Université de Montréal

Risk of elevated intraocular pressure after ranibizumab injection in patients with neovascular age-related macular degeneration

par

Solmaz Moghadaszadeh

Département de pharmacologie

Université de Montréal

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Solmaz Moghadaszadeh, Août 2016

Résumé

Risque d'élévation de la pression intraoculaire après des injections de ranibizumab chez des patients atteints de dégénérescence maculaire liée à l'âge de type néovasculaire.

Mots-clés : pression intraoculaire, ranibizumab, dégénérescence maculaire liée à l'âge

Objectif : Nous avons voulu évaluer le risque d'élévation chronique de la pression intraoculaire suite à des injections de ranibizumab dans le traitement de la dégénérescence maculaire liée à l'âge de type néovasculaire.

Méthode : Nous avons réalisé une étude rétrospective sur 161 patients ayant reçu des injections de ranibizumab dans un œil seulement. Les critères excluent les patients ayant du glaucome non contrôlé au départ (PIO>21mmHg) et ceux qui ont eu moins de 9 semaines de suivi après l'injection. L'élévation de la PIO est définie comme une augmentation de >5mmHg sur deux visites consécutives.

Résultats : Nous n'avons pas déterminé de différence de pourcentage entre les yeux ayant reçus des injections pour lesquels il y a une élévation de la PIO (n=8.5%), comparé au pourcentage de yeux n'ayant pas reçus d'injections pour lesquels il y a une élévation de la PIO (n=9.6%). Cependant, un plus grand nombre d'injections d'anti-VEGF est associé avec une élévation chronique de la PIO (P=0.032). D'autres facteurs de risque de l'élévation chronique de la PIO sont le diabète, une PIO faible au départ, et une PIO maximale plus élevée (P<0.05).

Conclusion : Un plus grand nombre d'injections semble augmenter le risque d'élévation de la PIO. Les patients atteints de diabète semblent être plus à risque et nécessiter une étroite surveillance.

Abstract

Risk of elevated intraocular pressure after ranibizumab injection in patients with neovascular age-related macular degeneration

Keywords: intraocular pressure, ranibizumab, age-related macular degeneration

Purpose: Conflicting evidence exists about the risk of chronic elevation of intraocular pressure (IOP) after ranibizumab injections for neovascular age-related macular degeneration. The goal of this study is to evaluate this risk.

Methods: A retrospective cohort study of 161 people. Inclusion criteria included receiving at least three ranibizumab injections in one eye only and having at least 9 weeks of follow-up. Exclusion criteria included the presence of uncontrolled glaucoma or ocular hypertension at baseline (IOP>=21mmHg). Chronic IOP elevation was defined as an increase >5mmHg of IOP on at least 2 consecutive visits.

Results: There was no difference in the percentage of injected eyes that experienced a chronic IOP increase (n=8, 5%) compared to the percentage of uninjected eyes that experienced an IOP increase (n=9, 6%). However, a greater number of anti-VEGF injections was associated with chronic IOP elevation (P=0.032). Other risk factors for chronic IOP elevation included diabetes, a lower baseline IOP, and a higher maximum IOP (P<0.05).

Conclusions: A greater number of injections appears to increase the risk of chronic IOP elevation. Also, diabetics appear to be more at risk and may need more careful follow-up or preventive pharmacological treatment.

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AMD	Age-Related Macular Degeneration
AREDS	Age-Related Eye Disease Study
ARMS	Age-Related Maculopathy Susceptibility
CFH	Complement Factor H
CNV	Choroidal Neovascularisation
FDA	Food and Drug Administration
GA	Geographic Atrophy
HTO	Ocular hypertension
IOP	Intraocular pressure
OR	Odds ratio
n	Number of subjects
SD	Standard Deviation
PDT	Photodynamic Therapy
ROS	Reactive Oxygen Species
RPE	Retinal Pigment Epithelium
TA	Triamcinolone acetonide
VEGF	Anti-Vascular Endothelial Growth Factor

To my beloved family

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CHAPTER 1. Age-related macular degeneration (AMD)

1.1 Prevalence

The prevalence of any age-related macular degeneration (AMD) is 6.5% and 0.8% for the late stage according to the 2005-2008 cohort of the National Health and Nutrition Examination Survey, based on a civilian and noninstitutionalized US population older than 40 years[1]. AMD is an important cause of irreversible visual impairment in the United States and Canada as well as in developed countries where it causes 50% of blindness in general[2]. AMD is the first leading cause of blindness in the United States, responsible of the blindness of 54.4% of the white persons in 2000[3] and in Canada, with 59.6% of blind persons age 40 years and older due to this disease in 2006[4].

1.2. Disease course

AMD is a degenerative disorder affecting the macula of the retina, the center, responsible for visual acuity and color vision[5]. The appearance of drusen is an early sign of the disease, characterized by yellowish extracellular deposits of proteins and lipids within and beneath the retinal pigment epithelium (RPE) of the central retina[6]. There are two forms of the late stage of AMD. Dry AMD (85% of AMD cases) is the geographic atrophy form (apoptosis of the RPE, loss of photoreceptors and choriocapillaris)[5] and causes a permanent central vision loss for which there is no effective treatment[7]. Wet neovascular AMD (15% of AMD cases)[6] leads to a choroidal neovascularisation (CNV), which is the growth of new blood vessels beneath and

within the retina[8]. Destroying Bruch's membrane and the RPE layer to the subretinal space, the pathologic neovascularization destroys the photoreceptors and causes vision damage[7].

In patients with late stage AMD, there is an increased lipofuscin accumulation in RPE cells[9]. Lipofuscin is a photoinductible generator of reactive oxygen species (ROS) that cause oxidative stress. Its accumulation in lysosomes reduces the autophagocytosis of RPE cells essential to their homeostasis and their phagocytic capacity, decreasing the capacity to remove damaged cellular proteins during ageing, resulting in RPE cell damage[10].

Oxidative stress with damage to the function of RPE induces chronic inflammation, and is involved in the pathogenesis of both forms of AMD[11].

