging is correlated with retinal structural and functional changes that predispose to the development of AMD. Moreover, it contributes to the additive effects of other risk factors with time. Joachim *et al.*, (2013), prospective studies revealed that age is strongly associated with geographic atrophy (GA) progression. Jonasson *et al.*, (2014), also confirmed that age is significantly correlated with the progression to GA and wet AMD.

3.6.2 Gender

Previously, it was believed that AMD is more prevalent in males, but several studies showed that the female gender is associated with an increased risk, as well as progression rate from early to late-stage AMD (McGuinness *et al.*, 2016; Merle *et al.*, 2017). Controversially, some studies reported a lack of relationship between gender and AMD progression (Sakurada *et al.*, 2019; Klein *et al.*, 2019). These conflicting results may be explained by follow-up differences, as well as to the females' greater life expectancy (Roth *et al.*, 2018). Other studies suggest a potential role of female sex hormones, such as estrogens, in AMD progression, since it may alter serum lipids levels and exert antioxidant activity (Fraser-Bell *et al.*, 2006).

3.6.3 Smoking

Smoking is a modifiable risk factor, accounting for a 2 – 4 fold-increased probability of contracting AMD (Connolly *et al.*, 2018), along with a faster development rate of GA (Yu *et al.*, 2012). Saunier *et al.*,(2018), reported an additive dose-response effect after comparing pack-years of smoking. Furthermore, ex-smokers have a modestly higher risk of AMD progression (Merle *et al.*, 2017), even though other studies did not confirm this association (Yip *et al.*, 2015; Hoffman *et al.*, 2016). Cigarette smoke contains various toxic substances that have negative pathological influences on biochemical pathways, such as the induction of oxidative stress and inflammation in the retinal RPE cells, as well as alterations in the choroidal vessels (Zinflou and Rochette, 2019).

3.6.4 Body Composition and Diet

Increased body mass index (BMI) was found to be associated with an increased risk of developing AMD in several studies (Yu *et al.*, 2012; Jonasson *et al.*, 2014), whilst no correlation was observed in others (Shim *et al.*, 2016; Saunier *et al.*, 2018). Elevated levels of pro-inflammatory factors and cytokines in obese people could alter RPE cells function. Moreover, the increase in carotenoids stored in adipocytes lowers the levels available in the macula (Leung *et al.*, 2005). Merle *et al.*, (2015), reported that an adherence to the Mediterranean diet, characterized by high contents of fruits, vegetables, grains, legumes, and nuts, typically rich in antioxidants, in addition to olive oil, which contains a high content of unsaturated fatty acids, could exert a protective role against the AMD progression.

3.6.5 Comorbidity

Well-established evidence in the literature supports the involvement of hypertension in AMD progression (Wang *et al.*, 2016). Chen *et al.*, (2018), reported a higher occurrence of all forms of AMD in chronic kidney disease patients than in healthy population. Furthermore, Chaker *et al.*, (2015), noted that hyperthyroidism might be a potential risk factor for the development of late-stage AMD. Additionally, Zhang *et al.*, (2011), mentioned that dyslipidemia and hyperglycemia, especially among diabetic people, could disturb the retinal homeostasis, caused by an increase in inflammation and oxidative stress, thus contributing to the progression of AMD.

3.7 Diagnosis

There are several diagnostic methods of AMD, however, Optical Coherence Tomography (OCT) is considered the most widely used method. It was developed as a non-invasive imaging technique for biological structures, and is based on the interferometric analysis of the low frequency light reflected from the retina structures (Huang et al., 1991), It allows quantification

of retinal morphology with a resolution that largely corresponds to the histology. OCT provides a 3D structural information of retinal imaging, which can be used in order to determine the presence of AMD (Yoo, Choi, Seo, Ramasubramanian, & Selvaperumal, 2018). Moreover, OCT is subcategorized in to; time domain and spectral domain. In time domain, the retinal information is taken after longitudinal translation in time of a reference arm, whereas in spectral domain, interferometric signal is detected using optical frequencies. This leads to a much faster imaging than that of time domain OCT (Forte, Cennamo, Finelli, & Crecchio, 2009). Rosenfeld *et al.*, (2006), mentioned that OCT has become the gold standard in anti-VEGF therapy monitoring.

Fluorescence angiography is another diagnostic method, where a fluorescent dye is injected intravenously, after which it's distribution in the eye vessels is monitored (Stürzlinger, Genser, & Fröschl, 2007). Moreover, Fluorescence angiography can distinguish between classic and occult CNV. Classic CNV usually can be found in the subretinal space, while the occult CNV remains beneath the retinal pigment epithelium (Hughes, Khan, & Kashani, 2005). According to (Yonekawa, Miller, & Kim, 2015), early stages of AMD is not usually detected by routine fundus examination, hence, it may not be used as a primary diagnostic tool of AMD.

3.8 Management Approaches

The vascular endothelial growth factor in AMD patients promotes abnormal proliferation of new blood vessels, thus leading to the development of wet AMD. Anti-VEGF agents has a significant role in wet AMD management. In the United States and Europe, clinical practice guidelines from ophthalmological organizations consider anti-VEGF agents as the first-line therapy of wet AMD. Notable improvement of both visual and anatomic outcomes, in comparison with other applied therapies, were observed (Bakri *et al.*, 2019).

Anti-VEGF treatment options available for wet AMD have immensely progressed. The first used intravitreal anti-VEGF (pegaptanib), approved by FDA in 2004, is no longer used in clinical practice, since no improvement in the visual acuity was clinically noted, especially in new-onset wet AMD. (Flaxel *et al.*, 2020). After that, the FDA approved the use of ranibizumab (RBZ) in 2006 and the use of aflibercept (AFB) in 2011 (Bakri *et al.*, 2019). Ranibizumab is FDA approved to use for wet AMD treatment, after its effectiveness was confirmed by several trials. The ANCHOR and MARINA trials confirmed its clinical efficacy and safety for wet AMD treatment (Ferro Desideri *et al.*, 2019). Off-label use of bevacizumab (BVZ) as an intraocular agent, is highly related to its cheap cost, in comparison with other agents, as well as it's comparable effect in the improvement of visual acuity in wet AMD patients, (J.B. *et al.*, 2017). The FDA approved the use of aflibercept in 2011 as a potent anti-VEGF, with a more prolonged

effect in comparison to the previously approved anti-VEGFs. Aflibercept is a recombinant fusion protein that contains the extracellular binding domains of VEGF receptor (Chhablani, Narayanan, Mathai, Yogi, & Stewart, 2016). Trials showed similar efficacy of aflibercept, compared to ranibizumab and bevacizumab (Hyman & Neborsky, 2002). A retrospective analysis, in which 134 patients were included, compared the efficacy of aflibercept to ranibizumab and bevacizumab. The results showed that aflibercept has comparable efficacy to ranibizumab and bevacizumab, and patients who were resistant to the latter two treatments could benefit from aflibercept (Cardoso et al., 2017). Ranibizumab is composed of the Fab chain of the antibody; bevacizumab resembles the whole monoclonal antibody, whereas aflibercept is a recombinant protein, resembling the receptor-binding site. The structural differences in addition to the properties of the three anti-VEGF therapies used in this study are compared in (Fig 3).

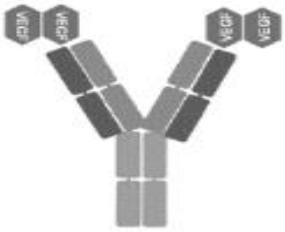
	Bevacizumab (BVZ)	Ranibizumab (RBZ)	Aflibercept (AFB)
Manufacturer	Avastin; Genetech, South San Francisco, CA, USA	Lucentis; Genetech, South San Francisco, CA, USA	Eylea; Regeneron Pharmaceuticals, Tarrytown, NY, USA
Type of molecule	Full-size recombinant humanized IgG1 kappa monoclonal antibody	Fab fragment of a recombinant humanized IgG1 kappa isotype murine monoclonal antibody	Fusion protein of the second Ig domain of human vascular endothelial growth factor receptor 1 (VEGFR-1) and the third Ig-binding domain of human VEGFR-2 with the constant fragment crystallizable portion of the human IgG1
Molecular weight	149 kDa	48 kDa	115 kDa
Picture			
Comments	N-glycosylated in its Fc region	Not glycosylated	NA
Production	Mammalian cell lines CHO DP-12	*Escherichia coli* cells, recombinant DNA technology	Hamster ovary cells
Receptor-ligand interaction	Against all isoforms of VEGF-A	Against all isoforms of VEGF-A	Binds to all isoforms of VEGF-A (higher affinity than BVZ and RBZ); also binding to VEGF-B and Placental Growth Factor (PlGF).
Authorization in the USA	FDA in 2005, colorectal and breast cancers, used in AMD off-label	FDA in 2006, AMD	FDA—2011, AMD

Fig. 3 Comparison between aflibercept, bevacizumab, ranibizumab. Adopted from (Plyukhova *et al.*, 2020).

Newly approved Anti-VEGFs are still emerging; one of these medications is brolucizumab, which was approved by US FDA in 2019. Brolucizumab, developed by the pharmaceutical company Novartis, is a low molecular weight anti-VEGF, which was approved to treat wet AMD (Markham, 2019). It is a humanized, single-chain variable fragment which inhibits VEGF-A,

with a high binding affinity, thus suggesting a better efficacy than the previous anti-VEGFs (Nguyen et al., 2020).

The frequency of administering the anti-VEGF agents is an important factor that determines the success of therapy; hence, several schemata were developed trying to achieve an optimal treatment outcome. Treat and extend (TnE) is one of the regimens used, in which the intervals for giving the anti-VEGF are scheduled regardless of the disease state (Skelly, Bezlyak, Liew, Kap, & Sagkriotis, 2019). On the other hand, Pro Re Nata (PRN) regimen, depends on the activity of disease, thus the anti-VEGF is given at flexible intervals depending on the case of the patient. In other words, the OCT is monthly performed, but an anti-VEGF treatment will be initiated only if a macular edema of bleeding was seen. According to Richard *et al* (2015), a scheduled treatment was found to be superior over the PRN regimen.

Numerous pioneering clinical trials have compared the visual-related outcomes in the use of various anti-VEGF agents in the treatment of exudative AMD. These include the “Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in AMD” (ANCHOR), the “Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD “(MARINA), the “Phase IIIb, multicenter, randomized, double-masked, sham injection controlled study of the efficacy and safety of ranibizumab in subjects with subfoveal CNV with or without classic CNV secondary to AMD” (PIER), the “Prospective OCT Study With Lucentis for Neovascular AMD” (PRONTO), the “VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD“ (VIEW 1 and 2), and the “Comparison of Age-related Macular Degeneration Treatment Trials” (CATT). All the above-mentioned trials showed no significant difference in the effect of the various anti-VEGF agents. Moreover, the findings revealed that the visual acuity gain is similar in all tested anti-VEGFs (Rao *et al.*, 2018).

Optical coherence tomography (OCT) is needed to regularly monitor and detect intraretinal cystoid fluid or subretinal fluid (SRF), which simulates the CNV activity, during the duration and maintenance of the therapy. (Flaxel *et al.*, 2020). OCT is used for measuring the central retinal thickness and detecting drusenoid pigment epithelial detachment (“The coalescence of small soft drusen into a large mass”), an important predictor for blindness (Blanco-Garavito *et al.*, 2018)., in addition to detecting any fluid accumulation, thus indicating the activity of AMD and the needs for a more intensive therapy (Schmidt-Erfurth, Kaiser, *et al.*, 2014).

3.9 Aim of this Work

Since the use of anti-VEGF therapy began, several “real-world” studies have been published. These revealed that the success rate of the treatment in the anti-VEGF approval studies

surpassed those observed in the clinical care setting. Due to the high morbidity rates of older populations, the administration of adequate therapy is not always possible.

The influence of anti-VEGF injections on retinal edema, which is determined by the central retinal thickness (CRT), is already available. This study aims mainly to examine the influence of the three different anti-VEGF agents (ranibizumab, bevacizumab, and aflibercept) on the visual acuity (VA), intraocular pressure (IOP), and most importantly the retinal nerve fiber layer thickness (RNFLT). Standard OCT parameters such as central retinal thickness (CRT) and total macular volume (TMV), were also examined, in order to investigate the effect of these anti-VEGF injections in a clinical setting. The key questions of this work are:

a) Will there be an improvement in the visual acuity, and if so, will it be maintained during the course of therapy?
b) What influence does the initial visual acuity have on the course of therapy?
c) Is the change in visual acuity different between the 3 examined anti-VEGF agents, and if so, is it reflected in the standard OCT parameters?
d) What is the effect of the three various anti-VEGF agents on the RNFLT? Is the change dependent on the number of injections? Was a change in IOP noticed?

4. Methodology

4.1 Patient Selection

In this retrospective study, a total of 120 patients (80 women and 40 men), started an anti-VEGF therapy for neovascular AMD, in the period extending from January 6, 2010, to November 6, 2018, at MVZ-Schlosscarree Eye clinic and Ophthalmology practice in Braunschweig, Germany. They participated and received at least six injections in the course of therapy. The included therapy regimes are Pro-Re-Nata (PRN) and Treat-and-Extend (TAE) for intravitreal injections of (0.5 mg) ranibizumab, (1.25 mg) bevacizumab or (0.5 mg) aflibercept, injected as a 0.05 ml solution. Fundus examination, fluorescence angiography (FAG), and OCT evaluation techniques were used to confirm the diagnosis. In patients that have received anti-VEGF treatment in both eyes, the eye that has received more intravitreal injections (IVI) was the one included in this study.

4.2 Exclusion Criteria

1. Patients diagnosed with glaucoma.
2. Ocular hypertension, with an IOP > 22 mmHg

3. High-grade myopia > – 6 Dpt.
4. Retinal laser coagulation and pars plana Vitrectomy.
5. Patients who developed fibrosis before therapy.
6. Patients who developed fibrosis during the therapy and an adequate analysis of the RNFL was no longer possible were also excluded.

