Diplomarbeit

Anti-VEGF pro re nata therapy in diabetic macular edema: a retrospective analysis of the number of injections applied in the first 2 years of treatment

eingereicht von

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zur Erlangung des akademischen Grades

Doktorin der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt an

Universitäts-Augenklinik

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Graz, 24. Januar 2020

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Acknowledgement

Firstly, I would like to express my special thanks to my advisors Dr. med. univ. Monja Michelitsch and Univ.-Prof. Dr. Andreas Wedrich who gave me the opportunity to write my thesis about a diabetic eye disease, were patient and supported me in writing. I would also like to thank Dr. Regina Riedl for the statistical analysis she had done.
I am also grateful to Kaspars Silins and my parents for their spiritually support throughout writing this thesis and my life in general.

Zuerst möchte ich herzlichst meinen Betreuern Dr. med. univ. Monja Michelitsch und Univ.-Prof. Dr. Andreas Wedrich für die Möglichkeit meine Diplomarbeit über die diabetische Augenerkrankung zu schreiben sowie für Ihre Geduld und Unterstützung beim Schreiben danken. Des Weiteren bedanke ich mich bei Dr. Regina Riedl für die statistische Auswertung, die sie durchführte.
Abschließend möchte ich mich bei Kaspars Silins und meinen Eltern für ihre Unterstützung während des Schreibens meiner Diplomarbeit sowie meines Lebens bedanken.

Сперва, я хочу поблагодарить руководителей моей научной работы Др. Монью Михелич и Профессора Андреаса Ведрих за возможность написать эту работу на тему диабетическое заболевание глаза, за их терпение и поддержку во время написания. Я хочу выразить отдельную благодарность Др. Регине Ридль за выполнение статистического анализа.
Также, я очень благодарна Каспару Силинчу и моим родителям за ментальную поддержку во время написания моей работы и в течении моей жизни.

Abstract

Background: Diabetic macular edema (DME) is one of the main causes of blindness worldwide. Intravitreal injections with anti-VEGF agents are the first-line treatment for central involved DME. In clinical trials treating DME with anti-VEGF good outcomes in visual acuity and central macular thickness were achieved with a high injection rate after treatment initiation. The aim of the following study was to evaluate the number of injections applied in the first two years, and its functional and anatomic outcomes, under real life conditions at the Department of Ophthalmology at Medical University of Graz.

Design and methods: This study is a retrospective analysis on eyes of patients with diagnosed central involved DME at the Department of Ophthalmology at Medical University of Graz receiving initial anti-VEGF treatment with Avastin® between the 1.1.2014 and the 31.12.2015. Patients medical records were followed over two years.

Outcome measures: Main outcome was to determine the number of intravitreal injections with Avastin® applied in eyes with intravitreal treatment-naive DME over two years, after treatment initiation. Secondary outcomes were the change in best corrected visual acuity (BVCA), central subfield thickness (CST), intraocular pressure and macular volume achieved after 1 year and 2 years. In addition, a separate analysis was conducted for the number of injections applied in eyes which received panretinal photocoagulation prior and during the study period.

Results: Data of 157 eyes of 110 patients were analyzed for this study. The median number of injections over 2 years was 4, 37.58% received 3 injections and 53.5% received more than 3 injections. The median number of injections in the second year was 1. In only 50 eyes visits after one and during two years was recorded. Mean follow-up was 9.9 months over a 2-year surveillance period. Eyes which received panretinal photocoagulation prior or during the study period had a median of 5 injections. Mean BCVA increased from 0.45±0.23 to 0.53±0.25 (P-value <0.001), mean CST decreased from 416.5±122.1 to 358.1±113.2 (P-value <0.001) μm for all eyes over a 2-year surveillance period.

Conclusion: The median number of intravitreal injections with Avastin® during two-years after treatment initiation for central involved DME at the Department of Ophthalmology at Medical University of Graz was lower than in published clinical

trials with pro re nata (PRN) regime, potentially reducing the chances in gaining better visual acuity.

Zusammenfassung

Hintergrund: Diabetisches Makulaödem (DMÖ) ist eines der häufigsten Ursachen für Erblindung weltweit. Intravitreale Injektionen von VEGF-Inhibitoren ist Therapie der Wahl beim zentralen DMÖ. In klinischen Studien, wo man das DMÖ mit VEGF-Inhibitoren behandelte, wurden bessere Ergebnisse bei Veränderung von Sehstärke und zentrale Makuladicke mit erhöhter Injektionsrate nach der Therapieinitialisierung erzielt. Das Ziel der folgenden Studie war es die Anzahl der in zwei Jahren an der Universitäts-Augenklinik Graz unter den realen Bedingungen durchgeführten Injektionen, sowie deren anatomische und funktionelle Ergebnisse auszuwerten.

Aufbau und Methoden: Diese klinische Studie ist eine retrospektive Analyse von Augen von Patientinnen und Patienten mit diagnostiziertem zentralen DMÖ an der Universitäts-Augenklinik Graz, die vorher noch keine Behandlung mit einem VEGF-Inhibitor erhielten und zwischen dem 1.1.2014 und dem 31.12.2015 eine Initialisierung einer Anti-VEGF-Behandlung mit Avastin® erhielten. Patientenakten wurden über zwei Jahre nachverfolgt.

Zielgrößen: Die Hauptzielgröße war die Anzahl von applizierten intravitrealen Injektionen von Avastin® in Augen mit intravitreal unbehandeltem DMÖ über einen Zeitraum von zwei Jahren nach Behandlungsbeginn. Nebenzielgrößen waren die Änderungen der bestkorrigierten Sehstärke, der zentralen Makuladicke, des Augeninnendrucks und des Makulavolumens nach einem und nach zwei Jahren. Zusätzlich wurde eine separate Analyse für die Anzahl der applizierten Injektionen in Augen durchgeführt, die vor und während des Beobachtungszeitraums eine Behandlung mit panretinaler Photokoagulation erhielten.

Ergebnisse: Es wurden Daten von insgesamt 157 Augen von 110 Patientinnen und Patienten erhoben. Die mediane Anzahl von intravitrealen Injektionen während zwei Jahren betrug 4, 37.58% von Patienten erhielten insgesamt 3 Injektionen, 53.5% erhielten insgesamt mehr als 3 Injektionen. Die mediane Anzahl der Injektionen sank im zweiten Beobachtungsjahr auf 1. In nur 50 Augen wurden Visiten nach einem Jahr und während zwei Jahren registriert. Die durchschnittliche Beobachtungszeit betrug 9.9 Monate. Augen, die vor oder während des Beobachtungszeitraums eine panretinale Photokoagulation erhielten, hatten eine mediane Injektionsanzahl von 5. Die mittlere bestkorrigierte Sehstärke stieg von 0.45±0.23 auf 0.53±0.25 (P-Wert <0.001) und die mittlere zentrale

Makuladicke verringerte sich von 416.5±122.1 auf 358.1±113.2 (P-Wert<0.001) μm für alle Augen in zwei Jahren.

Schlussfolgerung:

Die mediane Anzahl der intravitrealen Injektionen mit Avastin® während zwei Jahren nach Beginn der Behandlung vom zentralen DMÖ an der Universitäts-Augenklinik Graz war niedriger als in den veröffentlichten klinischen Studien mit pro re nata (PRN) Therapieschema, was möglicherweise die Wahrscheinlichkeit einer Verbesserung der Sehschärfe verringert.

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Abbreviations

AGE	Advanced Glycation End Products
Anti-VEGF	Anti- vascular endothelial growth factor
BCVA	Best corrected visual acuity
CST	Central subfield thickness
CSME	Clinically significant macular edema
DME	Diabetic macular edema
EPO	Erythropoetine
ETDRS	The Early Treatment of Diabetic Retinopathy Study
FA	Fluorescence angiography
FAZ	Foveal avascular zone
FDA	Food and drug administration
GAPDH	Glyceraldehydphosphat-dehydrogenase
ICAM-1	Inflammatory intracellular adhesion molecule 1
IOP	Intraocular pressure
NADPH	Nicotinamide adenine nucleotide phosphate hydrogen
NADP+	Nicotinamide adenine nucleotide phosphate
OCT	Optical coherence tomography
PARP	Poly-ADP-ribose polymerase
PGF	Placenta growth factor
PKC	Protein kinase C
PPV	Pars plana vitrectomy
PRN	Pro re nata
RAGE	Receptors for Advanced Glycation End Products
ROS	Reactive oxygen species
VCAM-1	Vascular cell adhesion molecule-1

1 Introduction

Diabetic retinopathy (DR) is a result of long-term diabetes mellitus and is one of the leading causes of blindness in western countries for the population in working age [1]. The prevalence of diabetes mellitus is increasing and is prognosed to rise from 382 million to 592 million people by year 2035 [2]. One of the causes for visual impairment in DR is diabetic macular edema (DME). Treatment with anti-vascular endothelial growth factor agents (Anti-VEGF) have become the standard of care during the last years [3]. Before end of 2015, Bevacizumab, an anti-VEGF agent, which is used as off label treatment for diabetic macular edema, was the prime anti-VEGF agent used at the Department of Ophthalmology at the Medical University of Graz. Patients with central involved DME receive a loading dose of three intravitreal injections with four-weekly intervals between them. Further treatment is continued according to the pro re nata (PRN) scheme. Criteria for further treatment after the loading dose are recorded increase of best corrected visual acuity (BCVA) and/or decrease in central subfield thickness (CST). If no further increase in BCVA and/or decrease in CST is achieved, depending on BCVA and CST, treatment with Avastin may be stopped or other treatment options such as laser photocoagulation, treatment with other anti-VEGF agent or treatment with steroids may be considered. Retreatment is applied, should BCVA decrease and/or CST increase over time.