1.3. Risk factors

AMD has a complex aetiology which is influenced by a combination of multiple genetic susceptibility factors and environmental components.

Genetic variation is the major risk factor of the disease with 71% of variation in the severity of disease[12]. Risk alleles include mostly complement factor H (CFH), complement factor I and age-related maculopathy susceptibility 2 (ARMS2), but also factor B, complement components 2 and 3, and newly associated complement component 9[13-15]. Age is an important risk factor of AMD as well. Depending on the risk factors, they influence the early or late AMD incidence. For age, CFH and ARMS polymorphisms, they are associated with both[15-17] .

For the early stage of the disease, the prevalence of AMD is 8% for patients age 43 to 54 years and 30% for those age 75 years or older in the population-based Beaver Dam Eye Study in the United States[18] and a higher risk is observed in women[19] .

For the late stage of the disease, the prevalence of AMD is 0.1% for patients age 43 to 54 years and 7.1% for those age 75 years or older[18] .

The increased risk of the progression of AMD is associated with current smoking and greater number of pack-years smoked[20] . Hypertension and cataract surgery are also associated with late AMD[15-17] . As for the ethnicity, AMD is more common in whites than blacks[17] or than Asians but only for the late AMD, as the prevalence is equal for early AMD[21].

The results of studies indicate a higher risk of AMD in people with light-coloured irises, cardiovascular disease and increased sunlight exposure[18]. Data for hypercholesterolaemia[10] are conflicting, as it is for cardiovascular risk factors including hypertension, body mass index, and atherosclerosis[22]. The risk of incident neovascular AMD has been shown to be 2 to 3 times higher in persons using long-term low-dose aspirin[23].

1.4. Prevention of the progression of early AMD to late AMD

Physical activity[24] or nutritional supplementation[2] are known to slow progression of AMD to CNV or central GA, combining antioxidant vitamins C and E, beta-carotene and zinc[25] as recommended by the AREDS (Age-Related Eye Disease Study)[26]. Certain contraindications exist such as for smokers for whom β-carotene is harmful. Treatment modifications may be recommended to decrease their dosages, or according to recent data, replacing it with omega-3 fatty acids, lutein and zeaxanthin[26] to have a comparable efficacy. Some studies have examined the interactions between risk factors. For example, it has been shown that there are no benefits of nutritional supplementation for patients at risk of AMD because of variations in their genotypes at the CFH and ARMS2[27].

Anecortave acetate (RETAANE, Alcon Laboratories, Inc., Fort Worth, Texas, USA) is a synthetic cortisene angiostatic steroid[25] that prevents CNV in patients without neovascular AMD at high risk of progressing to neovascular AMD[28]. Its utility is appreciated because of the dosing schedule of 15 mg every 6 months, the delivery as a posterior juxtascleral deposit and the low risk profile[29].

1.5. Treatments of CNV AMD

Because the pathogenesis of AMD is multifactorial and not completely understood, different therapeutic approaches are effective, depending on the complex interaction of metabolic, functional, genetic, and environmental factors involved[10].

Laser photocoagulation was the only approved treatment until 2000 and even if this treatment has been approved for ablating CNV, other studies have shown its cytodestructive properties concerning retinal neurons[29]. The limitation of laser photocoagulation is thermal damage of viable neurosensory retina overlaying the treated CNV[30], which make this treatment rarely used now, particularly in light of existing new drugs except in selected cases[31]. It can make drusen disappear but does not prevent the emergence of the advanced disease like the pathological neovascularization or the geographic atrophy associated with visual loss after a two year follow-up[32].

Thermal damage can be avoided by using photodynamic therapy (PDT) with intravenous injections of verteporfin (Visudyne, Novartis AG, Basel, Switzerland) introduced in 2000. Verteporfin is a photosensitizer that is activated by a laser that corresponds to its absorption peak, resulting in reactive oxygen that will selectively occlude vessels without damaging the surrounding tissue[30]. Studies in general show that PDT treatment stabilizes vision rather than

improving it[33]. Vision loss has been stopped by both thermal laser photocoagulation and PDT treatments for many patients in the last decade, but improvement in visual acuity has been rare[31].

Intravitreal injections of anti-vascular endothelial growth factor (VEGF) has revolutionized the treatment of AMD-related CNV, by targeting the molecule in angiogenesis that is responsible for the growth of new vessels created in the late stage of AMD, VEGF.

Pegaptanib sodium injection (Macugen, Eyetech Pharmaceuticals/Pfizer) is the first anti-VEGF treatment that has been on the market since 2004 and used since then with the dosing of 0.3mg every 6 weeks[6]. It's a 28-nucleotide RNA aptamer of 50 kDa[6] that binds VEGF and blocks its activity[34]. Pegaptanib will inhibit proliferative responses only to (VEGF-A)165, the VEGF isoform primarily responsible for pathological ocular neovascularization and vascular permeability. The production of this oligonucleotide is simple and cost-effective unlike antibodies using cell-based expression systems, yet it has the advantages of an antibody, which is to be versatile, highly selective and specific[35]. Its effect on vision loss is similar to PDT[31].

Antibodies are another class of anti-angiogenesis agents which act by binding the target and neutralizing it. One of them, bevacizumab (Avastin, Roche), is a full-length humanized monoclonal IgG antibody of 149 kDa binding all VEGF-A isoforms. It was first approved by the Food and Drug Administration (FDA) in 2004 for certain metastatic cancers and since then widely used off-label in the treatment of AMD at the recommended dose of 1.25 mg/month[6].

Ranibizumab (Lucentis, Novartis) is a Fab fragment of 48 kDa engineered from bevacizumab, which has been approved since 2006 for AMD treatment[6] and is used preferably at a dose of 0.5 mg/month[36], which is the maximum tolerated[37]. This recombinant,

humanized, monoclonal antibody fragment acts against all isoforms of VEGF-A which allows an easier penetration into the retina[6].

