Intravitreal Bevacizumab as Anti-Vascular Endothelial Growth Factor in the Management of Complications of Diabetic Retinopathy

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DECLARATION

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This dissertation includes 10 original papers published in peer reviewed journals or books and 1 unpublished publication. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and for each of the cases where this is not the case a declaration is included in the dissertation indication the nature and extent of the contributions of co-authors.

J. Fernando Arevalo, MD FACS Date: December 2018

Declaration by the candidate, J. Fernando Arevalo:

In each publication where I am first author, the manuscript complied with the different journal's requirements for author's contribution (Chapters 2 to 11). With regard to Chapter 2 to 12, the nature and scope of my contribution were as follows:

Chapter	**Nature of contribution**	**Extent of contribution**
Chapter 2	The PhD candidate saw patients in his center that were included in the study. In addition, performed design of the work, the acquisition, analysis, and interpretation of data for the work; wrote the chapter/revising it critically for important intellectual content; and final approval of the version to be published.	90%
Chapter 3	The PhD candidate saw patients in his center that were included in the study. In addition, performed design of the work, the acquisition, analysis, and interpretation of data for the work; wrote the chapter/revising it critically for important intellectual content; and final approval of the version to be published.	90%
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Chapter 9	The PhD candidate operated on all patients in his center that were included in the study. In addition, invented the new technique described in the article, performed conception and design of the work, the acquisition, analysis, and interpretation of data for the work; wrote the chapter/revising it critically for important intellectual content; and final approval of the version to be published.	95%
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Chapter 11	The PhD candidate saw patients in his center that were included in the study. In addition, performed conception and design of the work, the acquisition, analysis, and interpretation of data for the work; wrote the chapter/revising it critically for important intellectual content; and final approval of the version to be published.	90%
Chapter 12	The PhD candidate saw patients in his center that were enrolled in the study (the majority of patients). Contacted other centers to contribute cases. In addition, performed conception and design of the work, the acquisition, analysis, and interpretation of data for the work; wrote the chapter/revising it critically for important intellectual content; and final approval of the version to be published.	90%

Nature of contribution Extent of contribution (%)

The following co-authors have contributed to chapters 2 to 12:

Name	**e-mail address**	**Nature of Contribution**	**Extent of contribution (%)**
Juan Gonzalo Sanchez	[redacted]	Chapter 2: Provided comments on the protocol, help design graphs and images, and provided comments on the chapter.	10%

		Chapter 3: Provided comments, help design graphics, and provided comments on the chapter.	10%
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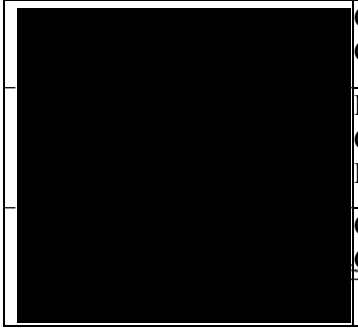
Declaration by co-authors:

The undersigned hereby confirm that

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to chapters 2-12,
2. no other authors contributed to chapters 2-12 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in chapters 2-12 of this dissertation.

Signature	**Institutional affiliation**	**Date**
	Clinica Clofan, Medellin, Colombia	7/31/2018
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Dedication

I dedicate this dissertation to my late father, Dr. Fernando Arevalo-Coutinho, for his love, and inspiration that have always guided my way.

Acknowledgements

I owe a great deal of gratitude to my promoter and friend, Prof David Meyer, for his support, encouragement and for always believing in me, and my co-investigators, members of the Pan-American Collaborative Retina Study Group. Together, we have changed retinal research in The Americas.

Last, but not least, I would like to thank my wife, Oly, and my son Fernando Andres for all their love, and infinite patience.

Summary

Bevacizumab is a complete full-length humanized antibody that binds to all subtypes of vascular endothelial growth factor (VEGF) and is used successfully in tumor therapy as a systemic drug. Recent studies have demonstrated the usefulness of an intravitreal injection of bevacizumab (IVB) in the reduction of macular edema secondary to central retinal vein occlusion, and choroidal neovascularization secondary to age-related macular degeneration (AMD). The drug is extremely cost-effective compared to similar anti-VEGF drugs on the market, hence the need to examine its effect in diabetic eye disease (the ever-growing global health epidemic challenge) for application in middle to low income countries.

The purpose of the current research is to determine if intravitreal bevacizumab (IVB) as anti-VEGF is helpful in the management of complications of diabetic retinopathy. We conducted several multicenter retrospective studies of eyes with complications from diabetic retinopathy treated with off-label IVB. Ten previously published studies (one prospective), and one unpublished prospective study are included here on the management of diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR). We progressively reported over the years our experienced as we followed patients with DME treated with IVB at 6 months, 12 months, and 24 months of follow up. In addition, 5 year follow up data was added later on. We found that primary IVB at doses of 1.25 to 2.5 mg seem to provide stability or improvement in best correct visual acuity (BCVA), optical coherence tomography (OCT), and fluorescein angiography (FA) in diffuse DME at 24 months. The results show no evident difference between IVB at doses of 1.25 or 2.5 mg. However, the early visual gains due to IVB were not maintained 5 years after treatment. Later, we provide evidence to support the use of primary IVB with or without grid laser photocoagulation (GLP) as treatment of diffuse DME. Primary IVB without GLP seems to be superior to GLP alone to provide stability or improvement in best-corrected visual acuity in patients with diffuse diabetic macular edema at 24 months. We showed first that IVB resulted in marked regression of retinal neovascularization (RN) in patients with PDR and previous pan retinal photocoagulation (PRP), and rapid resolution of vitreous hemorrhage in three naive eyes. Six-months results of intravitreal bevacizumab at doses of 1.25 or 2.5 mg in patients with PDR did not reveal any safety concerns. Later, we published that IVB resulted in marked regression of RN in patients with PDR and previous pan-retinal photocoagulation at 2 years. Intravitreal bevacizumab in naive eyes resulted in control or regression of 42.1% of eyes without adjunctive laser or vitrectomy during 24 months of follow-up. Meaning that a large number of patients (almost 58%) needed PRP or vitrectomy. Another one of our studies demonstrated the usefulness of using preoperative IVB during small-gauge vitreoretinal surgery in eyes with tractional retinal detachment (TRD) in PDR. This was a prospective non-comparative study and patients had significant anatomic and functional success. In addition, we reported for the first time ever that TRD may occur or progress shortly following administration of IVB in patients with severe PDR (5.2% and 3.2% in two studies). Based on our data, we now believe that extreme care must be taken in using a dose of 2.5 mg or more of bevacizumab in patients with PDR. In

addition, to have more than 15 years with a diagnosis of diabetes can increase the risk of TRD. Physicians must be prepared to perform the vitrectomy preferably before 13 days after the application of IVB and to perform a vitrectomy immediately on those patients in whom a TRD occurs. We recommend less than 5 days after injection as more than 80% of the retinal detachments developed after that period of time. Finally, in our prospective randomized clinical trial, pre-operative intravitreal bevacizumab therapy as adjuvant to PPV may be helpful and beneficial for patients with TRD secondary to severe PDR. Pre-operative IVB seems to reduce intraoperative bleeding, improving surgical visual field visualization, and reducing intraoperative and postoperative complications including iatrogenic retinal breaks and postoperative hemorrhage. In summary, IVB as anti-VEGF agent is helpful in the management of complications of diabetic retinopathy to prevent blindness with a more accessible drug worldwide.

Opsomming (Summary in Afrikaans)

Bevacizumab is ‘n vaskulêre endoteel groei faktor inhibitor. Dit is primêr geregistreer vir die binne-aarse gebruik as chemoterapeutiese middel vir verskeie kankers. Onlangs het die ongeregistreerde gebruik van die middel vir ouderdomsverwante makulêre degenerasie populêr geword. Die middel is baie koste-effektief vergeleke met soortgelyke anti-vaskulêre endoteel groei faktor inhibitore (anti-VEGF) op die mark. Dit het dus nodig geword om die effek van die middel op diabetiese oogsiekte, wat ‘n immers toenemende globale gesondheids uitdaging word, te bepaal. Veral middel tot lae inkomste lande sal hierby baat.

Die doel van die huidige navorsing is om te bepaal of intravitreale bevacizumab (IVB) as anti-VEGF behulpsaam is in die hantering van komplikasies van diabetiese retinopatie. Ons het verskeie multisenter retrospektiewe studies uitgevoer op oë met komplikasies van diabetiese retinopatie wat behandel was met IVB. Tien voorheen publiseerde studies (een prospektief) en een ongepubliseerde studie oor die hantering van diabetiese makulêre edeem (DME) en proliferatiewe diabetiese retinopatie (PDR) word hier ingesluit. Soos ons die IVB behandelde pasiente met DME opgevolg het oor 6, 12, 24 maande en later 5 jaar het ons progressief ons ervaring publiseer. Ons het gevind dat IVB in dosisse van 1.25 tot 2.5mg stabiliteit of verbetering gebring het in die bes gekorrigeerde visie, optiese koherensie tomografie en fluoresien angiografie teen die 24 maande merk. Die resultate het geen merkbare verskil getoon tussen die 1.25 en 2.5mg dosisse nie, maar die vroeë verbetering in visie kon nie volgehou word oor die 5 jaar periode nie. Latere studie verskaf bewys dat die gesamentlike gebruik van ‘rooster’ laser fototerapie (GLP) met die eerste IVB merkbaar beter is in diffuse DME. Primêre IVB sonder GLP blyk beter te vaar as GLP alleen ten einde stabiliteit of verbetering van die bes-gekorrigeerde visie teen 24 maande in pasiente met DME te verseker. In drie pasiente met PDR en vorige pan retinale fotokoagulasie (PRP) was ons die eerste om te toon dat IVB betekenisvolle regressive van retinale neovaskularisasie induseer sowel as om glasvogbloeding op te klaar. Geen veiligheidskwessies was ondervind ses maande na die toediening van IVB in dosisse van 1.25 of 2.5mg in pasiente met PDR nie. Later het ons publiseer dat in pasiente met PDR en vorige PRP die toediening van IVB lei tot betekenisvolle regressive van retinale neovaskularisasie na 2 jaar. Die toediening van IVB sonder adjuvante laser of vetrektomie het in onbehandelde oë gelei tot die beheer van regressive in 42.1% van oë na 24 maande se opvolg. Dit beteken dat byna 58% van pasiente wel PRP of vitrektomie benodig het. In ‘n ander studie het ons gewys hoe waardevol pre-operatiewe IVB is tydens klein insisie vitreoretinale chirurgie in oë met traksie retinale loslatings in PDR. Hierdie was ‘n prospektiewe nie-vergelykende studie. Pasiente het betekenisvolle anatomiese en funksionele suksesvolle uitkomste. In twee studies het ons voorts vir die eerste keer rapporteer dat traksie retinale loslatings (TRD) mag plaasvind of vererger na die toediening van IVB in pasiente met erge PDR (5.2% en 3.2% respektiewelik). Gebasseer op ons data, glo ons nou dat ‘n dosis van 2.5mg of meer nadelig kan wees in pasiente met

PDR. Voorts wys ons dat as pasiente diabetes het vir meer as 15 jaar is die risiko vir traksie loslatings van die retina verhoog.

Dokters moet bereid wees om vitrektomie chirurgie te doen verkieslik voor dag 13 nadat IVB toegedien is. As ‘n TRD vorm, moet onmiddellike chirurgie gedoen word. Omdat meer as 80% van die retinale loslatings ontwikkel het na 5 dae post IVB, beveel ons nou aan dat vitrektomie chirurgie binne 5 dae na die inspuit van bevacizumab uitgevoer word.

Laastens, in ons prospektiewe gerandomiseerde kliniese studie, het ons gewys dat IVB as adjuvant tot PPV behulpsaam en voordelig is in pasiente met traksie loslatings wat die gevolg is van erge PDR. In die studie het pre-operatiewe IVB intraoperatiewe bloeding verminder, chirurgiese gesigsveld en visualisasie verbeter en intra- en postoperatiewe kompliksasies soos iatrogene retinale gate en post-operatiewe bloedings, verminder. Opsommend is ons gevolgtrekking dat met ‘n middel wat geredelik wêreldwyd beskikbaar is in die vorm van IVB voordeel inhou in die hantering van die komplikasies van diabetiese retinopatie en so blindheid kan voorkom.

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Chapter 1: Introduction

We are currently witnessing a worldwide epidemic in diabetes mellitus (DM). Changing dietary habits and an increase in sedentarism are the main culprits of this epidemic. In the year 2014, more than 422 million people suffered from DM, this is almost double than in 1980. At this rate, by the year 2040 the number of people affected with DM in the world may double again, and everyone of them will be at risk of developing diabetic retinopathy (DR).[1] Vision loss due to diabetes mellitus is primarily caused by 2 mechanisms: diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR). Diabetic macular edema within 1 disk diameter of the fovea, leading to central vision loss, is present in about 9% of the diabetic population.[2] Proliferative diabetic retinopathy is present in about 1.5% of adults with diabetes,[3] and PDR can lead to vision loss by various mechanisms, such as retinal neovascularization, vitreous hemorrhage, neovascular glaucoma, and tractional retinal detachment (TRD). It has been shown that vision loss secondary to proliferative changes is more common in patients with type 1 diabetes, whereas vision loss secondary to DME is more common in patients with type 2 diabetes.[4] Prior to the advent of pan-retinal photocoagulation (PRP), PDR was the main cause of diabetic blindness. Since the development of PRP, DME has become the most common cause of visual loss in diabetic patients in the developed world.[5] Nevertheless, PDR is still a very important cause of blindness in diabetic patients.

More than 60 years ago, Michaelson speculated on the presence of a Factor X that was capable of inducing retinal angiogenesis or neovascularization.[6] Vascular endothelial growth factor (VEGF) appears to be the most likely candidate to be Michaelson's Factor X and the main molecular mediator in diabetic retinopathy. The underlying problem in diabetic patients is the progressive retinal ischemia caused by the metabolic disarray of chronic hyperglycemia. Hypoxia is a major inducer of VEGF gene transcription.[7] VEGF has been shown to be an endothelial cell-specific mitogen and an angiogenic inducer in a variety of in vitro and in vivo models.[7] Furthermore, it has been demonstrated to increase retinal vessel permeability by increasing the phosphorylation of tight junction proteins. Recent work has found elevated levels of VEGF in ocular fluids of patients with PDR.[8-11] In addition, intravitreal injection of VEGF into normal primate eyes induces the same pathological processes seen in diabetic retinopathy, including microaneurysm formation and increased vascular permeability.[12-13] Thus it makes sense to consider anti-VEGF treatments in the management of DME and PDR.

Several anti-VEGF agents are currently available in clinical practice. Pegaptanib sodium (Macugen®, Eyetech, NY, NY USA) is an aptamer against the VEGF-165 isoform.[14]

Ranibizumab (Lucentis®, Genentech, San Francisco, CA, USA) is a fragment of a humanized monoclonal antibody against all VEGF isoforms.[15] Bevacizumab (Avastin®, Genentech, San Francisco, CA, USA) is a humanized, recombinant monoclonal IgG antibody that binds and inhibits all VEGF isoforms.[16-18]Aflibercept, previously known as VEGF-Trap eye (Eylea®, Regeneron Pharmaceutics Inc., Tarrytown, NY) is a recombinant fusion protein that consists of portions of human VEGF receptors 1 and 2 that allows it to bind to VEGF-A, VEGF-B and placental growth factor.[19] All of these agents have been used to different extents in the management of DME and PDR.[20-30]

My group has long been interested in studying the effects of intravitreal bevacizumab in several vitreoretinal conditions including DME and PDR.[23-35] Given the off-label nature of intravitreal bevacizumab, its pharmacokinetics and safety have not been as thoroughly studied as other approved similar drugs that are more expensive and less accessible to most of the population worldwide. We have previously shown in an open label uncontrolled clinical study of 1265 patients that were injected with 4303 intravitreal injections of 1.25 mg or 2.5 mg of bevacizumab that intravitreal bevacizumab appears to be safe and well tolerated.[36]

Purpose of the research

***Central research question*:** The purpose of the current research is to determine if intravitreal bevacizumab as anti-vascular endothelial growth factor is helpful in the management of complications of diabetic retinopathy.

Hypothesis 1: Intravitreal bevacizumab (IVB) may have a beneficial anatomic (Optical Coherence Tomography [OCT]), and functional (visual acuity [VA]) effect on eyes with diffuse diabetic macular edema at 24 months of follow up. In addition, the lower dose (1.25 mg) may be as effective or more than the higher dose (2.5 mg) of IVB.

Hypothesis 2: IVB combined with grid laser photocoagulation may have a beneficial anatomic (OCT), and functional (VA) effect on eyes with diffuse diabetic macular edema at 24 months of follow up as compared to monotherapy. In addition, IVB combined with grid laser photocoagulation may decrease the number of injections if IVB necessary at 24 months.

Hypothesis 3: IVB may decrease retinal neovascularization in patients with PDR at 6 months of follow up. However, the effect may decrease at 24 months of follow up due to tachyphylaxis, and pan-retinal photocoagulation and/or vitrectomy will be necessary.

Hypothesis 4: Preoperative IVB may be beneficial for membrane dissection in diabetic tractional retinal detachment with minimally invasive vitreoretinal surgery

(23-gauge transconjunctival sutureless vitrectomy [TSV]). In addition, post-operative rebleeding may be decreased.

Hypothesis 5: Tractional retinal detachment (TRD) may occur following IVB as an adjuvant to vitrectomy for the management of severe PDR.

Hypothesis 6: Risk factors for the progression or development of TRD following IVB as an adjuvant to vitrectomy in severe PDR may include age, time from diabetes mellitus (DM) diagnosis, glycemic control, cholesterol levels, triglycerides levels, hemoglobin A1c (HbA1C), dose of bevacizumab, and time from injection to vitrectomy.

***Hypothesis 7 (*prospective unpublished study):** Intravitreal injection of 1.25 mg of bevacizumab as a pre-operative adjunct to PPV in eyes with TRD secondary to PDR will be safe and effective. IVB (compared to sham) will decrease intraoperative bleeding, total surgical time, post-operative vitreous hemorrhage, and visual acuity at 12 months.

Methods, and Ethical requirements of the research

We conducted several multicenter retrospective studies of eyes with complications from diabetic retinopathy treated with off-label IVB between September 2005 and July 2015 at nineteen institutions in 13 countries in Latin America (Venezuela, Colombia, Costa Rica, Brazil, Argentina, Peru, and Mexico). In addition, centers in Spain, and Saudi Arabia participated. Ten previously published studies (one prospective), and one unpublished prospective study are included here.

Approval was obtained from each participating center's Institutional Ethics Committee, and informed consent was obtained for these studies. In addition, these studies have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The off-label use of the drug and its potential risks and benefits were discussed extensively with all patients.

The following 11 chapters include the detailed methodology and results from our studies on DME and PDR including a prospective randomized clinical trial on *Pre-Operative Intravitreal Bevacizumab for Tractional Retinal Detachment Secondary to Proliferative Diabetic Retinopathy*. A final chapter 13 will summarize our conclusions.

Chapter 2: Intravitreal Bevacizumab for Diabetic Macular Edema at 6 months of follow up

Arevalo JF, Fromow-Guerra J, Quiroz-Mercado H, Sanchez JG, Wu L, Maia M, Berrocal MH, Solis-Vivanco A, Farah ME; Pan-American Collaborative Retina Study Group.
Primary intravitreal bevacizumab (Avastin) for diabetic macular edema: results from the Pan-American Collaborative Retina Study Group at 6-month follow-up. Ophthalmology. 2007 Apr;114(4):743-50.

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Hypothesis 1: Intravitreal bevacizumab (IVB) may have a beneficial anatomic (Optical Coherence Tomography [OCT]), and functional (visual acuity [VA]) effect on eyes with diffuse diabetic macular edema at 24 months of follow up. In addition, the lower dose (1.25 mg) may be as effective or more than the higher dose (2.5 mg) of IVB.

Purpose: To report the 6-month anatomic and best-corrected visual acuity (BCVA) response after primary intravitreal bevacizumab (Avastin) in patients with diabetic macular edema (DME)*Design:* Interventional retrospective multicenter study at 6 centers from 6 countries of patients with DME.

Participants: We reviewed the clinical records of 88 consecutive patients (110 eyes) with DME. Seventy- eight eyes of 64 consecutive patients with a minimum follow-up of 6 months and mean age of 59.7±9.3 years were included in this analysis.

Intervention: Patients were treated with at least one intravitreal injection of 1.25 mg or 2.5 mg of bevacizumab and underwent Early Treatment Diabetic Retinopathy Study (ETDRS) BCVA testing, ophthalmoscopic examination, optical coherence tomography (OCT), and fluorescein angiography (FA) at baseline and follow-up visits. Repeated-measures analysis of variance was used to compare mean values.

Main Outcome Measures: Changes in BCVA, OCT, and FA.

Results: Mean follow-up was 6.31±0.81 months (range, 6–9). Sixteen (20.5%) eyes needed a second injection at a mean of 13.8 weeks (range, 4–28), and 6 eyes needed a third injection (7.7%) at a mean of 11.5 weeks (range, 5–20). The mean baseline BCVA was 0.87 (logarithm of the minimum angle of resolution), and the final mean BCVA was 0.6, a difference that was statistically significant (P<0.0001). Final BCVA analysis by subgroups demonstrated that 32 (41.1%) eyes remained stable, 43 (55.1%) improved ≥2 ETDRS lines of BCVA, and 3 (3.8%) decreased ≥2 ETDRS lines of BCVA. Mean central macular thickness at baseline by OCT was 387.0±182.8 μm and decreased to a mean of 275.7±108.3 at end of follow-up (P<0.0001). No ocular or systemic adverse events were observed.

Conclusions: Primary intravitreal bevacizumab at doses of 1.25 to 2.5 mg seem to provide stability or improvement in VA, OCT, and FA in DME at 6 months. Follow-up is still short to make any specific treatment recommendations; however, the results appear promising. Evaluation in a multicenter randomized controlled clinical trial with longer follow-up is needed.