4.3 Procedure

All OCT evaluations were carried out with the Spectralis SD-OCT (Heidelberg Engineering, Heidelberg), software version 6.8.1). Sub- and/or intraretinal fluid seen with the OCT, new or persistent macular bleeding, as well as an increase in the pigment epithelium detachment, were used as signs of activity and progress of the CNV in exudative AMD. OCT changes signaling macular atrophy or fibrosis were also evaluated.

In the PRN regime, an initial series of three injections 4 weeks apart were first carried out, after which OCT, Visual Acuity (VA) and intraocular pressure (IOP) were measured 4 weeks after. The above-mentioned measurements for further treatment series, according to the activity of the disease, were also preformed four weeks after the last injection in the series was made.

In the Treat and Extend (TnE) regime, a loading phase, consisting of three anti-VEGF injections with a time span of four weeks in between, was implemented. OCT, Visual Acuity (VA) and intraocular pressure (IOP) were measured at the time of the third injection in order to determine the time interval for the next injection. If the OCT showed any of the above-mentioned activity signs, the next injection will be due in four weeks, on the other hand if no activity was noted, a two-weeks prolongation of the time interval will ensue, with the next injection taking place after 6 weeks. An OCT was done before each injection. If signs of disease activity were present, the interval between the injections were shortened by two weeks, up to a minimum time span of four weeks between each two injections. On the other hand, if no signs of disease activity were identified, an extra time extension of two weeks between the injections was carried out with a maximum of twelve weeks between two injections, indicating the end of the treatment. The disease progression was evaluated according to the recommendations of the following german ophthalmological und retinal societies: DOG (Deutsche Opthalmologische Gesellschaft), BVA (Berufsverband Augenärzte) and Retinologischer Gesellschaft .

4.4 SD-OCT (Spectralis)

A reliable evaluation of retinal structural integrity in both healthy and pathological conditions can be achieved through a qualitative comparison between hyper- and hyporeflective layers (Keane *et al.*, 2012). The use of Spectral-domain OCT (in this study SD-OCT, Spectralis ® Heidelberg Engineering, Heidelberg) with 20,000 axial scans (A-scan) and a resolution of approx. 5 µm ((Regatieri, Branchini and Duker, 2011), permits a reliable quantitative analysis of the standardized OCT parameters.

4.5 Measurement Parameters

The best corrected visual acuity (BCVA) was assessed using a decimal chart, after which it was converted to logMAR. As a part of the routine ophthalmological examination, the intraocular pressure (IOP) was measured using non-contact tonometry (NCT). In cases of an IOP >21 mmHg, and in order to rule out falsely high IOP, an applanation measurement using Goldmann applanation tonometer was carried out.

The quantitative assessment of retinal layer thicknesses is based on the grid that had been defined in the ETDR (Early Treatment Diabetic Retinopathy Study) (**Fig. 4)**. The innermost ring, defining the C1 area, lies within 0.5 mm of the fovea centralis. The two outer rings, having a radius of 1.5 and 3 mm consecutively, are divided into 8 segments; temporal, nasal, superior, and inferior parts. The central retinal thickness (CRT), also known as (foveal thickness), is the average thickness of the (C1) segment in the ETDRS grid. With the help of central retinal thickness (CRT) and total macular volume (TMV), macular edema can be measured and quantified, which is pivotal in monitoring the therapy of exudative AMD.

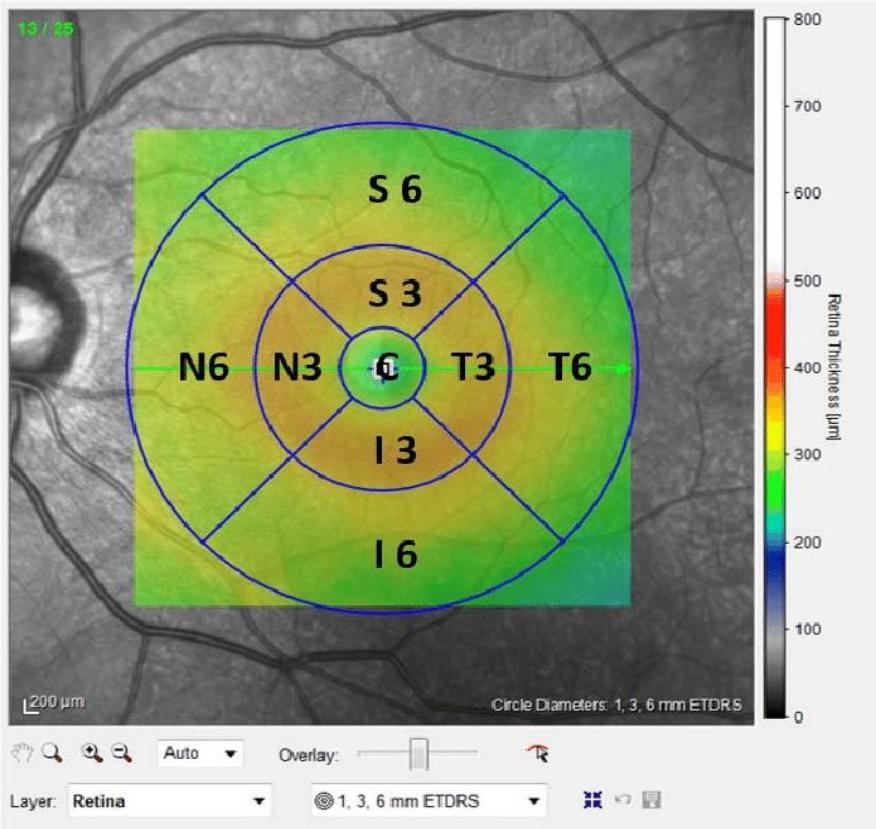


Fig.4 ETDRS grid in SD-OCT. Adopted from (J.T. Ferreira *et al*. 2016)

Standard data for the CRT was previously determined with the time-domain (TD) OCT. The analysis algorithm of the SD-OCT differs from that of TD-OCT, with the former identifying the RPE as the outermost limit. This resulted in a difference in the measured CRT of approximately 50 µm (Sayanagi, Sharma and Kaiser, 2009). Huang *et al.*,(2009), stated that CRT of healthy eyes using the Spectralis OCT lies at (270.2 ± 22.5 µm). Nearly similar results were obtained by other studies, (266 ± 23µm) by Grover *et al.*, (2009), and (271 ± 21) by Murthy *et al.*, (2015). The normal range of macular thickness was set between 225 µm and 315 µm. Values lying beneath or above this range are considered as macular atrophy or macular thickening respectively (Legarreta *et al.*, 2008).

CRT is used in several clinical studies to assess the extent of macular edema and thus CNV progression. The sum of all nine areas of the ETDRS grid is defined as the total macular volume (TMV), with a standard value of 10.1 ± 0.6 mm^3 (Li *et al.*, 2006). Although it is seldom used in clinical settings, it serves as an additional marker for macular edema, thus aiding in the detection of CNV, especially when a large part of the macula is affected. However, this can be disadvantageous if the changes involves only a small area. The CRT and TMV are both

determined automatically, but a simultaneous fundus examination to evaluate the corresponding retinal morphology is crucial. (Fig. 5).

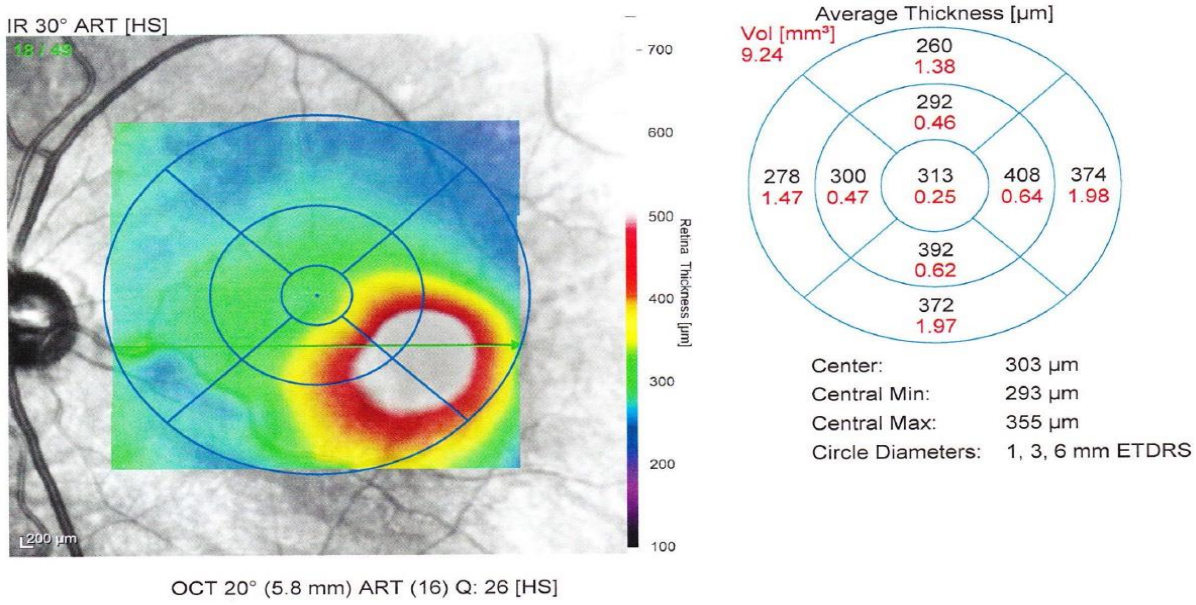


Fig.5: Determination of CRT and TMV with SD-OCT (Spectralis)

RNFLT was measured in the outer temporal segment (T6, 1.5-3.0 mm) of the ETDRS grid, after aligning it with the temporal margin of the optic nerve head. (ONH), and manual correction of the segmentation (Fig.6 and Fig. 7). This was done in order to produce objective RNFLT values , since the assessment of the RNFL is not a standard measurement in monitoring the treatment of neovascular AMD.

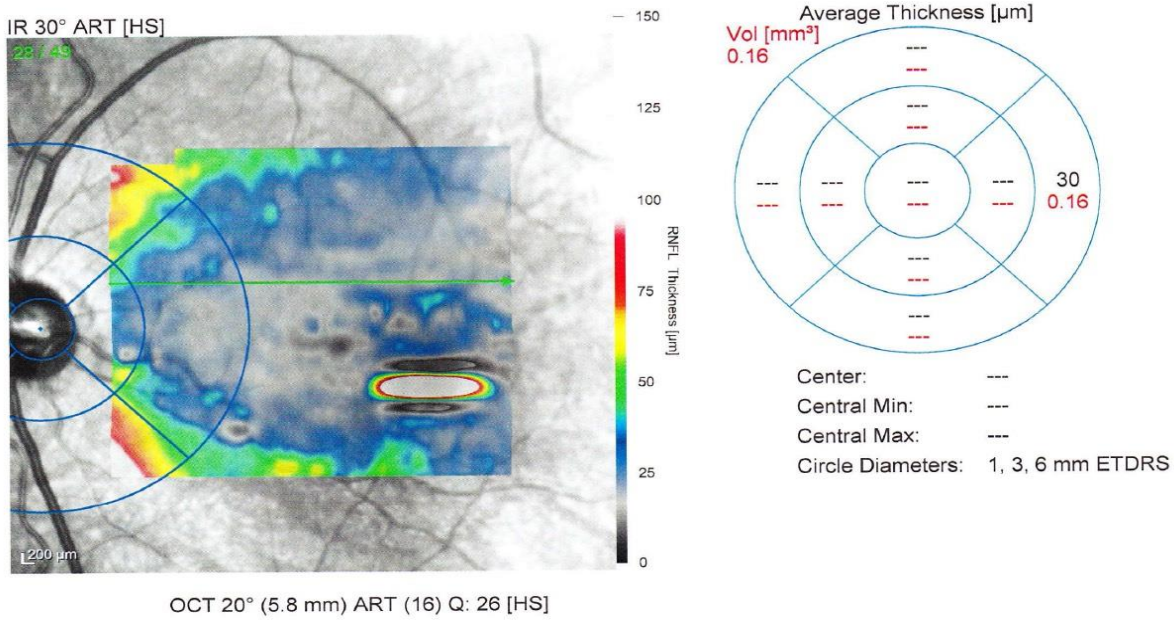


Fig. 6: Determination of an RNFL segment with a temporally papillary ETDRS grid

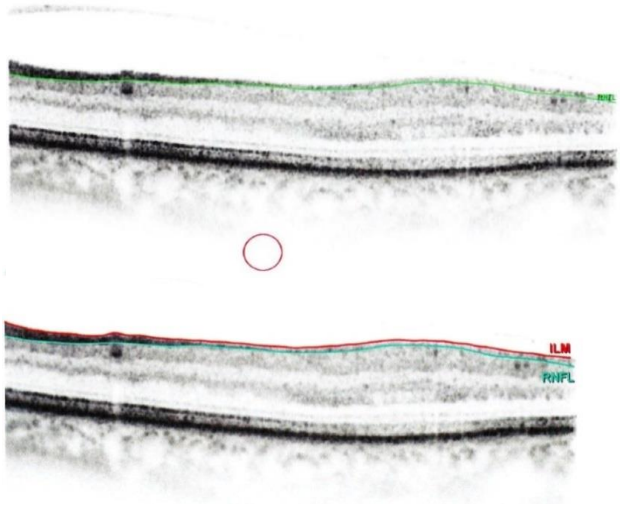


Fig. 7 Manual correction of the segmentation of the RNFL

4.6 Treatment Protocol

Intravitreal injections were administered according to a standardized protocol by a single ophthalmologist.

4.7 Statistical Analysis

A two-sample-t-test (GraphPad Prism 5.0), which compares the test group receiving the anti-VEGF injections (post-X injections) with the respective baseline, is used to analyze the measured parameters of the selected dependent group. Changes from the baseline value are considered with a corresponding p values of $p < 0.05$ = * significant, $p < 0.01$ = ** very significant, and $p < 0.001$ = *** highly significant.