Unlike the other treatments, both bevacizumab and ranibizumab can improve visual acuity[31, 38, 39]. The ANCHOR study showed that the 1-year efficacy of ranibizumab injections is better than PDT treatment, slowing the loss of vision and even gaining letters: 11.3 letters of increased mean visual acuity for the standard use of ranibizumab 0.5mg/injection compared with a decline of 9.5 letters in the verteporfin group ($P<0.001$ for each comparison)[33]. The two-year results were consistent with the 1-year results and showed no increased risk of adverse[36]. The CATT study showed that ranibizumab 0.5 mg/month and bevacizumab 1.25mg/month throughout the first year of follow-up, were equivalent in terms of visual acuity improvement with a gain of 8.0 and 8.5 letters respectively for monthly injections [40]. Improvement in the visual acuity by 15 or more letters was observed in 33.8% of the group receiving the standard dose of 0.5 mg ranibizumab with a mean increase of 7.2 letters, compared with 5% of the group receiving the sham injection with a decrease of 10.4 letters ($P<0.001$ for both comparisons)[39]. The proportion of patients experiencing serious adverse events was higher in the group taking bevacizumab than the group taking ranibizumab (24.1% vs. 19.0%; risk ratio, 1.29; 95% confidence interval, 1.01 to 1.66).

The price of ranibizumab is about 100 times the price per milligram of bevacizumab, which is the main reason explaining the off-label use of this drug despite the lack of long-term follow-up and the potentially higher risk of side effects[41].

Aflibercept (Eylea, Bayer) approved in 2011 for AMD treatment, is a VEGF Trap-Eye acting as a soluble receptor of 115 kDa including the Fc fragment of bevacizumab fused with the binding domains from VEGFR1 and VEGFR2. Thereby, it binds VEGFA more tightly than

all other anti-VEGF agents[31], but also VEGF-B and placental growth factors PLGF-1 and -2. The recommended dose is 2mg/month for the first three times followed by one injection every two months or bimonthly injections from the beginning[6].

Two double-masked and multicentered phase-3 studies with 2419 neovascular AMD patients concluded similar efficacy and adverse events between monthly, every-2-month aflibercept injection and monthly ranibizumab injection groups [42].

Steroids are antiangiogenic and anti-inflammatory in the treatment of neovascular AMD[43]. Triamcinolone acetonide (TA) is an intravitreal glucocorticoid injection effective for the treatment of AMD that affects both neurons and blood vessels. In contrast to anti-VEGF agents, which are suspected to be neurotoxic, TA prevents loss of Müller cells, glial cells, which serve as support cells for the neurons of the retina and affect photoreceptor degeneration, and prevent the development of retinal vascular lesions and leakage[44].

A combination of the previously described treatments brings new strategies to handle the different abnormalities of AMD, depending if it aims to address the photoreceptors, pigment epithelium (RPE), Bruch's membrane, and/or choriocapillaries to treat the lipofuscinogenesis, drusogenesis, inflammation, and/or neovascularization[10]. Studies show no superiority effect of steroids alone in the prevention of visual loss compared with placebo[29, 43], but their combination with another treatment of the choroidal neovascularization due to AMD can improve the efficacy of the monotherapy. For example, TA one week before PDT is more effective to stabilize visual acuity in the treatment of exudative AMD, compared with PDT and lead to less necessity for re-treatments with PDT[45]. When anti-VEGF monotherapy is unresponsive, it can be combined with triamcinolone acetonide as a safe and effective option with for example bevacizumab[46], or ranibizumab and result in fewer retreatments[47].

Anecortave acetate is not an appropriate monotherapy for active CNV but is currently evaluated in a combination therapy with ranibizumab[31].

The risks associated with frequent injections into the eye have brought about the development of other mechanisms of targeting angiogenesis than those already described. Examples include small interfering RNA that degrade VEGF mRNA, inhibitors of VEGF receptor tyrosine kinase, inhibitors of plasma membrane ion channels with downstream effects on VEGF, vitreoretinal surgery that removes the CNV, pigment epithelium-derived growth factor with anti-angiogenic, neurotrophic and neuroprotective properties, and antifibrotic agents. But the lack of available data from randomized placebo controlled or comparative studies make their role not well evaluated and not appropriate primary treatment strategies[31, 48]. Another area of research is treatments which decrease lipofuscinogenesis and maintain effective clearance systems in RPE cells, involved in the progression of AMD[10].

1.6. Our goal: understand long term safety on IOP

Intravitreal injection of anti-VEGF is the standard therapy for the treatment of neovascular AMD, with bevacizumab and ranibizumab the ones that are the most frequently used. They allow patients to maintain a stable or even improve visual acuity over the years. In general, the anti-VEGF drugs have a good safety profile. In fact, clinical trials did not indicate any safety concerns (ANCHOR, MARINA)[36, 39]. However, some observational studies have indicated that anti-VEGF drugs can cause chronic elevations in intraocular pressure (IOP) but the results are not consistent[49-54]. And as for most retina specialists working in both private and academic clinics, 55% of the 530 are convinced that sustained IOP elevation may be caused

by intravitreal anti-VEGF therapy[55]. Chapter 2 will highlight the literature on the risk of elevated IOP after anti-VEGF injection.

CHAPTER 2. Effect of anti-VEGF injections on IOP

2.1. Transient IOP increase

Patients undergoing treatment of neovascular AMD with intravitreal anti-VEGF injections almost uniformly face a transient rise in IOP[56]. This increase is short term and lasts 0 to 30 minutes after the injection[57]. It is not affected by the fact that the patient is phakic or pseudophakic. However, the position of the injection[50], the size of the needle[56], the history of glaucoma[56] and the axial length[57] will have an influence on the elevation of the IOP measured directly after the injection. For example, it takes more time to come back to the starting IOP in patients with history of glaucoma[56]. The risk of IOP elevation will be higher for a smaller needle[56], a tunneled scleral injection rather than straight scleral[50] which will have a vitreous reflux (loss of postinjectional drug) that is less important[58], patients with shorter eyes[57] and history of glaucoma[56]. These transient IOP increases are thought to be benign.