2 Diabetic macular edema

2.1 Definition

DME is a major cause of visual impairment in diabetic retinopathy, which is one of the microvascular complications of diabetes mellitus. It is defined as an accumulation of the extracellular fluid in the macula, followed by thickening of the central retina and failure of maintaining a normal retinal structure [4]. The accumulation of the extracellular fluid is caused by an imbalance between entry and exit of it due to pathophysiological mechanisms described below. Fluid can accumulate in cavities and build “cysts” or it can appear as subretinal fluid. A healthy retina remains relatively dehydrated and can provide a transparency for good light transmission, whereas a retina with diabetic macular edema cannot

sustain a normal vision. This pathological state of hydration interacts with photon transmission and disturbs vision [5].

2.2 Epidemiology

Based on a systemic literature review, it is estimated that DME affects about 21 million people worldwide, 6.8 % of all people with diabetes mellitus [6] and is the primary cause of loss of vision for patients with diabetes mellitus type 2 [7]. The incidence is continuously increasing because of the constant gain of diabetes mellitus patients and an ageing population. The prevalence increases with high levels of HbA1c and the duration of diabetes mellitus [8]. Furthermore, it is higher in patients with diabetes mellitus of type 1 (14.25 %) in comparison to those with diabetes mellitus of type 2 (5,57%) [6]. There is no significant difference between sexes in prevalence for DME. Analyzing the prevalence between different ethnic groups, it is highest among African Americans and lowest among South Asians [6,8].

2.3 Risk factors

Well known systemic risk factors for DME are not pharmacologically treated dyslipidemia, poorly regulated blood glucose, measured in high HbA1c values, arterial hypertension and long duration of diabetes mellitus [6, 9].
Anemia may also be a risk factor or DME: Erythropoetine (EPO) was effectively used in patients with anemia and DME for DME treatment, whose visual acuity was improved after the EPO treatment [10].
For diabetes mellitus type 1, male gender is a risk factor for the development and progression of DR and diabetic maculopathy.
Pregnancy is a further and relevant risk factor for female patients with present diabetes, but there is no evidence on gestational diabetes leading to diabetic retinopathy or macular edema [11].
Some studies show an association between present diabetic nephropathy and an increased risk for diabetic retinopathy, meanwhile there is no evidence for an increased risk of diabetic macular edema [12 ,13].

2.4. Pathogenesis

The mechanism of the development of diabetic macular edema is not yet fully understood. Studies proved that inflammatory and angiogenetic processes due to diabetic impact cause retinal damage [4,14,15]. Permanent exposure to high blood glucose levels causes microangiopathy with endothelial damage [15]. The endothelium is a main component of the blood retina barrier [4]. Hyperglycemia activates many intracellular pathways, one of them is linked up to protein kinase C (PCK): due to high blood glucose level the concentration of diacylglycerol remains high and it leads to increased concentration of protein kinase C. PKC activates endothelin, which is a vasoconstrictor, so the retinal blood flow is reduced. Furthermore, PKC induces expression of cytokines like ICAM-1 (inflammatory intracellular adhesion molecule), VCAM-1 (vascular cell adhesion molecule). ICAM-1 is an endothelial cell receptor for leucocytes, so an increased concentration of it would lead to more intense adhesion of leucocytes and inflammation. Capillaries get occluded and the endothelium gets damaged by leucocytes, which leads to vasoconstriction, hypoxia and ischemia [4,16]. This causes local expression of VEGF (vascular endothelial growth factor), which induces a breakup of the tight junctions of the blood retinal barrier, loss of pericytes and causing fenestration of the endothelium, with following increased vascular permeability and capillary leakage [5,14].

Oxidative stress belongs to main initiators of DME as well. ROS (reactive oxygen species) as superoxide, hydroxyradicals and peroxyradicals activate PARP (poly-ADP-ribose polymerase) and reduce GAPDH (glyceraldehydphosphat-dehydrogenase) activity, a glycolytic enzyme, which leads to activation of PKC, polyol- and Advanced Glycation End (AGE) Products pathways [17]. In the polyol pathway, unused glucose is reduced to sorbitol and due to this reaction NADPH is oxygenated to NADP+. Decreased level of NADPH can cause an accumulation of ROS [17]. AGESs are nonenzymatically glycated molecules and they bind to RAGE (receptors for AGEs), what causes cell damage. Expression of RAGE is increased by oxidative stress. Binding to RAGE, AGEs activate inflammatory processes [14].

2.5 Classification

DME has been classified by the Global Diabetic Retinopathy Project Group in mild, moderate and severe DME, the severity depends on the distance of thickening and exudates from the fovea [19]. Mild DME contains retinal thickening and hard exudates in posterior pole but still remote from the central macula. Moderate DME shows retinal thickening and hard exudates surrounding the center of the macula. In severe DME retinal thickening and hard exudates include the center of the macula [19].

The Early Treatment of Diabetic Retinopathy Study (ETDRS) classified DME in clinically significant macular edema (CSME) and clinically insignificant macular edema. CSME is existent by the following clinical findings within a slit lamp fundus biomicroscopy [20] :

- “thickening of the retina at or within 500 μm of the center of the macula” [20]
- “hard exudates at or within 500 μm of the center of the macula associated with thickening of adjacent retina” [20]
- “a zone of retinal thickening one disc area or larger, any part of which is within 1 disc diameter of the center of the macula” [20]

Using OCT (optical coherence tomography), DME has been attempted to be classified into different types due to morphological patterns by various authors.

Dr. Koleva-Georgeva defined three types of diabetic macular edema, referring to the thickness of the macula, retinal morphology and existence of macular traction [21].

- Type 1: Simple diabetic macular edema.
- Type 2: Cystoid diabetic macular edema: 3a, mild; 3b, intermediate; 3c, severe.
- Type 3: Serous macular detachment.

However, none of them have been implemented as classification system [22, 23].

Beside these definitions there is also a differentiation between focal and diffuse macular edema, two terms which again are inconsistently defined in the literature. The definitions were based on four common examination methods and depend on the proportion of leakage arising from microaneurysms and/or dilatated capillaries detected by fluorescein angiography [24].
Via fluorescein angiography focal macular edema is classified by the existence of ≥67% of leakage from microaneurysms, whereas diffuse macular edema correlates with <33% of leakage from microaneurysms and is caused by a generalized break-down of the blood-retinal barrier. The percentage of 33-67% leakage stands for intermediate DME [23, 25].

Diabetic macular ischemia is clinically defined as an enlargement and disruption of the foveal avascular zone (FAZ) and is characterized by loss of the capillary network. It is irreversible and can be diagnosed via fluorescein angiography. [26,27,28]. The severity of the diabetic macular ischemia increases with the enlargement of the foveal avascular zone. A progression of the diabetic macular ischemia is associated with loss of vision [26].

2.6 Diagnosis

Diagnostic means for the detection of DME are slit-lamp biomicroscopy with dilatated pupils, fluorescein angiography and OCT [3].
Fluorescein angiography (FA) has been for many years the main diagnostic tool, but nowadays it is more and more replaced by OCT. However, FA is essential in the diagnosis of diabetic macular ischemia, identifying an enlarged FAZ due to nonperfusion, and vascular leakage. [3]. FA also provides a better understanding of morphological damages of the peripheral retina.

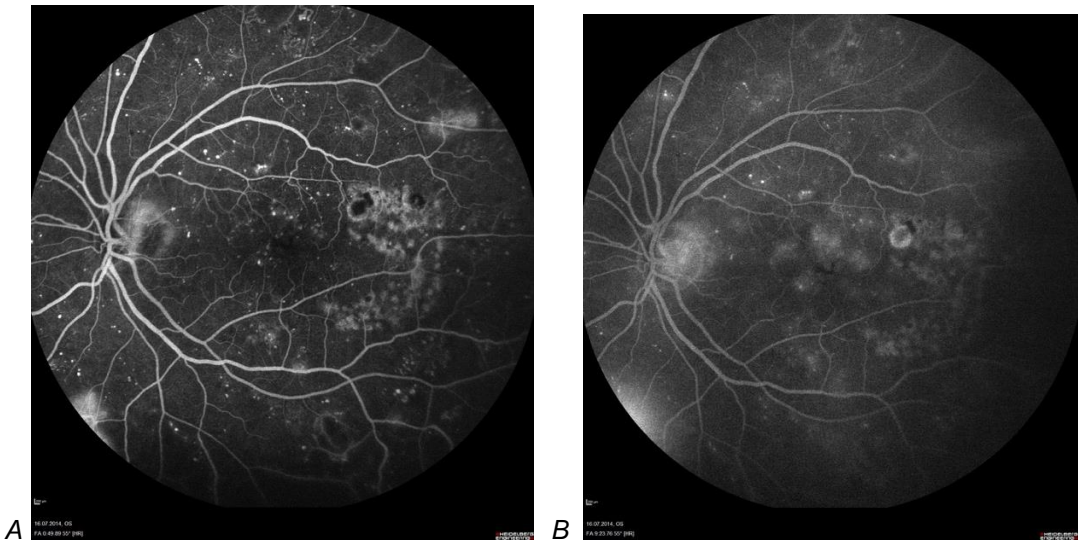


Figure 1: Fluorescein angiography of the retina in early (A) and late phase (B).