Primary Intravitreal Bevacizumab (Avastin) for Diabetic Macular Edema

Results from the Pan-American Collaborative Retina Study Group at 6-Month Follow-up

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Purpose: To report the 6-month anatomic and best-corrected visual acuity (BCVA) response after primary intravitreal bevacizumab (Avastin) in patients with diabetic macular edema (DME).

Design: Interventional retrospective multicenter study at 6 centers from 6 countries of patients with DME.

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Intervention: Patients were treated with at least one intravitreal injection of 1.25 mg or 2.5 mg of bevacizumab and underwent Early Treatment Diabetic Retinopathy Study (ETDRS) BCVA testing, ophthalmoscopic examination, optical coherence tomography (OCT), and fluorescein angiography (FA) at baseline and follow-up visits. Repeated-measures analysis of variance was used to compare mean values.

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Conclusions: Primary intravitreal bevacizumab at doses of 1.25 to 2.5 mg seem to provide stability or improvement in VA, OCT, and FA in DME at 6 months. Follow-up is still short to make any specific treatment recommendations; however, the results appear promising. Evaluation in a multicenter randomized controlled clinical trial with longer follow-up is needed. *Ophthalmology 2007;114:743–750 © 2007 by the American Academy of Ophthalmology.*

Diabetic retinopathy remains the major threat to sight in the working age population in the developed world. Furthermore, it is increasing as a major cause of blindness in other parts of the world, especially developing countries.[1] Diabetic macular edema (DME) is a manifestation of diabetic retinopathy that produces loss of central vision. Macular edema within 1 disc diameter of the fovea is present in 9% of the diabetic population.[2] Although visual loss secondary

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*For a complete listing of participating members, see "Appendix."

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to proliferative changes is more common in patients with type 1 diabetes, visual loss in patients with type 2 diabetes is more commonly due to macular edema.[3] Diabetic macular edema is caused by excessive vascular permeability, resulting in the leakage of fluid and plasma constituents, such as lipoproteins, into the retina, leading to its thickening.

Although the Early Treatment Diabetic Retinopathy Study (ETDRS)[4] demonstrated that immediate focal photocoagulation reduced moderate visual loss by 50% (from 24% to 12%, 3 years after initiation of treatment), 12% of treated eyes still lost ≥15 ETDRS letters at the 3-year follow-up interval. Approximately 40% of treated eyes that had retinal thickening involving the center of the macula at baseline still had thickening involving the center at 12 months, as did 25% of treated eyes at 36 months. Furthermore, only 3% of laser-treated eyes experienced a gain of ≥3 lines of vision. This suggests that a distinct subgroup of eyes exists with DME resistant to conventional laser photocoagulation. Other studies have reported a poor prognosis despite laser photocoagulation in eyes with diffuse DME.[4–6]

Vascular endothelial growth factor (VEGF) has been shown to be an endothelial cell-specific mitogen and an angiogenic inducer in a variety of in vitro and in vivo models.[7] Vascular endothelial growth factor, also known as vascular permeability factor, has been demonstrated to increase retinal vessel permeability by increasing the phosphorylation of tight junction proteins. Also, hypoxia has been shown to be a major inducer of VEGF gene transcription.[7] Recent work has found elevated levels of VEGF in ocular fluids of patients with proliferative diabetic retinopathy (PDR).[8–10] These studies also found that the growth of new vessels from the retina or optic nerve was thought to occur as a result of VEGF release into the vitreous cavity as a response to ischemia.[8–10] Furthermore, injection of VEGF into normal primate eyes induces the same pathological processes seen in diabetic retinopathy, including microaneurysm formation and increased vascular permeability.[11,12] Anti-VEGF treatments have been hypothesized as an alternative adjunctive treatment for DME.[13]

Bevacizumab (Avastin, Genentech Inc., San Francisco, CA) is a complete full-length humanized antibody that binds to all subtypes of VEGF and is used successfully in tumor therapy as a systemic drug.[14] Recent studies have demonstrated the usefulness of an intravitreal injection of bevacizumab in the reduction of macular edema secondary to central retinal vein occlusion, vascular permeability and fibrovascular proliferation in retinal neovascularization secondary to PDR, and choroidal neovascularization secondary to age-related macular degeneration (AMD).[15–18] The amount of human retinal penetration for a complete full-length anti-VEGF antibody is not known at present. However, full-thickness retinal penetration of intravitreal bevacizumab was observed in an animal model.[19,20] Additionally, intravitreal bevacizumab does not appear to be toxic to the albino rabbit retina at a concentration of up to 2.5 mg.[21]

Recently, Pieramici et al[22] reported a case of moderate anterior uveitis after repeated intravitreal injections of bevacizumab to treat choroidal neovascularization associated with AMD. In addition, Meyer et al[23] reported 2 patients who developed an acute retinal pigment epithelial tear after intravitreal bevacizumab. In an open-label uncontrolled clinical study of 1804 injections in human eyes with 1.25-mg or 2.5-mg intravitreal bevacizumab, our group reported 4 (0.3%) cases of endophthalmitis, 3 (0.3%) of elevation of intraocular pressure (IOP), 3 of tractional retinal detachment, and 1 (0.1%) of uveitis. No systemic adverse events were reported, and bevacizumab appears to be safe and well tolerated during the first 4 months (Wu et al, unpublished data).

The purpose of this retrospective study was to report the 6-month anatomic and best-corrected visual acuity (BCVA) response after primary intravitreal bevacizumab in patients with DME.

Patients and Methods

We conducted a multicenter retrospective study of eyes with DME treated with off-label intravitreal bevacizumab (Avastin) between September 2005 and August 2006 at 6 institutions in Venezuela, Mexico, Costa Rica, Brazil, Puerto Rico, and Colombia. We reviewed the clinical records of 88 consecutive patients (110 eyes) with DME treated with at least one intravitreal injection of 1.25 mg or 2.5 mg of bevacizumab. Institutional review board/ethics committee approval and patients' informed consent were obtained for this study at all 6 institutions. The off-label use of the drug and its potential risks and benefits were discussed extensively with all patients. Exclusion criteria included patients (eyes) with DME previously treated with laser photocoagulation or intravitreal triamcinolone, macular ischemia, and the presence of an epiretinal membrane or vitreomacular traction syndrome. Although not a formal exclusion criteria, patients with a history of uncontrolled hypertension and recent thromboembolic events were not usually injected with bevacizumab, but this decision was at the discretion of the treating physician.

Each patient underwent BCVA measurement with ETDRS charts and ophthalmic examination including slit-lamp biomicroscopy. Baseline central retinal characteristics were analyzed by optical coherence tomography (OCT) (Stratus III, Carl Zeiss, Dublin, CA) utilizing 6 diagonal slow 6-mm radial line scans, with software versions 3.0 and 4.0, through a dilated pupil performed by a retina specialist. The retinal thickness of the 1-mm central retina was obtained using the macular thickness map for our calculations.

A 0.18-ml aliquot of commercially available bevacizumab was prepared for each patient and placed in a tuberculin syringe using aseptic techniques. After the eye had been prepared in a standard fashion using 5% povidone/iodine, an eyelid speculum was used to stabilize the eyelids, and the injection of 1.25 mg (0.05 ml) or 2.5 mg (0.1 ml) of bevacizumab was performed 3.5 to 4 mm posterior to the limbus, through the inferotemporal pars plana with a 30-gauge needle under topical anesthesia or subconjunctival lidocaine. After the injection, IOP and retinal artery perfusion were checked, and patients were instructed to administer topical antibiotics for 7 days.

Patients were examined at 1 and 2 weeks and 1 month after the first injection and monthly thereafter. One, 3, and 6 months after the initial injection, ophthalmic examination included OCT and fluorescein angiograhy (FA). However, OCT or FA was performed earlier (weeks 1 and 2) in some patients according to the investigator's decision and preference.

Patients were included in this consecutive series only if there was a minimum of 6 months' follow-up. Fluorescein angiography was done at the discretion of the examiner and not at every postinjection evaluation, usually every 6 weeks. Patients received

Table 1. Distribution by Grade of Diabetic Retinopathy (DR) (78 Eyes)

Grade of DR	No. of Cases
Mild NDR	2 (2.6%)
Moderate NDR	10 (12.8%)
Severe NDR	20 (25.6%)
PDR	46 (58.9%)

NDR = nonproliferative DR; PDR = proliferative DR.

reinjections when there was a recurrence of DME. Recurrence was defined as a decrease of BCVA associated with an increase of intraretinal fluid due to macular edema on OCT and/or FA, after complete or partial resolution in previous follow-up visits.

Patients' ETDRS BCVAs were transferred from their records and converted to a logarithm of the minimum angle of resolution (logMAR) scale for analysis. Repeated-measures analysis of variance (ANOVA) was used to compare mean values to analyze mean retinal thickness and logMAR visual acuity (VA) statistically. An increase or decrease in BCVA was considered to have occurred if there was a change of ≥2 ETDRS lines. Main outcome measures included changes in BCVA, OCT, and FA. Interval data were analyzed at 1-, 3-, and 6-month follow-up time points. A P value < 0.05 was considered to be significant.

Results

Seventy-eight eyes (64 consecutive patients) with a minimum of 6 months' follow-up were included for analysis. Fifty-one (79.7%) patients were Hispanic and 12 (18.7%) were Caucasian. Our patients had a mean age of 59.7±9.3 years, and 54.7% were male (35 men, 29 women). Patients had a mean follow-up of 6.31±0.81 months (range, 6–9). Forty-four (56.4%) cases had PDR (Table 1). All of these 44 cases had had prior scatter photocoagulation at least 6 months before undergoing bevacizumab intravitreal injections. All eyes had clinically significant macular edema at the baseline biomicroscopy slit-lamp examination.[4]

Within 1 month after the initial bevacizumab injection, improvements in VA and central retinal thickness measurements were observed, and these significant changes continued throughout the 6-month follow-up. By 1 month, mean BCVA improved from 0.87 to 0.6, a difference that was statistically significant ($P<0.0001$). This BCVA was maintained throughout 6 months (Fig 1). At the 3- and 6-month follow-ups (data available for all 78 eyes), mean BCVA of 0.6 did not differ statistically ($P = 0.775$ and $P = 0.688$ respectively) from BCVA at 1-month follow-up. Final BCVA analysis by subgroups demonstrated that 32 (41.1%) eyes remained stable, 43 (55.1%) improved ≥2 ETDRS lines of BCVA, and 3 (3.8%) decreased ≥2 ETDRS lines of BCVA (Table 2, Fig 2).

Optical coherence tomography results were available for all 78 cases at 1-, 3-, and 6-month follow-ups. At 1 month, the mean 1-mm central retinal thickness measurements decreased from 387.0±182.8 μm to 287.9±102.4 μm ($P<0.0001$), and this overall improvement continued throughout the 6-month follow-up. At 3- and 6-month follow-ups, mean central macular thicknesses were 282.8±115.6 μm and 275.7±108.3, respectively, which were not significantly lower than the 1-month follow-up ($P = 0.678$ and $P = 0.371$, respectively) (Figs 3, 4).

We wished to compare response to treatment between patients with PDR and previous panretinal photocoagulation and those with

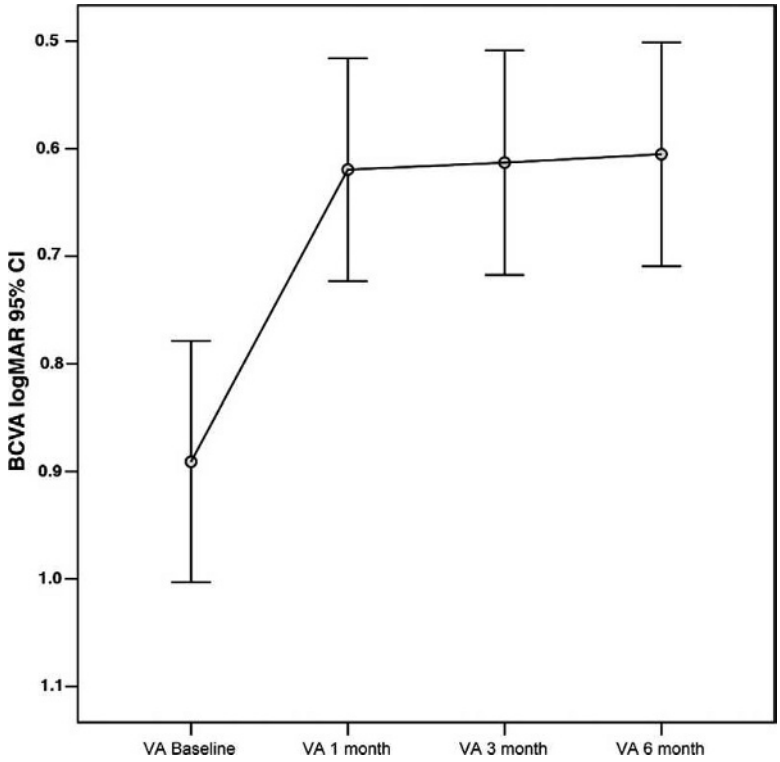

Figure 1. Changes in best-corrected visual acuity (BCVA) after intravitreal bevacizumab. Best-corrected visual acuity improved at 1 month from 0.87 to 0.6 (logarithm of the minimum angle of resolution), a difference that was statistically significant ($P<0.0001$); this level of BCVA was maintained throughout 3 and 6 months. The mean follow-up period was 6.31±0.81 months (range, 6–9). CI = confidence interval.

Table 2. Best-Corrected Visual Acuity (BCVA) Analysis by Subgroups (78 Eyes)

	First Month		Third Month		Sixth Month	
	No. of Eyes	*Percentage*	*No. of Eyes*	*Percentage*	*No. of Eyes*	*Percentage*
Decreased ≥2 ETDRS lines of BCVA	1	1.3	3	3.8	3	3.8
Remained stable	35	44.9	32	41.1	32	41.1
Improved ≥2 ETDRS lines of BCVA	42	53.8	43	55.1	43	55.1

ETDRS = Early Treatment Diabetic Retinopathy Study.

nonproliferative diabetic retinopathy and macular edema to see if there was any difference. However, when we ran the repeated-measures ANOVA to compare mean values to statistically analyze mean retinal thickness and logMAR VA adjusting for the grade of diabetic retinopathy as a covariate, we did not find statistical significance ($P = 0.565$ for BCVA and $P = 0.446$ for OCT retinal thickness).

All eyes received an intravitreal injection at the initial visit; however, recurrences were re-treated at the discretion of the treating physician. Sixty-three (80.8%) cases were treated with an intravitreal injection of bevacizumab at 2.5 mg, and 15 (19.2%) with a dose of 1.25 mg. Sixteen (20.5%) eyes needed a second injection at a mean of 13.8 weeks (range, 4–28), and 6 needed a third injection (7.7%) at a mean of 11.5 weeks (range, 5–20) (Fig 5). Numbers of eyes that needed reinjections were 13 of 63 (20.6%) treated with an intravitreal injection of bevacizumab at 2.5 mg and 3 of 15 (20%) treated with an intravitreal injection of bevacizumab at 1.25 mg. We did not observe statistically significant differences in changes of BCVA and macular thickness with OCT between doses of 1.25 and 2.5 mg of intravitreal bevacizumab.

There were no episodes of inflammation or severe decrease of vision immediately after an injection. At 6 months, no ocular or systemic adverse events such as thromboembolic events (cerebrovascular accidents, transient ischemic attacks, myocardial infarctions, or peripheral vascular disease) were reported.

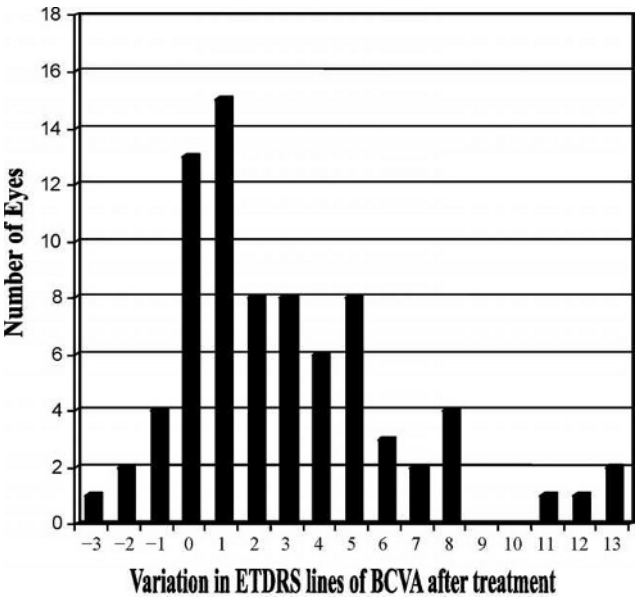

Figure 2. Number of patients losing, maintaining, or gaining Early Treatment Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) from baseline to final follow-up (mean, 6.31±0.81 months).

Discussion

Diabetic macular edema is a manifestation of diabetic retinopathy that produces loss of central vision. Although several treatment modalities are under investigation, the only demonstrated means to reduce the risk of vision loss from DME are laser photocoagulation, as demonstrated by the ETDRS[4]; intensive glycemic control, as demonstrated by the Diabetes Control and Complications Trial and the United Kingdom Prospective Diabetes Study; and blood pressure control, as demonstrated by the United Kingdom Prospective Diabetes Study.[24,25] Given that most eyes with DME that are treated with laser photocoagulation do not have an improvement in VA, there has been an interest in other treatment modalities such as pharmacologic therapy with oral protein kinase C inhibitors and the use of intravitreal corticosteroids.[26,27] The use of antibodies targeted at VEGF is another treatment modality that has generated considerable interest and is being investigated.

Diabetic macular edema is the most frequent cause of visual impairment in patients with nonproliferative diabetic retinopathy. However, the breakdown of endothelial tight junctions and loss of the blood–retina barrier that lead to DME can be associated with both nonproliferative diabetic retinopathy and PDR. Our study demonstrates a comparable population of PDR and nonproliferative diabetic retinopathy patients with macular edema. Due to the relatively small number of participants in the study, our series had no statistical power to determine significative differences in the treatment with intravitreal bevacizumab between different stages of diabetic retinopathy.

It was demonstrated recently that retinal hypoxia plays a role in DME,[28] and VEGF, which is upregulated by hypoxia, is likely to contribute to the excessive vascular permeability that results in macular edema in people with diabetes. To the best of our knowledge, there is only a pilot study in the literature on the intravitreal administration of antibodies against VEGF for DME as primary therapy. Chun et al reported that ranibizumab therapy has the potential to maintain or improve BCVA and reduce retinal thickness in patients with DME.[29] In addition, intravitreal injections of the aptamer pegaptanib sodium in patients with DME have been shown to improve VA and retinal thickening.[13] The Macugen Diabetic Retinopathy Study Group reported gains in VA of 10 letters in 34% and 15 letters in 18% of patients with DME after an intravitreal pegaptanib sodium injection in a randomized, double-masked, multicenter trial with a follow-up of 36 months.

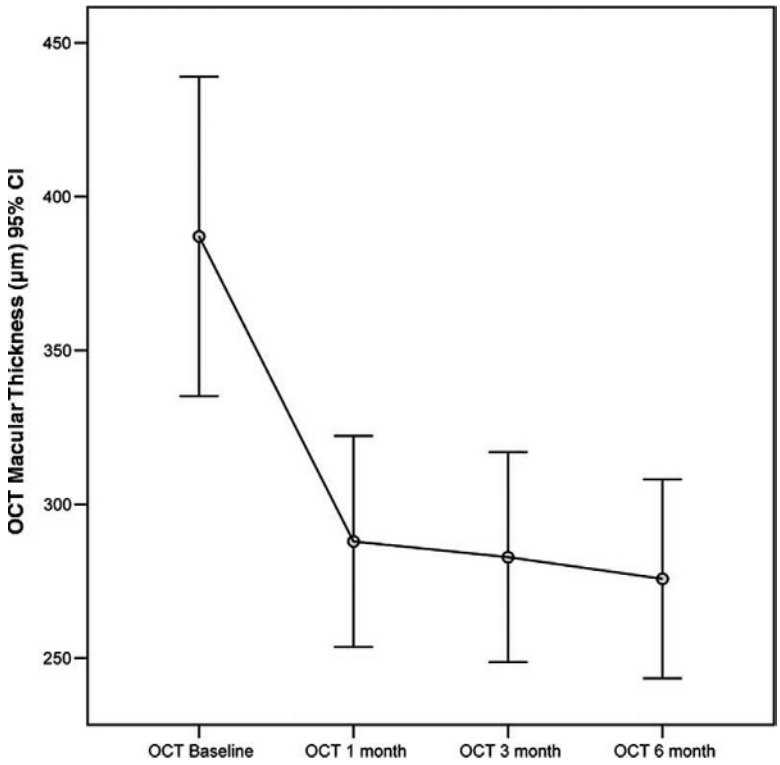

Figure 3. Changes in macular thickness with optical coherence tomography (OCT) during follow-up after intravitreal bevacizumab. The foveal thickness improved after 1 month, mean 1-mm central retinal thickness measurements decreased from 387.0±182.8 μm to 287.9±102.4 μm ($P<0.0001$), and this overall improvement continued at the 3- and 6-month time points. At 3- and 6-month follow-ups, mean central macular thicknesses were 282.8±115.6 μm and 275.7±108.3 μm, respectively, which were not significantly lower than the 1-month follow-up ($P = 0.678$ and $P = 0.371$, respectively). CI = confidence interval.

The 2 doses of bevacizumab evaluated in this study were 1.25 mg, which is the one that has been used most commonly in clinical practice, and 2.5 mg, which also has been used, though less commonly. Doses lower than 1.25 mg create difficulties with dilution and the accuracy of injection of a small volume. The results of our retrospective study demonstrated the efficacy of 1.25 mg or 2.5 mg of intravitreal bevacizumab as primary treatment of DME, as 55.1% of eyes showed anatomical and functional improvement. In addition, our results suggest a reduced risk of VA loss in eyes with DME treated with intravitreal bevacizumab (96.2% of eyes). Sixteen (20.5%) eyes needed a second injection at a mean of 13.8 weeks (range, 4–28), and 6 needed a third injection (7.7%) at a mean of 11.5 weeks (range, 5–20). We found that the anatomical and visual benefit of the intravitreal bevacizumab appears and reaches its maximum value during the first month and maintains itself over 6 months. Nevertheless, we did not find statistically significant differences in duration or anatomical or functional effectiveness between the 2 doses of bevacizumab evaluated.