2.2. Chronic IOP increase

By contrast, a chronic elevation of IOP is a cause for concern that must be treated in order to reduce the risk of glaucoma. In general, a chronic elevation of IOP is defined as an increase of >=5mmHg on two consecutives follow-up visits[49]. The literature will be presented dividing it into sections by study design and positive versus negative results.

2.2.1. Clinical trials showing no IOP elevation

Two 2-year multicenter randomized double-blind phase III studies, MARINA and ANCHOR, compared the use of two different dosages of ranibizumab with sham or PDT treatment. Neither trial indicated any major adverse effects associated with the use of ranibizumab and specifically no increased risk of IOP elevation was observed from the injections[33, 36, 39].

More specifically, MARINA, a sham-controlled study, enrolled 716 patients. The results showed an increase between preinjection IOP and postinjection IOP 1 hour after injections of 2.1 to 3.4 mm Hg with ranibizumab whereas it was 0.8 to 1.5 mm Hg in the sham-injection group. However, the IOP changes were transient as shown by comparing the monthly preinjection measurements over the follow-up. Few cases of adverse events such as endophthalmitis, cataract, arterial thromboembolic, hypertension, death, and IOP postinjection increases of more than 30, 40 and even 50 mmHg occurred. The adverse events were sometimes more frequent in groups with ranibizumab than in the sham group while for others the events were similar in all groups[39]. ANCHOR which compared two doses of ranibizumab with PDT treatment among 423 patients, showed the same kind of adverse events as the MARINA study with also cases of IOP increase, but also cases of rhegmatogenous retinal detachment, vitreous hemorrhage, and also back pain which was less common in the ranibizumab group[33].

2.2.2. Limitations of the clinical trials with no IOP increase results

In both the MARINA and ANCHOR trials, the clinical significance is unclear, given the fact that these clinical trials were not powered to detect differences between groups with low rates of adverse events[33, 36, 39].

Furthermore, those clinical trials have been criticized by an ad-hoc analysis[59]. For example, they compared the injected eye with a fellow eye that had received injections of steroids, known to increase the risk of long-term IOP elevation. Also, they didn't use any standardized techniques or account for the time of the measurement of IOP[59], knowing that the diurnal variations influence IOP. The other limitations to these studies are that there is no stratification at baseline for pre-existing IOP, glaucoma risk factors and medications, no central corneal thickness measurements, gonioscopy, visual fields, nerve fiber layer assessments, or detailed optic nerve examination[59].

2.2.3. Observational studies showing no IOP elevation

Observational studies have also examined the risk of IOP elevation after repeated anti-VEGF therapy. An increased number of ranibizumab injections was not associated with IOP changes, according to a retrospective study of 53 patients treated with intravitreal ranibizumab injections in the treatment of neovascular AMD, comparing frequently-treated study eyes (≥15 injections) with unfrequently-treated fellow control eyes (≤ five injections). The limitation of this study is the small amount of patients and the short follow-up period of 1 year[60].

A retrospective chart review of patients having AMD with or without glaucoma who received ranibizumab injections for more than 4 years compared 302 treated eyes and 226 control non injected fellow eyes. The results showed a low incidence of elevated IOP which is not clinically significative[61].

2.2.4. Observational studies showing an IOP elevation

A literature review of patients treated for wet AMD with anti-VEGF injections showed a significant IOP elevation in many studies, with an incidence of 3.45% to 11.6% depending on the study. Risk factors suggested are history of glaucoma, phakia, history of glucocorticoid use, and number of injections[51, 52, 62, 63].

IOP elevation occurred in patients without any history of glaucoma or ocular hypertension receiving injections of ranibizumab according to a case series of 4 patients. They all reached high IOP that goes even until 50mmHg and persisted after several visits so they needed interventions to control the IOP[53].

The chronic elevation of IOP defined as an IOP of >5 mmHg on ≥2 consecutive visits was related with the number of injections in a retrospective cohort study based on the charts of 207 patients. They undergo unilateral intravitreal ranibizumab and/or bevacizumab injections by a single physician for a period of 6 months. IOP elevation occured in 11.6% of injected eyes compared to 5.3% of control eyes without injections[49].

The chronic elevation of IOP depends on the choice of the anti-VEGF drug, according to a retrospective study with charts of 215 eyes with wet AMD. IOP elevation had a higher

incidence in patients who received bevacizumab injections only (9.9%) compared to 3.1% of eyes receiving only ranibizumab. A greater risk of IOP elevation had been correlated with pre-existing glaucoma for both treatments[52].

The risk of chronic elevation of IOP depends on the injection technique. A study on 45 patients with AMD showed a significantly IOP elevation followed by injections of anti-VEGF with a risk being significantly higher for the tunneled scleral injection compared to the standard straight scleral incision[50].

Other studies indicated an IOP elevation over the injections of anti-VEGF in the treatment of AMD, with negative results concerning the impact of other factors influencing the increase of IOP. In a cross-sectional physician survey on 530 retina specialists, a higher volume with a rapid injection technique is related to an IOP elevation but the drug of choice is not[55]. A study on 147 eyes with AMD receiving anti-VEGF injections conclude to an elevation of IOP without any influence of the axial length[54].

Knowing that the increase of IOP is a risk factor of the development of glaucoma, which is the second cause of blindness in developed countries, and that many other studies related the use of anti-VEGF with the persistent ocular hypertension (HTO), physicians should monitor their patients as a prevention[64].

2.2.5. Mechanism of IOP elevation

There are several hypotheses explaining the possible chronic increase of IOP. Contributing factors may include the injection procedure, the volume injected into the posterior

chamber, the reflux and the transient elevation of IOP, a direct effect of the VEGF blockade particles in the drug or the syringe that will cause obstruction or damage to the trabecular meshwork[51, 54, 55]. Studies showing a significant association of the total number of intra-vitreal anti-VEGF injections but not with the number of intra-vitreal steroid injections on the chronic increase of IOP, tend to suggest that the effect of the intra-vitreal injection procedure is negligible [65]. However, other studies have found a significant relationship between the number of intra-vitreal steroid injections and a chronic increase in IOP[66]. Therefore, it is not currently clear whether the increase may be due to the drug, the injection procedure, or both.