OCT Angiography (OCT-A) is a non-invasive, transpupillar method to produce in vivo images of retinal capillary layers and to evaluate the perfusion of the retina. An advantage of OCT-A, over the FA is to observe retinal layers in depth [3]. It is also an important method in identifying diabetic macular ischemia [27].
OCT should be used for monitoring of treatment response [28]. It is also useful to differentiate diabetic eye changes from nondiabetic eye diseases such as vitreofoveal traction syndrome, epiretinal gliosis and age-related macular degeneration [29].
However, it cannot be used to diagnose foveal ischemia and widening of FAZ, which are relevant for the outcome [3].

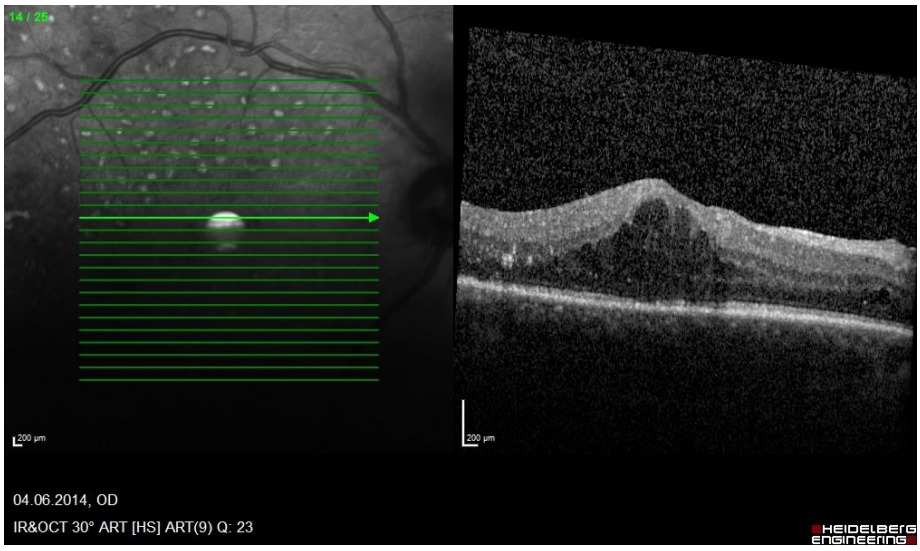


Figure 2: OCT-image of a diabetic macular edema.

2.7 Therapy

Treatment approach for every person with diabetic macular edema is an efficient blood sugar and blood pressure control in addition to lifestyle modification. Eliminating or controlling these risk factors via losing weight, antihypertensives and lipid-lowering agents are relevant in the regression of DME [15]. Nowadays three therapy onsets exist, which are medical treatment, laser treatment and surgical management.

2.7.1 Intravitreal medication

Due to pathophysiological mechanisms anti-VEGF treatment is the main initial therapy for DME involving the macular center. Multiple clinical trials have shown a significant improvement in DME with anti-VEGF agent treatment [30,31,32,33, 34,35]. Three agents, Ranibizumab, Aflibercept and Bevacizumab have been proven as safe treatment, the first two agents are approved by the Food and Drug administration (FDA) for treatment. Ranibizumab (Lucentis®) is a recombinant humanized monoclonal antibody fragment which binds to VEGF-A [36]. Aflibercept (Eylea®) is a human fusion protein consisting of key domains for VEGF receptor 1 and 2, as well as Placenta Growth Factor (PGF) and constant region (Fc) of human immunoglobulin IgG1 [37]. Bevacizumab (Avastin®), a solid humanized monoclonal antibody binding to all VEGF-A isoforms and with comparable efficacy to Aflibercept and Ranibizumab, is used off-label [37, 38]. Protocol T of the DRCR Network provides a comparison of the treatment results of these three agents presenting a mean improvement in visual acuity and reduction of central subfield thickness (CST). Figure 1 presents a summary of the 1 year results from the Protocol T study and figure 2 presents a summary of the 2 year results. As noticeable, the use of Bevacizumab has similar results in change in visual acuity and central subfield thickness as Ranibizumab and Aflibercept for eyes with visual acuity at baseline of 69 or more letters after one year and two years. For eyes with visual acuity less than 69 letters, Aflibercept showed a significant improvement in visual acuity gain in comparison to the other two agents after one year and remaining significant in comparison to Bevacizumab after two years. Comparing all eyes without dividing them by baseline visual acuity, Aflibercept had better results after 1 year compared to the other two agents and had better results after 2 years

compared to Bevacizumab but was similar to Ranibizumab. It is possible to compare different visual acuity scales roughly (Figure 2). Visual acuity EDTRS letter score of less than 69 (equivalent to 20/50 or worse) corresponds approximately to a decimal notation of 0.4 and less, 78 to 69 letters (equivalent to 20/32 to 20/40) correspond approximately to 0.5-0.8 [30, 31, 39].

There is an important factor to mention, which are the costs for a single intravitreal injection. The cost for a single injection of Aflibercept lies by $1,950, for Bevacizumab by $50 and for Ranibizumab by $1,200 [31].

	Aflibercept	Bevacizumab	Ranibizumab
VA improvement after 1 year from Baseline in letter score	+13.3	+9.7	+11.2
VA change after 1 year for eyes with Baseline VA < 69 ± SD	+18.9±11.5	+11.8±12.0	+14.2±10.6
VA change after 1 year for eyes with Baseline VA ≥ 69 ± SD	+8.0±7.6	+8.7.5±7.4	+8.3±6.8
CST decrease after 1 year from Baseline ± SD	169±138	101±121	147±134
CST decrease after 1 year for eyes with Baseline VA < 69 ± SD	210±151	135±152	176±151
CST decrease after 1 year for eyes with Baseline VA ≥ 69 ± SD	129±110	67±65	119±109
Median number of injections during 1 year, no.	9	10	10

Table 1: Summary of the DRCRnet Protocol T study 1 year results.

	Aflibercept	Bevacizumab	Ranibizumab

VA improvement after 2 years from Baseline in letter score	+12.8	+10.0	+12.3
VA change after 2 years for eyes with Baseline VA < 69 ± SD	+18.1±13.8	+13.3±13.4	+16.1±12.1
VA change after 2 years for eyes with Baseline VA ≥ 69 ± SD	+7.8±8.4	+6.8±8.8	+8.6±7.0
CST decrease after 2 years from Baseline ± SD	171±141	126±143	149±141
CST decrease after 2 years for eyes with Baseline VA < 69 ± SD	211±155	185±158	174±159
CST decrease after 2 years for eyes with Baseline VA ≥ 69 ± SD	133±115	68±98	125±118
Median number of injections during 2 years, no.	15	16	15

Table 2: Summary of the DRCRnet Protocol T study 2 years results.

Snellen score	**Decimal notation**
20/20	**1.0**
20/25	**0.8**
20/32	**0.63**
20/40	**0.5**
20/50	**0.4**
20/63	**0.32**
20/80	**0.25**
20/100	**0.2**
20/125	**0.16**
20/160	**0.125**
20/200	**0.1**

Table 3: Visual acuity conversion table.

Guidelines for the management of diabetic macular edema by the European Society of Retina Specialists (EURETINA) recommend following therapy scheme

with anti-VEGF agents: Ranibizumab and Aflibercept are the drugs of choice for BCVA under 69. In eyes with BCVA of 69 or more all three agents can be applied. The treatment with Ranibizumab (Lucentis®) should start with monthly injections and continued until achievement of stability in BCVA and OCT is reached. Afterwards a monthly monitoring should occur during the first year, on the basis of monitoring the interval of injections and the monitoring itself can be evaluated individually. If BCVA is below 69 letters Aflibercept (Eylea®) should be considered for treatment of DME and should initially be injected monthly as a loading dose, followed either by bimonthly injections or monthly monitoring with injections applied in a PRN regimen [3].