The optimum dosing and sequence for intravitreal bevacizumab in DME is still undetermined. We elected to defer reinjections until there was a recurrence. Recurrence was defined as a decrease of BCVA associated with an increase of intraretinal fluid due to macular edema on OCT and/or FA, after complete or partial resolution in previous follow-up visits. It is possible that a different dosing schedule, such as a series of injections every 12 weeks for an extended period followed by retreatment only for recurrences, may be superior to the method used in this study; however, we chose to err on the side of undertreatment until further toxicity data are obtained. It was interesting that, over time, the number of patients needing reinjections diminished from 20.5% to 7.7%. Probably, bevacizumab has a beneficial cumulative effect for DME, for which future studies are necessary.

Focal and grid laser photocoagulation are the primary treatments for DME.[4] However, although the ETDRS[4] demonstrated that immediate focal photocoagulation reduced moderate visual loss by 50%, 12% of treated eyes still lost ≥15 ETDRS letters at the 3-year follow-up interval, and 24% of immediately treated eyes had thickening involving the center of the macula at 36 months. In addition, laser treatment of eyes with diffuse macular edema has been disappointing.[30] Our results indicate that intravitreal bevacizumab injections may have a beneficial effect on macular thickness and VA, independent of the type of macular edema that is present (focal vs. diffuse). Therefore, in the future this new treatment modality could replace or complement focal/grid laser photocoagulation. Furthermore, focal/grid laser photocoagulation could be used to consolidate the results obtained with one intravitreal bevacizumab injection and decrease the need for reinjections.

Recently, Chun et al reported the biologic activity and adverse events of multiple intravitreal injections of ranibi-

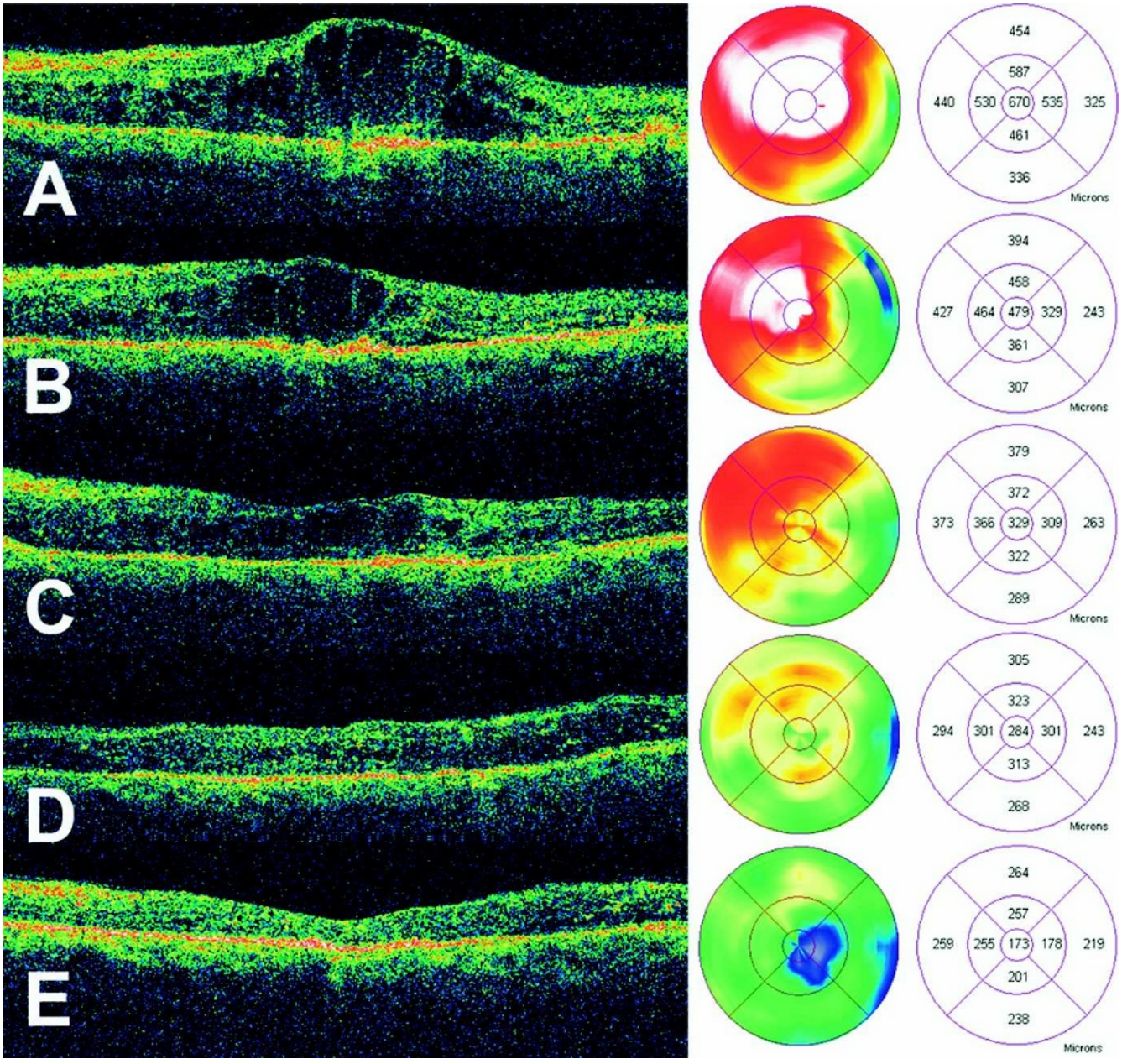

Figure 4. **A**, A horizontal optical coherence tomography (OCT) scan obtained through the fovea revealed loss of the normal foveal contour, diffuse macular thickening, and areas of low intraretinal reflectivity consistent with intraretinal cysts and fluid accumulation. The retinal map analysis revealed a foveal thickness of 670 μm. The patient underwent an intravitreal injection of bevacizumab at a dose of 2.5 mg in this eye. **B**, One week after the injection, an OCT scan showed that the foveal thickness had decreased to 479 μm. **C**, One month after the injection, the cystic spaces had resolved almost completely, and the patient's visual acuity (VA) improved to 20/100. Foveal thickness had decreased to 329 μm. **D**, Optical coherence tomography done 3 months after the injection showed complete resolution of intraretinal cysts. Visual acuity improved to 20/63 and foveal thickness decreased to 284 μm. **E**, Six months after the injection, an OCT scan showed normal macular anatomical architecture. The patient's VA was 20/63, and foveal thickness decreased to 173 μm.

zumab (0.3 mg or 0.5 mg each injection) in 10 eyes (patients) with DME.[29] Of the 10 patients enrolled, 5 received 0.3-mg and 5 received 0.5-mg ranibizumab. No systemic adverse events were reported, and 5 occurrences of mild to moderate ocular inflammation were reported. At month 3, 4 (40%) of 10 eyes gained $\geq$3 ETDRS lines of BCVA, and 5 (50%) of 10 gained $\geq$2 ETDRS lines of BCVA. At month 3, mean decreases in retinal thickness of the center point of the central subfield were 45.3$\pm$196.3 μm for the low-dose group and 197.8$\pm$85.9 μm for the high-dose group. The current study showed similar results of improved BCVA and OCT and no reported episodes of inflammation in a larger number of patients.

Limitations of our study include that it is short term, nonrandomized, uncontrolled, and retrospective, which preclude any estimation of the long-term efficacy or safety of

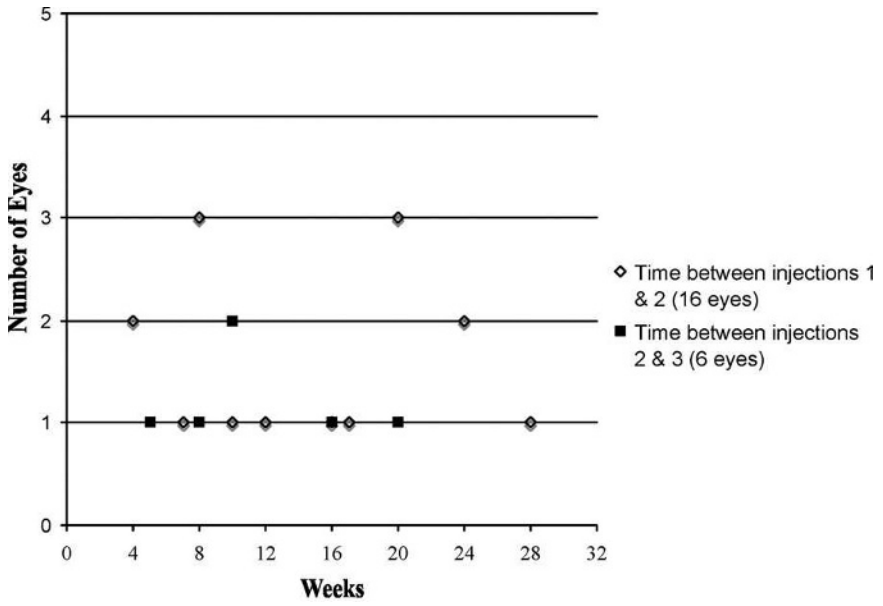

Figure 5. Distribution of time between the initial injection and subsequent injections. Sixteen (20.5%) eyes needed a second injection at a mean of 13.8 weeks, and 6 needed a third injection (7.7%) at a mean of 11.5 weeks.

intravitreal bevacizumab. In addition, because no control group is present we cannot rule out the possibility that some of the improvement in macular edema might be associated with improvement in systemic health. It is not uncommon that additional attention is directed towards improving systemic health when patients get involved in a clinical trial or new treatment. However, the results were very promising and suggest the need for further investigation. Furthermore, we can safely assume with a 95% confidence that the true rate of ocular complications is $<3.8\%$, and that the true rate of systemic complications is $<4.6\%$ in our study.[31]

In summary, primary intravitreal bevacizumab at doses of 1.25 mg or 2.5 mg seems to provide stability and improvement in VA, OCT, and FA in DME at 6 months. Follow-up is still too short to make any specific treatment recommendations; however, the results are promising. Evaluation in a multicenter randomized controlled clinical trial with longer follow-up is needed to evaluate the safety and efficacy of this new treatment.

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Appendix: Pan-American Collaborative Retina Study Group Investigators

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Chapter 3: Intravitreal Bevacizumab for Diabetic Macular Edema at 12 months of follow up

Arevalo JF, Sanchez JG, Fromow-Guerra J, Wu L, Berrocal MH, Farah ME, Cardillo J, Rodríguez FJ; Pan-American Collaborative Retina Study Group (PACORES). Comparison of two doses of primary intravitreal bevacizumab (Avastin) for diffuse diabetic macular edema: results from the Pan-American Collaborative Retina Study Group (PACORES) at 12-month follow-up. Graefes Arch Clin Exp Ophthalmol. 2009 Jun;247(6):735-43. doi: 10.1007/s00417-008-1034-x. Epub 2009 Feb 3.

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Hypothesis 1: Intravitreal bevacizumab (IVB) may have a beneficial anatomic (Optical Coherence Tomography [OCT]), and functional (visual acuity [VA]) effect on eyes with diffuse diabetic macular edema at 24 months of follow up. In addition, the lower dose (1.25 mg) may be as effective or more than the higher dose (2.5 mg) of IVB.

BACKGROUND: To report the 12-month anatomic and ETDRS best-corrected visual acuity (BCVA) response after primary intravitreal bevacizumab (Avastin) (1.25 mg or 2.5 mg) in patients with diffuse diabetic macular edema (DDME). In addition, a comparison of the two different doses of intravitreal bevacizumab (IVB) utilized was made.

METHODS: We reviewed the clinical records of 82 consecutive patients (101 eyes) with DDME in this interventional retrospective multicenter study. All patients with a minimum follow-up of 12 months (mean 57.6 +/- 8.4 weeks) were included in this analysis. Patients underwent ETDRS best-corrected visual acuity (BCVA) testing, ophthalmoscopic examination, optical coherence tomography (OCT), and fluorescein angiography (FA) at baseline and follow-up visits.

RESULTS: The mean age of our patients was 59.7 +/- 9.3 years. The mean number of IVB injections per eye was three (range: one to six injections) at a mean interval of 14.1 +/- 10.5 weeks. In the 1.25 mg group at 1 month BCVA improved from 20/190, logMAR = 0.97 to 20/85, logMAR 0.62, a difference that was statistically significant ($p = 0.0001$). This improvement was maintained throughout the 3-, 6-, and 12-month follow-up. The mean final BCVA at 12 months was 20/76, logMAR = 0.58 ($p < 0.001$), a statistically significant difference from baseline BCVA. Similar BCVA changes were observed in the 2.5 mg group. In the 1.25 mg group, the mean central macular thickness (CMT) decreased from 419.1 +/- 201.1 microm at baseline to 295.11 +/- 91.5 microm at 1 month, 302.1 +/- 124.2 microm at 3 months, 313.4.1 +/- 96.3 microm at 6 months, and 268.2 +/- 95.5 microm at 12 months ($p < 0.0001$). Similar CMT changes were observed in the 2.5 mg group. Adverse events included transient high blood pressure in one patient (1.2%), transient increased intraocular pressure in one eye (1%), and tractional retinal detachment in one eye (1%).

CONCLUSIONS: Primary IVB at doses of 1.25 to 2.5 mg seem to provide stability or improvement in BCVA, OCT, and FA in DDME at 12 months. There seems to be no difference in our results between intravitreal bevacizumab at doses of 1.25 mg or 2.5 mg. In addition, our results suggest the need for at least three injections a year to maintain the BCVA results.

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RETINAL DISORDERS

Comparison of two doses of primary intravitreal bevacizumab (Avastin) for diffuse diabetic macular edema: results from the Pan-American Collaborative Retina Study Group (PACORES) at 12-month follow-up

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Abstract
Background To report the 12-month anatomic and ETDRS best-corrected visual acuity (BCVA) response after primary intravitreal bevacizumab (Avastin®) (1.25 mg or 2.5 mg) in patients with diffuse diabetic macular edema (DDME). In addition, a comparison of the two different doses of intravitreal bevacizumab (IVB) utilized was made.
Methods We reviewed the clinical records of 82 consecutive patients (101 eyes) with DDME in this interventional retrospective multicenter study. All patients with a minimum

For a complete listing of participating members of PACORES see Appendix.

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follow-up of 12 months (mean 57.6±8.4 weeks) were included in this analysis. Patients underwent ETDRS best-corrected visual acuity (BCVA) testing, ophthalmoscopic examination, optical coherence tomography (OCT), and fluorescein angiography (FA) at baseline and follow-up visits.
Results The mean age of our patients was 59.7±9.3 years. The mean number of IVB injections per eye was three (range: one to six injections) at a mean interval of 14.1± 10.5 weeks. In the 1.25 mg group at 1 month BCVA improved from 20/190, logMAR=0.97 to 20/85, logMAR 0.62, a difference that was statistically significant (p= 0.0001). This improvement was maintained throughout the 3-, 6-, and 12-month follow-up. The mean final BCVA at 12 months was 20/76, logMAR=0.58 (p<0.001), a statistically significant difference from baseline BCVA. Similar BCVA changes were observed in the 2.5 mg group. In the 1.25 mg group, the mean central macular thickness (CMT) decreased from 419.1±201.1 μm at baseline to 295.11± 91.5 μm at 1 month, 302.1±124.2 μm at 3 months, 313.4.1±96.3 μm at 6 months, and 268.2±95.5 μm at 12 months (p<0.0001). Similar CMT changes were observed in the 2.5 mg group. Adverse events included transient high blood pressure in one patient (1.2%), transient increased intraocular pressure in one eye (1%), and tractional retinal detachment in one eye (1%).
Conclusions Primary IVB at doses of 1.25 to 2.5 mg seem to provide stability or improvement in BCVA, OCT, and FA in DDME at 12 months. There seems to be no difference in our results between intravitreal bevacizumab at doses of 1.25 mg or 2.5 mg. In addition, our results suggest the need for at least three injections a year to maintain the BCVA results.

Keywords Avastin · Bevacizumab · Diffuse diabetic macular edema · Intravitreal injections · OCT · Primary treatment

Introduction

Diabetic retinopathy remains the major threat to sight in the working-age population in the developed world. Furthermore, it is increasing as a major cause of blindness in other parts of the world, especially developing countries [1]. Diabetic macular edema (DME) is a manifestation of diabetic retinopathy that produces loss of central vision, and is now the principal cause of vision loss in persons with diabetes. Macular edema within 1 disc diameter of the fovea is present in 9% of the diabetic population [2]. Although visual loss secondary to proliferative changes is more common in patients with type 1 diabetes, visual loss in patients with type 2 diabetes is more commonly due to macular edema [3]. However, DME can occur at any stage of diabetic retinopathy, and it is caused by excessive vascular permeability, resulting in the leakage of fluid and plasma constituents, such as lipoproteins, into the retina, leading to its thickening.

There is good evidence that focal laser treatment preserves vision in eyes with DME. The Early Treatment Diabetic Retinopathy Study (ETDRS) [4] randomized 1,490 eyes with DME to receive focal laser treatment or observation. At 3 years, treatment significantly reduced moderate visual loss as compared with observation [4], with the greatest benefits in eyes with clinically significant DME [5]. Although the ETDRS [1] demonstrated that immediate focal photocoagulation reduced moderate visual loss by 50% (from 24% to 12%, 3 years after initiation of treatment), 12% of treated eyes still lost >15 ETDRS letters at the 3-year follow-up interval. Approximately 40% of treated eyes that had retinal thickening involving the center of the macula at baseline still had thickening involving the center at 12 months, as did 25% of treated eyes at 36 months. Furthermore, only 3% of laser-treated eyes experienced a gain of ≥3 lines of vision. This suggests that a distinct subgroup of eyes exists with DME resistant to conventional laser photocoagulation. In addition, some reports have indicated that diffuse diabetic macular edema (DDME) is refractory to macular photocoagulation [4–7]. Lee and Olk [8] demonstrated that with modified grid laser macular photocoagulation, visual acuity was stabilized in 60.9%, decreased in 24.6%, and increased in only 14.5% of eyes with DDME. Therefore, alternative or adjunct treatments for DME such as intravitreal triamcinolone acetonide [9–13], and anti–vascular endothelial growth factor (VEGF) therapy have been the focus recently [14–17].

Vascular endothelial growth factor (VEGF) has been demonstrated to increase retinal vessel permeability. Also, hypoxia has been shown to be a major inducer of VEGF gene transcription [18, 19]. It has been shown that VEGF-A levels are considerably higher in DME patients with extensive leakage in the macular region than in patients with minimal leakage [20]. Recent work has found elevated levels of VEGF in ocular fluids of patients with proliferative diabetic retinopathy (PDR) [21–24]. These studies also found that the growth of new vessels from the retina or optic nerve was thought to occur as a result of VEGF release into the vitreous cavity as a response to ischemia [21, 22].

Currently used anti-VEGF drugs are pegaptanib sodium (Macugen; OSI Eyetech Pharmaceuticals, Melville, NY, USA), ranibizumab (Lucentis, Genentech Inc., San Francisco, CA, USA), and bevacizumab (Avastin; Genentech Inc.). Bevacizumab is a complete full-length humanized antibody that binds to all subtypes of VEGF, and is used successfully in tumor therapy as a systemic drug [25]. Studies have demonstrated the usefulness of an intravitreal injection of bevacizumab, with promising effects in the reduction of macular edema secondary to central retinal vein occlusion, vascular permeability and fibrovascular proliferation in retinal neovascularization secondary to PDR, rubeosis iridis,

retinopathy of prematurity, choroidal neovascularization secondary to age-related macular degeneration (AMD) and in the treatment of DME [26–34].

A recetly published multi-center study, funded by the National Eye Institute and conducted through the Diabetic Retinopathy Clinical Research Network, studied 840 eyes of 693 subjects with DME involving the fovea and with visual acuity of 20/40 to 20/320. This 2-year study demonstrated that focal/grid photocoagulation is more effective and has fewer side effects than 1-mg or 4-mg doses of preservative-free intravitreal triamcinolone for most patients with DME who have characteristics similar to the cohort in this clinical trial. Though the evidence currently supports focal/grid photocoagulation as the most effective treatment, the authors commented that combining laser therapy with corticosteroids might prove useful [35]. Therefore, the same may be true for anti-VEGF therapies, and combination therapies should be considered. However, some unresolved issues such as the ideal regimen, duration of treatment, potential of combination treatments, and safety concerns with long-term VEGF inhibition deserve further investigation.

The purpose of this retrospective study was to report the 12-month anatomic and ETDRS best-corrected visual acuity (BCVA) response after primary intravitreal bevacizumab (1.25 mg or 2.5 mg) in patients with DDME. This report comprises a series of 101 eyes, including 38 eyes with DDME from a previously reported series with longer follow up [34]. In addition, a comparison of the two different doses of intravitreal bevacizumab (IVB) utilized was made.

Patients and methods

Approval was obtained from each participating center's Institutional Ethics Committee, and informed consent was obtained for this study. In addition, this study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. This was a multicenter retrospective study of eyes with DDME treated with off-label intravitreal bevacizumab (Avastin) between September 2005 and February 2007 at 8 institutions in Venezuela, Mexico, Costa Rica, Puerto Rico, Brazil, Colombia and Argentina. We reviewed the clinical records of 82 consecutive patients (101 eyes) with DDME treated with at least one intravitreal injection of 1.25 mg or 2.5 mg of bevacizumab. The dose of 1.25 mg or a dose of 2.5 mg to be used to treat a patient was determined at the discretion of the treating physician. If a patient received one of the doses at baseline, the same dose was delivered throughout the study. The off-label use of the drug and its potential risks and benefits were discussed extensively with all patients.