Monomer antibodies, as ranibizumab, might act like aggregated proteins and lead to inflammation in the trabecular meshwork, affecting the aqueous outflow channels and leading to subsequent elevation in IOP[67]. Another pathway that has to be investigated is that anti-VEGF might decrease the endothelial nitric oxide in the anterior segment by increasing the constriction of the trabecular meshwork, leading to the increasing IOP from repeated injections[62].

2.2.6. Specific aims

We conducted a retrospective cohort study of 161 patients undergoing unilateral ranibizumab injection to determine whether the injections increase the risk of elevated IOP by comparing the IOP changes in injection eyes to non-injection eyes. We considered all the factors that can influence the IOP : age, gender, ethnicity, family history of glaucoma, number of ranibuzumab injections, other ocular comorbidity (diabetes, arterial hypertension, atherosclerosis), systemic and ocular medications (history of steroid, IOP-lowering drop,

photodynamic therapy, bevacizumab or pegaptanib), injection doctor, lens implant, pseudoexfoliative material on lens capsule and any prior non cataract intraocular surgery or laser (trabeculoplasty, filtration surgery, yag capsulotomy, iridotomy) during the time in which the patient was actively receiving intravitreal injections. The next chapter consists of a manuscript that contains the methods and results of the project.

CHAPTER 3. RESULTS

Risk of chronically elevated intraocular pressure after ranibizumab injection in patients with age-related macular degeneration

Solmaz Moghadaszadeh, BSc[1,2], Paul Harasymowycz, MD, MSc[1,2,3],

Sébastien Olivier, MD[1,2], Gisèle Li, MD, MSc[1,2], Ellen E. Freeman, PhD[1,2]

1. Department of Ophthalmology, University of Montreal; 2. Research Centre, Maisonneuve-Rosemont Hospital; 3. Clinique d'ophtalmologie Bellevue; Montreal, Canada

3.1. Abstract

Purpose: Conflicting evidence exists about the risk of chronic elevation of intraocular pressure after ranibizumab injections for neovascular age-related macular degeneration. The goal of this study is to evaluate this risk.

Methods: A retrospective cohort study of 161 people was conducted using medical charts from Bellevue Ophthalmology Clinic in Montreal. Inclusion criteria included receiving at least three ranibizumab injections in one eye only and having at least 9 weeks of follow-up. Exclusion criteria included the presence of uncontrolled glaucoma or ocular hypertension at baseline (IOP>=21mmHg). Chronic IOP elevation was defined as an increase >5mmHg of IOP on at least 2 consecutive visits.

Results: There was no difference in the percentage of injected eyes that experienced a chronic IOP increase (n=8, 5%) compared to the percentage of uninjected eyes that experienced an IOP increase (n=9, 6%). However, a greater number of anti-VEGF injections was associated with chronic IOP elevation (P=0.032). Other risk factors for chronic IOP elevation included diabetes, a lower baseline IOP, and a higher maximum IOP (P<0.05).

Conclusions: A greater number of injections appears to increase the risk of chronic IOP elevation. Also, diabetics appear to be more at risk and may need more careful follow-up or preventive pharmacological treatment.

3.2. Introduction

Ranibizumab is an ocular anti- vascular endothelial growth factor (VEGF) treatment that dramatically reduces vision loss associated with neovascular age-related macular degeneration

(AMD), a leading cause of blindness in Canada[68]. However, there is some concern about the effect of ranibizumab and other anti-VEGF drugs on intraocular pressure (IOP). Randomized clinical trials of ranibizumab did not show an increased risk of chronically elevated IOP[33, 39, 61]. Research from observational studies on this topic is preliminary and shows conflicting results, as some studies indicated sustained increased intraocular pressure after ranibizumab[49, 50, 53], while others found no association[60, 61]. Some of these observational studies have been underpowered or uncontrolled.

Our goal was to conduct a controlled analysis of the risk of chronic IOP elevation after ranibizumab injection in one eye only. Our hypothesis was that eyes that have had injections of ranibizumab will be at a greater risk of an IOP increase than the fellow eye, and that this increase will be higher with a higher number of injections.

3.3. METHODS

3.3.1. Study Population

A retrospective cohort study of patients receiving anti-VEGF injection treatment in one eye only at Bellevue Ophthalmology Clinic in Montreal was performed. Data were abstracted from the medical charts of 161 patients with neovascular AMD receiving at least 3 anti-VEGF injections of ranibizumab in one eye only. Occasionally, a patient may have switched to a different anti-VEGF agent such as bevacizumab or aflibercept but this was rare. Exclusion criteria included the presence of uncontrolled glaucoma or ocular hypertension at baseline (IOP>=21mmHg) and having less than 9 weeks of care after the injection. We collected medical chart data on patients for up to 5 years. Patients had their injections approximately once a month

and the IOP was measured the same day right before the injection by a nurse. We only included data from people who had injections in one eye only. If a person began injection therapy in the second eye too, we no longer collected additional information on that person. Ethics approval was obtained by the ethics committee at Maisonneuve-Rosemont Hospital.

3.3.2. Data Collection

All data were abstracted from the electronic medical records at Bellevue Ophthalmology Clinic and were entered into a Microsoft Excel database. Intraocular pressure is routinely measured by Goldman applanation tonometry at Bellevue Ophthalmology Clinic after ranibizumab injection. Data that were abstracted besides intraocular pressure included demographic information, family history of glaucoma, number of ranibizumab injections, dates of injections, dates of IOP readings, other ocular comorbidity, systemic and ocular medications, and injection doctor.

3.3.3. Outcomes

The primary outcome was the percentage of eyes with an IOP elevation of >5mmHg on at least two consecutive visits.