The mean change from baseline value is used to describe the study parameters. Since the baseline group, in this retrospective study, is different for each analyzed band (Post 3-18), these values should be considered separately. It should also be noted that the baseline groups are not independent of one another, since patients with a higher number of injections are always included in the previous groups.

4.8 Patients Demographics and Clinical Characteristics

A total of 120 (80 females) patients diagnosed with exudative AMD, with an average age of 78.5 years, were included in the study. The patients were classified in to three groups, according to the anti-VEGF agent administered: bevacizumab (n=41), ranibizumab (n=52) and aflibercept (n=27). Each patient received an average of 10 injections, and the total number of participants decreased with increasing number of injections, as noted in Table 1. The remaining demographic, clinical and ophthalmologic characteristics of the patients included in the study are summarized in Table 2.

Table 1. Number of patients in the active substance subgroups.

Therapy	*Bevacizumab*	*Ranibizumab*	*Aflibercept*	*Total*
Post 3	41	52	27	120
Post 6	36	52	27	115
Post 9	24	39	15	78
Post 12	15	24	8	47
Post 15	6	15	5	26
Post 18	5	6	2	13

Table 2: Patients Characteristics

	Bevacizumab n=41	Ranibizumab n=52	Aflibercept n=27	Total n=120
Age − n (%)				
60−69 Years	*3 (7.3%)*	*5 (9.3%)*	*1 (3.7%)*	*9 (7.4%)*
70−79 Years	*18 (43.9%)*	*28 (51.9%)*	*10 (37.1%)*	*56 (45.9%)*
80−89 Years	*16 (39.0%)*	*18 (33.3%)*	*15 (55.5%)*	*49 (40.2%)*
≥90 Years	*4 (9.8%)*	*3 (5.5%)*	*1 (3.7%)*	*8 (6.5%)*
Mean Age	*79.7 ± 6.8*	*76.6 ± 8.7*	*80.7 ± 6.1*	*78.5 ± 7.8*
Sex − n (%)				
Females	*31 (75.6%)*	*32 (61.5%)*	*17 (63.0%)*	*80 (66.7%)*
Males	*10 (24.4%)*	*20 (38.5%)*	*10 (37.0%)*	*40 (33.3%)*
AMD Subtype – n (%)				
Occult	*20 (48.8%)*	*20 (38.5%)*	*8 (29.6%)*	*48 (40.0%)*
Classic	*9 (22.0%)*	*23 (44.2%)*	*11 (40.7%)*	*43 (35.8%)*
Mixed form	*12 (29.3%)*	*9 (17.3%)*	*8 (29.6%)*	*29 (24.2%)*
DM Type 2 − n (%)	8 (19.5%)	9 (17.3%)	5 (18.5%)	22 (18.3%)
Cataract-Operation − n (%)	23 (56.1%)	27 (51.9%)	23 (85.2%)	73 (60.8%)

Before Treatment	*7 (17.1%)*	*15 (48.1%)*	*21 (77.8%)*	*43 (35.8%)*
During Treatment	*16 (39.0%)*	*12 (23.1%)*	*2 (7.4%)*	*30 (25.0%)*
Mean number of Injections pro Eye	9,3	10,8	9,3	10,0
BCVA − logMAR	0.55 ± 0.28	0.43 ± 0.23	0.53 ± 0.32	0.49 ± 0.29
IOP − mmHg	15.5 ± 2.6	16.1 ± 3.0	15.1 ± 3.5	15.7 ± 2.9
RNFLT − µm	51.2 ± 9.9	54.5 ± 12.2	48.7 ± 9.4	52.1 ± 11.8
CRT − µm	396 ± 100	389 ± 92	375 ± 100	389 ± 98
TMV − mm^3	9.2 ± 1.7	9.0 ± 1.3	9.0 ± 1.6	9.0 ± 1.4

Abbreviations: n = Number of patients; (%) = percentage; CRT = Central retinal thickness; TMV = Total macular volume; RNFLT= Retinal Nerve Fiber Layer Thickness; IOP = Intraocular Pressure; logMAR = logarithm of the minimum angle of resolution.
All ± values are standard deviations

5. Results

5.1 Visual Acuity

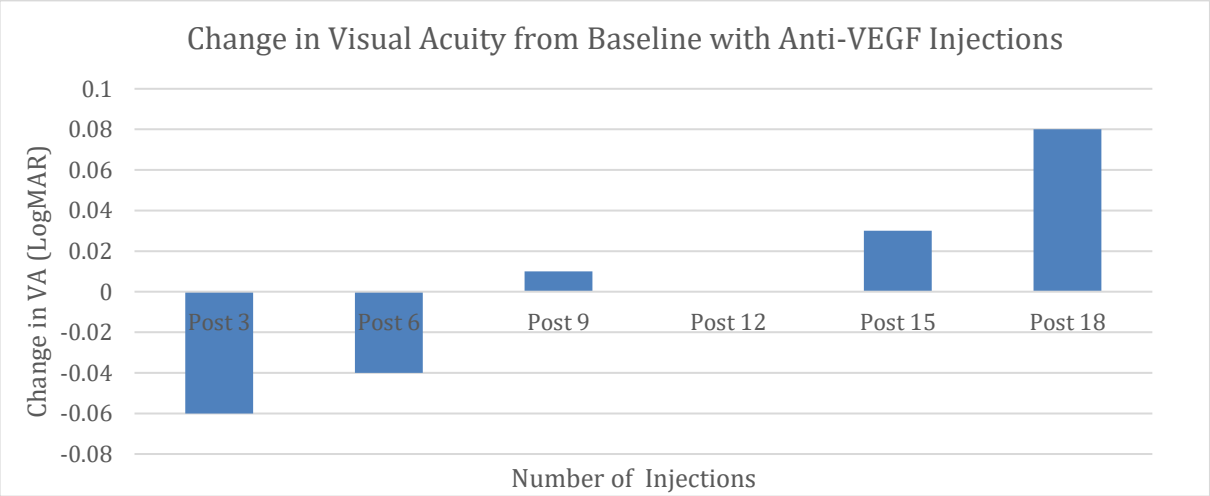
The mean BCVA of all three anti-VEGF groups was 0.49 logMAR. A statistically significant improvement of the visual acuity with anti-VEGF injections was only in the post 3 subgroup measured, which showed a gain of (-0.06 logMAR). The visual acuity remained almost unchanged in the till after the 12^{th} injection, after which a higher number of injections tended to worsen the visual acuity.

Ranibizumab post 3 group had the best baseline visual acuity of 0.42 logMAR, but no significant increase of visual acuity was noted. The groups treated with bevacizumab and aflibercept benefited significantly during the first three injections, where a decrease of 0.02 logMAR for bevacizumab and 0.09 logMAR for aflibercept were recorded. A significant improvement was still observed after 6 injections in the aflibercept subgroup, after which the visual acuity in the other aflibercept subgroups remained stable, with no significant improvement or worsening.

In the bevacizumab subgroups, worsening of the visual acuity in the post 15 and post 18 subgroups was noted. These are likely non-representative values, which can be explained by the small sample size of N = 6 and N = 5.

The change in visual acuity from baseline with anti-VEGF therapy (all three agents), as well as it's change with each of them is summarized in Tables 3.1-3.4

Table 3.1	**Visual Acuity (logMAR) with Anti-VEGF Therapy**				
Therapy	*Baseline*	*SD Baseline*	*Anti-VEGF*	*SD Anti-VEGF*	*p-Value*
Post 3	0.50	0.29	0.44	0.31	**0.031***
Post 6	0.48	0.28	0.44	0.32	0.218
Post 9	0.50	0.30	0.51	0.34	0.895
Post 12	0.48	0.29	0.48	0.34	0.930
Post 15	0.39	0.19	0.42	0.35	0.693
Post 18	0.35	0.11	0.43	0.35	0.408



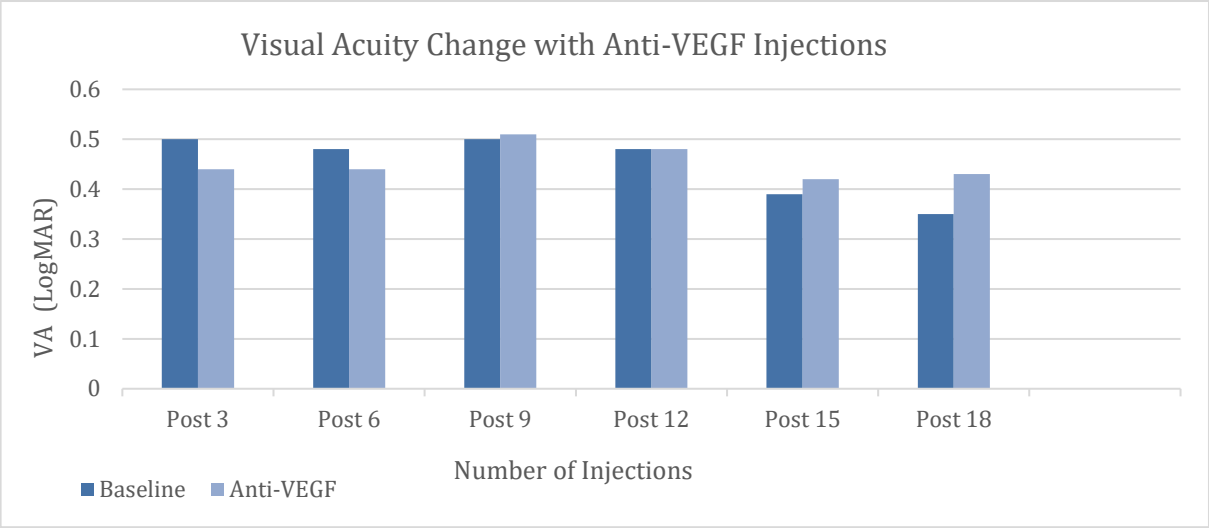
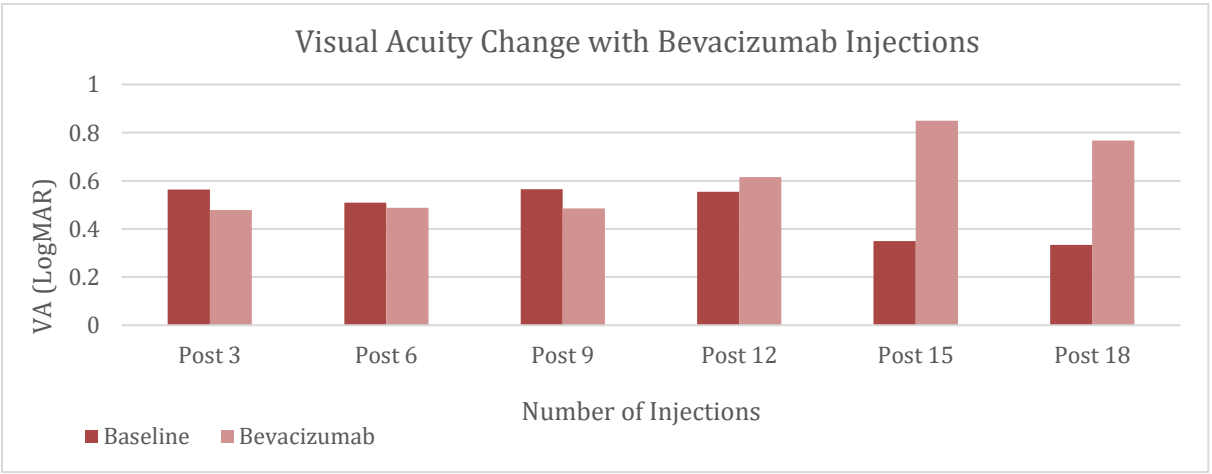


Table 3.2	**Visual Acuity (logMAR) in Bevacizumab Group**				
Therapy	*Baseline*	*SD Baseline*	*Bevacizumab*	*SD BVZ*	*p-Value*
Post 3	0.564	0.277	0.479	0.338	**0.020***
Post 6	0.509	0.230	0.488	0.299	0.246
Post 9	0.565	0.274	0.485	0.320	0.468
Post 12	0.554	0.257	0.615	0.287	0.416
Post 15	0.350	0.100	0.850	0.265	0.051
Post 18	0.333	0.115	0.767	0.351	0.186