The DDME had to show evidence of diffuse retinal thickening involving the center of the macula on biomicroscopy, and diffuse fluorescein leakage involving the center of the macula on fluorescein angiography (FA). In addition, a significant reduction in the reflectivity of the outer retinal layers, and/or subretinal fluid collection on by optical coherence tomography (OCT) (Stratus OCT, Carl Zeiss, Dublin, CA, USA) should be present. Exclusion criteria included patients (eyes) with DDME previously treated with laser photocoagulation or intravitreal triamcinolone, macular ischemia, intraocular inflammation, uncontrolled intraocular pressure (IOP), cataract surgery within the past 6 months, or a prior history of vitreoretinal surgery, and the presence of an epiretinal membrane or vitreomacular traction syndrome. Although not a formal exclusion criteria, patients with a history of uncontrolled hypertension and recent thomboembolic events were not usually injected with bevacizumab, but this decision was at the discretion of the treating physician.

Each patient underwent best-corrected visual acuity (BCVA) measurement with ETDRS charts, and ophthalmic examination including slit-lamp biomicroscopy. Baseline central retinal characteristics were analyzed by OCT utilizing six diagonal slow 6-mm radial line scans, with software version 4.0, through a dilated pupil performed by a retina specialist. The retinal thickness of the 1-mm central retina was obtained using the macular thickness map for our calculations. The scans were reviewed, and manual caliper-assisted measurements were used in case of delineation errors.

A 0.18-ml aliquot of commercially available bevacizumab was prepared for each patient, and placed in a tuberculin syringe using aseptic techniques. After the eye had been prepared in a standard fashion using 5% povidone/iodine, an eyelid speculum was used to stabilize the eyelids, and the injection of 1.25 mg (0.05 ml) or 2.5 mg (0.1 ml) of bevacizumab was performed 3.5 to 4 mm posterior to the limbus, through the inferotemporal pars plana with a 30-gauge needle under topical anesthesia or subconjunctival lidocaine. After the injection, IOP and retinal artery perfusion were checked, and patients were instructed to administer topical antibiotics for 7 days.

Patients were examined at 1 and 2 weeks and 1 month after the first injection, and monthly thereafter. One, 3, 6 and 12 months after the initial injection, ophthalmic examination included OCT and FA. However, OCT was performed earlier (weeks 1 and 2) in some patients, according to the investigator's decision and preference. In addition, FA was done at the discretion of the examiner and not at every post injection evaluation, usually every 6 weeks.

Patients were included in this consecutive series only if there was a minimum follow-up of 12 months. Patients received reinjections when there was a recurrence of DDME. Recurrence was defined as a decrease of BCVA associated with an increase of intraretinal fluid due to macular edema on OCT ($\geq$ 50 μ in central macular thickness) and/or FA, after complete or partial resolution in previous follow-up visits.

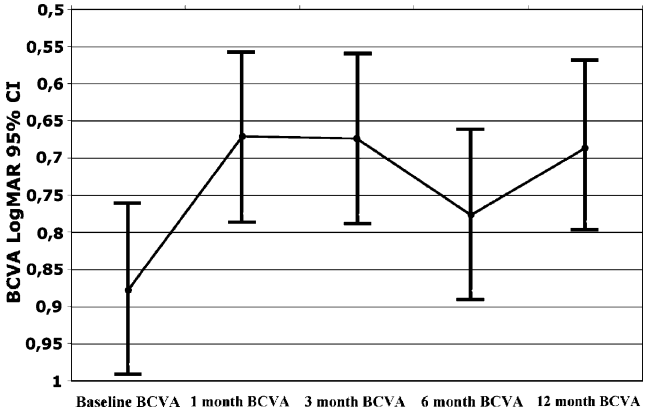

Fig. 1 Changes in best-corrected visual acuity (BCVA) after intravitreal bevacizumab. BCVA improved at 1 month from logMAR=0.87 to logMAR=0.67, a difference that was statistically significant ($p<0.001$); this level of BCVA was maintained throughout 3, 6, and 12 months

Patients' ETDRS BCVAs were transferred from their records and converted to a logarithm of the minimum angle of resolution (logMAR) scale for analysis. Repeated-measures analysis of variance (ANOVA) was used to compare mean values to analyze mean retinal thickness and logMAR visual acuity (VA) statistically. An increase or decrease in BCVA was considered to have occurred if there was a change of 2 ETDRS lines. Main outcome measures included changes in BCVA, OCT, and FA. Interval data were analyzed at 1-, 3-, 6 and 12-month follow-up time points. A p value<0.05 was considered to be significant.

Results

We reviewed the clinical records of 82 consecutive patients (101 eyes) with DDME. All patients had a minimum follow-up of 12 months. Sixty-five patients (79.3%) were Hispanic, and 12 (14.6%) were Caucasian. Our patients had a mean age of 59.7±9.3 years, and 53.7% were female (38 men, 44 women). Patients had a mean follow-up of 57.6±8.4 weeks (range: 54–68 weeks). Fifty-three cases (52.4%) had quiescent PDR. All of these 53 cases had had prior scatter panretinal photocoagulation (PRP) at least 6 months before undergoing bevacizumab intravitreal injections. All the eyes had DDME diagnosed by biomicroscopy slit-lamp examination, FA, and OCT at baseline.

Within 1 month after the initial bevacizumab injection, improvements in BCVA and central retinal thickness measurements were observed, and these significant changes continued throughout the 12-month follow-up. At 1 month, BCVA improved from logMAR=0.87 to 0.67, a difference that was statistically significant ($p=0.0001$). This improvement in BCVA was maintained throughout the 3-, 6-, and 12-month follow-up (Fig. 1). At the 6-month follow-up time point, we noticed a worsening of vision with a mean BCVA of 20/115, logMAR=0.77, still a difference statistically significant from BCVA at baseline. In addition, the mean final BCVA at 12 months was 20/96, logMAR=0.68 ($p<0.001$) a statistically significant difference from baseline BCVA. Final BCVA analysis by sub-groups demonstrated that 33 eyes (32.7%) remained stable, 50 eyes (49.5%) improved 2 or more ETDRS lines of BCVA, and 18 eyes (17.8%) decreased 2 or more ETDRS lines of BCVA (Table 1).

Optical coherence tomography results were available for all 101 eyes at 1-, 3-, 6- and 12-month follow-ups. At 1 month, the mean 1-mm central macular thickness (CMT) measurements decreased from 401.8 µm±180 µm to 290.2 µm±119.6 µm ($p<0.001$), and this overall improvement continued throughout the 12-month follow-up (Fig. 2). At 3-, 6- and 12-month follow-ups, mean CMT were 309.5.1 µm±147.3 µm, 309.3 µm±123.6 µm, and 281.6 µm±105.0 µm respectively, which were significantly different from baseline ($p<0.001$).

We wished to compare the response to treatment between patients with PDR and previous PRP and those with nonproliferative diabetic retinopathy and DDME, to see if there was any difference. However, when we ran the repeated measures ANOVA to compare mean values to statistically analyze mean retinal thickness and logMAR

Table 1 BCVA analysis by sub-groups (101 eyes)*

Dose	1st month		3rd month		6th month		12 month	
	1.25 mg	2.5 mg	1.25 mg	2.5 mg	1.25 mg	2.5 mg	1.25 mg	2.5 mg
	# eyes (%)	# eyes (%)	# eyes (%)	# eyes (%)	# eyes (%)	# eyes (%)	# eyes (%)	# eyes (%)
Decreased 2 or more ETDRS lines of BCVA	0 (0%)	5 (9.4%)	0(0%)	6 (11.3%)	2 (4.2%)	13 (24.5%)	3 (6.3%)	15 (28.3%)
Remained stable	11 (23%)	21 (39.6%)	15 (31.2%)	19 (35.8%)	16 (33.3%)	15 (28.3%)	17 (35.4%)	16 (30.1%)
Improved 2 or more ETDRS lines of BCVA	37 (77%)	27 (51%)	33 (68.8%)	28 (52.8%)	30 (62.5%)	25 (47.1%)	28 (58.3%)	22 (41.5%)

* BCVA = best-corrected visual acuity, ETDRS = Early Treatment Diabetic Retinopathy Study.

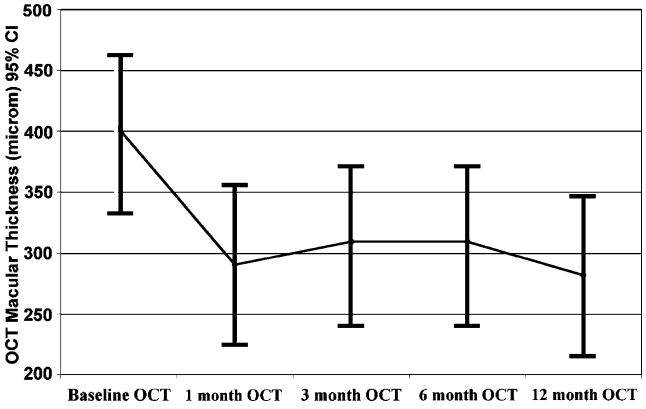

Fig. 2 Changes in macular thickness with optical coherence tomography (OCT) during follow-up after intravitreal bevacizumab. At 1 month, the mean 1-mm central macular thickness (CMT) measurements decreased from 401.8 μm±180 μm to 290.2 μm± 119.6 μm ($p<0.001$), and this overall improvement continued throughout the 12-month follow-up

VA, adjusting for the grade of diabetic retinopathy as a covariate, we did not find statistical significance ($p=0.565$ for BCVA and $p=0.446$ for CMT).

All eyes received an intravitreal injection at the initial visit; however, recurrences were re-treated at the discretion of the treating physician. A total of 244 IVB injections were performed. The mean number of IVB injections per eye was three (range: one to six injections) at a mean interval of 14.1±10.5 weeks. Thirty-seven of 101 eyes (36.6%) received one injection, 16.8% of eyes (17/101) received two injections, 29.7% of eyes (30/101) received three injections, 5% of eyes (5/101) received four injections, 8.9% of eyes (9/101) received five injections, and 3% of eyes (3/101) received six injections (Table 2).

Forty-eight cases (47.5%) were treated with an intravitreal injection of 1.25 mg of bevacizumab and fifty-three cases (52.5%) with at a dose of 2.5 mg of bevacizumab. Sixty-four eyes (63.4.%) needed a second injection at a mean interval of 15.7±11.9 weeks (range: 4 to 64 weeks) between injections, 47 eyes (46.5%) required a third injection at a mean interval of 14.8±7.9 weeks (range: 4 to 34 weeks) between injections, 17 eyes (16.8%) required a fourth injection at a mean interval of 11.2±6.8 weeks (range: 4 to 20 weeks) between injections, 12 eyes (11.9%) required a fifth injection at a mean interval of 20±10.3 weeks (range: 8 to 26 weeks) between injections, and three eyes (3.0%) required a sixth injection mean at a mean interval of 8± 6 weeks (range: 4 to 8 weeks) between injections.

Adverse events included transient arterial hypertension in one patient (1.2%), transient increased intraocular pressure in one eye (1.0%), and tractional retinal detachment in one eye (1.0%). At 12 months, no systemic adverse events such as thromboembolic events (cerebrovascular accidents, transient ischemic attacks, myocardial infarctions, or peripheral vascular disease) were reported.

Analysis of visual acuity and central macular thickness by doses of 1.25 mg and 2.5 mg

We did not observe statistically significant differences in changes of BCVA and between doses of 1.25 and 2.5 mg of intravitreal bevacizumab (Table 3) (Fig. 3). In the 1.25 mg group, at 1 month BCVA improved from 20/190, logMAR= 0.97 to 20/85, logMAR 0.62, a difference that was statistically significant ($p=0.0001$). This improvement was maintained throughout the 3-, 6-, and 12-month follow-up. At the 6-month follow-up we noticed worsening of vision, with a mean BCVA of 20/112, logMAR=0.70 ($p<0.001$), a difference that was still statistically significant from BCVA at baseline. However, BCVA improved again at 12 months with mean final BCVA of 20/76, logMAR=0.58 ($p<0.001$), a statistically significant difference from baseline BCVA (Fig. 3). Similar BCVA changes were observed in the 2.5 mg group; at 1 month, BCVA improved from 20/120, logMAR=0.76 to 20/76, logMAR=0.58, a difference that was statistically significant ($p=0.0001$). This improvement in BCVA was maintained throughout the 3-, 6-, and 12-month follow-up. At the 6-month follow-up we noticed worsening of vision with a mean BCVA of 20/106,

Table 2 Injections analysis by sub-groups 1.25 mg (48 eyes) and 2.5 mg (53 eyes)

Injections required by eye during 12 months	Sub-group 1.25		Sub-group 2.5	
	Frequency	Percent	Frequency	Percent
No re-injection	25	52.1%	12	22.6%
Two injections	5	10.4%	12	22.6%
Three injections	7	14.6%	23	43.4%
Four injections	1	2.1%	4	7.6%
Five injections	7	14.6%	2	3.8%
Six injections	3	6.2%	0	0%

Table 3 BCVA in eyes injected with doses of 1.25 and 2.5 mg of IVB at 12-month follow-up*

	Sub-group 1.25 mg		Sub-group 2.5 mg	
	BCVA	LogMar	BCVA	LogMar
Baseline	20/190	0.97	20/120	0.76
1 month	20/85	0.62	20/76	0.58
3 months	20/90	0.65	20/80	0.6
6 months	20/112	0.74	20/106	0.72
12 months	20/76	0.58	20/85	0.62

* BCVA = best-corrected visual acuity, IVB = intravitreal bevacizumab.

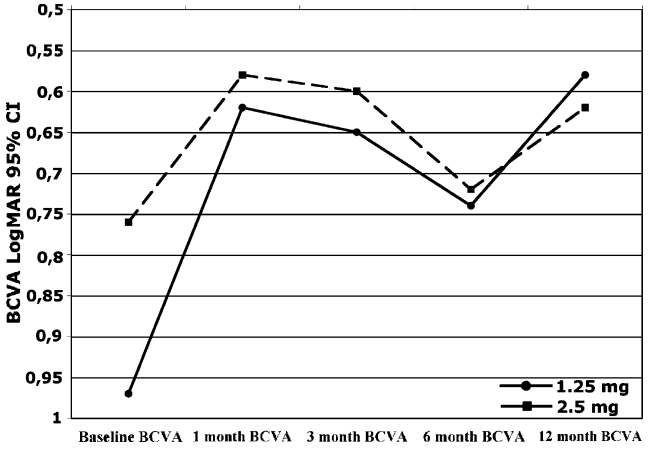

Fig. 3 Changes in best-corrected visual acuity (BCVA) between doses of 1.25 and 2.5 mg of intravitreal bevacizumab. In the 1.25 mg group at 1 month, BCVA improved from logMAR=0.97 to logMAR 0.58, a difference that was statistically significant (p=0.0001). This improvement in BCVA was maintained throughout the 3-, 6-, and 12-month follow. At the 6-month follow-up a worsening of vision was noted, with a mean logMAR = 0.74 (p<0.001), a difference statistically significant from BCVA at baseline. Similar BCVA changes was observed in the 2.5 mg group; at 1 month BCVA improved from logMAR=0.76 to logMAR=0.62, a difference that was statistically significant (p=0.0001). This improvement in BCVA was maintained throughout the 3-, 6-, and 12-month follow-up. At the 6-month follow-up a worsening of vision was noted, with a mean logMAR=0.72 (p<0.001), a difference statistically significant from BCVA at baseline

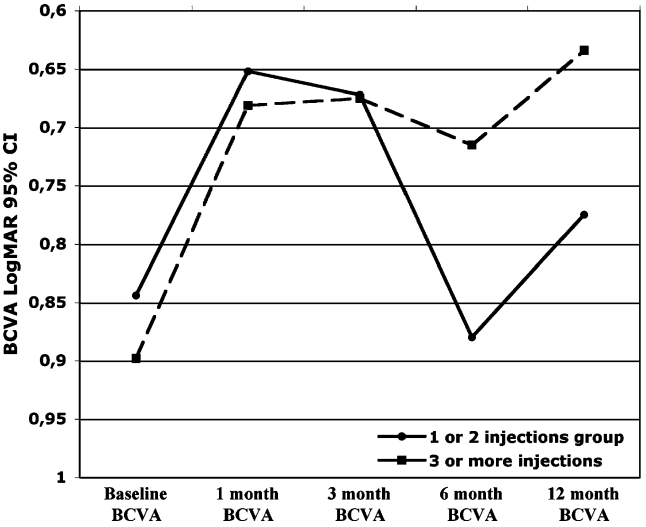

Fig. 4 When we analyzed our data comparing eyes that had one or two injections against those eyes that had three or more injections, there was a significant drop in best-corrected visual acuity (BCVA) at 6 months in the "one or two injections" group, and not in the "three or more injections" group. This suggests the need for at least three injections a year to maintain the BCVA results

logMAR=0.72 (p<0.001), a difference still statistically significant from BCVA at baseline. However, BCVA improved again at 12 months, with mean final BCVA of 20/85, logMAR=0.62 (p<0.001), a statistically significant difference from baseline BCVA (Fig. 3). In addition, in the 1.25 mg group, at 1 month there was an average gain of 3.2±2.9 lines of BCVA, at 3 months 2.7±2.6 lines of BCVA, at 6 months 2.1±3.4 lines of BCVA, and 2.9±3.6 lines of BCVA at 12 months (p<0.001). In the 2.5 mg group, at 1 month eyes gained 2.0±4.0 lines of BCVA, 1.9±2.9 lines of BCVA at 3 months, 0.6±5.5 lines of BCVA at 6 months, and 0.7±5.5 lines of BCVA at 12 months (p<0.01).

We wondered about the reason for a temporary decrease of BCVA at the 6-month time point, and if the number of reinjections had any influence on our results. When we analyzed our data comparing eyes that had one or two injections against those eyes that had three or more injections, there was a significant drop in BCVA at 6 months in the "one or two injections" group, and not in the "three or more injections" group (Fig. 4).

We did not observe statistically significant differences in macular thickness with OCT between doses of 1.25 and 2.5 mg of intravitreal bevacizumab (Fig. 5). In the 1.25 mg group, the mean CMT decreased from 419.1±201.1 μm at baseline to 295.11±91.5 μm at 1 month, 302.1±124.2 μm at 3 months, 313.4.1±96.3 μm at 6 months, and 268.2±95.5 μm at 12 months (p<0.001). In the 2.5 mg group, the mean CMT decreased from 387.7±162 μm at baseline to 287±136 μm at 1 month, 316.9±169.8 μm at 3 months, 306.7±139.6 μm at 6 months, and 295.5±113.9 μm at 12 months (p<0.001).

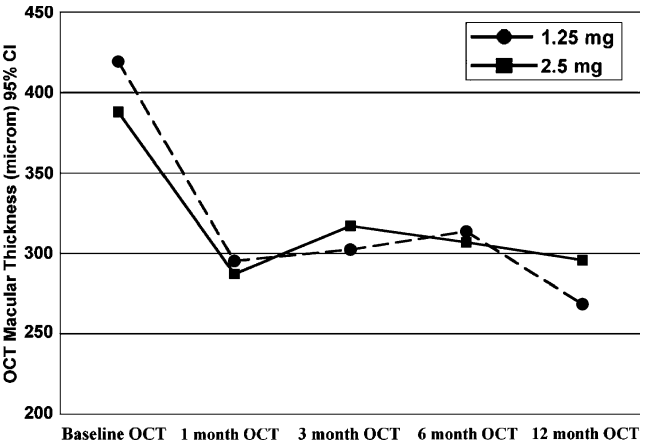

Fig. 5 Changes in macular thickness with optical coherence tomography (OCT) during follow-up between doses of 1.25 and 2.5 mg of intravitreal bevacizumab. In the 1.25 mg group, the mean central macular thickness (CMT) decreased from 419.1 μm to 295.11±91.5 μm at 1 month, 302.1 μm at 3 months, 313.4 μm at 6 months, and 268.2 μm at 12 months (p<0.001). In the 2.5 mg group, the mean CMT decreased from 387.7 μm at baseline to 287 μm at 1 month, 316.9 μm at 3 months, 306.7 μm at 6 months, and 295.5 μm at 12 months (p<0.001)

Discussion

Diabetic macular edema is a manifestation of diabetic retinopathy that produces loss of central vision. Although several treatment modalities are under investigation, the only demonstrated means to reduce the risk of vision loss from DME are: (1) laser photocoagulation, as demonstrated by the ETDRS [4], (2) intensive glycemic control, as demonstrated by the Diabetes Control and Complications Trial and the United Kingdom Prospective Diabetes Study, and (3) blood pressure control, as demonstrated by the United Kingdom Prospective Diabetes Study [36, 37]. Taking into account that most eyes with DDME that are treated with laser photocoagulation do not have an improvement in VA [8], there has been an interest in other treatment modalities such as pharmacologic therapy with oral protein kinase C inhibitors and the use of intravitreal corticosteroids [38, 39]. The use of antibodies targeted at VEGF is another treatment modality that has generated considerable interest and is being investigated [9–17].

We report on 101 consecutive eyes with DDME treated with intravitreal bevacizumab, which resulted in both anatomic and functional improvement. Interestingly, the reduction of retinal thickness and improvement of BCVA were detected within the first 4 weeks after the injection in most of the patients. In addition, both doses (1.25 and 2.5 mg) were associated with improvement of BCVA and a greater reduction in CMT, and no differences between the groups were found. Ocular tolerance of the two different doses of IVB was demonstrated, and no serious systemic adverse events were noticed during the study.

There are several studies in the literature on the intravitreal administration of antibodies against VEGF for DDME. However, none of them deal with anti-VEGF as a primary treatment with a 12-month follow-up. Haritoglou et al. reported that intravitreal ranibizumab has the potential to maintain or improve BCVA and reduce retinal thickness in patients with DDME not responding to previous treatments such as photocoagulation, intravitreal injection of triamcinolone, or vitrectomy. Their follow-up period was too short (6 weeks) to provide specific treatment recommendations [16]. Kumar and Sinha reported results of 20 eyes with DDME treated with IVB at dose of 1.25 mg that had not responded to previous photocoagulation. Their follow-up period was 6 months. They concluded that IVB resulted in a significant decrease in macular thickness and improvement in VA at 3 months, but the effect was somewhat blunted, though still statistically significant, at the end of 6 months [40]. Our study compares favorably with these reports, and confirms their findings with longer follow-up, and a larger number of patients. Furthermore, at the 6-month follow-up time point we noticed a small worsening of vision, as described by Kumar and Sinha [40]. When we analyzed our data comparing eyes that had one or two injections against those eyes that had three or more injections, there was a significant drop in BCVA at 6 months in the "one or two injections" group, and not in the "three or more injections" group. This suggests the need for at least three injections a year to maintain the BCVA results. Sixty-four eyes (63.4.%) needed at least a second injection at a mean of 15.7±11.9 weeks (range: 4 to 64 weeks).