As inflammatory pathways are assumed being co-responsible for the development of DME, corticosteroids (triamcinolone acetonide, which is an off-label treatment option, dexamethasone and fluocinolone acetonide) can be another option for medical treatment, especially for pseudophakic patients, patients with poor response to a series of anti-VEGF treatments, ones with a history of major cardiovascular events, multimorbid and/or non-compliant patients. However, intravitreal corticosteroids are associated with increasing IOP (intraocular pressure), cataract progression and seem to have an increased risk for endophthalmitis after intravitreal injection in comparison to anti-VEGF treatments [40,41].

2.7.2 Laser photocoagulation and surgery

Laser treatment was the gold standard therapy for CSME for many years recommended by the ETDRS [3]. Today there is no evidence about the benefit of laser photocoagulation over pharmacological treatments, so it is no longer first line therapy and it has been replaced by anti-VEGF agents [3]. The use of laser alone is less effective than the use of an anti-VEGF agent with a focal or grid laser [42, 43]. Laser can be considered for microaneurysms and capillary leakage and if central macular thickness is less than 300 μm or DME is accompanied with persistent vitreal macular adhesion. Subthreshold grid laser can be useful at the beginning of the development of DME, as long as the visual acuity is still good [3].

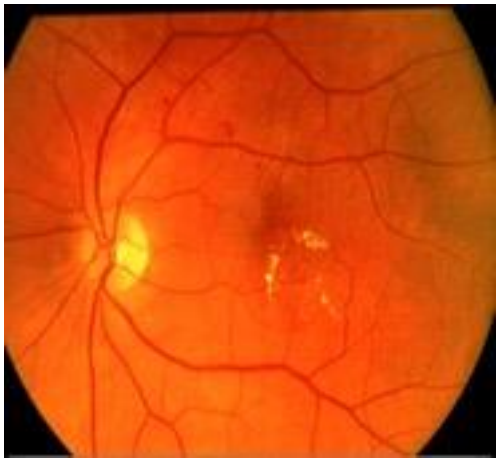
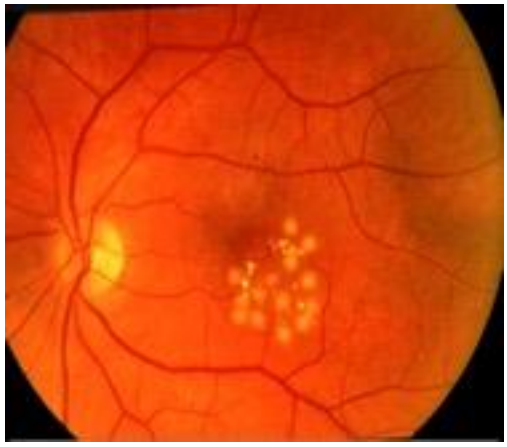
A  B 

Figure 3: Fundus photography in diabetic macular edema before treatment with focal laser photocoagulation (A) and after focal laser photocoagulation treatment (B).

Pars plana vitrectomy (PPV) is a potential therapy alternative, if epiretinal membrane or vitreomacular traction and diffuse DME are present and intravitreal therapy with anti-VEGF or steroids was ineffective and in eyes without posterior vitreous detachment [3]. Predictive factor for a successful PPV is a poor preoperative BCVA which is associated with better BCVA improvement after surgery [44].

3 Study

3.1 Purpose

The purpose of the following study was to compare real-life anti-VEGF therapy for diabetic macular edema at the Medical University of Graz with the results of clinical trials, which have strict requirements and relatively optimal conditions. At the Medical University Graz mainly Avastin® and Eylea® are used as anti-VEGF agents. Before autumn 2015, eyes with central involved diabetic macular edema received as first-line therapy primarily Avastin®. Since autumn 2015 eyes with an initial visual acuity of 0.4 or lower are treated with Eylea®.

The clinic follows a PRN scheme, beginning in most cases with a loading dose of three injections and continue treatment as needed by monitoring the clinical

features best corrected visual acuity (BCVA) and central subfield thickness (CST) of patients. Second line therapy is intravitreal injection with steroids, predominantly Ozurdex®.

The main outcome of this study is to evaluate the number of injections with Avastin® applied in the first two years in eyes with intravitreal treatment-naive diabetic macular edema. The secondary outcomes are changes of BCVA, CST, macular volume and IOP during the first two years of treatment with Avastin®. In addition, an analysis of the number of injections in eyes with a pretreatment with panretinal photocoagulation before intravitreal anti-VEGF treatment and with a treatment with panretinal photocoagulation during intravitreal anti-VEGF treatment was performed.

This study will help to evaluate the efficiency of anti-VEGF treatment under a PRN scheme. The outcomes of this study will be compared with existing literature, with particular interest in Protocol T of the DRCR Network, which compared the three anti-VEGF agents Aflibercept, Bevacizumab and Ranibizumab over one and two years.

3.2 Methods

This retrospective study was conducted according to the guidelines of the declaration of Helsinki and was approved by the ethic committee of the Medical University of Graz.

This study included patients who were diagnosed with diabetic macular edema and received initial treatment with Avastin® between the 01.01.2014 and the 31.12.2015 and were treated only with Avastin® over the following 2 years. Patients medical records were followed for 2 years. Hence, the last possible date of visit was the 31.12.2017. Inclusion criteria was diagnosed diabetic macular edema requiring anti-VEGF treatment with Avastin®. Exclusion criteria was a previous treatment with intravitreal anti-VEGF agents and/or intravitreal corticosteroids, a change in intravitreal medication (anti-VEGF agents and/or corticosteroids) other than Avastin® over 2 years, patients under the age of 18 and

other macular diseases influencing visual acuity, e.g. age-related macular degeneration, retinal vein/arterial occlusions ect.

All of patients' data used in this study were collected in a Microsoft Excel 2016 sheet. Each patient and each eye received an ID. Patients data contained information on gender, age, stage of diabetic retinopathy, diabetes mellitus type, duration and therapy and HbA1c level at baseline visit (date on which initial treatment with Avastin® was recommended for DME), as well as pathological events associated with diabetes mellitus, such as stroke, myocardial infarction, renal insufficiency and arterial hypertension. Pretreatment of the eyes with panretinal or central laser, use of glaucoma medication and if the lens was phakic or pseudophakic at baseline visit, were also recorded.

The baseline visit is defined as date of first visit on which initial treatment with Avastin® was recommended for DME. Patients were treated and monitored as required, at baseline and on each follow-up visit BCVA (expressed in decimal numbers), intraocular pressure (IOP), CST and macular volume (measured by OCT) were collected. CST or foveal thickness was defined as the average thickness in the central 1000-µm diameter of the Early Treatment Diabetic Retinopathy Study layout [45]. Macular volume was defined as the sum of all nine sections of the macula [46]. Treatments applied within the study period, including injections with Bevacizumab (Avastin®), initiation of glaucoma treatment, panretinal photocoagulation, central photocoagulation and cataract surgery, were documented.

Statistical analysis of the data was performed with SAS version 9.4. in cooperation with Dr. Riedl from the Institute of medical informatics, statistics and documentation. The significance threshold was set at a P value less than 0.05.

This study used two models to compare the secondary outcomes. The first model compared the secondary outcomes BCVA, CST, macular volume and IOP from the baseline visit with the last control visit of the first year and the last control visit

of the second year. The second model compared secondary outcomes from the baseline visit with the last control visit of the entire surveillance period.

3.3 Results

Patients

The study included 157 eyes of 110 patients with intravitreal treatment naïve diagnosed DME requiring Anti-VEGF therapy. 70 (63.6%) of them were male, 40 (36.4%) were female. The age of the patients ranged from 29 to 90 years, mean age was 67.3 years. Their mean diabetes duration was 17.2 years and their mean HbA1c was 7.6 %. Most of the patients were type 2 diabetics, 11 (10%) suffered from diabetes mellitus type 1 and 99 (90%) from diabetes mellitus type 2.

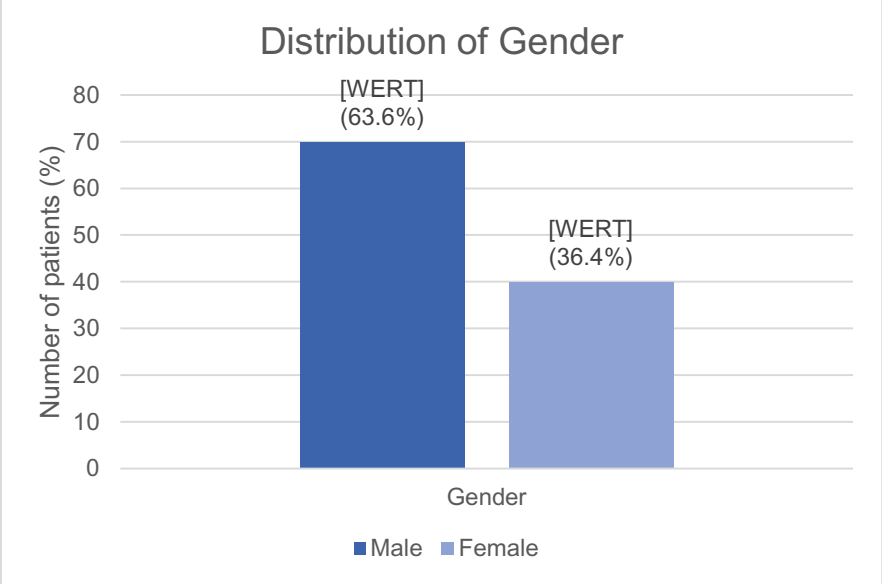


Figure 4: Distribution of gender of patients.