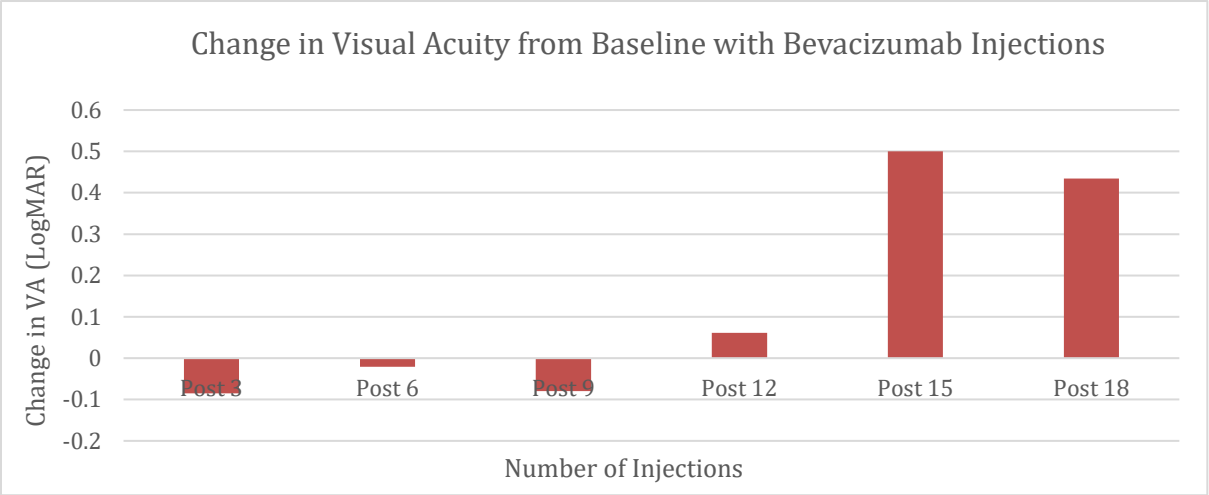
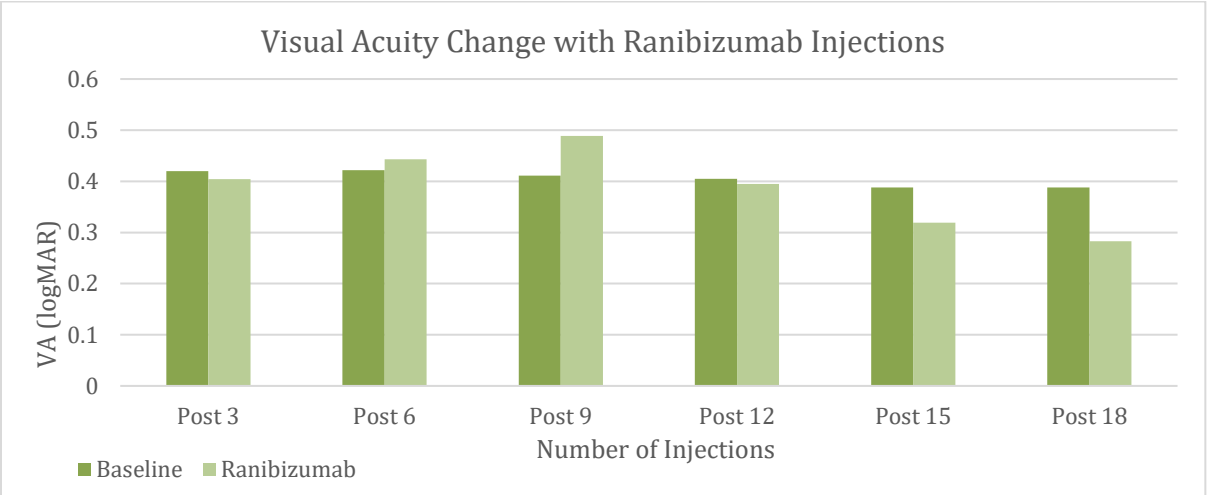


Table 3.3	**Visual Acuity (logMAR) in Ranibizumab Group**				
Therapy	*Baseline*	*SD Baseline*	*Ranibizumab*	*SD RBZ*	*p-Value*
Post 3	0.420	0.227	0.404	0.284	0.834
Post 6	0.422	0.230	0.443	0.328	0.587
Post 9	0.411	0.200	0.489	0.349	0.203
Post 12	0.405	0.167	0.395	0.307	0.881
Post 15	0.388	0.150	0.319	0.283	0.281
Post 18	0.388	0.121	0.283	0.271	0.383



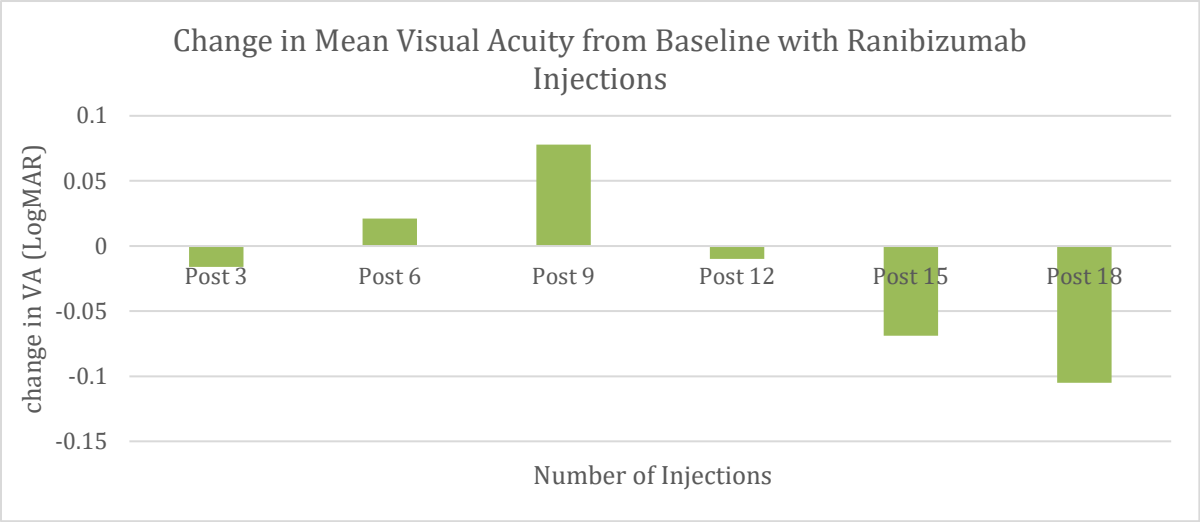
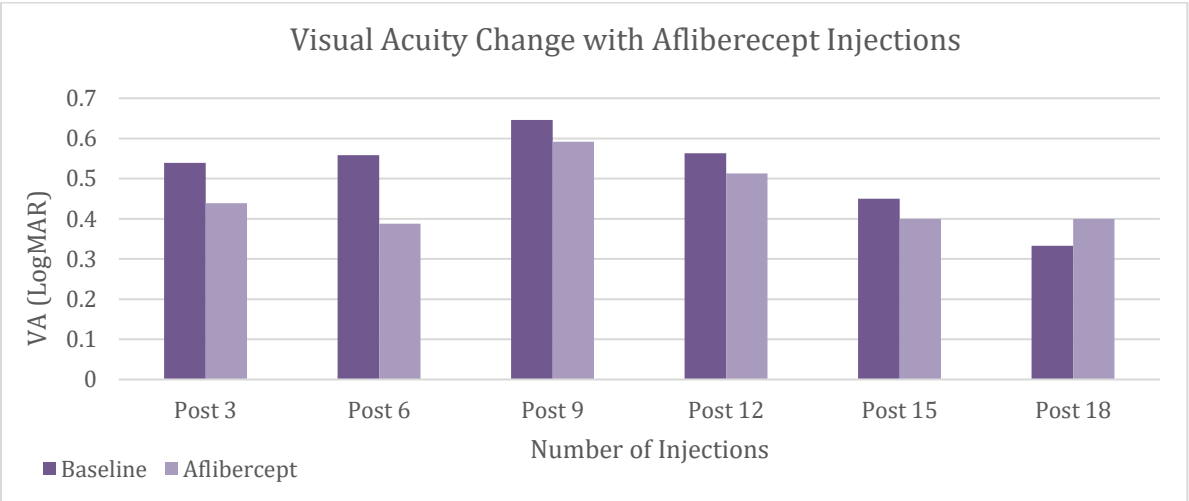
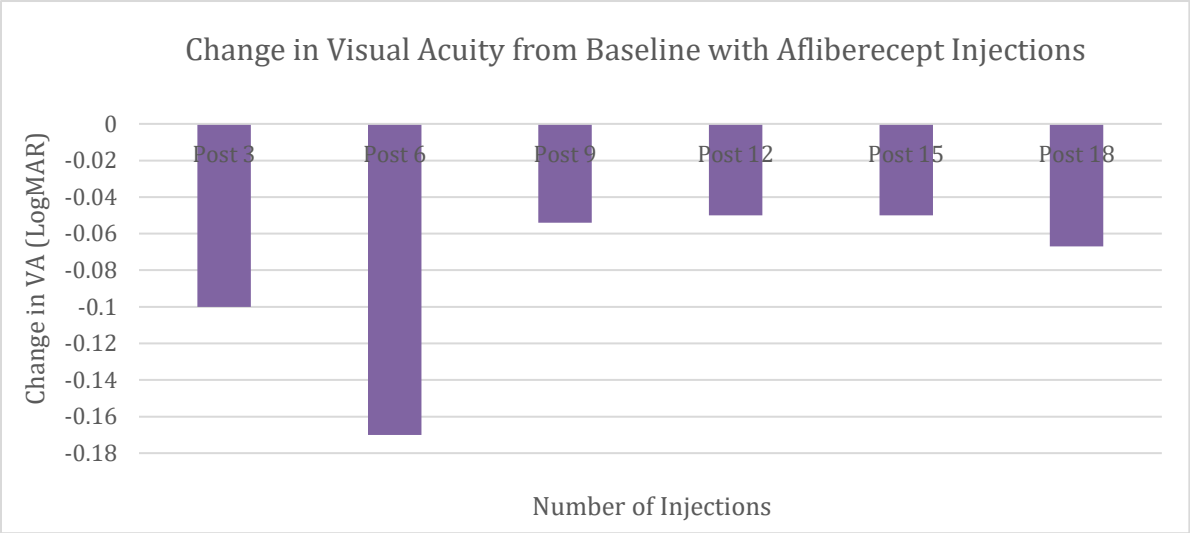


Table 3.4	Visual Acuity (logMAR) in Aflibercept Group				
Therapy	*Baseline*	*SD Baseline*	*Aflibercept*	*SD AFB*	*p-Value*
Post 3	0.539	0.321	0.439	0.299	**0.088***
Post 6	0.558	0.327	0.388	0.257	**0.021***
Post 9	0.646	0.353	0.592	0.331	0.680
Post 12	0.563	0.312	0.513	0.307	0.814
Post 15	0.450	0.257	0.400	0.331	0.703
Post 18	0.333	0.116	0.400	0.333	0.742





5.2 Intraocular Pressure

No significant change in the intraocular pressure (IOP) with anti-VEGF therapy was noticed. In general, no significant IOP differences were observed between bevacizumab, ranibizumab and aflibercept groups or their subgroups.

Table 4.1	**IOP in mmHg in Anti-VEGF Group**				
Therapy	*Baseline*	*SD Baseline*	*Anti-VEGF*	*SD-Anti-VEGF*	*p-value*
Post 3	15.7	2.9	15.8	3.0	0.647
Post 6	15.8	3.0	15.9	3.4	0.655
Post 9	15.8	2.8	15.8	2.8	0.853
Post 12	16.1	2.8	15.8	2.7	0.511
Post 15	15.8	2.5	15.6	2.1	0.779
Post 18	15.6	3.0	15.2	2.8	0.669

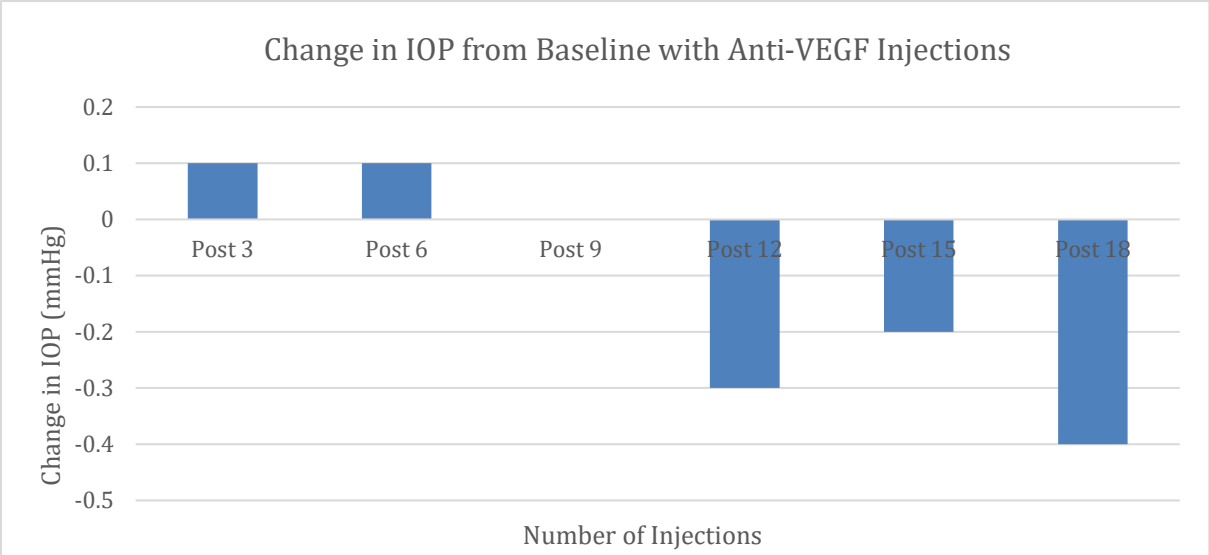


Table 4.2	Change in IOP (mmHg) in Bevacizumab Group				
Therapy	*Baseline*	*SD Baseline*	*Bevacizumab*	*SD BVZ*	*p-Value*
Post 3	15.4	2.6	15.6	2.9	1.00
Post 6	15.5	2.7	15.2	2.5	0.48
Post 9	15.2	2.7	15.1	2.6	0.84
Post 12	15.1	3.2	14.7	2.6	0.94
Post 15	16.3	3.4	14.3	1.4	0.20
Post 18	17.0	3.5	14.2	2.7	0.19