A recent study has suggested that repeated intravitreal injections of bevacizumab in exudative AMD may be associated with decreased bioefficacy. This phenomenon has been termed tachyphylaxis [41]. Our study was not designed to investigate whether repeated intravitreal injection of bevacizumab can induce a decrease in biological response in DDME. However, we found no statiscally significant difference in OCT CMT after each IVT bevacizumab injection.

The two doses of bevacizumab evaluated in this study were 1.25 mg (which is the one that has been used most commonly in clinical practice) and 2.5 mg, which also has been used, though less commonly. The results of our retrospective study demonstrated the efficacy of 1.25 mg or 2.5 mg of IVB as primary treatment of DDME, as 49.5% of eyes showed anatomical and functional improvement. In addition, our results suggest a reduced risk of VA loss in eyes with DDME treated with IVB (82.2% of eyes). We found that the anatomical and visual benefit of intravitreal bevacizumab appears and reaches its maximum value during the first month, and maintains itself over 12 months. Nevertheless, we did not find statistically significant differences in duration or anatomical or functional effectiveness between the two doses of bevacizumab evaluated.

Our results indicate that IVB injections may have a beneficial effect on macular thickness and BCVA in DDME. Therefore, in the future this new treatment modality could complement focal/grid laser photocoagulation. Furthermore, focal/grid laser photocoagulation could be used to consolidate the results obtained with one IVB injection and decrease the need for reinjections.

Limitations of our study include that it is nonrandomized, uncontrolled, and retrospective, which precludes any estimation of the long-term efficacy or safety of IVB. In addition, because no control group is present we cannot rule out the possibility that some of the improvement in macular edema might be associated with improvement in systemic health. It is not uncommon that additional attention is directed towards improving systemic health when patients get involved in a clinical trial or new treatment. However, the results were very promising and suggest the need for further investigation. Furthermore, we can safely assume with a 95% confidence that the true rate of ocular complications in our study was <2.9%, and that the true rate of systemic complications was <3.6% [42].

In summary, primary intravitreal bevacizumab at doses of 1.25 mg or 2.5 mg seems to provide stability and improvement in BCVA, OCT, and FA in DDME at 12 months. Follow-up is still short; however, the results are promising. There seems to be no difference in our results between IVB at doses of 1.25 mg or 2.5 mg. Therefore, lower doses than 2.5 mg should be preferred. In addition, our results suggest the need for at least three injections a year to maintain the BCVA results. Evaluation in a multicenter randomized controlled clinical trial with longer follow-up is needed to evaluate the safety and efficacy of this treatment modality.

Appendix

The following investigators belong to the Pan-American Collaborative Retina Study Group (PACORES):

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Chapter 4: Intravitreal Bevacizumab for Diabetic Macular Edema at 24 months of follow up

Arevalo JF, Sanchez JG, Wu L, Maia M, Alezzandrini AA, Brito M, Bonafonte S, Lujan S, Diaz-Llopis M, Restrepo N, Rodríguez FJ, Udaondo-Mirete P; Pan-American Collaborative Retina Study Group. Primary intravitreal bevacizumab for diffuse diabetic macular edema: the Pan-American Collaborative Retina Study Group at 24 months. Ophthalmology. 2009 Aug;116(8):1488-97, 1497.e1. doi: 10.1016/j.ophtha.2009.03.016. Epub 2009 Jul 9.

DOI: 10.1016/j.ophtha.2009.03.016

Hypothesis 1: Intravitreal bevacizumab (IVB) may have a beneficial anatomic (Optical Coherence Tomography [OCT]), and functional (visual acuity [VA]) effect on eyes with diffuse diabetic macular edema at 24 months of follow up. In addition, the lower dose (1.25 mg) may be as effective or more than the higher dose (2.5 mg) of IVB.

PURPOSE: To report the 24-month anatomic and Early
Treatment Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) response after primary intravitreal bevacizumab (Avastin; Genentech, Inc., San Francisco, CA; 1.25 or 2.5 mg) in patients with diffuse diabetic macular edema (DDME). In addition, a comparison of the 2 different doses of intravitreal bevacizumab (IVB) used is presented.

DESIGN: Retrospective, multicenter, interventional, comparative case series.

PARTICIPANTS: The clinical records of 115 consecutive patients (139 eyes) with DDME at 11 centers from 8 countries were reviewed.

METHODS: Patients were treated with at least 1 intravitreal injection of 1.25 or 2.5 mg of bevacizumab. All patients were followed up for 24 months. Patients underwent ETDRS BCVA testing, ophthalmoscopic examination, optical coherence tomography (OCT), and fluorescein angiography (FA) at the baseline, 1-, 3-, 6-, 12-, and 24-month visits.

MAIN OUTCOME MEASURES: Changes in BCVA and OCT results.

RESULTS: The mean age of the patients was 59.4+/-11.1 years. The mean number of IVB injections per eye was 5.8 (range, 1-15 injections). In the 1.25-mg group at 1 month, BCVA improved from 20/150 (0.88 logarithm of the minimum angle of resolution [logMAR] units) to 20/107, 0.76 logMAR units (P<0.0001). The mean BCVA at 24 months was 20/75 (0.57 logMAR units; P<0.0001). Similar BCVA changes were observed in the 2.5-mg group: at 1 month, BCVA improved from 20/168 (0.92 logMAR units) to 20/118 (0.78 logMAR units; P = 0.02). The mean BCVA at 24 months was 20/114 (0.76 logMAR units; P<0.0001). In the 1.25-mg group, the mean central macular thickness (CMT) decreased from 466.5+/-145.2 microm at baseline to 332.2+/-

129.6 microm at 1 month and 286.6+/-81.5 microm at 24 months (P<0.0001). Similar results were obtained in the 2.5-mg group.

CONCLUSIONS: Primary IVB at doses of 1.25 to 2.5 mg seem to provide stability or improvement in BCVA, OCT, and FA in DDME at 24months. The results show no evident difference between IVB at doses of 1.25 or 2.5 mg.

Primary Intravitreal Bevacizumab for Diffuse Diabetic Macular Edema

The Pan-American Collaborative Retina Study Group at 24 Months

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Purpose: To report the 24-month anatomic and Early Treatment Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) response after primary intravitreal bevacizumab (Avastin; Genentech, Inc., San Francisco, CA; 1.25 or 2.5 mg) in patients with diffuse diabetic macular edema (DDME). In addition, a comparison of the 2 different doses of intravitreal bevacizumab (IVB) used is presented.

Design: Retrospective, multicenter, interventional, comparative case series.

Participants: The clinical records of 115 consecutive patients (139 eyes) with DDME at 11 centers from 8 countries were reviewed.

Methods: Patients were treated with at least 1 intravitreal injection of 1.25 or 2.5 mg of bevacizumab. All patients were followed up for 24 months. Patients underwent ETDRS BCVA testing, ophthalmoscopic examination, optical coherence tomography (OCT), and fluorescein angiography (FA) at the baseline, 1-, 3-, 6-, 12-, and 24-month visits.

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Conclusions: Primary IVB at doses of 1.25 to 2.5 mg seem to provide stability or improvement in BCVA, OCT, and FA in DDME at 24 months. The results show no evident difference between IVB at doses of 1.25 or 2.5 mg.

Financial Disclosure(s): The author(s) have no proprietary or commercial interest in any materials discussed in this article. *Ophthalmology 2009;116:1488–1497 © 2009 by the American Academy of Ophthalmology.*

Group members of PACORES listed in Appendix 1 (available at http://aaojournal.org).

Diabetic retinopathy remains the major threat to sight in the working-age population in the developed world. Furthermore, it is increasing as a major cause of blindness in other parts of the world, especially developing countries.[1] Diabetic macular edema (DME) is a manifestation of diabetic retinopathy that produces loss of central vision and is now the most common cause of moderate vision loss in persons with diabetes.[2] Macular edema within 1 disc diameter of the fovea is present in 9% of the diabetic population.[3] Although visual loss secondary to proliferative changes is more common in patients with type 1 diabetes, visual loss in patients with type 2 diabetes more commonly is the result of macular edema.[4] Diabetic macular edema can occur at any stage of diabetic retinopathy and is caused by excessive vascular permeability resulting in the leakage of fluid and plasma constituents, such as lipoproteins, and a secondary thickening and distortion of the central retina, together with stretching of neurons and an initial reversible loss of vision. Because in the course of time these disturbed neurons can die off, permanent sight reduction also can result.[2]

There is good evidence that focal laser treatment preserves vision in eyes with DME. The Early Treatment Diabetic Retinopathy Study (ETDRS)[5] randomized 1490 eyes with DME to receive focal laser treatment or observa-

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tion. At 3 years, treatment significantly reduced moderate visual loss as compared with observation,[5] with the greatest benefits in eyes with clinically significant DME.[6] Although the ETDRS[5] demonstrated that immediate focal photocoagulation reduced moderate visual loss by 50% (from 24% to 12%, 3 years after initiation of treatment), 12% of treated eyes still lost 15 ETDRS letters or more at the 3-year follow-up. Approximately 40% of treated eyes that had retinal thickening involving the center of the macula at baseline still had thickening involving the center at 12 months, as did 25% of treated eyes at 36 months. Furthermore, only 3% of laser-treated eyes experienced a gain of 3 lines of vision or more. This suggests that a distinct subgroup of eyes exists with DME resistant to conventional laser photocoagulation. In addition, some reports have indicated that diffuse diabetic macular edema (DDME) is refractory to macular photocoagulation.[6–8] Lee and Olk[9] demonstrated that with modified grid laser macular photocoagulation, visual acuity was stabilized in 60.9%, decreased in 24.6%, and increased in only 14.5% of eyes with DDME. The low frequency of improvement, gain of significant vision (≥3 lines), or both, after focal laser photocoagulation for DME has prompted interest in alternative or adjunct treatments, such as intravitreal triamcinolone acetonide,[10–14] pars plana vitrectomy,[15] and antibodies directed against vascular endothelial growth factor (VEGF).[16–19]

Vascular endothelial growth factor has been shown to be an endothelial cell-specific mitogen and an angiogenic inducer in a variety of in vitro and in vivo models.[20] Vascular endothelial growth factor, also known as vascular permeability factor, has been demonstrated to increase retinal vessel permeability by increasing the phosphorylation of tight junction proteins. Also, hypoxia has been shown to be a major inducer of VEGF gene transcription.[20] All variants of VEGF (particularly VEGF-A) have been implicated in the occurrence of increased vascular permeability by affecting endothelial tight-junction proteins in ocular vascular diseases such as DME.[21] It has been shown that VEGF-A levels are considerably higher in DME patients with extensive leakage in the macular region than in patients with minimal leakage.[22,23] Recent work has found elevated levels of VEGF in the ocular fluids of patients with proliferative diabetic retinopathy (PDR).[24–26] These studies also found that the growth of new vessels from the retina or optic nerve occurred as a result of VEGF release into the vitreous cavity as a response to ischemia.[24–26] Furthermore, injection of VEGF into normal primate eyes induces the same pathologic processes seen in diabetic retinopathy, including microaneurysm formation and increased vascular permeability.[27,28]

Human VEGF-A is found in at least 9 isoforms. Currently used anti-VEGF drugs are pegaptanib sodium (Macugen; OSI Eyetech Pharmaceuticals, Melville, NY), ranibizumab (Lucentis; Genentech, Inc., San Francisco, CA), and bevacizumab (Avastin; Genentech, Inc.). Bevacizumab is a complete full-length humanized antibody that binds to all subtypes of VEGF and is used successfully in tumor therapy as a systemic drug.[29] Studies have demonstrated the usefulness of intravitreal bevacizumab (IVB) with promising effects in the reduction of macular edema secondary to central retinal vein occlusion, vascular permeability, fibrovascular proliferation in retinal neovascularization secondary to PDR, rubeosis iridis, retinopathy of prematurity, choroidal neovascularization secondary to age-related macular degeneration and in the treatment of DME.[30–43] The amount of human retinal penetration for a complete full-length anti-VEGF antibody is not known at present. However, full-thickness retinal penetration of IVB was observed in an animal model.[44,45] Additionally, IVB does not seem to be toxic to the albino rabbit retina at a concentration of up to 2.5 mg.[46] The use of anti-VEGF drugs is becoming increasingly prevalent; however, some unresolved issues such as the ideal regimen or dose, duration of treatment, potential of combination treatments, and safety concerns with long-term VEGF inhibition deserve further investigations.

The purpose of this retrospective study was to report the 24-month anatomic and ETDRS best-corrected visual acuity (BCVA) response after primary IVB (1.25 or 2.5 mg) in patients with DDME. This report comprises a series of 139 eyes, including 38 eyes with DDME from a previously reported series[43] with longer follow-up. In addition, a comparison of the 2 different doses of IVB used was carried out.

Patients and Methods

A multicenter, retrospective study was conducted of eyes with DDME treated with off-label IVB between September 2005 and July 2006 at 11 institutions in Venezuela, Colombia, Costa Rica, Brazil, Argentina, Spain, Peru, and Mexico. The clinical records were reviewed of 115 consecutive patients (139 eyes) with DDME treated with at least 1 intravitreal injection of 1.25 or 2.5 mg bevacizumab. All patients were followed up for 24 months. Whether a dose of either 1.25 or 2.5 mg was to be used to treat a patient was determined at the discretion of the treating physician. If a patient received one of the doses at baseline, the same dose was delivered throughout the study. Approval was obtained from each participating center's institutional ethics committee, and informed consent was obtained for this study. In addition, this study

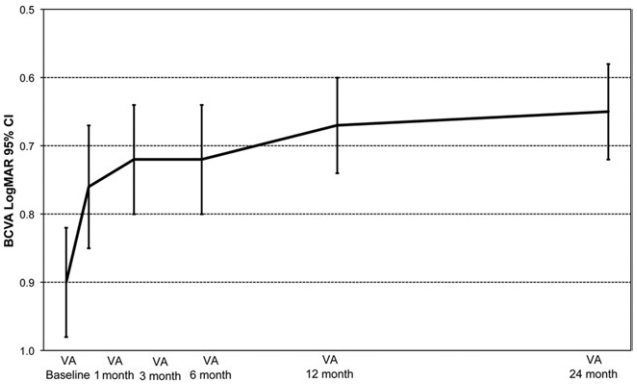

Figure 1. Graph showing changes in best-corrected visual acuity (BCVA) after intravitreal bevacizumab. The BCVA improved at 1 month from 0.90 to 0.76 logarithm of the minimum angle of resolution (logMAR) units, a difference that was statistically significant ($P<0.001$). This level of BCVA was maintained throughout 3, 6, 12, and 24 months of follow-up. CI = confidence interval.

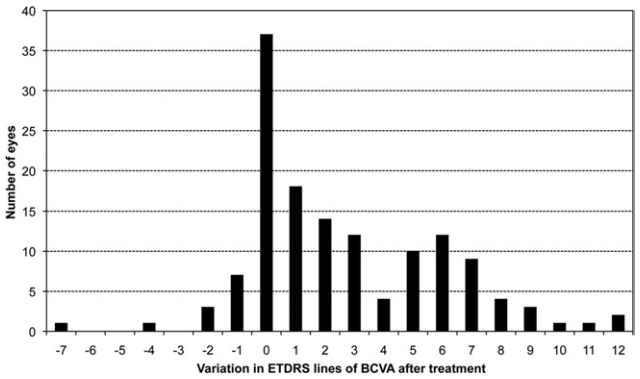

Figure 2. Bar graph showing the number of patients losing, maintaining, or gaining Early Treatment Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) from baseline to the 24-month follow-up.

has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The off-label use of the drug and its potential risks and benefits were discussed extensively with all patients.

The definition of DDME required evidence of diffuse retinal thickening, hard exudates (without a circinate ring pattern) involving the center of the macula (clinically significant DME as defined by the ETDRS on slit-lamp biomicroscopic examination), or both, and diffuse fluorescein leakage involving the center of the macula on fluorescein angiography (FA) with less than 33% of leakage associated with microaneurysms.[47] In addition, a significant reduction in the reflectivity (cysts) of the outer retinal layers, subretinal fluid collection by optical coherence tomography (OCT; Stratus OCT; Carl Zeiss, Dublin, CA), or both, should be present.[48] Exclusion criteria included patients (eyes) with DDME previously treated with laser photocoagulation or intravitreal triamcinolone, macular ischemia, intraocular inflammation, uncontrolled intraocular pressure, cataract surgery within the past 6 months or a prior history of vitreoretinal surgery, and the presence of an epiretinal membrane or vitreomacular traction syndrome. Although not a formal exclusion criterion, patients with a history of uncontrolled hypertension and recent thromboembolic events usually were not injected with bevacizumab, but this decision was left at the discretion of the treating physician.

Each patient underwent BCVA measurement with ETDRS charts and ophthalmic examination, including slit-lamp biomicroscopy. Baseline central retinal characteristics were analyzed by OCT using 6 diagonal slow 6-mm radial line scans, with software version 4.0, through a dilated pupil performed by a retina specialist. The retinal thickness of the 1-mm central retina was obtained using the macular thickness map for the calculations. The scans were reviewed and manual caliper-assisted measurements were used in case of delineation errors.

A 0.18-ml aliquot of commercially available bevacizumab was prepared for each patient and placed in a tuberculin syringe using aseptic techniques. After the eye had been prepared in a standard fashion using 5% povidone–iodine, an eyelid speculum was used to stabilize the eyelids, and the injection of 1.25 mg (0.05 ml) or 2.5 mg (0.1 ml) bevacizumab was performed 3.5 to 4 mm posterior to the limbus through the inferotemporal pars plana with a 30-gauge needle under topical anesthesia or subconjunctival lidocaine. After the injection, retinal artery perfusion was checked with the indirect ophthalmoscope (no anterior chamber paracentesis was necessary), and patients were instructed to administer topical antibiotics for 7 days.

Patients were examined at 1 and 2 weeks and 1 month after the first injection, and monthly thereafter. One, 3, 6, 12, and 24 months after the initial injection, the ophthalmic examination included OCT and FA. However, OCT was performed earlier (weeks 1 and 2) in some patients according to the investigator's decision and preference. In addition, FA was performed at the discretion of the examiner and not at every postinjection evaluation, rather, usually every 6 weeks.

Patients received reinjections whenever there was a recurrence of DDME. Recurrence was defined as a decrease of BCVA associated with an increase of intraretinal fluid because of macular edema on OCT ($\geq$50 μm in central macular thickness [CMT]), FA, or both, after complete or partial resolution in previous follow-up visits.

All data were collected in a Microsoft Excel 2003 spreadsheet (Microsoft Corporation, Unterschleissheim, Germany) and were analyzed using SPSS software version 13.0 for Windows (SPSS, Inc., Chicago, IL). For statistical analysis, the Friedman test was performed and $P<0.05$ was considered significant. Interval data were analyzed at the 1-, 3-, 6-, 12-, and 24-month follow-up time points. Patients' ETDRS BCVAs were transferred from their records and were converted to a logarithm of the minimum angle of resolution (logMAR) scale for analysis. Repeated measures of the analysis of variance were used to compare mean values to analyze mean retinal thickness and logMAR visual acuity (VA) statistically. An increase or decrease in BCVA was considered to have occurred if there was a change of 2 or more ETDRS lines. Main outcome measures included changes in BCVA and CMT measured by OCT.

Results

The clinical records of 115 consecutive patients (139 eyes) with DDME were reviewed. All patients had a minimum follow-up of 24 months. Seventy-six (66.1%) patients were Hispanic, 37

Table 1. Best-Corrected Visual Acuity Analysis by Subgroup (139 eyes)

	First Month, No. Eyes (%)		Third Month No. Eyes (%)		Sixth Month, No. Eyes (%)		Twelfth Month, No. Eyes (%)		Twenty-fourth Month, No. Eyes (%)	
	1.25 mg	*2.5 mg*	*1.25 mg*	*2.5 mg*	*1.25 mg*	*2.5 mg*	*1.25 mg*	*2.5 mg*	*1.25 mg*	*2.5 mg*
Decreased 2 or more ETDRS lines of BCVA	6 (8.1)	5 (6.8)	5 (6.8)	4 (6.2)	9 (12.2)	11 (16.9)	3 (4.0)	8 (12.3)	2 (2.7%)	3 (4.6)
Remained stable	29 (39.2)	40 (54.1)	31 (41.9)	36 (55.4)	19 (25.7)	32 (49.2)	21 (28.4)	31 (47.7)	25 (33.8)	37 (56.9)
Improved 2 or more ETDRS lines of BCVA	39 (52.7)	20 (27.0)	38 (51.3)	25 (38.4)	46 (62.1)	22 (33.8)	50 (67.6)	26 (40.0)	47 (63.5)	25 (38.5)

BCVA = best-corrected visual acuity; ETDRS = Early Treatment Diabetic Retinopathy Study.

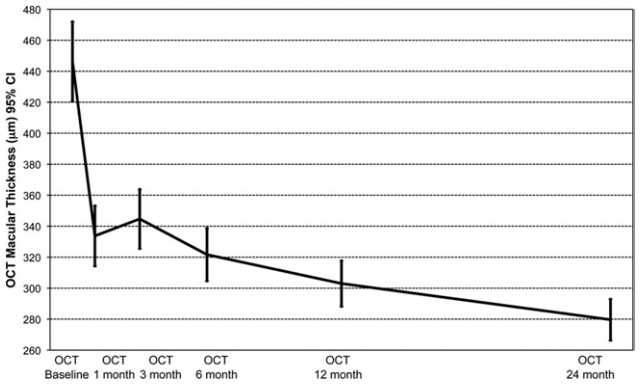

Figure 3. Graph showing the changes in macular thickness with optical coherence tomography (OCT) during follow-up after intravitreal bevacizumab. The foveal thickness improved after 1 month, mean 1-mm central macular thickness (CMT) measurement decreased from 446.4±154.4 μm to 333.75±117 μm ($P<0.001$), and this overall improvement continued throughout the 24-month follow-up. At 3-, 6-, 12-, and 24-month follow-up, CMT measurements were 344.7±115.3 μm, 321.7±102.7 μm, 303±89.1 μm, and 279.7±80 μm, respectively, which were significantly lower than baseline ($P<0.001$). CI = confidence interval.