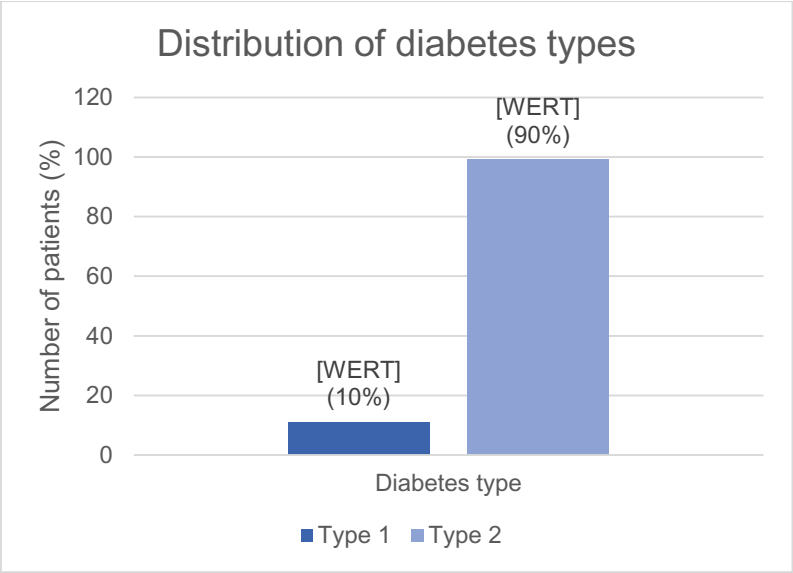


Figure 5: Distribution of diabetes mellitus type of patients.

Concerning the treatment management of diabetes, 5 (4.6%) patients had no therapy at all or were on a diet, 30 (27.5%) had a therapy with oral antidiabetic agents, 70 (64.2%) had a therapy with either insulin injections alone or in combination with oral antidiabetic agents and in 4 (3.7%) patients the therapy was inconclusive.

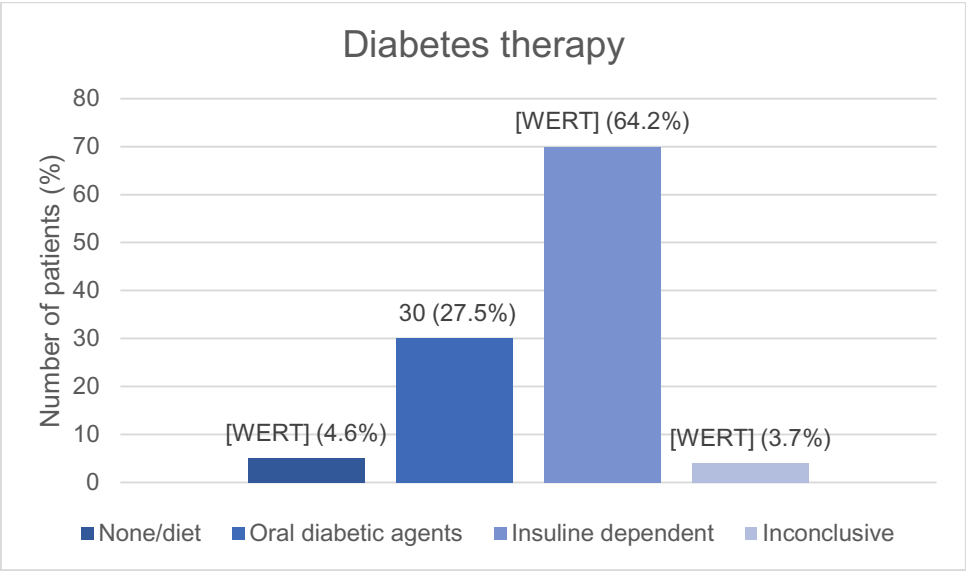


Figure 6: Distribution of different therapy modes among the patients.

83 (75.5%) patients suffered from arterial hypertension, 23 (20.9%) patients had no arterial hypertension and in 4 (3.6%) patients it was inconclusive.
6 (5.5%) patients suffered from renal insufficiency, 22 (20.0%) patients had no renal insufficiency and in 82 (74.5%) patients it was inconclusive.

7 (6.4%) patients suffered a stroke at least once in their life, 103 (93.6%) patients had no history of a stroke.
8 (7.3%) patients suffered a myocardial infarction at least once in their life, 102 (92.7%) had no history of a myocardial infarction.
66 (42%) eyes had a mild DR, 32 (20.4%) a moderate DR, 18 (11.5%) a severe DR and 41 (26.1%) a proliferative DR.

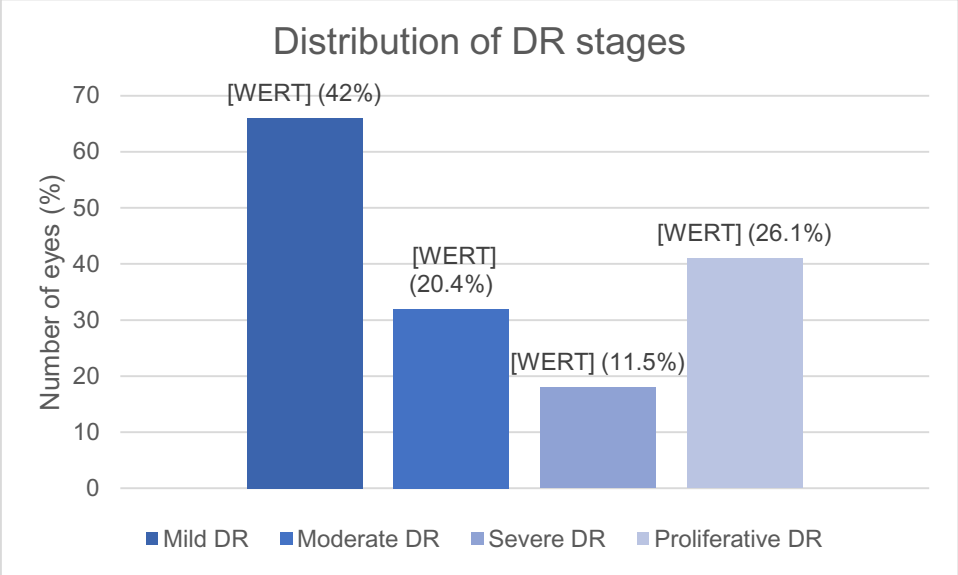


Figure 7: Distribution of diabetic retinopathy stages among the eyes.

38 (24.2%) received panretinal photocoagulation treatment prior to study inclusion, 119 (78.8%) eyes had no pretreatment with panretinal photocoagulation.
22 (14.0%) had at least one focal laser photocoagulation treatment prior to study inclusion, 135 (86.0%) eyes had no pretreatment with focal laser photocoagulation.
101 (91.8%) had no glaucoma therapy at the baseline visit, 9 (8.2%) already had a glaucoma therapy at baseline.
116 (73.9%) eyes were phakic, 41 (26.1%) pseudophakic.

Surveillance period

Surveillance period was defined as the period from the first visit to the last available data, within 2 years after treatment initiation, including both last control visit or last injection visit.

In 22 (14%) the last visit was an injection visit. The mean surveillance period was 9.9 ± 7,2 months (0.23-23.92). In the first 12 months 157 eyes were treated and monitored, in the following 12 months there were data from only 50 eyes. Based on this fact, a subgroup analysis for these 50 eyes, concerning secondary outcomes, was conducted.

The median follow-up time for the first year was 6.5 months and 19.2 months for the second year. The median follow-up for all patients over two years was 6.9 months with a minimum of 2.0 months and a maximum of 23.9 months.

Intravitreal injections

The 157 eyes received a total of 719 injections during the study period of 24 months. The mean number of injections per eye was 4.6 ± 2.4 (1-13). 59 (37.58%) eyes received 3 injections, 73 (46.5%) eyes received 1-3 injections and 84 (53.5%) received more than 3 injections. During the first 12 months, 157 eyes received 645 injections altogether, the mean number of injections was 4.1 ± 1.8 (1-9), the majority of eyes, 67 (42.68%), received 3 injections over the first year. In the next 12 months, 50 eyes received a total of 74 injections, mean number of injections was 1.48 ± 1.8 (0-7) and 22 (44%) of these 50 eyes received no injection at all in the second year.