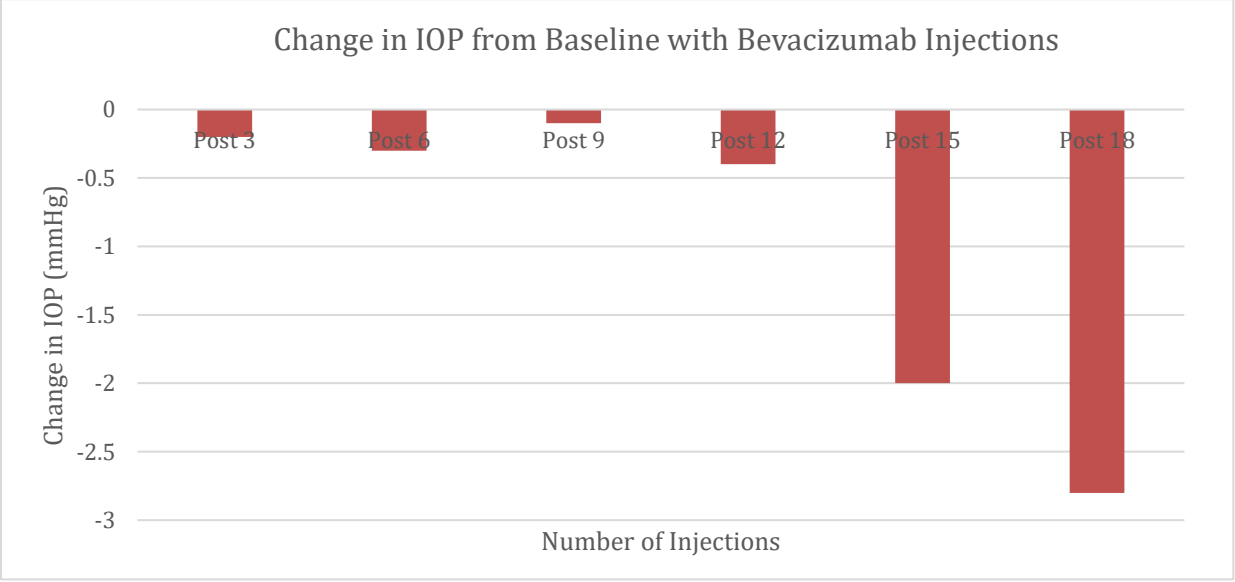


Table 4.3	Change in IOP (mmHg) in Ranibizumab Group				
Therapy	*Baseline*	*SD Baseline*	*Ranibizumab*	*SD RBZ*	*p-Value*
Post 3	16.1	3.0	16.2	3.0	0.75
Post 6	16.2	3.1	16.5	3.8	0.46
Post 9	16.3	2.7	16.4	2.7	0.96
Post 12	16.7	2.9	17.0	2.5	0.72
Post 15	15.5	2.4	16.1	2.2	0.32
Post 18	14.0	2.4	16.3	2.8	0.11

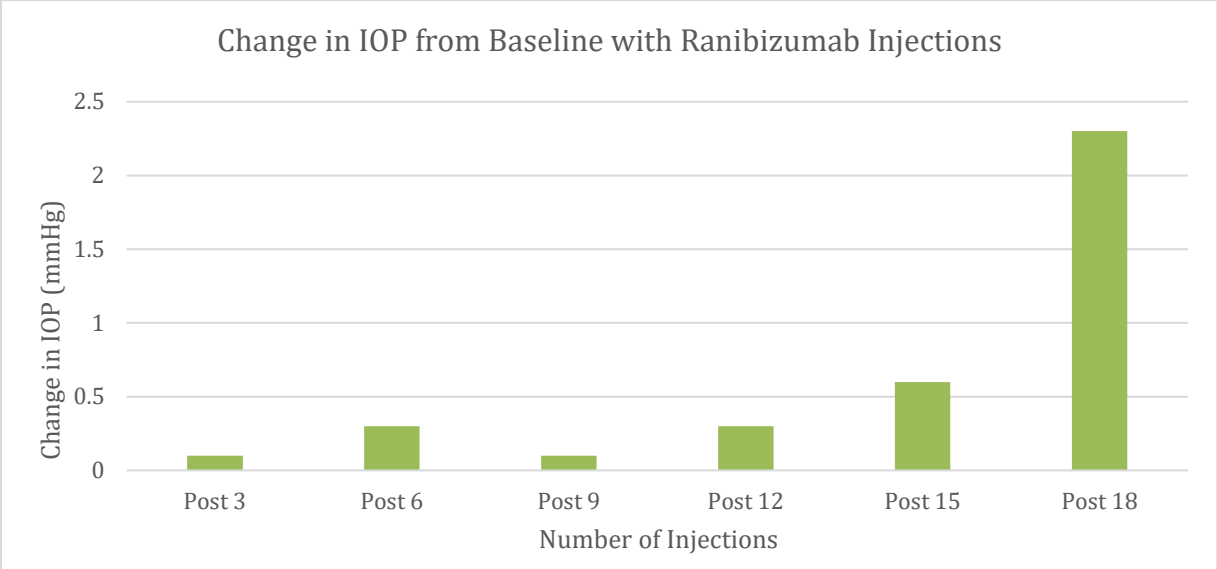
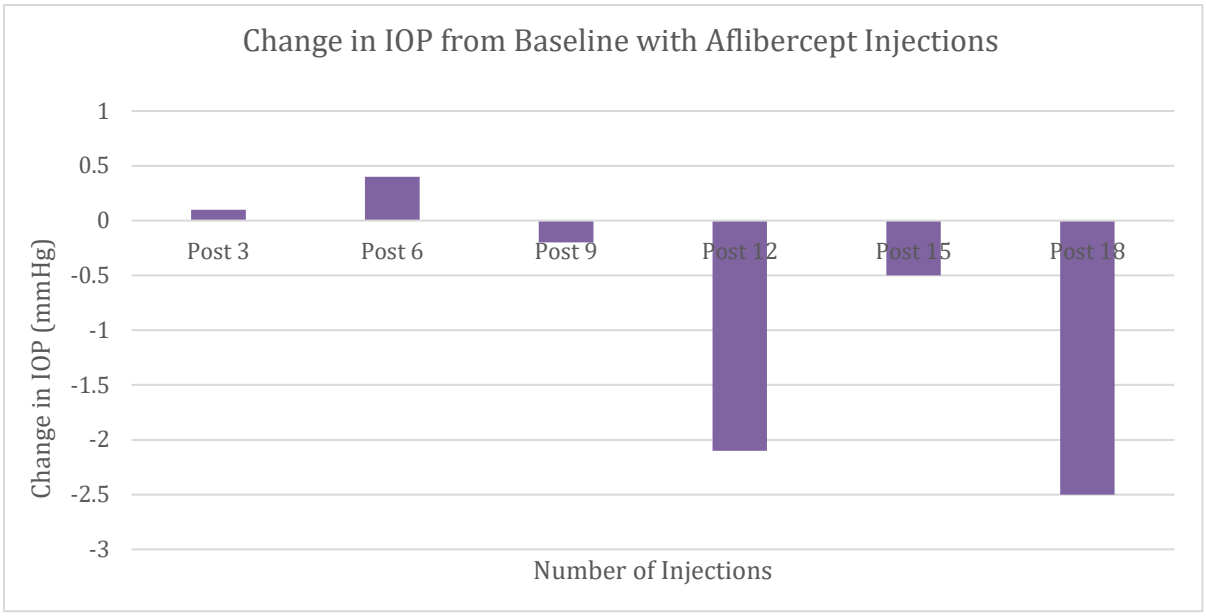


Table 4.4	Change in IOP in mmHg in Aflibercept Group				
Therapy	*Baseline*	*SD Baseline*	*Aflibercept*	*SD AFB*	*p-Value*
Post 3	15.3	3.6	15.4	3.1	0.83
Post 6	15.2	3.6	15.6	3.4	0.55
Post 9	15.5	4.1	15.3	4.1	0.79
Post 12	16.4	4.2	14.3	3.8	0.10
Post 15	16.0	5.1	15.5	4.6	0.70
Post 18	16.5	5.9	14.0	5.0	0.34



5.3 Central Retinal Thickness

Before starting with anti-VEGF therapy, an average central retinal thickness (CRT) of 389 ± 98µm was measured. A consistent and statistically significant reduction in CRT, ranging from -64 µm to -88 µm, among all patients receiving anti-VEGF was noticed.

Bevacizumab showed the highest CRT reduction, with a decrease of 119 µm, while the least reduction was observed with aflibercept. All three anti-VEGFs groups showed a significant reduction in macular edema with nine injections. Moreover, a significant decrease was still detected up to the 15th injection in patients treated with ranibizumab or bevacizumab. Although a significant change was no longer recorded with the post 18 subgroups, even though a reduction was still seen, this is attributed to the smaller sample sizes.

Table 5.1 CRT in µm in Anti-VEGF Injection Group

Therapy	*Baseline*	*SD Baseline*	*Anti-VEGF*	*SD Anti-VEGF*	*p-Value*
Post 3	386	96	307	74	**<0.001 *****
Post 6	388	97	324	93	**<0.001 *****
Post 9	399	103	323	84	**<0.001 *****
Post 12	394	95	329	86	**<0.001 *****
Post 15	370	78	283	43	**<0.001 *****
Post 18	380	79	303	77	**<0.01 ****

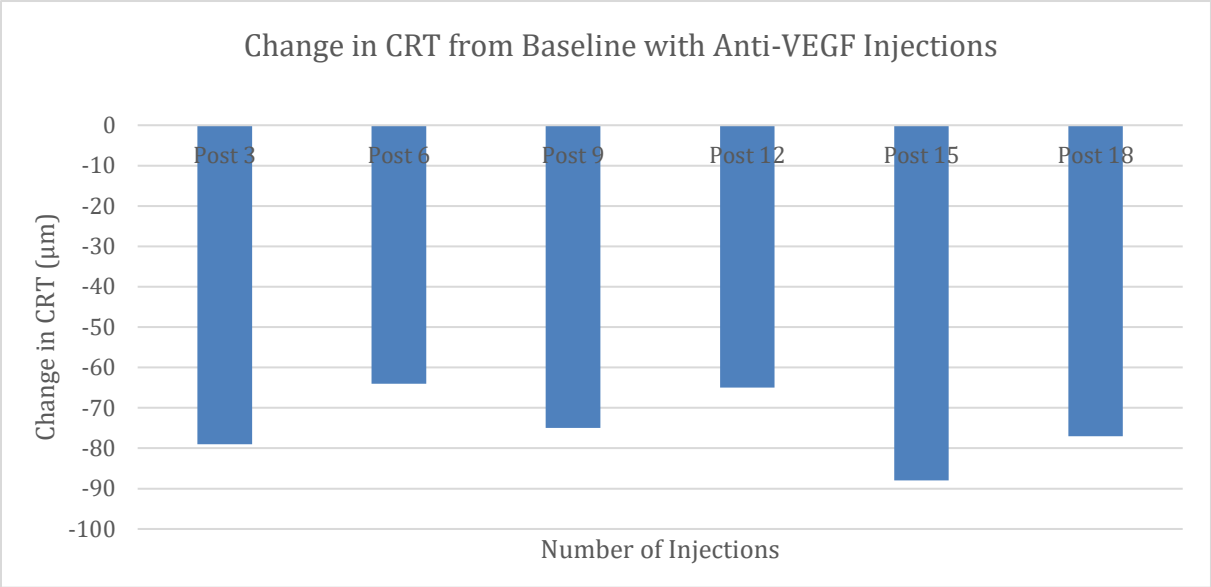


Table 5.2 CRT in µm in Bevacizumab Subgroup

Therapy	*Baseline*	*SD Baseline*	*Bevacizumab*	*SD BVZ*	*p-Value*
Post 3	397	102	307	73	**<0.001 *** **
Post 6	395	97	312	79	**<0.01 ** **
Post 9	419	108	306	56	**<0.01 ** **
Post 12	400	84	327	73	**0.04 ***
Post 15	397	107	277	47	**0.04 ***
Post 18	370	105	266	54	0.065

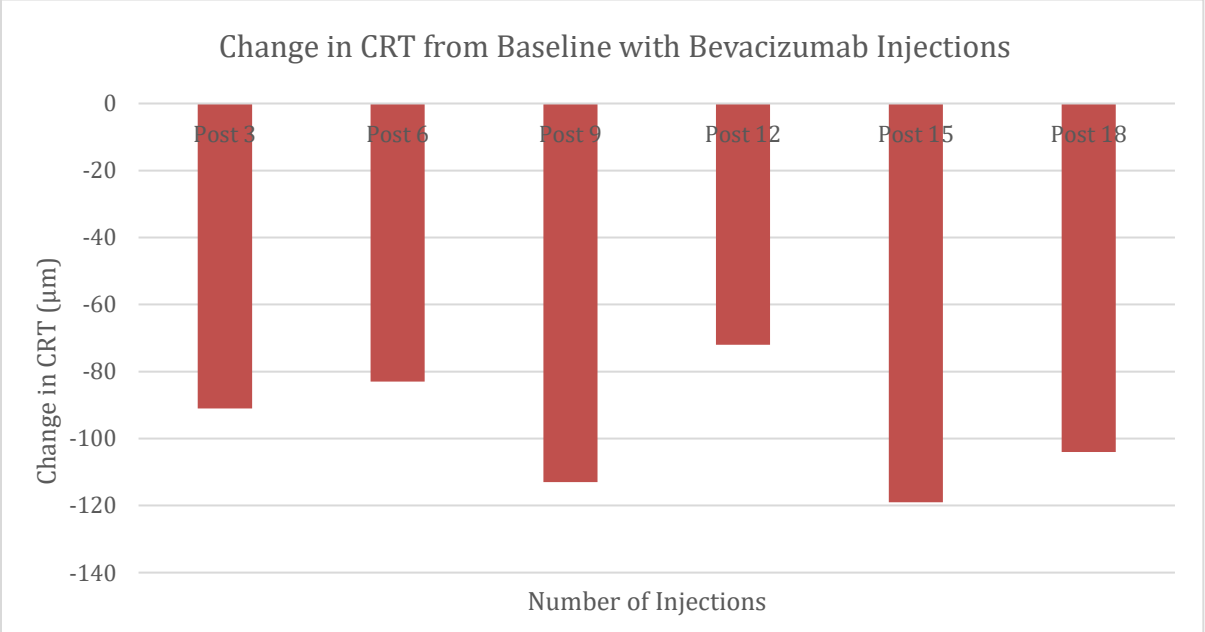


Table 5.3 CRT in µm in Ranibizumab Subgroup					
Therapy	*Baseline*	*SD Baseline*	*Ranibizumab*	*SD RBZ*	*p-Value*
Post 3	382	84	314	80	**<0.001 *** **
Post 6	391	94	338	95	**<0.01 ** **
Post 9	404	96	349	84	**<0.01 ** **
Post 12	407	98	332	77	**<0.01 ** **
Post 15	381	68	291	43	**<0.01 ** **
Post 18	415	65	359	76	0.166