(32.2%) were white, and 2 (1.7%) were black. The patients had a mean age of 59.4±11.1 years, and 51.3% were male (59 men, 56 women). In the current study, patients had a glycosylated hemoglobin mean of 9.1±1.86%. Regarding the severity of diabetic retinopathy (DR), 17 (12.2%) eyes had mild DR, 25 (18%) eyes had moderate DR, 39 (28.1%) eyes had severe DR, and 58 (41.7%) eyes had PDR. All these 58 cases with PDR had undergone prior scatter panretinal photocoagulation at least 6 months before undergoing IVB. All eyes had DDME diagnosed by biomicroscopic slit-lamp examination, FA, and OCT at baseline.

Within 1 month after the initial bevacizumab injection, improvements in BCVA and CMT measurements were observed, and these significant changes continued throughout the 24-month follow-up. At 1 month, BCVA improved from 0.90 to 0.76 logMAR units, a difference that was statistically significant ($P = 0.0001$). This improvement in BCVA was maintained throughout the 3-, 6-, 12-, and 24-month follow-up (Fig 1). In addition, the mean BCVA at 24 months was 20/100 (0.70 logMAR units; $P<0.001$), a statistically significant difference from baseline BCVA. Twenty-four–month BCVA analysis by subgroups demonstrated that 62 (44.6%) eyes remained stable, 72 (51.8%) eyes

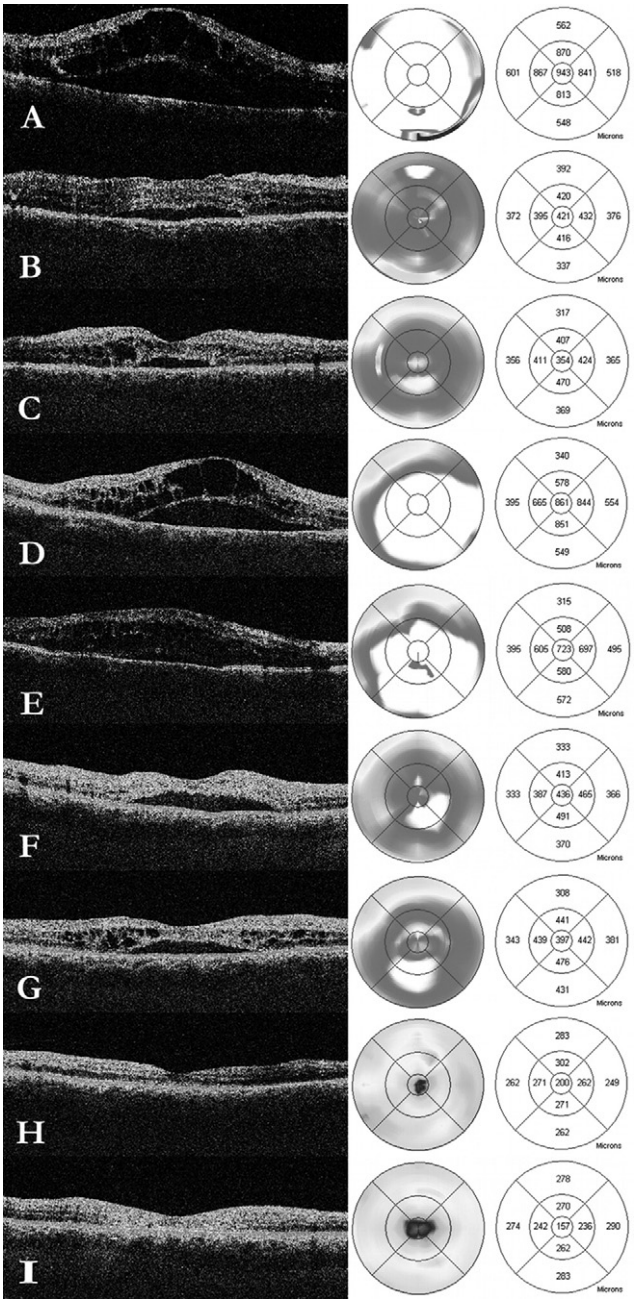

Figure 4. Sequential optical coherence tomography (OCT) of a 32-year-old diabetic man with a 3-month history of loss of vision to counting fingers (CF) in his right eye, in which diabetic macular edema (DME) had developed. **A**, Horizontal OCT scan obtained through the fovea revealing loss of the normal foveal contour, diffuse macular thickening, areas of low intraretinal reflectivity consistent with intraretinal cysts, and subretinal fluid (SRF). The retinal map analysis revealed a foveal thickness of 943 μm. The patient underwent an intravitreal injection of bevacizumab at a dose of 2.5 mg in this eye. **B**, Optical coherence tomography image revealing decrease of macular edema and SRF at 1 month after bevacizumab injection. The retinal map analysis indicates a central foveal thickness of 421 μm. Visual acuity (VA) improved to 10/200. **C**, Three months after the injection, OCT scan showing improvement in foveal thickness (354 μm) and almost complete resolution of the SRF. The VA improved to 20/200. **D**, Four months after the first injection, VA diminished to 20/400 and OCT scan demonstrated the reappearance of macular edema associated with an increase of intraretinal cysts and SRF. Central foveal thickness increased to 861 μm. He received a second injection of intravitreal bevacizumab at a dose of 2.5 mg at this point. **E–G**, Optical coherence tomography scans obtained at (**E**) 5, (**F**) 6, and (**G**) 9 months showing a progressive decrease in macular edema, intraretinal cysts, and SRF, which were confirmed with decrease of central foveal thickness (723 μm, 436 μm, and 397 μm, respectively). The VA also improved progressively (20/200, 20/160, and 20/125, respectively). **H**, Twelve months after the first injection, OCT scan showed resolution of DME, with complete reabsorption of SRF and restoration of foveal anatomy. Central foveal thickness decreased to 200 μm, and visual acuity was 20/80. **I**, Optical coherence tomography scan obtained at 24 months showing a marked resolution of DME, with complete reabsorption of SRF and restoration of foveal anatomic features. Central foveal thickness was 157 μm, and the visual acuity improved to 20/50.

improved 2 or more ETDRS lines of BCVA, and 5 (3.6%) eyes decreased 2 or more ETDRS lines of BCVA (Fig 2 and Table 1).

Optical coherence tomography results were available for all 139 eyes at the 1-, 3-, 6-, 12-, and 24-month follow-up examinations. At 1 month, the mean 1-mm CMT measurements decreased from 446.4±154.4 μm to 333.75±117 μm ($P<0.001$), and this overall improvement continued throughout the 24-month follow-up (Figs 3–5). At the 3-, 6-, 12-, and 24-month follow-up examinations, mean CMTs were 344.7±115.3 μm, 321.7±102.7 μm, 303±89.1 μm, and 279.7±80 μm, respectively, which were significantly different from baseline ($P<0.001$).

The response to treatment between patients with PDR and previous panretinal photocoagulation were compared with that of patients with nonproliferative diabetic retinopathy and DDME to see if there was any difference. However, when the repeated-measures analysis of variance was carried out to compare mean values to analyze statistically the mean retinal thickness and logMAR VA adjusting for the grade of diabetic retinopathy as a covariate, no statistical significance ($P = 0.511$ for BCVA and $P = 0.483$ for CMT) was found.

All eyes received an intravitreal injection at the initial visit; however, recurrences were retreated at the discretion of the treat-

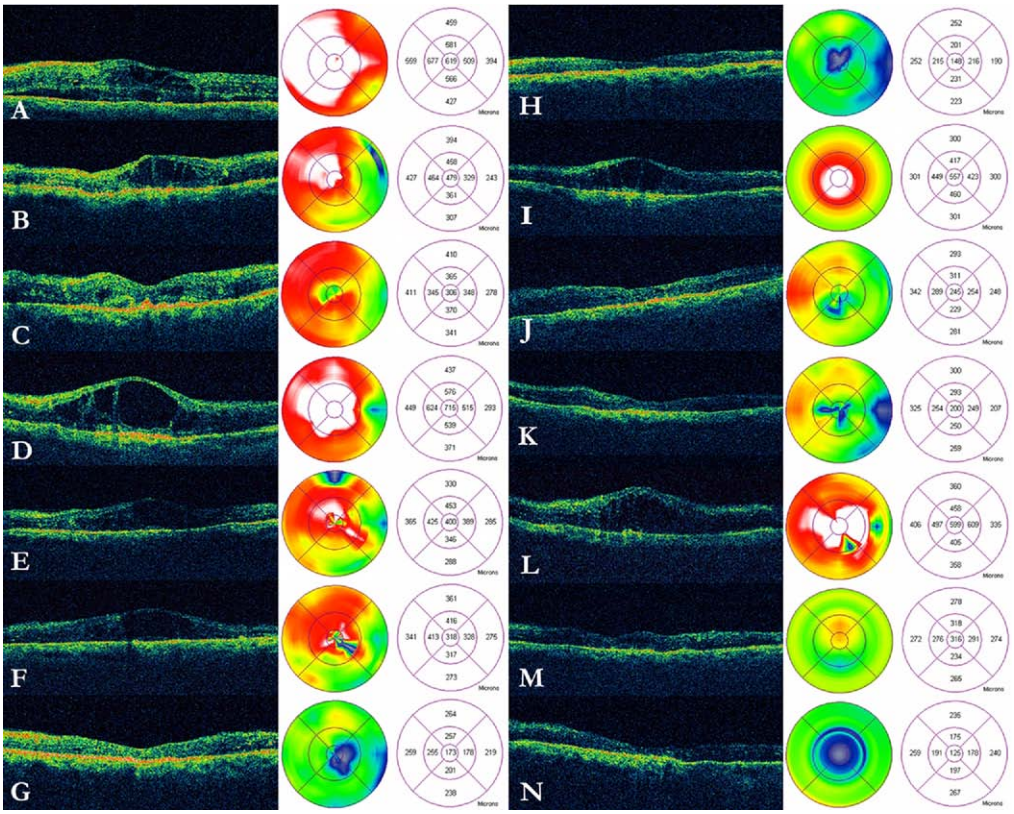

Figure 5. Sequential optical coherence tomography (OCT) imaging of a 69-year-old diabetic woman with a 6-month history of loss of vision to counting fingers (CF) in her left eye, in which diabetic macular edema (DME) had developed. **A**, Horizontal OCT scan obtained through the fovea revealing loss of the normal foveal contour, diffuse macular thickening, areas of low intraretinal reflectivity consistent with intraretinal cysts, and subretinal fluid (SRF). The retinal map analysis revealed a foveal thickness of 619 μm. The patient underwent an intravitreal injection of bevacizumab at a dose of 2.5 mg in this eye. **B**, Optical coherence tomography image revealing partial resolution of intraretinal macular edema and complete reabsorption of SRF at 1 month after bevacizumab injection. The retinal map analysis indicates a central foveal thickness of 479 μm. Visual acuity (VA) improved to 20/400. **C**, Three months after the injection, OCT scan showing improvement in foveal thickness (306 μm). The VA improved to 20/200. **D**, Four months after the first injection, VA diminished to CF, and OCT scan showed the reappearance of macular edema associated to increase of intraretinal cysts. Central foveal thickness increased to 715 μm. She received a second injection of intravitreal bevacizumab at a dose of 2.5 mg at this point. **E–G**, At month 6, she received a third injection of intravitreal bevacizumab at dose of 2.5 mg. The OCT scans at (**E**) 5, (**F**) 6, and (**G**) 9 months showed a progressive resolution in macular edema and intraretinal cysts, which were confirmed with decrease of central foveal thickness (400 μm, 318 μm, and 173 μm, respectively). Her VA also improves progressively (20/200, 20/200, and 20/125, respectively). **H**, Twelve months after the first injection, the OCT scan showed resolution of DME, with complete reabsorption of SRF and restoration of foveal anatomic features. Foveal thickness decreased to 148 μm, and visual acuity was 20/125. **I**, Sixteen months after the first injection, VA diminished to 20/400, and the OCT scan showed a reappearance of macular edema associated to increase of intraretinal cysts. Central foveal thickness increased to 557 μm. She received a fourth injection of intravitreal bevacizumab at dose of 2.5 mg. **J**, Optical coherence tomography scan obtained at 17 months showing a resolution in macular edema and intraretinal cysts. Central foveal thickness decreased to 245 μm and VA was 20/160. **K**, Eighteen months after the first injection (2 months after the previous injection), OCT scan showing improvement in foveal thickness (200 μm). The VA improved to 20/125. **L**, Nineteen months after the first injection, her visual acuity diminished to 20/400. Optical coherence tomography scan showing the reappearance of macular edema. The retinal map analysis indicates a central foveal thickness of 599 μm. She received a fifth injection of intravitreal bevacizumab at a dose of 2.5 mg at this point. **M**, Optical coherence tomography scan obtained at 20 months showing resolution in macular edema and intraretinal cysts. Central foveal thickness decreased to 316 μm. The VA improved to 20/200. **N**, Twenty-four months after the first injection, OCT showing a marked resolution in macular edema and restoration of foveal anatomic features. Central foveal thickness was 125 μm, and VA improved to 20/160.

Table 2. Analysis of the Frequency of Injections by Subgroups 1.25 mg (74 Eyes) and 2.5 mg (65 Eyes)

Injections Required by Eye during 24 Months	1.25 mg		2.5 mg		Both Groups	
	Frequency	*Percentage*	*Frequency*	*Percentage*	*Frequency*	*Percentage*
No reinjection	7	9.5	0	0	7	5
2 Injections	17	23	6	9.2	23	16.5
3 Injections	3	4.1	7	10.8	10	7.2
4 Injections	9	12.2	14	21.5	23	16.5
5 Injections	20	27.1	6	9.2	26	18.7
6 Injections	5	6.7	8	12.3	13	9.3
7 Injections	4	5.4	5	7.7	9	6.4
8 Injections	6	8.1	5	7.7	11	7.9
9 Injections	1	1.3	7	10.8	8	5.7
10 Injections	0	0	1	1.5	1	0.7
11 Injections	2	2.7	2	3.1	4	2.8
12 Injections	0	0	1	1.5	1	0.7
14 Injections	0	0	1	1.5	1	0.7
15 Injections	0	0	1	1.5	1	0.7

ing physician. There were a total of 807 IVB injections performed. The mean number of IVB injections per eye was 5.8 (range, 1–15 injections) at a mean interval of 12.2±10.4 weeks (Tables 2 and 3). Seventy-four (53.2%) cases were treated with an intravitreal injection of 1.25 mg bevacizumab and 65 (46.8%) cases were treated with a 2.5-mg dose of IVB.

Adverse events included transient high blood pressure in 1 (0.9%) patient, cerebrovascular accident in 1 (0.9%) patient, heart attack in 1 (0.9%) patient, transient increased intraocular pressure in 7 (5%) eyes, cataract in 5 (3.6%) eyes, and tractional retinal detachment in 1 (0.7%) eye.

Analysis of Visual Acuity and Central Macular Thickness by Doses of 1.25 and 2.5 mg

No statistically significant differences in changes of BCVA between doses of 1.25 and 2.5 mg of IVB were observed (Table 4 and Fig 6). In the 1.25-mg group at 1 month, BCVA improved from 20/150 (0.88 logMAR units) to 20/107 (0.73 logMAR units), a difference that was statistically significant ($P<0.0001$). This improvement was maintained throughout the 3-, 6-, 12-, and 24-month follow-up. The mean BCVA at 24 months was 20/75 (0.57 logMAR units; $P<0.0001$), a statistically significant difference from baseline BCVA (Fig 6). Similar BCVA changes were observed in the 2.5-mg group: at 1 month, BCVA improved from 20/168 (0.92 logMAR units) to 20/118 (0.78 logMAR units), a difference that was statistically significant ($P = 0.02$). This improvement in BCVA was maintained throughout the 3-, 6-, 12-, and 24-month follow-up. The mean BCVA at 24 months was 20/114 (0.76 logMAR units; $P<0.0001$), a statistically significant difference from baseline BCVA (Fig 6). In addition, in the 1.25-mg group, at 1 month there was an average gain of 3.1±2.1 lines of BCVA, at 3 months there was an average gain of 3.2±2.5 lines of BCVA, at 6 months there was an average gain of 2.4±1.8 lines of BCVA, at 12 months there was an average of 3.8±2.6 lines of BCVA, and at 24 months there was an average gain of 2.4±1.6 lines of BCVA ($P<0.001$). In the 2.5-mg group, at 1 month eyes gained 4.2±3.3 lines of BCVA, 1.8±1.3 lines of BCVA at 3 months, 3.0±2.1 lines of BCVA at 6 months, 2.6±2.3 lines of BCVA at 12 months, and 2.4±2.2 lines of BCVA at 24 months ($P<0.01$).

No statistically significant differences in macular thickness with OCT were observed between doses of 1.25 and 2.5 mg of IVB (Fig 7). In the 1.25-mg group, the mean CMT decreased from 466.5±145.2 μm at baseline to 332.2±129.6 μm at 1 month, 358.8±111.8 μm at 3 months, 317.6±87.7 μm at 6 months, 299.1±79.4 μm at 12 months, and 286.6±81.5 μm at 24 months ($P<0.0001$). In the 2.5-mg group, the mean CMT decreased from 423.4±163.5 μm at baseline to 335.5±102.8 μm at 1 month, 328.7±118.8 μm at 3 months, 326.3±118.7 μm at 6 months, 307.5±99.9 μm at 12 months, and 271.8±78.8 μm at 24 months ($P<0.0001$).

Table 3. Interval between Reinjections (132 Eyes)

Reinjections Required by Eye during 24 Months	No. of Eyes	Mean±Standard Deviation (Wks)	Range (Wks)	Percentage*
2 Injections	132	10.9±11.5	1–52	94.9
3 Injections	109	13.3±4.8	4–75	78.4
4 Injections	99	15.2±13.7	4–83	71.2
5 Injections	76	16.1±10.9	3–83	54.7
6 Injections	50	18.4±12.3	4–86	36
7 Injections	37	13.3±5.1	4–24	26.6
8 Injections	28	18.4±12.3	4–86	20
9 Injections or more	16	15.7±4.8	4–40	11.5

*Total percentage is more than 100% because 132 eyes needed 2 or more injections.

Discussion

Diabetic macular edema is a manifestation of DR that produces loss of central vision. Although several treatment methods are under investigation, the only demonstrated means to reduce the risk of vision loss from DME are laser photocoagulation, as shown by the ETDRS[5]; intensive glycemic control, as reported by the Diabetes Control and Complications Trial and the United Kingdom Prospective Diabetes Study; and blood pressure control, as demonstrated by the United Kingdom Prospective Diabetes Study.[49,50] Taking into account that most eyes with DDME treated with laser photocoagulation show no improvement in VA,[9] there has been an interest in other treatment methods such as pharmacologic therapy with oral protein kinase

Table 4. Best-Corrected Visual Acuity in Eyes Injected with Doses of 1.25 and 2.5 mg Intravitreal Bevacizumab at 24 Months of Follow-up

	1.25 mg		2.5 mg	
	Best-Corrected Visual Acuity	*Logarithm of the Minimum Angle of Resolution Units*	*Best-Corrected Visual Acuity*	*Logarithm of the Minimum Angle of Resolution Units*
Baseline	20/150	0.88	20/168	0.92
1 month	20/107	0.73	20/118	0.78
Third month	20/100	0.70	20/114	0.76
Sixth month	20/94	0.67	20/118	0.78
Twelfth month	20/80	0.60	20/114	0.76
Twenty-fourth month	20/75	0.57	20/114	0.76

C inhibitors and the use of intravitreal corticosteroids.[51,52] The use of antibodies targeted at VEGF is another treatment method that has generated considerable interest and is being investigated.[10–19]

Retinal hypoxia and various rheological disturbances play a role in DME. Several medical articles point to leukocyte dynamics as one of the causes of diabetic retinopathy.[53–54] Leukocytes have decreased deformability,[55] increased activation,[44] and increased adhesiveness to vascular endothelium in diabetes.[54] The levels of intercellular adhesion molecule 1 immunoreactivity were reported to be elevated in the retina of diabetic patients.[54] A previous study demonstrated that the vitreous levels of intercellular adhesion molecule 1 and VEGF were significantly higher in DME patients than in control patients.[23] Leukocyte entrapment, which is promoted by intercellular adhesion molecule 1 expression, is considered the critical early event in the pathogenesis of diabetic retinopathy. The trapped leukocytes cause transient or permanent microcirculatory disturbances and release cytotoxic products, such as cytokines, oxygen-free radicals, or proteolytic enzymes, and result in vascular endothelial cell damage and promote vascular permeability.[53,54] Long-term circulatory disturbance may lead to functional vascular obstruction, relative retinal ischemia, and release of cytokines such as VEGF. In 2 studies, Funatsu et al[22,23] reported that the levels of VEGF were elevated in the vitreous fluid of subjects with DME. Vascular endothelial growth factor causes conformational changes in the tight junctions of retinal endothelial cells[56,57] and plays a major role in increasing vascular permeability and in the progression of DME.[58]

This study reports on 139 consecutive eyes with DDME treated with intravitreal bevacizumab, which resulted in both anatomic and functional improvement. In most of the patients, the reduction of retinal thickness and improvement of BCVA were detected within the first 4 weeks after the injection. In addition, both doses (1.25 and 2.5 mg) were associated with improvement of BCVA and a greater reduction in CMT, and no differences in between were found. Ocular tolerance of the 2 different doses of IVB was dem-

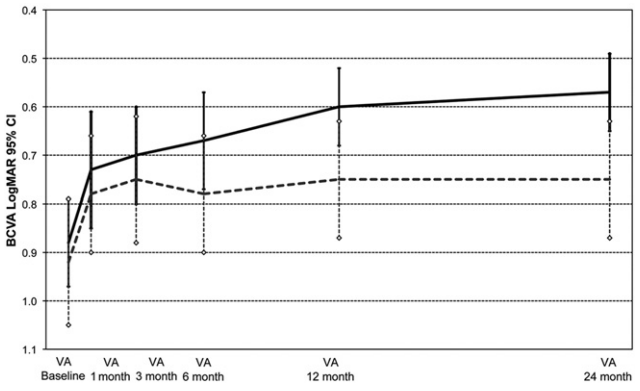

Figure 6. Graph showing changes in best-corrected visual acuity (BCVA) between doses of 1.25 and 2.5 mg of intravitreal bevacizumab. In the 1.25-mg group (solid line), BCVA at 1 month improved from 0.88 to 0.73 logarithm of the minimum angle of resolution (logMAR) units, a difference that was statistically significant ($P<0.0001$). This improvement was maintained throughout the 3-, 6-, 12-, and 24-month follow-up examinations. The mean BCVA at 24 months was 0.57 logMAR units ($P<0.0001$), a statistically significant difference from baseline BCVA. Similar BCVA changes were observed in the 2.5-mg group (doted line): at 1 month, BCVA improved from 0.92 to 0.78 logMAR units, a difference that was statistically significant ($P = 0.02$). This improvement in BCVA was maintained throughout the 3-, 6-, 12-, and 24-month follow-up examinations. The mean BCVA at 24 months was 0.76 logMAR units ($P<0.0001$), a statistically significant difference from baseline BCVA. CI = confidence interval; — = 1.25 mg; --- = 2.5 mg.