3.3.4. Statistical Analysis

A trajectory graph was made for each person showing the IOP at each injection date. The percentage of eyes with an IOP elevation of >5mmHg was compared between the injected

eyes and the uninjected fellow eyes. Differences were tested using McNemar's test for paired data. Then, in injected eyes only, we examined risk factors for having a chronic IOP increase using unpaired t-tests, chi-square tests, and Fisher's exact tests. SAS and R were used to conduct the analyses and to make the graphs.

3.3.5. Statistical Power

With 161 injected eyes and 161 uninjected eyes, we had 80% power to detect an odds ratio of 2.9 assuming a Type 1 error of 5% and a probability of chronic IOP elevation in the uninjected eyes of 6%.

3.4. Results

There was no difference in the percentage of injected eyes that experienced an IOP increase (n=8, 5%) compared to the percentage of uninjected eyes that experienced an IOP increase (n=9, 6%) (P=0.74).

However, a greater number of anti-VEGF injections was associated with chronic IOP elevation (P=0.032) (Table I and Figure 1). Those with chronic IOP elevation had an average of 28 injections (SD=12) compared to 18 injections (SD=13) in those without IOP elevation (Table I). Also, those with diabetes were more likely to have a chronic IOP elevation (15%) compared to those without diabetes (3%) (P=0.028). Those with a lower baseline IOP were more likely to have a chronic IOP elevation as were those who had a higher maximum IOP elevation (P<0.01).

The small number of people who experienced a chronic IOP elevation (n=8) did not allow for regression modeling.

Sensitivity analyses were done to determine if excluding those with missing IOP values at the beginning of follow-up (n=49) changed our conclusions. The conclusions were the same whether we included or excluded these people so they were included in the main results.

3.5. Discussion

Eyes that had anti-VEGF injections, regardless of the number, were not more at risk of chronically elevated IOP than uninjected fellow eyes. However, of injected eyes, eyes that had a higher number of injections were at a greater risk than eyes that had fewer injections. Also, diabetics were more at risk of developing chronically elevated IOP. Other risk factors included lower baseline IOP and a higher maximum IOP which indicate a greater variability in IOP in those who developed chronically elevated IOP.

Our results largely confirmed a recent paper by Hoang et al which also found number of injections to increase the risk of chronic IOP elevation[49]. They also found borderline evidence (P=0.09) that diabetes was a risk factor for chronic IOP elevation.

A review by Dedania et al speculated about potential mechanisms regarding why anti-VEGF injections could chronically increase the IOP[51]. One hypothesis was that silicone microdroplets and protein aggregates from the anti-VEGF injection could clog the trabecular meshwork. It is thought that bevacizumab, which has three times the molecular weight as ranibizumab, would be more likely to obstruct the trabecular meshwork. Another hypothesis is that the injection may cause subclinical inflammation which could cause scar formation and

fibroblast proliferation thereby obstructing the trabecular meshwork. Others believe that transient elevations in IOP which occur due to the volume of the liquid being injected into the eye could over time result in chronic elevations in IOP. Researchers are investigating whether preventing these transient increases in IOP might reduce the risk of chronic elevations.

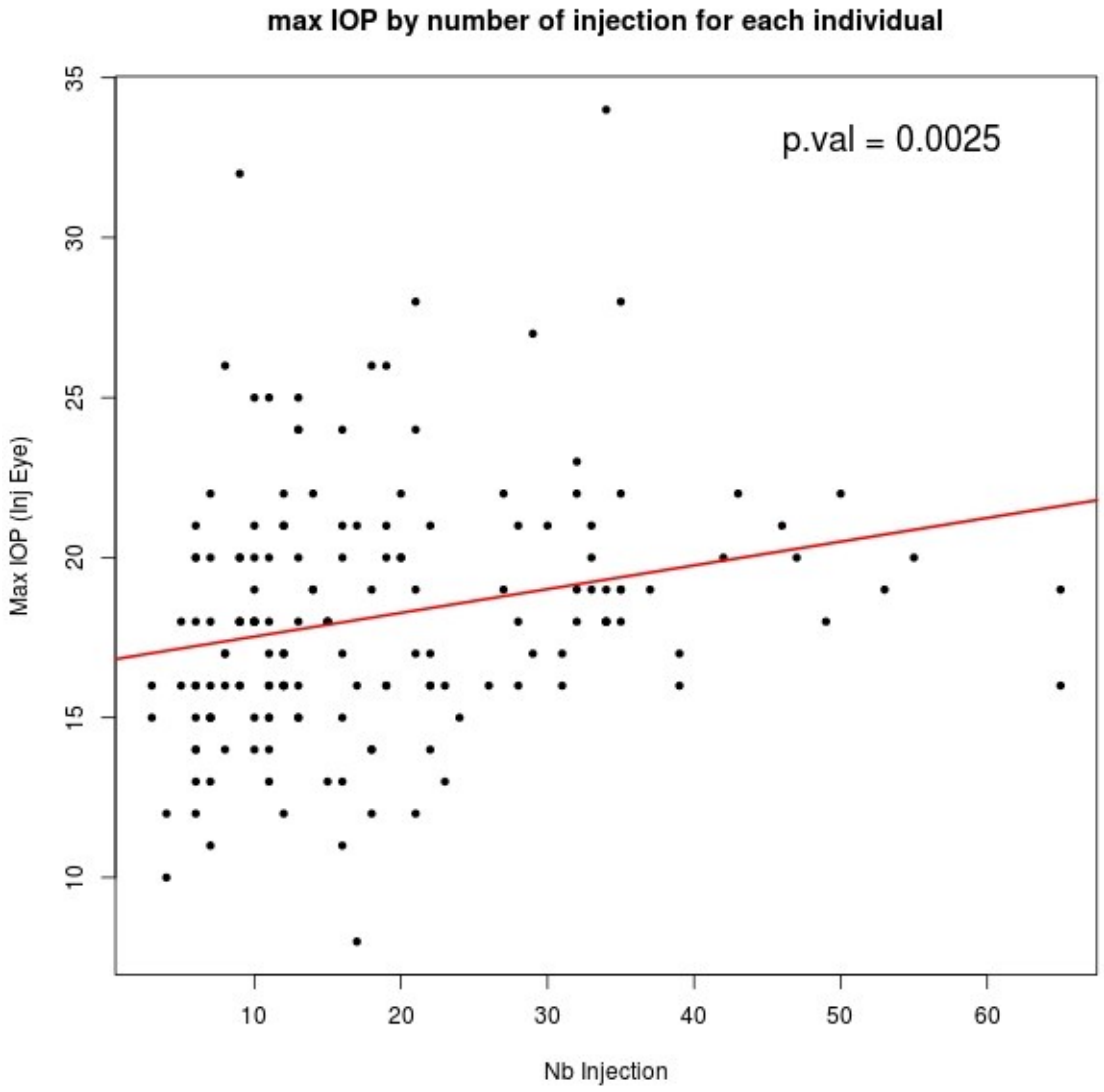
Strengths of this study include the controlled nature of the design, the use of a cohort study of people with controlled IOP at baseline, and the fairly routine measurement of IOP on the day of injection at Bellevue clinic. A limitation is that we were not able to achieve the sample size that we had intended because not enough people had only one injection. With 161 people, we only had 8 people who developed chronic IOP elevation. This limited our power to detect associations with risk factors and made regression impossible. For example, we only had 30% power to detect the potential association between history of ocular hypertension and chronic IOP elevation. Another limitation is that retrospective chart reviews often have missing data and ours was no exception. Some individuals had missing IOP data at the start of their follow-up. However, sensitivity analyses indicated that this did not affect the results.