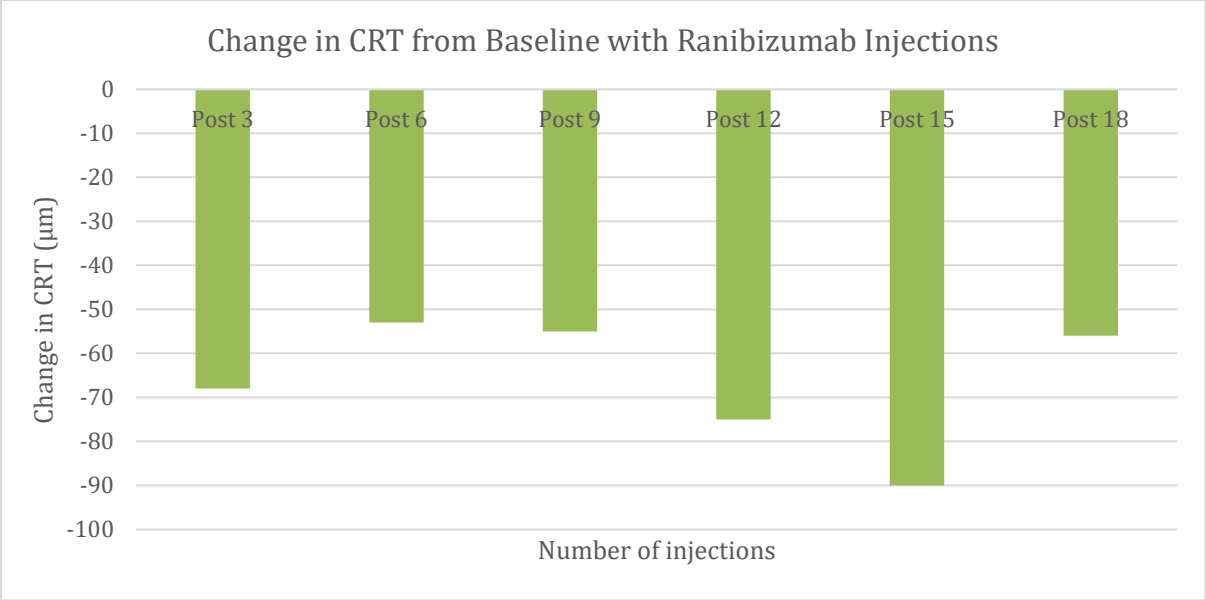
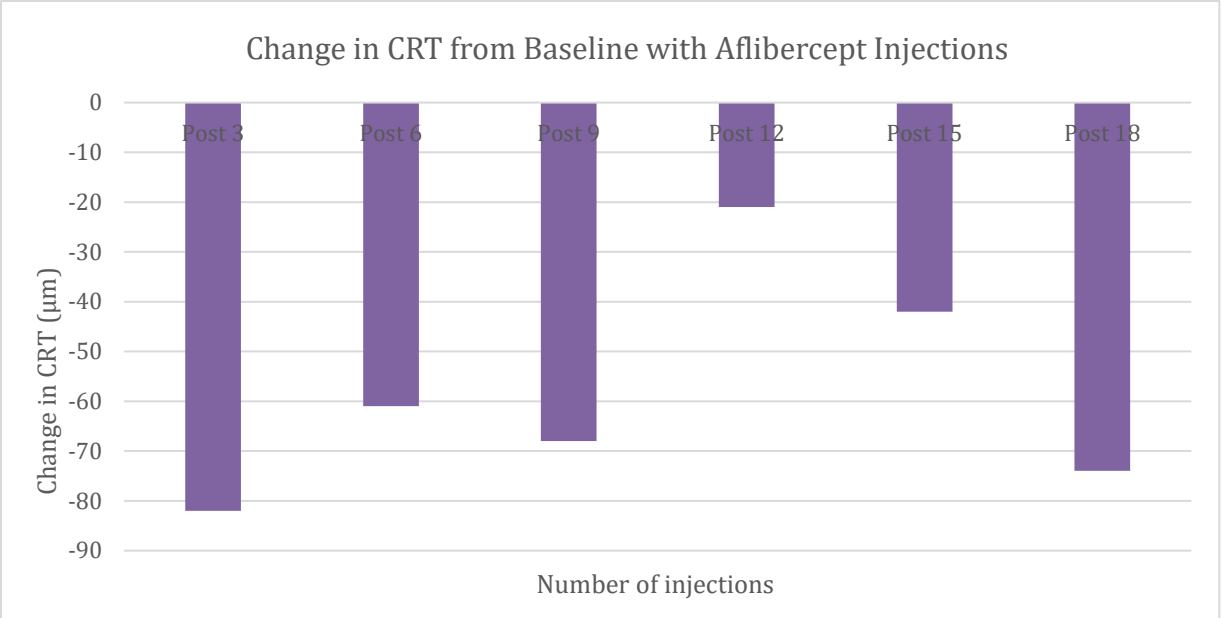


Table 5.4 CRT in µm in Aflibercept Subgroup					
Therapy	*Baseline*	*SD Baseline*	*Aflibercept*	*SD AFB*	*p-Value*
Post 3	375	100	293	70	**<0.001 *** **
Post 6	374	103	313	98	**<0.01 ** **
Post 9	354	100	286	96	**<0.01 ** **
Post 12	344	102	323	110	0.43
Post 15	309	108	267	82	0.143
Post 18	329	120	255	83	0.074



5.4 Total Macular Volume

An average total macular volume (TMV) of 9.0 mm^3 with anti-VEGF therapy was measured. A consistent and statistically significant reduction in the TMV (with a range between -0.55 and -0.75 mm^3), was observed.

The greatest TMV reduction was seen in the afliberecept group, while the least was in the ranibizumab group. The first six injections of the three anti-VEGF agents, bevacizumab, ranibizumab and aflibercept, showed a significant decrease of the TMV, after which further significant TMV reduction in only the afliberecpt group till the 15th injection was noted.

Table 6.1 TMV in mm^3 with anti-VEGF-Injections

Therapy	*Baseline*	*SD Baseline*	*Anti-VEGF*	*SD Anti-VEGF*	*p-Value*
Post 3	9.01	1.43	8.27	0.98	**<0.001 *****
Post 6	9.02	1.45	8.32	1.02	**<0.001 *****
Post 9	9.09	1.65	8.34	0.86	**<0.01 ****
Post 12	8.98	1.43	8.44	0.71	**<0.01 ****
Post 15	8.73	1.24	8.06	0.55	**<0.01 ****
Post 18	8.84	1.28	8.15	0.72	**0.05 ***

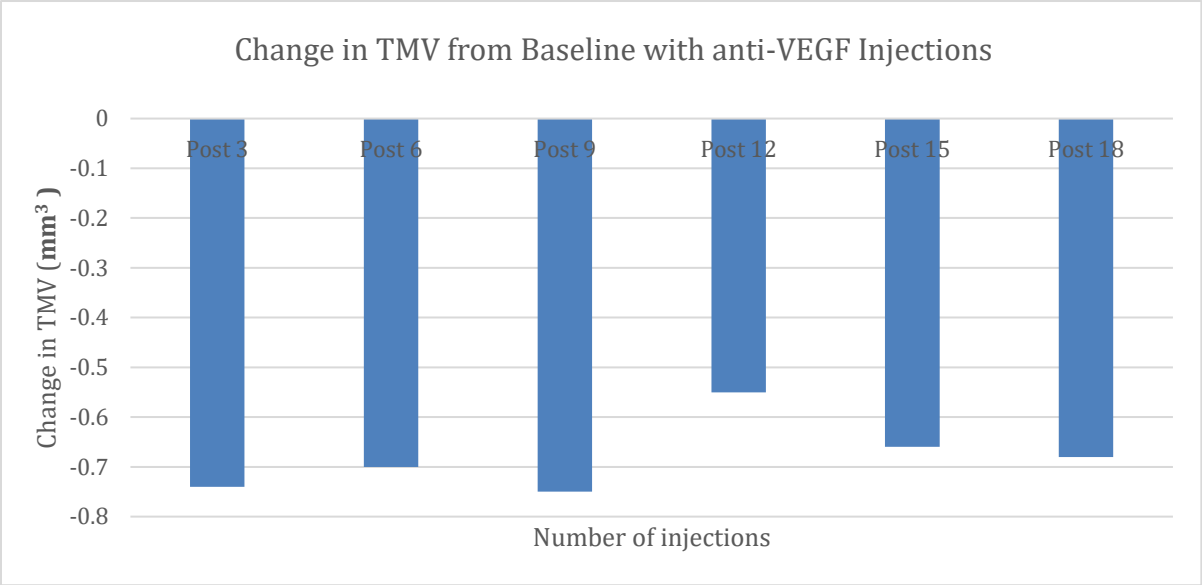


Table 6.2 TMV in mm^3 in Bevacizumab Group

Therapy	*Baseline*	*SD Baseline*	*Bevacizumab*	*SD BVZ*	*p-Value*
Post 3	9.16	1.77	8.16	1.11	**<0.01 ****
Post 6	9.19	1.84	8.33	0.73	**0.02***
Post 9	9.38	2.13	8.29	0.62	0.06
Post 12	9.02	1.28	8.45	0.46	0.34
Post 15	8.61	1.93	7.97	0.35	0.45
Post 18	8.58	1.99	8.02	0.46	0.67

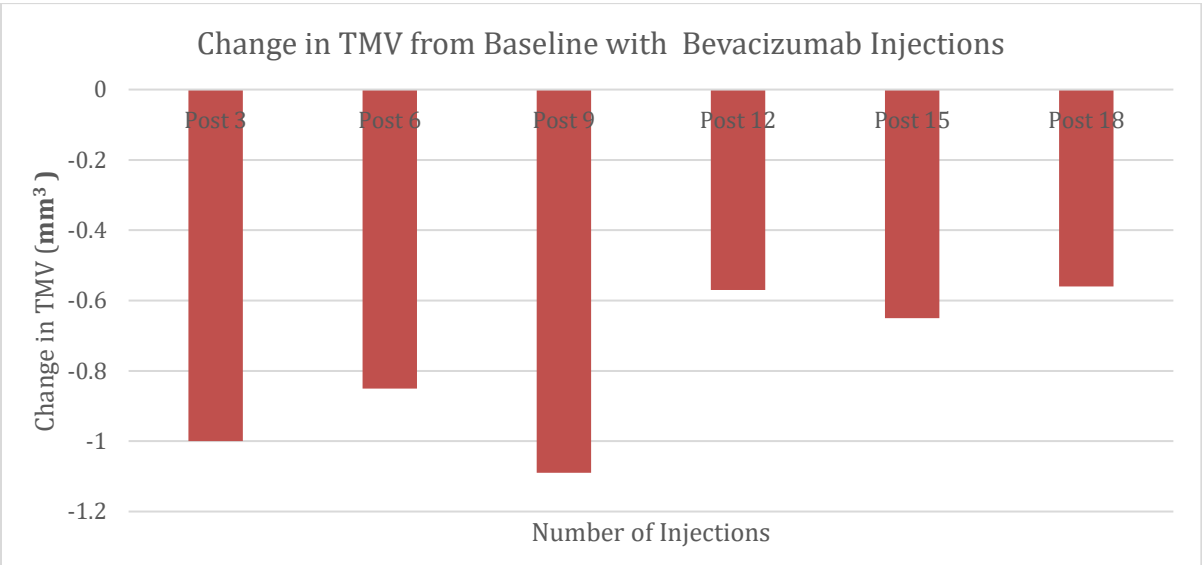


Table 6.3 TMV in mm^3 in Ranibizumab Group					
Therapy	*Baseline*	*SD Baseline*	*Ranibizumab*	*SD RBZ*	*p-Value*
Post 3	8.91	1.28	8.42	0.94	**<0.01 ****
Post 6	8.94	1.31	8.40	1.26	**<0.01 ****
Post 9	8.93	1.43	8.57	0.97	0.07
Post 12	8.86	1.51	8.56	0.75	0.30
Post 15	8.83	1.13	8.23	0.54	0.03
Post 18	9.32	0.67	8.58	0.67	0.10