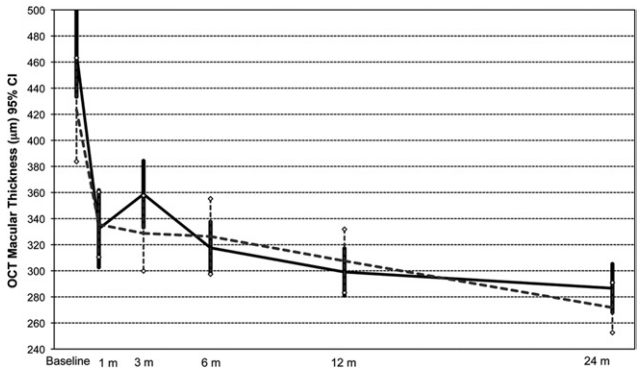

Figure 7. Graph showing changes in macular thickness with optical coherence tomography (OCT) during follow-up between doses of 1.25 and 2.5 mg intravitreal bevacizumab. In the 1.25-mg group (solid line), the mean central macular thickness (CMT) decreased from 466.5±145.2 μm at baseline to 332.2±129.6 μm at 1 month, 358.8±111.8 μm at 3 months, 317.6±87.7 μm at 6 months, 299.1±79.4 μm at 12 months, and 286.6±81.5 μm at 24 months ($P<0.0001$). In the 2.5-mg group (dotted line), the mean CMT decreased from 423.4±163.5 μm at baseline to 335.5±102.8 μm at 1 month, 328.7±118.8 μm at 3 months, 326.3±118.7 μm at 6 months, 307.5±99.9 μm at 12 months, and 271.8±78.8 μm at 24 months ($P<0.0001$). CI = confidence interval; — = 1.25 mg; --- = 2.5 mg.

onstrated, and no seriously adverse systemic events were noted during the study.

There are several studies in the literature on the intravitreal administration of antibodies against VEGF for DDME. Two studies previously reported IVB as a primary treatment for DME, including a previously reported series at 6 months[43] and a study by Soheilian et al[59] that reported 103 eyes with 12 weeks follow-up comparing IVB alone or combined with intravitreal triamcinolone versus macular focal or grid laser photocoagulation. They reported better results with IVB regarding visual outcome than with laser photocoagulation, although it was not associated with a significant decrease in CMT. No further beneficial effect of intravitreal triamcinolone could be demonstrated in their study.[59] Haritoglou et al[18] reported that intravitreal ranibizumab has the potential to maintain or improve BCVA and to reduce retinal thickness in patients with DDME not responding to previous treatments such as photocoagulation, intravitreal injection of triamcinolone, or vitrectomy. Their follow-up period was too short (6 weeks) to provide specific treatment recommendations. Kumar and Sinha[60] reported results of 20 eyes with DDME treated with IVB at a dose of 1.25 mg that had not responded to previous photocoagulation. Their follow-up period was 6 months. They concluded that IVB resulted in a significant decrease in macular thickness and improvement in VA at 3 months, but that the effect was somewhat blunted, although still statistically significant, at the end of 6 months. The results of the current study compare favorably with those of these reports and confirm their findings with longer follow-up and a larger number of patients.

The 2 doses of bevacizumab evaluated in this study were 1.25 mg, which is the one that has been used most commonly in clinical practice, and 2.5 mg, which also has been used, although less commonly. The results of this retrospective study demonstrated the efficacy of 1.25 or 2.5 mg of IVB as primary treatment of DDME, because 51.8% of eyes showed anatomic and functional improvement. In addition, the results suggest a reduced risk of VA loss in eyes with DDME treated with IVB (97.1% of eyes). The anatomic and visual benefit of intravitreal bevacizumab appears and reaches its maximum value during the first month and is maintained over 24 months. This study had an 80% power with an α of 5% to detect a 25% difference between the 1.25-mg and the 2.5-mg groups with respect to BCVA and CMT variations. No statistically significant differences in duration or anatomic or functional effectiveness were found between the 2 doses of bevacizumab evaluated.

These results indicate that IVB injections may have a beneficial effect on macular thickness and BCVA in DDME. Therefore, in the future, this new treatment method may replace or complement focal or grid laser photocoagulation. Furthermore, focal or grid laser photocoagulation may be used to consolidate the results obtained with 1 IVB injection and may decrease the need for reinjections. A recently published multicenter study, funded by the National Eye Institute and conducted through the Diabetic Retinopathy Clinical Research Network, studied 840 eyes of 693 subjects with DME involving the fovea and with VA of 20/40 to 20/320. This 2-year study demonstrated that focal or grid photocoagulation is more effective and has fewer side effects than 1-mg or 4-mg doses of preservative-free intravitreal triamcinolone for most patients with DME who have characteristics similar to the cohort in this clinical trial.[61] This study showed that at 2 years, focal or grid photocoagulation is more effective than expected. Schachat[62] recently pointed out 2 main reasons for these findings: longer-term follow-up than in previous intravitreal triamcinolone studies and the fact that in the ETDRS, subjects mainly had good vision at baseline. If the subject starts out with VA better than 20/40, it is harder to gain 5, 10, or 15 letters or 1 to 3 ETDRS lines than if the patient has worse vision at baseline. Therefore, we should all consider laser therapy for patients with DME while we await advances and better outcomes from new therapies still under investigation. Although the evidence currently supports focal or grid photocoagulation as the most effective treatment, the authors from the Diabetic Retinopathy Clinical Research Network study commented that combining laser therapy with corticosteroids may prove useful.[61] The same may be true for anti-VEGF therapies, and combination therapies should be considered.

Limitations of this study include that it is nonrandomized, uncontrolled, and retrospective, that is, features that preclude any estimation of the long-term efficacy or safety of IVB. In addition, because no control group is present, the possibility that some of the improvement in macular edema may be associated with improvement in systemic health cannot be ruled out. It is not uncommon that additional attention is directed toward improving systemic health when patients become involved in a clinical trial or new treatment. Furthermore, there was no standardized adverse event form to collect the safety data. However, the results are very promising and suggest the need for further investigation. In addition, it can be safely assumed (with 95% confidence) that the true rate of ocular complications in this study was less than 4.3% and that the true rate of systemic complications was less than 2.6%.[56,63]

In summary, primary IVB at doses of 1.25 or 2.5 mg seem to provide stability and improvement in BCVA, OCT, and FA results in DDME at 24 months. No difference in outcomes between IVB at doses of 1.25 or 2.5 mg was identified. Therefore, doses lower than 2.5 mg should be preferred. Evaluation in a multicenter, randomized, controlled clinical trial comparing IVB and focal or grid photocoagulation is needed to evaluate the safety and efficacy of this treatment method.

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Footnotes and Financial Disclosures

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Chapter 5: Intravitreal Bevacizumab plus Grid Laser Photocoagulation for Diabetic Macular Edema at 24 months of follow up

Arevalo JF, Lasave AF, Wu L, Diaz-Llopis M, Gallego-Pinazo R, Alezzandrini AA, Berrocal MH; Pan-American Collaborative Retina Study Group (PACORES). Intravitreal bevacizumab plus grid laser photocoagulation or intravitreal bevacizumab or grid laser photocoagulation for diffuse diabetic macular edema: results of the Pan-american Collaborative Retina Study Group at 24 months. Retina. 2013 Feb;33(2):403-13. doi: 10.1097/IAE.0b013e3182695b83.

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Hypothesis 2: IVB combined with grid laser photocoagulation may have a beneficial anatomic (OCT), and functional (VA) effect on eyes with diffuse diabetic macular edema at 24 months of follow up as compared to monotherapy. In addition, IVB combined with grid laser photocoagulation may decrease the number of injections if IVB necessary at 24 months.

PURPOSE: To evaluate the anatomical and functional outcomes at 24 months in patients with diffuse diabetic macular edema treated with primary intravitreal bevacizumab (IVB) plus grid laser photocoagulation (GLP) or primary IVB alone or GLP alone.

METHODS: Retrospective, interventional, comparative, multicenter study. We included in this analysis 141 eyes of 120 patients with diffuse diabetic macular edema treated with primary IVB alone (Group A), 120 eyes of 94 patients with GLP therapy (Group B), and 157 eyes of 104 patients treated with IVB plus GLP (Group C).

RESULTS: In all 3 groups, the authors observed improvement of Early Treatment Diabetic Retinopathy Study best-corrected visual acuity from baseline to 24-month follow-up ($P < 0.0001$). The improvement rate in Group A was statistically significantly better than in Group B (analysis of variance, $P = 0.013$). The authors also found a decrease in central macular thickness in all groups from baseline to the 24-month follow-up ($P < 0.0001$). The comparison among 3 groups showed higher central macular thickness decrease in Group A than in Groups B and C (analysis of variance, $P < 0.001$).

CONCLUSION: The study provides evidence to support the use of primary IVB with or without GLP as treatment of diffuse diabetic macular edema. Primary IVB without GLP seems to be superior to GLP alone to provide stability or improvement in best-corrected visual acuity in patients with diffuse diabetic macular edema at 24 months.

INTRAVITREAL BEVACIZUMAB PLUS GRID LASER PHOTOCOAGULATION OR INTRAVITREAL BEVACIZUMAB OR GRID LASER PHOTOCOAGULATION FOR DIFFUSE DIABETIC MACULAR EDEMA

Results of the Pan-American Collaborative Retina Study Group at 24 Months

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Conclusion: The study provides evidence to support the use of primary IVB with or without GLP as treatment of diffuse diabetic macular edema. Primary IVB without GLP seems to be superior to GLP alone to provide stability or improvement in best-corrected visual acuity in patients with diffuse diabetic macular edema at 24 months.

RETINA 33:403–413, 2013

Diabetic retinopathy is the leading cause of blindness among the working population in industrially developed countries.[1,2] Diabetic macular edema (DME) is a common manifestation of diabetic retinopathy that can occur at any stage of the disease and produce loss of central vision. The Wisconsin Epidemiologic Study of Diabetic Retinopathy[3] estimated that in people whose age at the time of diagnosis of diabetes was 30 years or older, the prevalence rate of DME was approximately 28% in both Type 1 and Type 2 diabetes if they had more than 20 years of known diabetes.[3]

Although several treatment modalities are currently under investigation, until recently, the only demonstrated means to reduce the risk of vision loss from DME were laser photocoagulation, as demonstrated by the Early Treatment Diabetic Retinopathy Study (ETDRS)[4]; intensive glycemic control, as demonstrated by the Diabetes Control and Complications Trial and the U.K. Prospective Diabetes Study; and blood

pressure control, as demonstrated by the U.K. Prospective Diabetes Study.[5–7]

Although the ETDRS[4] demonstrated that immediate focal photocoagulation reduced moderate visual loss by 50% (from 24 to 12%, 3 years after initiation of treatment), 12% of treated eyes still lost 15 or more ETDRS letters at the 3-year follow-up interval. Approximately 40% of treated eyes that had retinal thickening involving the center of the macula at baseline still had thickening involving the center at 12 months, as did 25% of treated eyes at 36 months. Furthermore, only 3% of laser-treated eyes experienced a gain of 3 or more lines of vision. This suggests that a distinct subgroup of eyes exists with DME resistant to conventional laser photocoagulation. As well as, some studies have reported a poor prognosis despite laser photocoagulation in eyes with diffuse diabetic macular edema (DDME).[4,8,9] The Diabetic Retinopathy Clinical Research Network (DRCR.net) showed that focal/grid photocoagulation in eyes with center-involved DME and visual acuity $\leq$ 20/40 produces gradual visual acuity improvement of $\geq$2 lines in approximately one third of eyes after 2 years of follow-up, although approximately 20% of laser-treated eyes worsen by $\geq$2 lines.[10] Thus, different additional therapies have been proposed for the treatment of DME.[11,12]

It has been suggested that DME is caused by excessive vascular permeability, resulting in the leakage of fluid and plasma constituents, such as lipoproteins into the retinal layers, leading to thickening of the retina. It was recently demonstrated that retinal hypoxia plays a role in DME,[13] and vascular endothelial growth factor (VEGF), which is upregulated by hypoxia, is likely to contribute to the excessive vascular permeability that results in macular edema in people with diabetes. Several studies have demonstrated not only a correlation of VEGF levels with the severity of diabetic retinopathy[14] but also a reduction in levels after successful laser treatment of proliferative diabetic retinopathy.[15] If VEGF has a role in the development or exacerbation of DME,[14,16] a rational approach to treat macular edema in these patients would include the use of anti-VEGF agents.

Chun et al[17] reported that ranibizumab (RBZ) (Lucentis; Genentech, Inc, San Francisco, CA) therapy has the potential to maintain or improve best-corrected visual acuity (BCVA) and reduce retinal thickness in patients with DME. In addition, intravitreal injections of the aptamer pegaptanib sodium (Macugen; OSI Eyetech Pharmaceuticals, Melville, NY) in patients with DME have been shown to improve visual acuity and retinal thickening. Cunningham et al[18] reported gains in visual acuity of 10 letters in 34% and 15 letters in 18% of patients with DME after an intravitreal pegaptanib sodium injection in a randomized, double-masked, multicenter trial with a follow-up of 36 months. Our group has also reported the 24-month anatomical and BCVA response after primary intravitreal bevacizumab (IVB) in patients with DME. These results demonstrated the efficacy of 1.25 mg or 2.5 mg of IVB as primary treatment of DME as 51.8% of eyes showed anatomical as well as functional improvement.[19] Recently, the Diabetic Retinopathy Clinical Research Network[20] showed that intravitreal RBZ with prompt or deferred laser is more effective through at least 2 years compared with prompt laser alone for the treatment of DME involving the central macula.

Solaiman et al[21] published that combined therapy with IVB and sequential grid laser photocoagulation (GLP) 3 weeks later appeared to be superior to GLP or IVB alone in reducing macular thickening and improving visual acuity. However, at 6 months of follow-up, there was no significant improvement of BCVA. The RESTORE Study[22] demonstrated recently that RBZ monotherapy and combined with laser provided superior visual acuity gain over standard laser in patients with visual impairment because of DME. At 1 year, no differences were detected between the RBZ and RBZ plus laser arms. They concluded that RBZ monotherapy and combined with laser had a safety profile in DME similar to that in age-related macular degeneration.

Because IVB and GLP achieve their effect on different pathways, a combination therapy may yield more favorable results than either therapy alone. We wished to know whether intravitreal therapy of bevacizumab has a synergistic effect when combined with GLP to produce better visual outcomes or greater decrease in central macular thickness (CMT) in patients with DDME and to compare this combination with primary IVB therapy or GLP alone.

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For a complete listing of participating members of PACORES, see Appendix 1.

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The purpose of this retrospective study was to evaluate the anatomical and functional outcomes in patients with DDME treated with primary IVB (1.25 or 2.5 mg) plus GLP or primary IVB alone or GLP alone at 24 months of follow-up.

Patients and Methods

This is a retrospective, comparative, multicentric, and interventional study performed at 5 centers from Venezuela, Costa Rica, Argentina, Spain, and Puerto Rico between May 2006 and May 2009. All patients with DDME treated with primary IVB (1.25 or 2.5 mg) plus GLP or primary IVB alone or GLP alone were included in this study. We reviewed the clinical records of 318 consecutive patients (418 eyes) with DDME treated with primary IVB alone (1.25 or 2.5 mg), GLP, or combined IVB plus GLP. Each pattern of treatment was separated into different groups. One hundred and forty-one (141) eyes of 120 patients with DDME were treated with at least 1 intravitreal injection of 1.25 mg or 2.5 mg of bevacizumab alone (Group A). The dose of 1.25 mg or a dose of 2.5 mg to be used to treat a patient was determined at the discretion of the treating physician. If a patient received one of the doses at baseline, then the same dose was delivered throughout the study. One hundred and twenty (120) eyes of 94 patients were treated with GLP therapy (Group B), and 157 eyes of 104 patients were treated with IVB plus GLP (Group C). In Group A, 75 eyes (53.2%) were treated with an intravitreal injection of 1.25 mg of bevacizumab and 66 eyes (46.8%) with a dose of 2.5 mg of bevacizumab. In Group C, 96 eyes (61.1%) were treated with an intravitreal injection of 1.25 mg of bevacizumab and 61 eyes (38.9%) with a dose of 2.5 mg of bevacizumab.

Approval was obtained from each participating center's Institutional Ethics Committee, and informed consent was obtained for this study. In addition, this study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The off-label use of the drug and its potential risks and benefits were discussed extensively with all patients.

Our definition of DDME required evidence of diffuse retinal thickening and/or hard exudates (without a circinate ring pattern) involving the center of the macula (clinically significant DME as defined by the ETDRS on slit-lamp biomicroscopic examination) and diffuse fluorescein leakage involving the center of the macula on fluorescein angiography (FA) with $<33\%$ of leakage associated with microaneurysms.[23] In addition, a significant reduction in the reflectivity (cysts) of the outer retinal layers and/or subretinal fluid collection by optical coherence tomography (OCT) should be present.[24]

All cases with proliferative diabetic retinopathy had had previous scatter panretinal photocoagulation at least 6 months before undergoing IVB or GLP. All eyes had DDME diagnosed by biomicroscopic slit-lamp examination, FA, and OCT (Stratus OCT; Carl Zeiss, Dublin, CA) at baseline.

Patients were excluded if they had other conditions known to cause macular edema, such as branch retinal vein occlusion, central retinal vein occlusion, exudative macular degeneration, or previous radiation. In addition, patients (eyes) with DDME previously treated with intravitreal triamcinolone, macular ischemia, intraocular inflammation, uncontrolled intraocular pressure, cataract surgery within the past 6 months, or a history of vitreoretinal surgery were excluded. Although not a formal exclusion criterion, patients with a history of uncontrolled hypertension and recent thromboembolic events were not usually injected with bevacizumab but this decision was left at the discretion of the treating physician.

Each patient underwent BCVA measurement with ETDRS charts and ophthalmic examination including slit-lamp biomicroscopy. Baseline central retinal characteristics were analyzed by OCT using 6 diagonal slow 6-mm radial line scans, with software version 4.0, through a dilated pupil performed by a retina specialist. The retinal thickness of the 1-mm central retina was obtained using the macular thickness map for our calculations. The scans were reviewed, and manual caliper-assisted measurements were used in case of delineation errors.

A 0.18-mL aliquot of commercially available bevacizumab was prepared for each patient included in Group A or Group C. Bevacizumab was placed in a tuberculin syringe using aseptic techniques. After the eye had been prepared in a standard fashion using 5% povidone/iodine, an sterile eyelid speculum was used to stabilize the eyelids and the injection of 1.25 mg (0.05 mL) or 2.5 mg (0.1 mL) of bevacizumab was performed 3.5 mm to 4 mm posterior to the limbus, through the inferotemporal pars plana with a 30-gauge needle under topical anesthesia or subconjunctival lidocaine. After withdrawal of the needle, sterile cotton-tipped applicator was used to apply pressure over the injection site for 1 minute and then retinal artery perfusion was checked with the indirect ophthalmoscope (no anterior chamber paracentesis was necessary). All patients were instructed to administer topical antibiotics for 7 days.

In patients treated with laser therapy (Groups B and C), GLP was performed with an argon or green laser

delivering 2 to 3 rows of 75 μm spots and 100 μm apart in the parafoveal region guided by FA and OCT. Then, 150 μm to 200 μm spots were applied 200 μm apart to the remaining areas of retinal thickening and capillary nonperfusion. Focal leaks outside or within the zones of diffuse leakage were treated with 100 μm to 150 μm spots to achieve a mild whitening of the microaneurysms. Treatment was placed up to 500 μm from the foveal center.

Patients were examined 1 month after the initial therapy and monthly thereafter. At each visit, patients underwent a complete ophthalmic examination using the same procedures performed at baseline except FA that was done at the discretion of the examiner.

Patients' ETDRS BCVAs were transferred from their records and converted to a logarithm of the minimal angle of resolution (logMAR) scale for analysis.

An increase or decrease in BCVA was considered to have occurred if there was a change of two or more ETDRS lines. Main outcome measures included changes in BCVA and CMT measured by OCT.

Patients received retreatment whenever there was a recurrence of DDME. Recurrence was defined as a decrease of BCVA associated with an increase of intraretinal fluid because of macular edema on OCT ($\geq$50 μm in CMT) and/or an increase of diffuse fluorescein leakage involving the center of the macula on FA, after complete or partial resolution in previous follow-up visits. Retreatment in Group B was GLP even if full laser was already in place. Retreatment in Group C was IVB.

All data were collected in a Microsoft Office Excel 2007 spreadsheet (Microsoft Corporation, Unterschleissheim, Germany) and statistically analyzed by MedCalc Software for Windows 8.2.0.3 (MedCalc, Mariakerke, Belgium). The paired sample *t*-test was performed to compare the CMT and the BCVA with baseline values within each treatment group. Correlation was considered significant with a P value < 0.05. Repeated measures analysis of variance (ANOVA method) were used to compare mean values to statistically analyze mean retinal thickness and logMAR visual acuity among the three treatment groups.