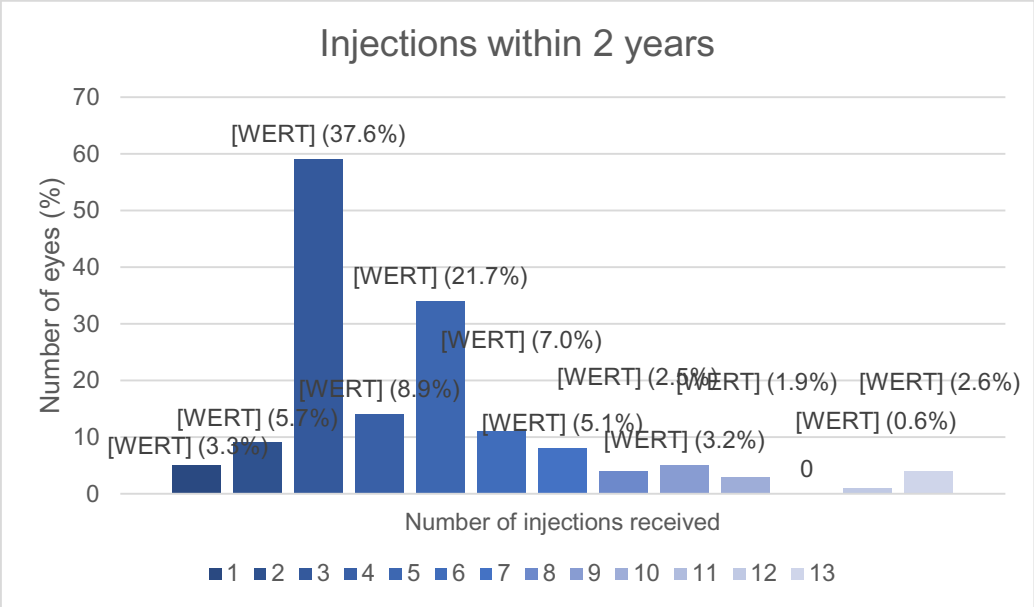


Figure 8: Distribution of the number of injections over 2 years.

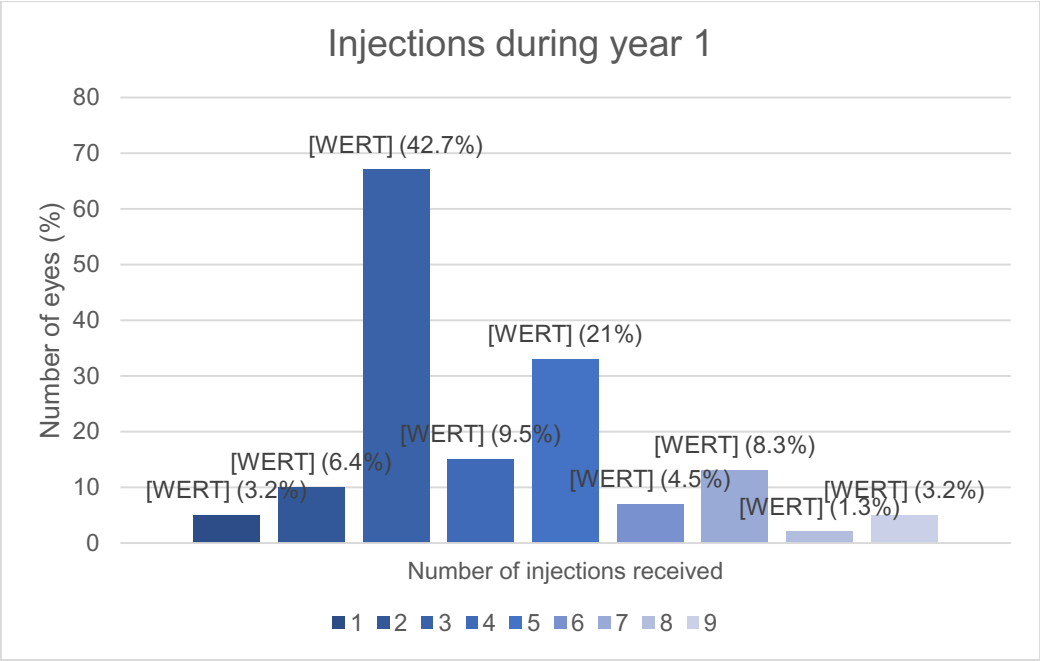


Figure 9: Distribution of the number of injections within the first year.

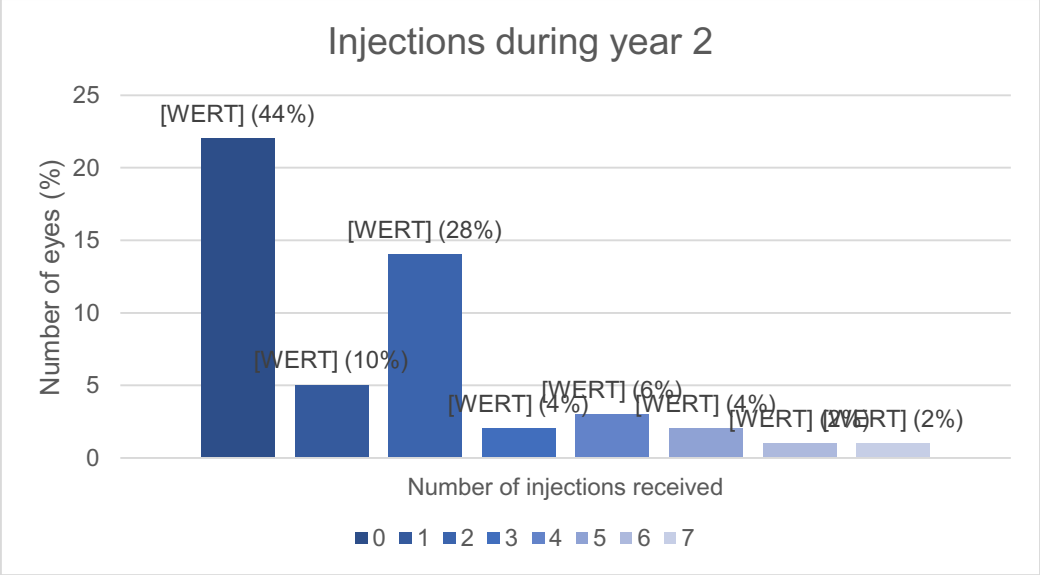


Figure 10: Distribution of the number of injections within the second year.

38 (24.2%) eyes had been pretreated with panretinal laser photocoagulation (PRP) before baseline visit and had a median number of 5 Avastin® injections. In comparison, 119 (75.8%) eyes, who had no previous treatment with PRP, had a median number of 4 Avastin® injections. In both groups the total number of injections per eye ranged from 1 to 13 injections.

27 (17,2%) eyes which were treated with PRP for proliferative diabetic retinopathy during the surveillance period had a median of 5 Avastin® injections. The total number of injections per eye ranged from 2 to 9 injections. 130 eyes received no PRP treatment during the surveillance period and had a median number of 4 Avastin® injections.

PrePRP	No. of eyes	No. of all injections	Mean no. of injections ± SD	Median	Minimum	Maximum
No PRP pretreatment	119	534	4.5 ± 2.3	4	1	13
PRP pre-treatment	38	185	4.9 ± 2.7	5	1	13

Table 4: Number of injections applied in 2 years in eyes which were pretreated with PRP at the baseline and in eyes without a PRP pretreatment.

PRP01	No. of eyes	No. of all injections	Mean no. of injections ± SD	Median	Minimum	Maximum
No PRP treatment	130	590	4.5 ± 2.5	4	1	13
PRP treatment	27	129	4.8 ± 1.9	5	2	9

Table 5: Number of injections applied in 2 years in eyes which were treated with PRP during the treatment with Bevacizumab and in eyes without a PRP treatment during Bevacizumab treatment.

BCVA

Mean BCVA at baseline was 0.45±0.23. In the first model there was an increase of BCVA during the first year from baseline to 0.54 ± 0.25 (P<0.001) and an increase of BCVA from baseline to 0.51 ± 0.24 (P=0.017) during the second year.

The second model showed similar results. BCVA increased from 0.45 ± 0.23 to 0.53 ± 0.25 (P<0.001) during the second year.

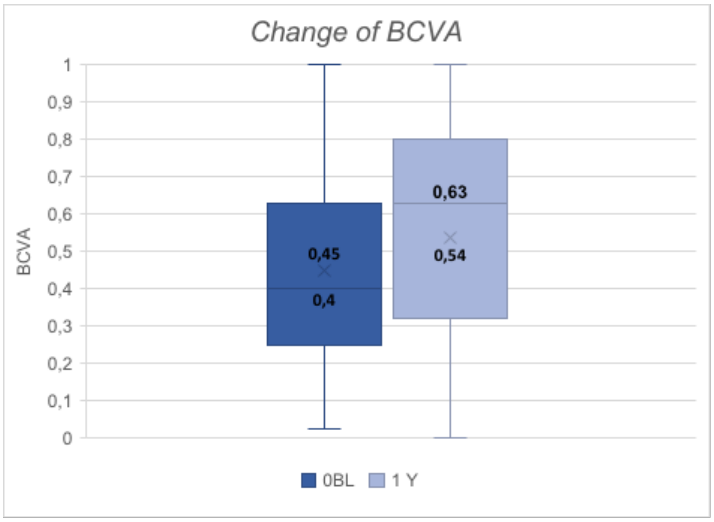


Figure 11: Change of BCVA at baseline and during the first year for all eyes

Subgroup:

In the subgroup of 50 eyes similar results were noticed. The mean BCVA at baseline was 0.45 ± 0.22 (0.1-1.0), which increased during the first year to 0.54 ± 0.25 (0.13-1.0, P=0.003) and increased to 0.51± 0.24 (0.05-1.0, P=0.05) over the second year.