Our results in combination with other studies indicate that some patients are at risk of chronic IOP elevation after anti-VEGF injection and need to be watched closely to prevent long-term damage to the optic nerve and blindness due to glaucoma. Strategies to prevent this chronic IOP elevation deserve greater research.

Table I: Characteristics of those who did and did not experience a chronic IOP elevation

	Chronic IOP elevation, n=8 Mean (SD) or %	**No Chronic IOP Elevation, n=153 Mean (SD) or %**	**P-Value**
Age, Years	78.9 (11.7)	78.1 (8.9)	0.814
Female Sex Male Sex	4% 7%	96% 93%	0.687
Number of Injections	28.0 (11.9)	18.2 (12.5)	0.032
Glaucoma Family History No Glaucoma Family History	8% 4%	92% 96%	0.343
Baseline IOP, mmHg	11.4 (2.5)	14.7 (3.5)	0.010
Maximum IOP, mmHg	22.3 (5.0)	18.0 (3.8)	0.002
Visual Acuity, logMAR	0.56 (0.36)	0.60 (0.42)	0.793
Intraocular Steroid Injections No Steroid Injections	5% 5%	95% 95%	1.00
History of Diabetes No Diabetes	15% 3%	85% 97%	0.028
History of Ocular Hypertension No Ocular Hypertension History	15% 4%	85% 96%	0.138
History of Glaucoma No History of Glaucoma	8% 5%	92% 95%	0.623

Figure 1: Relationship between number of injections and maximum IOP



Legend Figure 1: Relationship between the number of anti-VEGF injections and the maximum IOP observed in the injection eye of each individual.

CHAPTER4. DISCUSSION

The general objective of this master's thesis was to evaluate the risk of chronically elevated intraocular pressure after ranibizumab injection in patients with age-related macular degeneration. The hypothesis tested was that eyes that have had injections of ranibizumab will be at a greater risk of an IOP increase than the uninjected fellow eye, and that this increase will be higher with a higher number of injections. The results didn't show any difference in the percentage of injected eyes that experienced an IOP increase compared to the percentage of uninjected eyes that experienced an IOP increase. However, a greater number of anti-VEGF injections was associated with chronic IOP elevation.

We also wanted to determine if certain groups are at higher risk for chronically elevated IOP. We found that those with diabetes and those with a lower baseline IOP were more likely to have a chronic IOP elevation. No other factors were statistically significant although those with a history of ocular hypertension were somewhat more likely to develop a chronically elevated IOP compared to those without (15% versus 4%, P=0.138).

Several points related to the results have been discussed in the manuscript in Chapter 3. This discussion will elaborate more on our results and will compare them to results from other scientific studies. Table II summarizes the results from our study and 7 other studies done on this topic.

Table II: Comparison of our results with other literature

First Author (Year)	Study Design	Association with Number of Injections	Association with Diabetes	Association with History of Ocular Hypertension
Our study	Retrospective chart review of 161 patients	Association Mean of 28 injections in eyes with chronic IOP elevation vs 18.2 in eyes without IOP elevation (P=0.032)	Association	No association
Hoang et al. (2012)[49]	Retrospective chart review of 207 patients	Association For ≥29 injections compared to <12, OR=5.75, P=0.03	No association although borderline No odds ratio given; P=0.09	No association OR= 1.08, P=0.86
Hoang (2013)[68]	Retrospective study of 328 patients	Association ≥29 injections compared with ≤12 injections, OR = 16.1, P = 0.008	Did not assess	No association

Choi et al. (2011)[69]	Retrospective chart review of 127 patients; case series	No association	Did not assess	Did not assess
Adelman et al. (2010)[64]	Retrospective case series of 116 patients	Did not assess	Did not assess	Did not assess (the 4 cases who showed an elevated IOP do not have any history of HTO)
Bakri et al. (2008)[54]	Case series of 4 patients	Did not assess	Did not assess	Did not assess (Did not have any history of ocular hypertension)

Good et al. (2011)[53]	Retrospective chart review of 215 patients; case series	Did not assess	Did not assess	Did not assess
Bakri et al. (2014)[60]	Post hoc analysis of 1125 patients (Reanalysis of IOP data from MARINA and ANCHOR clinical trials)	Did not assess	Did not assess	Did not assess

4.1. Comparison of injected eyes versus uninjected fellow eyes

Not all the prior studies compared injected eyes to uninjected fellow eyes. Most of the prior studies were uncontrolled in that they only examined injected eyes. The lack of a difference in the incidence of chronically elevated IOP between injected and uninjected eyes was a surprise to us and was different from what Hoang et al found[49]. They found the incidence of chronically elevated IOP to be 11.6% in injected eyes compared to 5.3% in uninjected fellow eyes while we found the incidence of chronically elevated IOP to be 5% in injected eyes and 6% in uninjected fellow eyes. We had very limited numbers of people who had chronically elevated IOP in each group so it is possible that these percentages are unstable estimates of the true percentage of chronically elevated IOP in the underlying population. The lack of an

association comparing injected eyes to uninjected fellow eyes may be because ranibizumab does not increase the risk of chronically elevated IOP. In contrast, the lack of an association could be due to the fact that ranibizumab has systemic effects on the uninjected fellow eye as well as on the injected eye. We think that this is unlikely with ranibizumab because of a very short half-life time of 0.09 days[70].