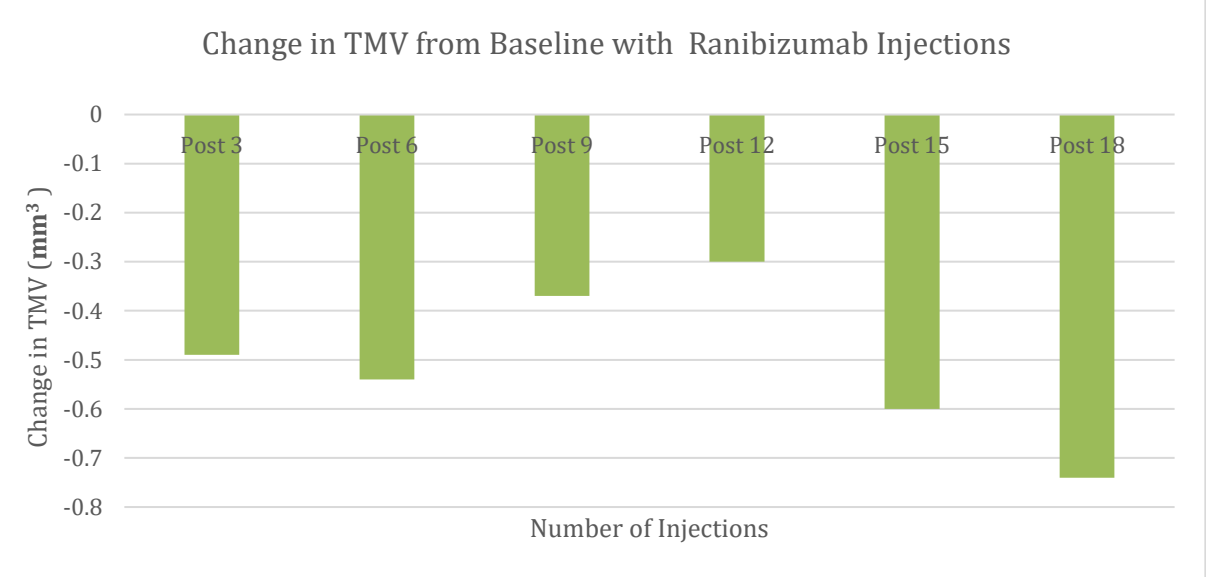
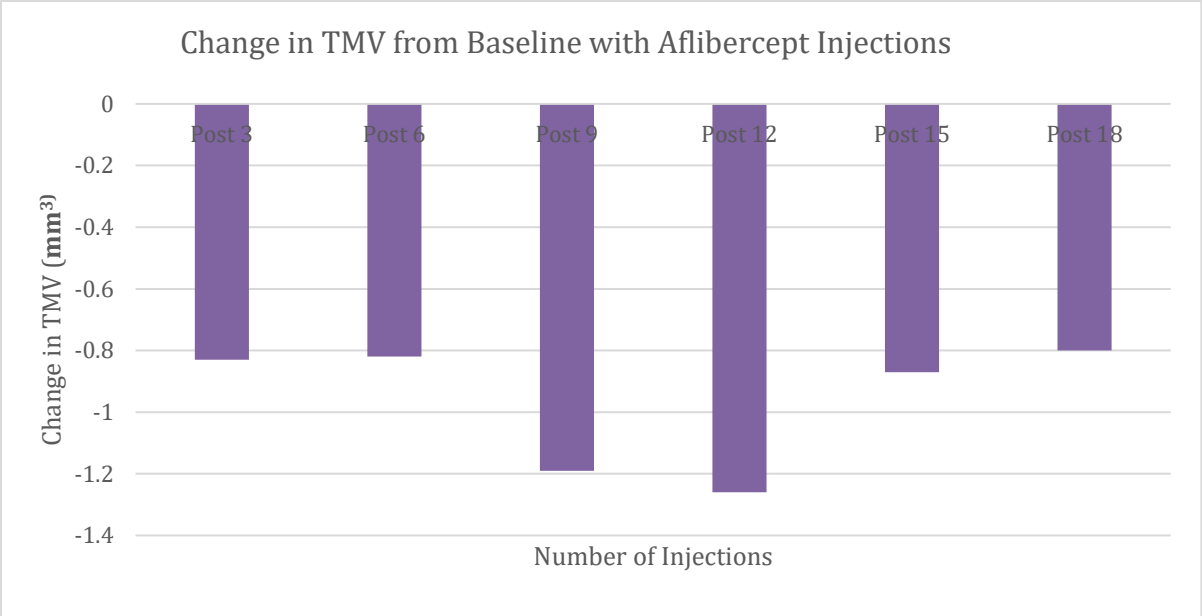


Table 6.4 TMV in mm^3 in Aflibercept Group					
Therapy	*Baseline*	*SD Baseline*	*Aflibercept*	*SD AFB*	*p-Value*
Post 3	8.98	1.60	8.15	1.38	**<0.001 *****
Post 6	8.94	1.46	8.12	1.46	**<0.001 *****
Post 9	9.03	1.63	7.85	1.63	**<0.001 *****
Post 12	9.31	1.98	8.04	1.98	**0.03 ***
Post 15	8.57	2.42	7.70	2.24	**0.01 ***
Post 18	8.31	2.62	7.51	2.62	0.11



5.5 Retinal Fiber Layer Thickness

An average retinal fiber layer thickness (RNFLT) of 51.9 µm was measured with anti-VEGF therapy. A significant RNFLT reduction was noticed in the post 3 anti-VEGF injection, while a significant increase in the RNFLT was observed after the 15th anti-VEGF injection.
Ranibizumab group was associated the highest RNFLT reduction of -1.4 µm after the 3rd injection, while a significant increase of +1.67 µm was noticed after the 15th bevacizumab injection. There was no significant change in the RNFLT with aflibercept.

Table 7.1 RNFLT in µm with Anti-VEGF-Injections

Therapy	*Baseline*	*SD Baseline*	*Anti-VEGF*	*SD Anti-VEGF*	*p-Value*
Post 3	51.86	11.82	51.22	11.11	**0.038 ***
Post 6	51.87	11.93	51.58	11.95	0.342
Post 9	51.80	12.66	51.35	12.48	0.426
Post 12	51.60	12.28	52.47	13.36	0.255
Post 15	47.81	10.69	49.42	12.50	**0.030***
Post 18	47.00	6.00	47.79	4.68	0.499

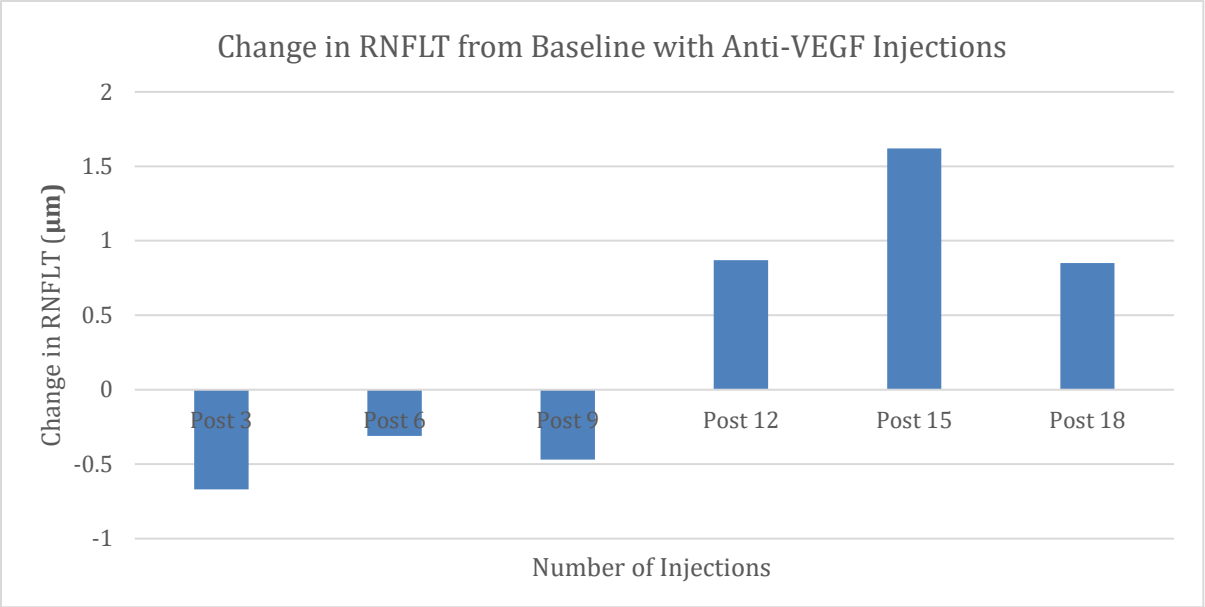


Table 7.2 RNFLT in µm in Bevacizumab Group

Therapy	*Baseline*	*SD Baseline*	*Bevacizumab*	*SD BVZ*	*p-Value*
Post 3	51.17	9.69	51.05	9.01	0.41
Post 6	50.92	10.40	51.11	10.01	0.53
Post 9	52.96	10.80	52.74	9.72	0.71
Post 12	50.13	8.78	51.13	8.53	**0.05***
Post 15	40.50	4.46	42.17	4.79	0.30
Post 18	44.20	7.66	45.60	6.62	0.51

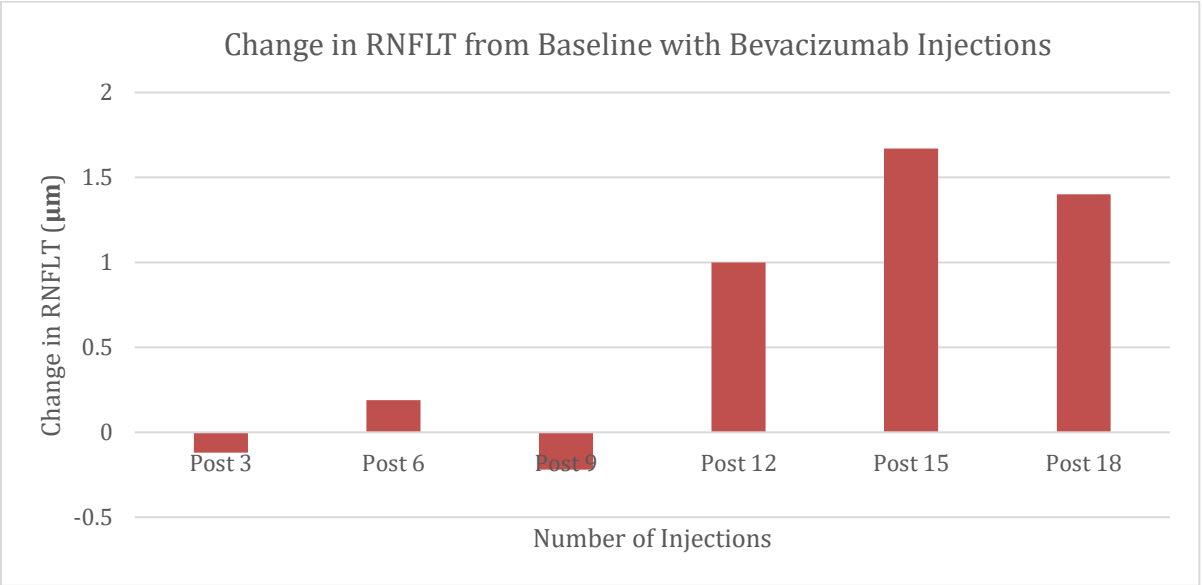


Table 7.3 RNFLT in µm in Ranibizumab Group					
Therapy	*Baseline*	*SD Baseline*	*Ranibizumab*	*SD RBZ*	*p-Value*
Post 3	54.21	12.42	52.81	11.77	**0.03***
Post 6	54.43	12.73	53.76	12.71	0.28
Post 9	53.50	12.07	52.62	12.03	0.42
Post 12	53.71	13.84	54.88	15.93	0.41
Post 15	51.33	12.53	53.47	14.99	0.07
Post 18	49.17	5.42	49.83	3.19	0.78

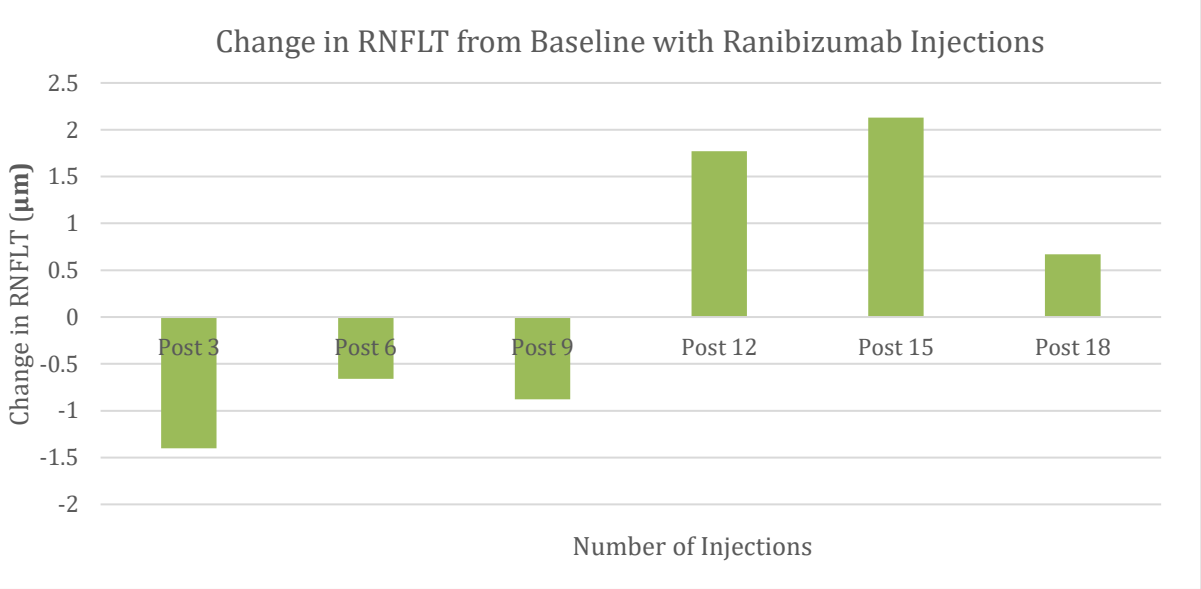
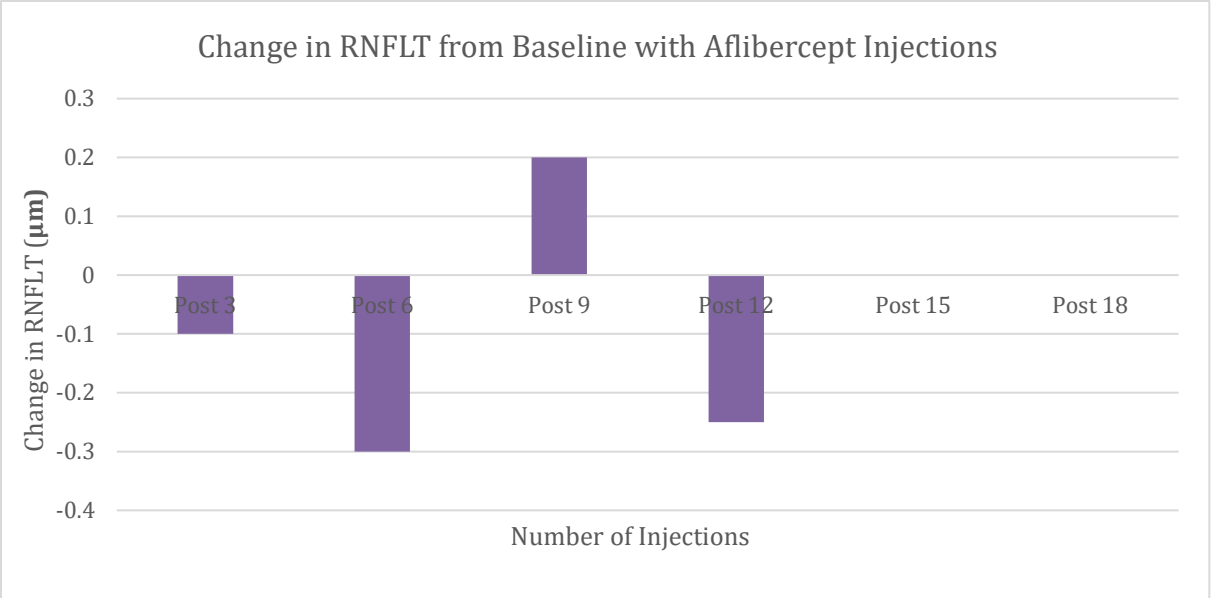


Table 7.4 RNFLT in µm in Aflibercept Group					
Therapy	*Baseline*	*SD Baseline*	*Aflibercept*	*SD AFB*	*p-Value*
Post 3	48.66	9.40	48.55	9.34	0.77
Post 6	48.04	9.39	47.74	9.30	0.47
Post 9	45.73	9.80	45.93	9.84	0.72
Post 12	48.00	11.64	47.75	11.35	0.75
Post 15	46.00	13.14	46.00	13.06	1.00
Post 18	47.33	13.68	47.33	13.85	1.00



5.6 Head-to-Head Comparison

In this section, a head-to-head comparison between the three anti-VEGFs bevacizumab, ranibizumab and aflibercept in terms of their effect on visual acuity, intraocular pressure, central retinal thickness (CRT), total macular volume (TMV) and retinal nerve fiber layer thickness (RNFLT) is discussed.