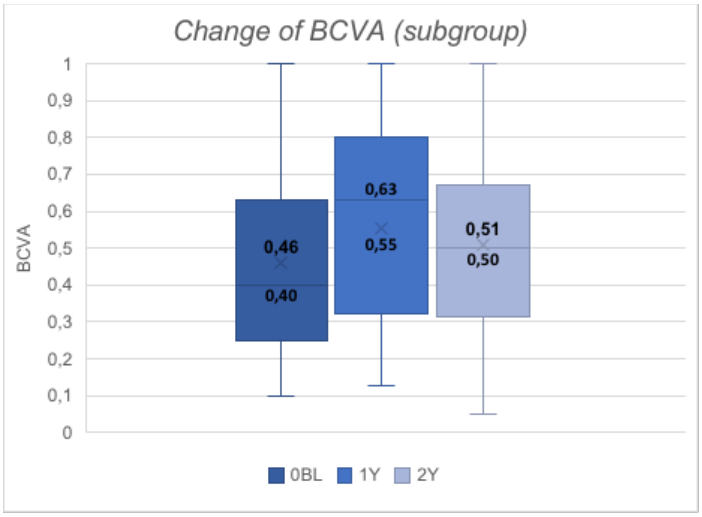


Figure 12: Change of BCVA at baseline, during the first and second year for the subgroup

IOP

Outcomes for IOP were also analyzed with the two above mentioned models. There was no significant variation of IOP in both models. Mean baseline IOP was 15.1 ± 3.3 mmHg (8.0-25.0). In the first model mean IOP during the first year was 14.6 ± 3.7 mmHg (5.0-28.0, P=0.28), in the second year mean IOP was 14.9 ± 4.9 mmHg (9.0-32.0, P=0.8).

The second model showed similar results. Mean IOP during the complete two years was 14.6 ± 4.7 mmHg (5.0-32.0, P=0.36).

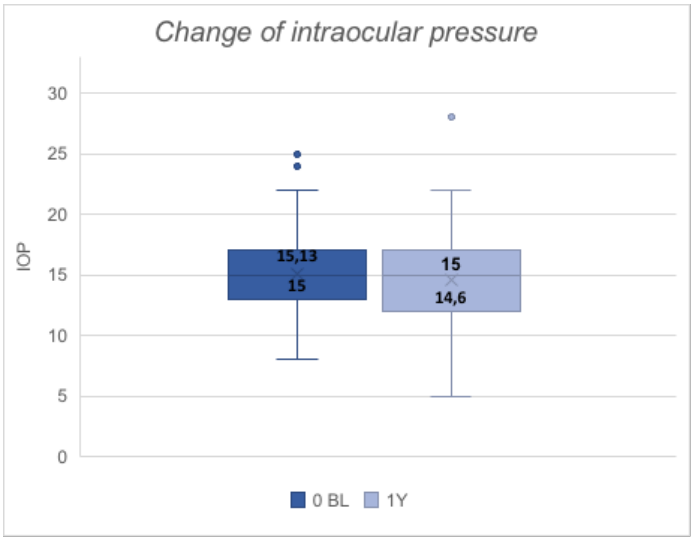


Figure 13: Change of IOP at baseline and during the first year for all eyes

Subgroup:

The subgroup also showed no significant changes in IOP compared to all eyes. IOP at the baseline was 15.46 ± 3.91 mmHg (8.0-25.0). In the first year the mean IOP was 14.42 ± 3.29 mmHg (5.0-21.0, P=0.3), in the second year mean IOP was 14.87 ± 4.90 mmHg (9.0-32.0, P=0.76).

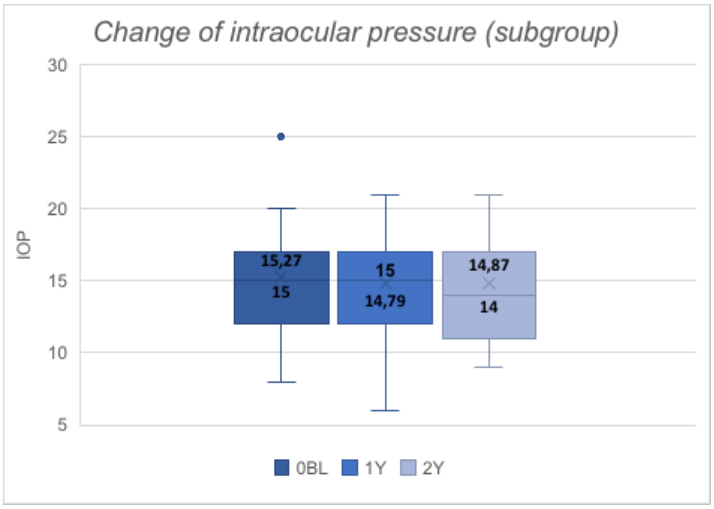


Figure 14: Change of IOP at baseline, during the first and second year for the subgroup

CST

Mean baseline CST was 416.5 ± 122.1 μm (226.0-905.0). In the first model, mean CST decreased to 356.1 ± 103.9 μm (149.0-706.0, P<.0000001) during the first year, during the second year mean CST decreased to 347.2 ± 132.2 μm (196.0-862.0, P=0.002). The second model showed a decrease in mean CST to 358.1 ± 113.2 μm (196.0-862.0, P=0.0), the mean change in CST was 58,4 μm.

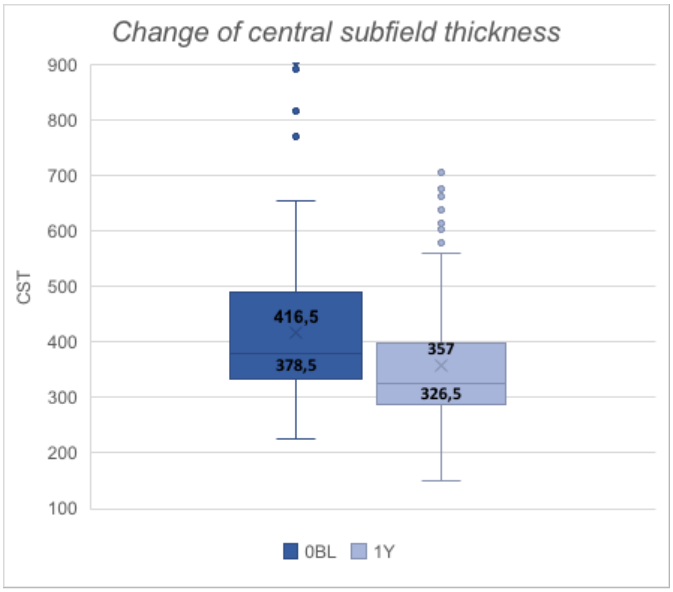


Figure 15: Change of the mean central subfield thickness at baseline and during the first year for all eyes

Subgroup:

Regarding the mean CST, the subgroup showed similar results to all eyes.

The baseline CST was 441.51 ± 133.82 μm (226.0-905.0). Mean CST decreased during the first year to 367.51 ± 103.98 μm (149.0-679.0, P=0.001) and decreased during the second year to 374.19 ± 132.25 μm (196.0-862.0, P=0.004).

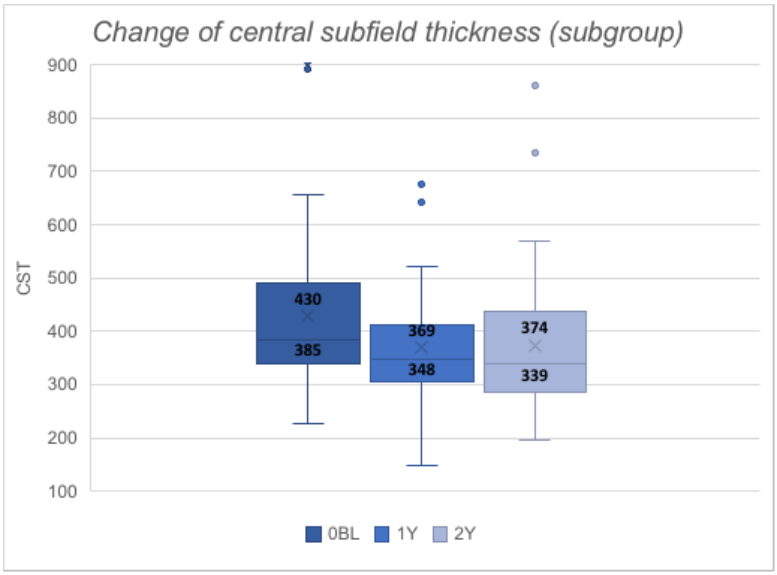


Figure 16: Change of central subfield thickness at baseline, during the first and second year for the subgroup.