4.2. Association with the number of injections

Despite not finding a difference in chronic IOP elevation between injected and uninjected eyes, we did find an association between number of injections and chronic IOP elevation in injected eyes. There was a mean of 28 injections in eyes with chronic IOP elevation compared with 18 in eyes without IOP elevation (P=0.032).

In a retrospective study by Hoang et al[49] with 207 patients, the risk of sustained elevated IOP defined by an IOP more than 5mmHg on at least 2 consecutives visits, was associated with a greater number of injections of ranibizumab and/or bevacizumab. The increased odds ratio of experiencing IOP elevation in patients receiving ≥29 injections compared with ≤12 injections was 5.75 (P = 0.03).

Another retrospective study of 328 patients realized by Hoang et al[65] with chronically elevated IOP defined by an absolute IOP >25 mmHg with increase above baseline >10 mmHg, or IOP of >21 mmHg with increase of >5 mmHg, was associated with a greater number of injections of ranibizumab and/or bevacizumab. The increased odds ratio of experiencing IOP

elevation in patients receiving ≥29 injections compared with ≤12 injections was 16.1 (P = 0.008).

4.3. No association with the number of injections

However, not all studies have found an association between number of injections and chronic IOP elevation. The retrospective chart review realized by Choi et al on 127 patients receiving bevacizumab, ranibizumab or pegaptanib defined sustained elevated IOP as an IOP >25 mmHg on 2 separate visits requiring glaucoma medication or surgery. Seven patients (5.5%) developed chronically elevated IOP[71], but there was no association with the number of injections. This study may not have had adequate power to detect an association with number of injections as their sample size was quite small at 127.

4.4. Diabetes

We found that diabetes was a risk factor for chronic IOP elevation. Most other studies did not assess diabetes as a risk factor. One study that did, Hoang et al[49], reported a borderline association between diabetes and chronic IOP elevation (P=0.09) although they did not give an odds ratio or the percentages in those with and without diabetes who experienced chronic IOP elevation. It makes sense that diabetes is a risk factor for chronic IOP elevation after ranibizumab injection because it has been frequently identified as a risk factor for glaucoma[69].

Some experimental articles have attempted to explain the biochemical mechanism linking diabetes and intraocular pressure or glaucoma. One study examined fibronectin expression in bovine trabecular meshwork cells grown in normal or high glucose conditions. Under the high glucose conditions, fibronectin expression increased and tracular meshwork cell numbers decreased indicating that diabetic patients may be at risk of altered aqueous humor outflow dynamics causing increased intraocular pressure[72]. Another researcher hypothesized that the mechanism linking Type 2 diabetes, a chronic inflammatory disease, and glaucoma has to do with the anti-inflammatory response mediated by Interleukin-10 (IL-10) and its downstream signaling protein the Signal transducer and activator of transcription 3 (STAT3). The IL-10/STAT3- mediated anti-inflammatory response inhibits apoptosis of retinal ganglion cells (RGC) leading to greater survival. High glucose levels contributing to the pathogenesis of Type 2 diabetes (T2D) are responsible for the impairment of the IL-10/STAT3- which by consequence leads to the apoptosis of retinal ganglion cells, which is also initiated by the elevated intraocular pressure in the neurodegenerative disorder which is glaucoma[73].

High hemoglobin A1c levels, which represent a poor glycemic control, contribute to increased intraocular pressure levels in long-term diabetic patients. Hypertension, dyslipidemia and insulin resistance are significant predictors for diabetes, elevated IOP and glaucoma. The mechanism of increased oxidative stress caused by the vasodilator nitric oxide, contributes to vascular dysregulation, inflammation and apoptosis of RGCs in diabetes and glaucoma. Another link between these two diseases is the dysfunction of the glial cell, responsible for supporting and protecting neurons in the central nervous system that includes the retina and optic nerve. Clinically, it has been showed that metformin, used to treat insulin resistance in type 2 diabetes, decreases the risk of developing open-angle glaucoma (POAG). Genetic polymorphisms

associated with pancreatic beta-cell function in T2D showed an increase of the risk of POAG[74].

4.5. History of Ocular Hypertension or Glaucoma

We found a borderline association (P=0.09) between history of ocular hypertension and chronic IOP elevation. In general, studies did not assess the history of ocular hypertension as a risk factor. Adelman et al found that 4 out of 116 patients with AMD (3.45%) developed chronically elevated IOP after multiple bevacizumab and/or ranibizumab injections, with none of them having any history of glaucoma or ocular hypertension[63]. Good et al[52] found that 3.1% (3/96) of eyes receiving ranibizumab experienced elevation of IOP. They found a higher risk in patients with pre-existing glaucoma compared to no pre-existing glaucoma (33% versus 3% respectively, P<0.001) but did not assess the association with history of ocular hypertension.

Conclusion

Our study, one of the few controlled studies that has been done, has added valuable data on the topic of whether ranibizumab injections increase the risk of chronic IOP elevation. Routine IOP measurements should be taken when giving anti-vegf injections to ensure that the IOP has not become chronically elevated, particularly in those with a high number of previous injections and in those with diabetes. Further research should be done on how to prevent chronic IOP elevation.

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