4.6.1 Bevacizumab and Ranibizumab

When it comes to visual acuity, subgroups of both agents showed no statistically significant improvement, except for the post 3 bevacizumab subgroup. As a general comparison, patients that received bevacizumab injections tended to profit more in terms of visual acuity than those that received ranibizumab in the post 3, post 6 and post 9 subgroups. It is worthy to note that for these subgroups, the baseline visual acuity for the bevacizumab subgroups was worse than that of ranibizumab. On the contrary, the ranibizumab post 15 and the post 18 subgroups were associated with a better outcome in terms of visual acuity than those of bevacizumab.

Regarding intraocular pressure, no significant increase or decrease was detected with either agent.

Bevacizumab subgroups had slightly higher baseline CRT values compared to those of ranibizumab. A statistically significant CRT reduction was observed with both agents up to the 15th injection, with the bevacizumab subgroups showing a greater decrease than those of

ranibizumab. Even though both agents elicited further CRT reductions, they were found to be statistically non-significant, most likely due to the small sample sizes.

Similar findings were noticed regarding the TMV, in which bevacizumab generally showed a greater decline in TMV than ranibizumab. However, a significant TMV decrease was witnessed only in the post 3 and post 6 subgroups of both agents.

The measured RNFLT baseline values were higher for ranibizumab. In general, both agents tended to cause a decrease in the RNFLT up to the 9th injection, after which an increase was noted with increasing number of injections. However, both bevacizumab and ranibizumab showed no significant change in RNFLT, except for two subgroups. A significant decrease was associated with the post 3 ranibizumab subgroup, and a significant increase in the RNFLT was observed after the 12th injection of bevacizumab.

4.6.2 Bevacizumab and Aflibercept

Both above-mentioned anti-VEGF agents showed a significant improvement in the visual acuity after the 3rd injection, which further significantly increased only with the aflibercept till after the 6th injection. Moreover, aflibercept subgroups generally showed a better visual acuity gain than those of bevacizumab.

There was no significant change in intraocular pressure noticed in any of the subgroups of both agents.

Bevacizumab, as well as aflibercept, showed a significant reduction in the CRT up to the 9th injection, after which, although a reduction could be observed with both treatments, it was only statistically significant till the 12th bevacizumab injection. Generally, a greater reduction in CRT was observed with bevacizumab than aflibercept.

However, aflibercept showed a larger reduction in TMV than Bevacizumab. Both agents caused a significant decrease in TMV up to the 6th injection, after which a significant reduction up to the 15th aflibercept injection was seen.

When talking about RNFLT, both bevacizumab and aflibercept did not show a significant change, except for one subgroup, where bevacizumab showed increase in the RNFLT.

4.6.3 Ranibizumab and Aflibercept

Regarding the visual acuity, only the aflibercept subgroups post 3 and post 6 showed a significant increase, but no ranibizumab subgroup showed a significant improvement. Additionally, treatment with aflibercept showed a larger gain in visual acuity than ranibizumab.

A significant intraocular pressure was not observed in any of the subgroups and both anti-VEGF therapies.
Even though all ranibizumab and aflibercept subgroups showed a reduction in CRT, a significant decrease was seen till the 15th ranbizumab injection and 9th aflibercept injection. Aflibercept post 3, post 6 and post 9 subgroups were associated with a slightly greater decrease in CRT than that seen with the corresponding ranibizumab subgroups.

On the other hand, all aflibercept subgroups showed a greater reduction in TMV than those of ranibizumab. Even though all subgroups of both agents showed a reduction in TMV, a statistically significant decrease was seen till the 15th aflibercept injection, but only till the 6th ranibizumab injection.

RNFLT was not significantly changed by aflibercept and a slight decrease was observed till the 6th injection. On the other hand, one ranibizumab subgroup, the post 3, showed a statistically significant decrease in RNFLT. A slight decrease was further seen till the 9th injection, after which a slight increase was observed with increasing number of ranibizumab injections.

5.7 OCT Morphological Changes

5.7.1 Atrophy

It was noticed that the incidence of macular atrophy increased with increasing treatment duration and number of anti-VEGF injections. However, it is not clear if this deterioration is caused by the anti-VEGF treatment or due to the progression of the AMD.

5.7.2 Fibrosis

Anti-VEGF treatment did not hinder or induce fibrosis.

5.7.3 Pigment Epithelium Detachment (PED)

The anti-VEGF treatment did not cause or prevent PED development.

6. Discussion

6.1 General

A sample of 120 patients diagnosed with neovascular AMD and treated with one of three different anti-VEGF agents, were included in this study. Visual acuity and intraocular pressure were regularly tested. CRT and TMV were used as markers for the macular edema and RNFLT as marker for the integrity of the retinal nerve fibers. The results of this study showed that the visual acuity significantly improved after 3 injections of anti-VEGF agents by 0.06 logMAR, however, with further injections the visual acuity stabilized compared to the baseline visual acuity. Therefore, only an initial improvement in visual acuity was noticed in this study, which was not consistent with the outcomes of the randomized controlled trials (ANCHOR, MARINA, CATT, IVAN). Furthermore, it was observed that the change in visual acuity depended on the baseline visual acuity; patients with lower visual acuity experienced a larger improvement under anti-VEGF therapy, while patients with a better baseline visual acuity gained less in terms of visual acuity.

Regarding the change in intraocular pressure with anti-VEGF agents, this study showed no notable long-term change in IOP, even with high numbers of injections. This contradicts the findings of other studies, such as that of Good *et al.,* 2011, which has showed an increased risk of sustained high intraocular pressure after treatment with anti-VEGF agents, even leading to medical or laser treatment.

Macular edema was clearly reduced, throughout the treatment, which can be seen with the significant decrease in CRT and TMV. These results stratifies the effectiveness of anti-VEGF agents, where a morphological improvement was observed in this study, even after 18 injection, with no resistance to treatment. This leads to the conclusion, that anti-VEGF is a potent treatment of exudative AMD.

When discussing the effect of anti-VEGF agents on the RNFLT, a significant decrease from the baseline was observed after the third injection, while a significant increase was seen after the fifteenth injection. When further looking at the effect of individual anti-VEGF agents, it

clearly shows that a significant decrease was seen only after the third ranibizumab injection, whereas this was not the case with the other agents.

6.2 Visual Acuity

As mentioned earlier, the visual acuity showed only slight and not significant improvement in most of the subgroups, while some aflibercept subgroups showed significant improvement in terms of visual acuity. However, Van Asten *et al.*, (2018), mentioned that in wet AMD treatment, both AFB and RBZ are associated with comparable efficacies with fewer injections for best-corrected visual acuity (BCVA) over two years. While BVZ and RBZ are associated with equivalent efficacy for BCVA.

Rasmussen *et al.*,(2017), compared the efficacy between 559 wet AMD patients with intravitreal ranibizumab (RBZ) treatment in 2011–2012 and 468 patients with aflibercept (AFB) in 2013–2014, starting with three injections as a fixed loading dose followed by pro re nata (PRN) regimen. For RBZ and AFB, a significant increase in BCVA was observed between baseline and after one year of treatment with a 15% less number of injections given within the first year for aflibercept compared to the number of injections for ranibizumab. Moreover, in the United States Lotery *et al.*, (2017), confirmed the comparable efficacy of RBZ and AFB, among (3350) ranibizumab and (4300) aflibercept therapies for 12 months. The mean change in the VA score was 0.30 for RBZ and 0.19 for AFB. The mean injections number for ranibizumab was 6.70 and for aflibercept was 7.00.

Furthermore, Chakravarthy *et al.*, (2019), revealed from their 24-month, retrospective, nonrandomized, comparative, matched cohort study, that patients switched from RBZ to AFB resulted in no VA differences compared to patients that remained on RBZ only. Brown *et al.*, (2017), mentioned that no head-to-head comparison between bevacizumab and aflibercept had not been established yet.

6.3 Intraocular Pressure

In this study, no significant change in intraocular pressure was noticed, which was consistent with most of the literature. Where Sengul *et al.*, (2016), studied the effect of RBZ among 168 patients with wet AMD, the results showed no significant difference between the mean IOP of the injected eyes.

However, some studies reported an increase in the IOP as a temporary side effect of anti-VEGF agents (Good, Kimura, Mandava, & Kahook, 2010; Puerto, Juan, & Rebolleda, 2019).

6.4 Macular Edema

The macular edema reduction induced by anti-VEGF treatment was clear in this study, which was evident by reduction in CRT and TMV where Bevacizumab showed the greatest reduction in CRT while Aflibercept showed the greatest TMV reduction among the used agents.

A recent meta-analysis by Nguyen *et al.*, (2018), reported that seven previous trials among 2825 AMD patients assessed the CMT mean change at 12-months follow-up and the results suggested that ranibizumab is better in reducing CMT with no heterogeneity. Three studies including 1538 patients reported the mean change in CMT at 24-months follow-up confirmed no significant variance between bevacizumab and ranibizumab therapies. Other 2 trials including 2412 patients on aflibercept and ranibizumab treatments, confirmed comparable improvements in BCVA as well as a comparable reduction in CMT at 1-year, TMV is much less common than CRT as a parameter for assessing therapeutic success so that only a few studies are available.

Ma et al., (2015), studied the efficacy of 1.25 mg of Bevacizumab in nAMD patients. BVCA, TMV and CRT where assessed before and after the injections. Patients were selected from the intravitreal injection clinic. The results showed a significant decrease in the logMAR, in addition to decrease in TMV and CRT.

6.5 Retinal Nerve Fiber Layer Thickness

Treatment with anti-VEGF agents did not significantly decrease the RNFLT, except for one RBZ subgroup

These results were consistent. Kim *et al.*, (2019), retrospective study that included 50 eyes were treated with aflibercept, and 40 with ranibizumab, the results demonstrated that retinal nerve fiber layer thickness did not significantly differ among study groups. However, Soheilian *et al.*, (2017), observed a significant change in RNFL after 3 months of treatment with bevacizumab. While according to Enders & Altay, 2017, a moderate reduction in the RNFLT was noticed after the treatment with anti-VEGF agents.

7. Limitations

As in all retrospective studies, especially those held in a clinical setting, this work also faced some limitations. One of the most important is the patient adherence to the treatment plan, thus receiving the injections as scheduled. The general health state, age as well as the comorbidities of the patients involved in this study played an important role in patient non-compliance. In fact the patients who were adherent to the treatment regime and those with better baseline factors, had better visual and morphological outcomes.

Another limitation faced was the decreasing number of participants with increasing numbers of injections, so that a statistically significant change was hard to elicit. A decreasing sample size is expected with increasing number of injections, since either a switch to another anti-VEGF agent was made, or the therapy was aborted.

Varying number of patients receiving each of the three anti-VEGF agents led to different sample sizes. In Germany, the off-label intravitreal use of bevacizumab is not covered by all public health insurances, while some allow it under certain circumstances.

Moreover, patients treated following both regimens, PRN and TnE, were included in this study, without examining in detail the used treatment regimens and the exact treatment periods. It is challenging to evaluate the long-term outcomes in participants treated following the PRN regime, especially that the number of injections is not related to the therapy duration.

8. Additional Studies

The effect of newer anti-VEGF medications, such as brolucizumab, on the CRT, TMV and RNFLT, as well as on the improvement in visual acuity, and it's comparison with the older anti-VEGF agents, should be the focus of new studies. This can aid in optimizing the treatment of exudative AMD.

Treatment regimens play an important role on the effect anti-VEGF agents. In this study, patients treated according to both Pro Re Nata and Treat and Extend regimens were included. A study, in which the effect of different anti-VEGFs following only a single regimen is examined, is needed to better investigate the outcomes of the various agents.

It is a well-known fact that glaucoma causes a decrease in the RNFLT, and so it is worthy to investigate the effect of anti-VEGF on the RNFLT in glaucoma patients, since patients suffering from glaucoma were excluded from this study.

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10. Attachment

10.1 List of Figures

10.2 List of Tables