Macular volume

Mean baseline macular volume was 10.1 ± 2.1mm^3 (1.5-17.1). In the first model, there was a decrease in mean macular volume during the first year to 9.3 ± 1.7 mm^3 (1.4-14.2, P<.0000001) and a decrease during the second year to 9.6 ± 2.0 mm^3 (3.8-17.1, P=0.0005). According to the second model, mean macular volume during the 2 years was 9.3 ± 1.9 mm^3 (1.4-17.1, P=0.0000).

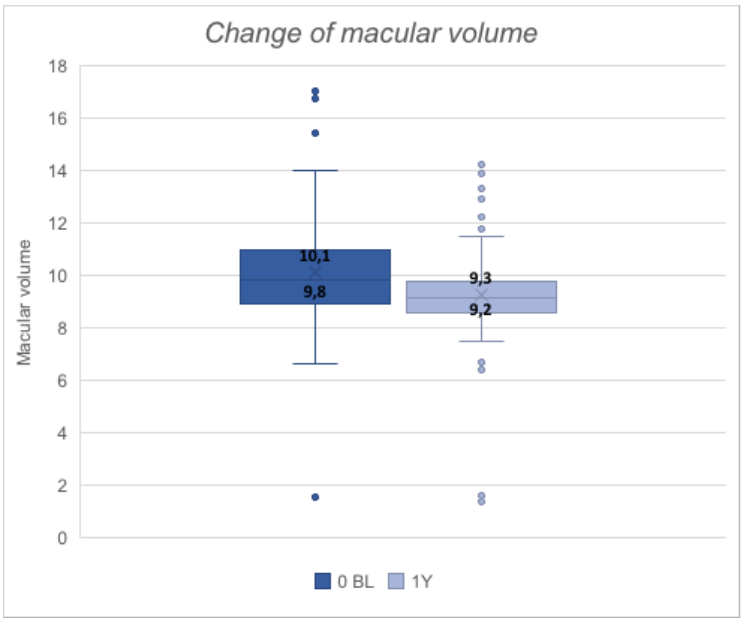


Figure 17: Change of the macular volume at baseline and during the first year for all eyes

Subgroup:

Again the results were similar to all eyes. The mean baseline macular volume of 50 eyes was 10.45 ± 1.71 mm^3 (8.17-16.77). There was a decrease during the first year to 9.65 ± 1.35 mm^3 (7.73-14.23, P=0.002) and remaining at 9.64 ± 1.95 mm^3 (9.47-17.10, P=0.0025) during the second year.

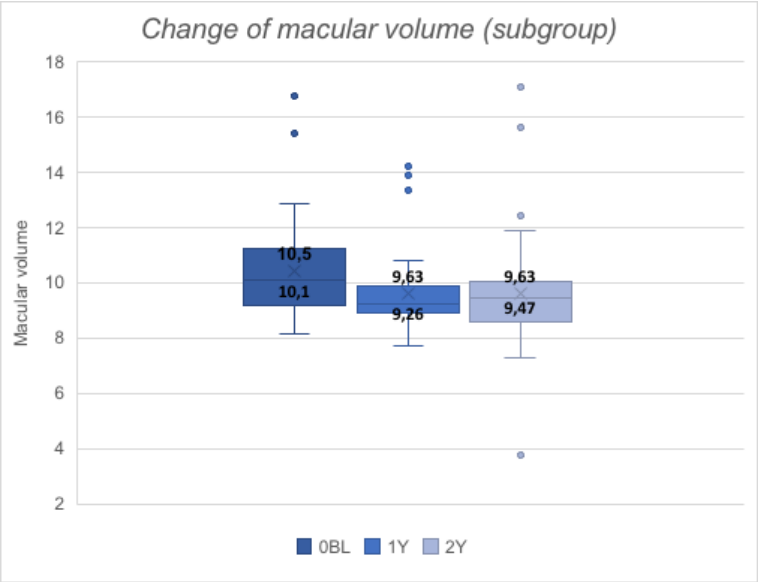


Figure 18: Change of macular volume at baseline, during first and second year for the subgroup.

3.4. Discussion

The 157 eyes of 110 patients with diabetic macular edema, treated with intravitreous bevacizumab injections, showed an improvement in visual acuity and a reduction in central subfield thickness, which was statistically significant. However, when compared with clinical trials the improvement was not as sufficient. Median number of injections during the first year was 3, which is less than in other clinical trials like Protocol T of the DRCR Network (median number of bevacizumab injections was 10) [31], RETAIN (median number of Ranibizumab injections was 7) [47], RESTORE (median number of Ranibizumab injections was 7) [33] and BOLT (median number of Bevacizumab injections was 9) [48].Though the number of injections in this study was similar to the outcomes in the POLARIS study. The POLARIS study was a retrospective study, under a non-clinical trial setting in a German population, which had a median number of 4 injections [49]. POLARIS is an international observational study which analyzed the influence of treatment and control intervals in clinical practice. Median number of injections during the two years in this study was 4, which is again less compared to 16 for

Bevacizumab in Protocol T of the DRCR Network [30], 10 in the RETAIN (PRN group) and RESTORE studies [47,33], and 13 in the BOLT study [48]. 46.5% of eyes received 3 or less injections during the surveillance period.
Baseline characteristics, age, duration of diabetes, diabetes type and HbA1c of the patients in this study, were similar to other clinical studies such as RETAIN, RELIGHT, RESTORE and BOLT [47,50,33,48]. In this retrospective study visual acuity was collected in decimals. Due to the use of a different visual acuity scale, comparison of baseline BCVA and its changes over one- and two-years with other studies was limited. However, after converting the decimals into Snellen score or ETDRS, baseline BCVA in this study was similar to the Protocol T study [30].

Concerning baseline ocular characteristics, mean BCVA with 0.45 ± 0.23, approximately 20/50-20/40 Snellen equivalent or 65-70 EDTRS letter score was similar to slightly better, than in other studies [30, 33, 47,48, 50]. Mean baseline CST with 416.50+-122.05 µm was similar to slightly less, than in other studies [30, 33, 47,48,50].

Mean BCVA increased over two years when comparing BCVA at baseline (0.45 ± 0.23) with the last available visit (0.53 ± 0.25), which is approximately a gain of one line in the visual acuity scale. In Protocol T the Avastin® group had a mean gain in visual acuity of 10 EDTRS letters, approximately 2 lines, for Eylea® and Lucentis® it was more than 2 lines [29]. In the BOLT study Bevacizumab showed a mean gain of 8.6 EDTRS letters after two years [48]. In the POLARIS study, Ranibizumab had a gain of 4.1 ± 12.4 EDTRS letters after one year, which is similar to this study [49]. Mean BCVA for the subgroup of 50 eyes at the end of the first year was 0.54 ± 0.25 higher than towards the end of the second year with 0.51 ± 0.24.

Mean CST decrease over two years was 58.4 µm. This result was significant less compared to other abovementioned clinical trials, where mean CST decrease was 126 ± 143 µm for Bevacizumab in Protocol T [30], 146 ± 171 µm for Bevacizumab in the BOLT study [48] and 102,1 ± 162 µm in the POLARIS study [49].

In Protocol T there is a differentiation between two groups of eyes with baseline BVCA of a letter score between 78 and 69 and with BCVA of a letter score less than 69. The group with baseline BCVA of a letter score less than 69 achieved significant better results under the treatment with Aflibercept and Ranibizumab than with Bevacizumab. The group with baseline BCVA of a letter score from 78 to 69 achieved slightly better results under the treatment with Aflibercept and Ranibizumab than with Bevacizumab (Table 1 and 2).

50% percent of the eyes in this study had a baseline BCVA of 0.4 or less, which is currently an indication for intravitreal injection with Eylea® in the Eye Clinic of Graz. These eyes may have potentially reached a better BCVA, if they would have been treated with Eylea®.

An important fact should be considered, that patients were lost to follow-up, either after injections or control visits due to unknown reasons such as incompliance, hospitalization and death. Furthermore, it is unknown if patients that were sent in care of registered ophthalmologists within the 2-year surveillance period, might have required further treatment due to recurring DME.

4 Conclusion

Median number of intravitreal injections of Avastin® at the University Eye Clinic in Graz in the surveillance period of 2 years was 4, which is significantly lower than the number of injections in studies mentioned in the discussion above. All the same, these results are well comparable with the POLARIS cohort study of Germany, a trial to analyze the real-life treatment situation of patients with DME in Germany.

Because of the real-life setting and hindered logistics it might be impossible to achieve similar results concerning the number of applied injections as in prospective trials, thereby it might be unrealistic to get similar outcomes in BCVA and CST as in these trials. However, it is necessary to apply routinely more injections so that patients can benefit from better visual acuity.

Limitations of this study are a retrospective nature and a small number of patients, especially during the second year of the study. An accurate comparison and evaluation of the results regarding BCVA is not possible due to the use of different visual acuity scales. Therefore, it should be considered for future studies at the Eye Clinic of Graz to use ETDRS charts for routine patients.

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