Results

We reviewed the clinical records of 318 consecutive patients (418 eyes) with DDME. All patients had 24 months of follow-up. One hundred and forty-one (141) eyes of 120 patients with DDME were treated with primary IVB alone (Group A), 120 eyes of 94 patients with GLP therapy (Group B), and 157 eyes of 104 patients with IVB plus GLP (Group C). The baseline ocular and systemic characteristics of all three groups were comparable. Baseline characteristics of patients in each treatment group are presented in Table 1.

The total number of injections was 5.8 ± 3.2 in Group A and 6.2 ± 4.9 in Group C. The interval between the first and second injection was 11.2 ± 9.4 weeks in Group A and 12.4 ± 18.8 weeks in Group C. The number of macular GLP sessions was 2.2 ± 1.4, and the interval between the first and second laser application was 20.2 ± 14.1 weeks in Group B. In Group C, the time of laser application was prompt (within 1 week) in 75 eyes (47.8%), intermediate (1 week $\leq$ 24 weeks) in 55 eyes (35%), and deferred (>24 weeks) in 27 eyes (17.2%).

In all 3 groups, we observed improvement of BCVA from baseline to 24 months of follow-up (Figures 1 and 2). In the Group A (IVB therapy), at 1 month after intravitreal injection of bevacizumab, BCVA improved significantly and these changes were maintained throughout 24 months of follow-up. In this group, the mean BCVA improved from baseline 20/160, logMAR 0.87 ± 0.4 (range, 0.1–1.8), to 20/100, logMAR 0.72 ± 0.5 (range, 0.1–1.8; $P < 0.0001$) at 1 month of follow-up. At 3 months and 6 months, the mean BCVA were 20/100, logMAR 0.70 ± 0.5 (range, 0.1–1.8; $P < 0.0001$). At 12 months and 24 months of follow-up, the mean BCVAs were 20/100, logMAR 0.65 ± 0.5 (range, 0.1–1.8) at both time points ($P < 0.0001$). Twenty-four months of BCVA analysis by subgroups demonstrated that 62 eyes (44.6%) remained stable, 74 (52.5%) eyes improved 2 or more ETDRS lines of BCVA, and 5 eyes (3.6%) decreased 2 or more ETDRS lines of BCVA. Mean BCVA letter score from baseline to Month 1 was 7.48 ± 14.49 (range, −35 to +60) and 8.55 ± 16.48 (−30 to +65) at 3 months of follow-up. At 6, 12, and 24 months of follow-up, the mean BCVA letter scores were 8.16 ± 17.37 (range, −40 to +60), 11.13 ± 17.65 (range, −50 to +65), and 11.84 ± 17.18 (range, −20 to +30), respectively.

In Group B (GLP therapy), there was no statistically significant difference from baseline BCVA until the first 6 months of treatment. In this group, the mean BCVA slightly improved from baseline 20/125, logMAR 0.77 ± 0.34 (range, 0.3–1.4), to 20/100, logMAR 0.75 ± 0.32 (range, 0.2–1.5; $P = 0.1257$), and 0.73 ± 0.32 (range, 0.2–1.8) ($P = 0.1162$) at 1 month and 3 months of follow-up. However, we found that 6 months after GLP, BCVA improved significantly ($P<0.05$) from baseline, and these changes were maintained throughout the 24 months of follow-up. At 6, 12, and 24 months of follow-up, the mean BCVA was 20/100, logMAR 0.72 ± 0.4 ($P = 0.0258$), logMAR

Table 1. Baseline Characteristics of Patients with DDME Included in Each Treatment Group

	Group A (IVB Therapy)	Group B (GLP Therapy)	Group C (IVB plus GLP)	*P*
Number of patients	120	94	104	—
Number of eyes	141	120	157	—
Sex (%)				
Male	63 (52.5)	46 (48.9)	43 (41.3)	0.379
Female	57 (47.5)	48 (51.1)	61 (58.7)	0.982
Age (mean ± SD), years	59.4 ± 10.8	64.3 ± 9.0	62.2 ± 8.7	0.229
Mean BCVA: baseline	20/160	20/125	20/125	0.061
Mean OCT baseline scores, μm	446.2 ± 155.8	379.1 ± 91	415.5 ± 144.8	0.001*
Hypertension (%)	69 (57.5)	52 (55.3)	59 (57)	0.898
Systemic glycemic control, n (%)				
Insulin	71 (59.1)	49 (52.1)	79 (75.9)	0.0127†
Oral hypoglycemic	59 (49.1)	65 (69.1)	42 (40.3)	0.1573
Combined therapy	0%	0%	34 (21.7%)	—
None, n (%)	11 (7.8)	6 (5)	0 (0)	0.158
HbA_{1c}, %	8.9 ± 1.6	9.7 ± 1.7	9.1 ± 1.6	0.064‡
Grade of DR, n (%)				
Mild NPDR	17 (12.1)	10 (8.3)	10 (6.4)	0.283
Moderate NPDR	27 (19.1)	27 (22.5)	49 (31.2)	0.155
Severe NPDR	38 (27)	27 (22.5)	44 (28)	0.707
PDR	59 (41.8)	30 (25)	54 (34.4)	0.130

Values represent number and percentages.

*Optical coherence tomography baseline score comparison among 3 groups showed a greater basal central macular thickening in Groups A and C than in Group B (ANOVA, $P < 0.001$).

†Chi-square test. The differences in the comparison of proportions of insulin treatment between Group A and Group C, $P = 0.010$ and between Group B and Group C, $P = 0.0007$, were observed.

‡In Groups A, B, and C, glycosylated hemoglobin data were obtained in 72% (86/120), 41% (39/94), and only in 56% (58/104) of patients, respectively. Chi-square test $P = 0.066$.

DR, diabetic retinopathy; HbA_{1c}, glycosylated hemoglobin; n, number of patients; NPDR, no proliferative diabetic retinopathy; PDR, proliferative diabetic proliferative.

0.71 ± 0.4 (range, 0.1–3; $P = 0.0107$), and logMAR 0.65 ± 0.4 (range, 0.2–4; $P = 0.0020$), respectively. Twenty-four months of BCVA analysis by subgroups demonstrated that 59 eyes (49.2%) remained stable, 36 eyes (30.0%) improved 2 or more ETDRS lines of BCVA, and 25 eyes (20.8%) decreased 2 or more ETDRS lines of BCVA. Mean BCVA letter scores from baseline to Month 1 were 1.25 ± 7.2 (range, −15 to +25) and 3.29 ± 9.8 (range, −15 to +35) at 3 months of follow-up. At 6, 12, and 24 months of follow-up, the mean BCVA letter scores were 4.1 ± 11.3 (range, −15 to +30), 4.41 ± 11.7 (range, −35 to +40), and 4.82 ± 12.9 (range, −30 to +45), respectively.

In Group C (IVB plus GLP), the mean BCVA improved from baseline 20/125, logMAR 0.76 ± 0.44 (range, 0.1–1.8), to 20/100, logMAR 0.70 ± 0.43 (range, 0.1–1.8; $P < 0.0001$) at 1 month of follow-up. At 3, 6, 12, and 24 months of follow-up, the mean BCVAs were 20/100, logMAR 0.69 ± 0.39 ($P = 0.0150$); 20/80, logMAR 0.67 ± 0.4 ($P = 0.0002$); 20/80, logMAR 0.65 ± 0.44 ($P = 0.0001$); and 20/80, logMAR 0.60 ± 0.43 ($P < 0.0001$), respectively. Twenty-four months of BCVA analysis by subgroups demonstrated that 78 eyes (49.7%) remained stable, 58 eyes (36.9%) improved 2 or more ETDRS lines of BCVA, and 21 eyes (13.4%) decreased 2 or more ETDRS lines of BCVA. Mean BCVA letter scores from baseline to Month 1 were 3.44 ± 9.07 (range, −15 to +30) and 3.66 ± 12.27 (range, −35 to +30) at 3 months of follow-up. At 6, 12, and 24 months of follow-up, the mean BCVA letter scores were 4.9 ± 16.37 (range, −30 to +30), 7.44 ± 16.73 (range, −25 to +30), and 8.18 ± 16.96 (range, −25 to +30), respectively. Best-corrected visual acuity analysis by subgroups of all eyes included in this study is shown in Table 2.

In Groups A and C, there were statistically significant differences from baseline BCVA at all time points of follow-up ($P < 0.0001$). The improvement rate in Group A was statistically significantly better than in Group B (ANOVA, $P = 0.013$). However, the improvement rate was not statistically significantly different between Groups A and C (ANOVA, $P = 0.167$) or between Groups B and C (ANOVA, $P = 0.092$).

Optical coherence tomography results were available for all 418 eyes at 1-, 3-, 6-, 12-, and 24-month follow-ups. We also found a decrease in CMT in all groups from baseline to the 24-month follow-up (Figure 3). In Group A (IVB therapy), there was a decrease from 446.2 ± 155.8 μm (range, 222–1082 μm) to 330.2 ± 115.6 μm (range, 198–841 μm; $P <$ 0.0001) at the first month after IVB. At 3 months and 6

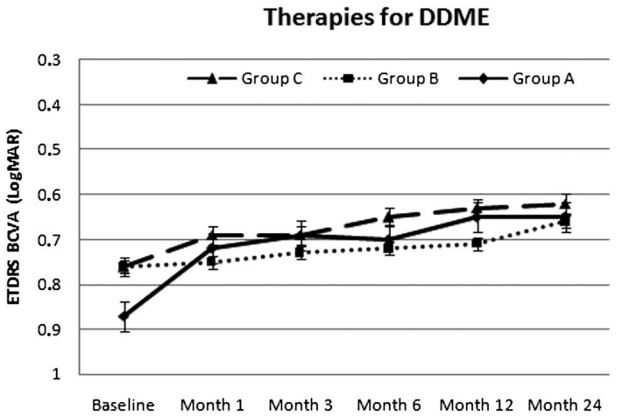

Fig. 1. Changes in BCVA after IVB alone (Group A), GLP (Group B), and IVB plus GLP (Group C) in patients with DDME at 24 months of follow-up. In Group A, the mean BCVA improved from baseline logMAR 0.87 ± 0.4 to logMAR 0.72 ± 0.5 ($P < 0.0001$) at 1 month of follow-up. At 3 months and 6 months, the mean BCVAs were logMAR 0.70 ± 0.5 ($P < 0.0001$). At 12 months and 24 months of follow-up, the mean BCVAs were logMAR 0.65 ± 0.5 ($P < 0.0001$). In Group B, the mean BCVA slightly improved from baseline logMAR 0.77 ± 0.34 to logMAR 0.75 ± 0.32 ($P = 0.1257$) and 0.73 ± 0.32 ($P = 0.1162$) at 1 month and 3 months of follow-up. At 6, 12, and 24 months of follow-up, the mean BCVAs were logMAR 0.72 ± 0.4 ($P = 0.0258$), logMAR 0.71 ± 0.4 ($P = 0.0107$), and logMAR 0.65 ± 0.4 ($P = 0.0020$), respectively. In Group C, the mean BCVA improved from baseline logMAR 0.76 ± 0.44 to 0.70 ± 0.43 ($P < 0.0001$) at 1 month of follow-up. Best-corrected visual acuity improved significantly (<0.05) from baseline, and these changes were maintained throughout the 24 months of follow-up. At 3, 6, 12, and 24 months of follow-up, the mean BCVAs were logMAR 0.69 ± 0.39 ($P = 0.0150$), logMAR 0.67 ± 0.4 ($P = 0.0002$), logMAR 0.65 ± 0.44 ($P = 0.0001$), and logMAR 0.60 ± 0.43 ($P < 0.0001$), respectively.

months, the mean CMT measurements were 341.5 ± 114.9 μm (range, 174–715 μm; $P < 0.0001$) and 356.1 ± 103.9 μm (range, 175–705 μm; $P < 0.0001$), respectively. At 12 months and 24 months of follow-up, the mean CMT measurements were 302.8 ± 89.6 μm (range, 150–524 μm; $P < 0.0001$) and 273.8 ± 79.5 μm (range, 135–583 μm; $P < 0.0001$), respectively.

In Group B (GLP therapy), there was a decrease from 379.1 ± 91 μm (range, 220–763 μm) to 368.9 ± 84.4 μm (range, 230–689 μm; $P = 0.1370$) at the first month after grid laser therapy. At 3 months and 6 months, the mean CMT measurements were 351.7 ± 86.2 μm (range, 216–640 μm; $P = 0.0010$) and 333.7 ± 103.9 μm (range, 202–581 μm; $P < 0.0001$), respectively. At 12 months and 24 months of follow-up, the mean CMT measurements were 303.5 ± 89.6 μm (range, 169–531 μm; $P < 0.0001$) and 271.2 ± 78.6 μm (range, 156–579 μm; $P < 0.0001$), respectively.

In the Group C (IVB plus GLP), there was a decrease from 415.5 ± 144.8 μm (range, 222–1,076 μm) to 377 ± 118.6 μm (range, 155–900 μm; $P < 0.0001$) at the first month after combined therapy. At 3 months and 6 months, the mean CMT measurements were 314.9 ± 135.9 μm (range, 150–1055 μm; $P < 0.0001$) and 364.4 ± 140.1 μm (range, 144–1,465 μm; $P < 0.0001$), respectively. At 12 months and 24 months of follow-up, the mean CMT measurements were 345.1 ± 118.5 μm (range, 142–1,033 μm; $P < 0.0001$) and 333 ± 138.5 μm (range, 132–608 μm; $P < 0.0001$), respectively (Figures 4 and 5). The comparison among 3 groups showed higher CMT decrease in Group A than in Groups B and C (ANOVA, $P < 0.001$).

In the IVB groups, we did not find any differences in the effectiveness between the doses of 1.25 mg and 2.5 mg in terms of BCVA or CMT. In Groups A and C, there were no complications related to the intravitreal injection during the 24 months of follow-up. No ocular or systemic adverse events were observed in all groups.

Discussion

This is a retrospective, comparative, multicentric, and interventional study that evaluates the anatomical and functional outcomes at 24 months of follow-up in patients with DDME treated with primary IVB alone (Group A), GLP (Group B), or primary IVB plus GLP (Group C).

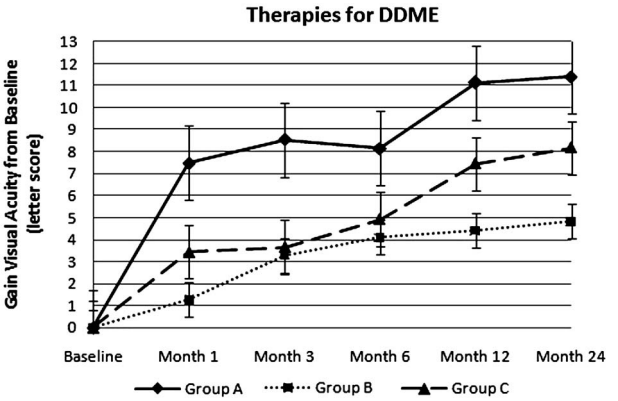

Fig. 2. Mean gain in BCVA letter score from baseline to 24 months of follow-up in all groups of treatment. In Group A (IVB therapy), mean BCVA letter score from baseline to Month 1 was 7.48 ± 14.49 (range, −35 to +60) and 8.55 ± 16.48 (−30 to +65) at 3 months of follow-up. At 6, 12, and 24 months of follow-up, the mean BCVA letter scores were 8.16 ± 17.37 (range, −40 to +60), 11.13 ± 17.65 (range, −50 to +65), and 11.84 ± 17.18 (range, −20 to +30), respectively. In Group B (GLP therapy), mean BCVA letter scores from baseline to Month 1 were 1.25 ± 7.2 (range, −15 to +25) and 3.29 ± 9.8 (range, −15 to +35) at 3 months of follow-up. At 6, 12, and 24 months of follow-up, the mean BCVA letter scores were 4.1 ± 11.3 (range, −15 to +), 4.41 ± 11.7 (range, −35 to +40), and 4.82 ± 12.9 (range, −30 to +45), respectively. In Group C (IVB plus GLP), mean BCVA letter scores from baseline to Month 1 were 3.44 ± 9.07 (range, −15 to +30) and 3.66 ± 12.27 (range, −35 to +30) at 3 months of follow-up. At 6, 12, and 24 months of follow-up, the mean BCVA letter scores were 4.9 ± 16.37 (range, −30 to +30), 7.44 ± 16.73 (range, −25 to +30), and 8.18 ± 16.96 (range, −25 to +30), respectively.

Table 2. Variation of BCVA at 24 Months after IVB, GLP, and IVB plus GLP Therapy for the Treatment of DDME

BCVA (logMAR) Results	Group A (IVB Therapy)	Group B (GLP Therapy)	Group C (IVB plus GLP)
Improved 2 or more lines, n (%)	74 (52.5)	36 (30)	58 (36.9)
Remained stable, n (%)	62 (44.6)	59 (49.2)	78 (49.7)
Decreased 2 or more lines, n (%)	5 (3.6)	25 (20.8)	21 (13.4)
Mean gain letter score	+11.84	+4.82	+8.18

Values represent number and percentages.
n, number of patients.

It is known that focal/grid photocoagulation has potential side effects, including laser scar expansion, paracentral scotoma, elevation of central visual field thresholds, and secondary choroidal neovascularization and subretinal fibrosis.[25–27] In addition, given that most eyes with DME that are treated with laser photocoagulation do not have an improvement in visual acuity, there has been an interest in other treatment modalities, and thus, newer approaches continue to be studied. Recent studies showed that adding anti-VEGF to laser therapy for DME achieved a visual acuity better than laser-alone therapy at 1 year to 2 years of follow-up.[28–30]

The DRCR.net study compared RBZ plus prompt or deferred laser with Early Treatment Diabetic Study type laser alone. At 1 year, the mean change in visual acuity letter score compared with baseline was significantly greater in the RBZ plus prompt laser group (8 letters) and RBZ plus deferred laser group (9 letters) but only 3 letters in the laser-alone group. At 2 years, the data were stable and similar with 7, 9, and 3 letters, respectively.[20,28]

The READ-2 study[29,30] analyzed RBZ alone, laser alone, and RBZ plus laser. The mean improvement in BCVA was about 7, 0.5, and 4 letters at the 6-month primary end point compared with 8, 5, and 7 letters at Month 24, for the RBZ alone, combined, and laser-only groups, respectively.

Recently, results of the randomized controlled RESOLVE study have been published. RESOLVE had about 50 subjects in a sham arm and about 100 in RBZ arms. Ranibizumab subjects had three initial monthly injections, and rescue laser was permitted in each group. For 1-year primary outcome, RBZ subjects improved about 10 letters and sham subjects had lost about 1 letter.[31]

The results from the RESTORE Study[22] also demonstrated that treatment with RBZ as monotherapy and combined with laser treatment provided superior visual acuity gain over standard laser in patients with visual impairment because of DME at 12 months of follow-up.

Solaiman et al[21] showed that combined therapy with IVB and sequential GLP 3 weeks later appeared to be superior to GLP or IVB alone in reducing macular thickening and improving visual acuity at 6 months of follow-up. However, no significant improvement in BCVA occurs 6 months after treatment. The authors showed that a combination of IVB and sequential GLP could be used as an initial treatment of DDME.

A prospective randomized trial of IVB or laser therapy in the management of DME (BOLT study 2) showed that IVB group gained a median of 8 ETDRS letters, whereas the laser group lost a median of 0.5 ETDRS letters ($P = 0.0002$) at 12 months of follow-up. At 12 months, CMT decreased from 507 ± 145 μm

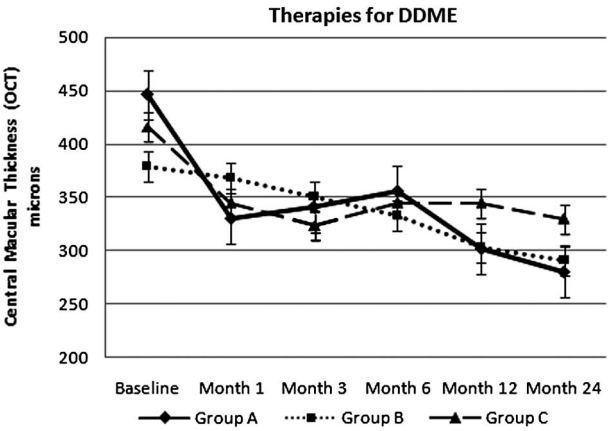

Fig. 3. Changes in macular thickness with OCT after IVB alone (Group A), GLP (Group B), and IVB plus GLP (Group C) in patients with DDME at 24 months of follow-up. In Group A, there was a decrease from 446.2 ± 155.8 μm to 330.2 ± 115.6 μm ($P < 0.0001$) at the first month after intravitreal therapy of bevacizumab. At 3 months and 6 months, the mean CMT measurements were 341.5 ± 114.9 μm ($P < 0.0001$) and 356.1 ± 103.9 μm ($P < 0.0001$), respectively. At 12 months and 24 months, the mean CMT measurements were 302.8 ± 89.6 μm ($P < 0.0001$) and 273.8 ± 79.5 μm ($P < 0.0001$), respectively. In Group B, there was a decrease from 379.1 ± 91 μm to 368.9 ± 84.4 μm ($P = 0.1370$) at the first month after grid laser therapy. At 3 months and 6 months, the mean CMT measurements were 351.7 ± 86.2 μm ($P = 0.0010$) and 333.7 ± 103.9 μm ($P < 0.0001$), respectively. At 12 months and 24 months of follow-up, the mean CMT measurements were 303.5 ± 89.6 μm ($P < 0.0001$) and 271.2 ± 78.6 μm ($P < 0.0001$), respectively. In Group C, there was a decrease from 415.5 ± 144.8 μm to 377 ± 118.6 μm ($P < 0.0001$) at the first month after combined therapy. At 3 months and 6 months, the mean CMT measurements were 314.9 ± 135.9 μm ($P < 0.0001$) and 364.4 ± 140.1 μm ($P < 0.0001$), respectively. At 12 months and 24 months of follow-up, the mean CMT measurements were 345.1 ± 118.5 μm ($P < 0.0001$) and 333 ± 138.5 μm ($P < 0.0001$), respectively.

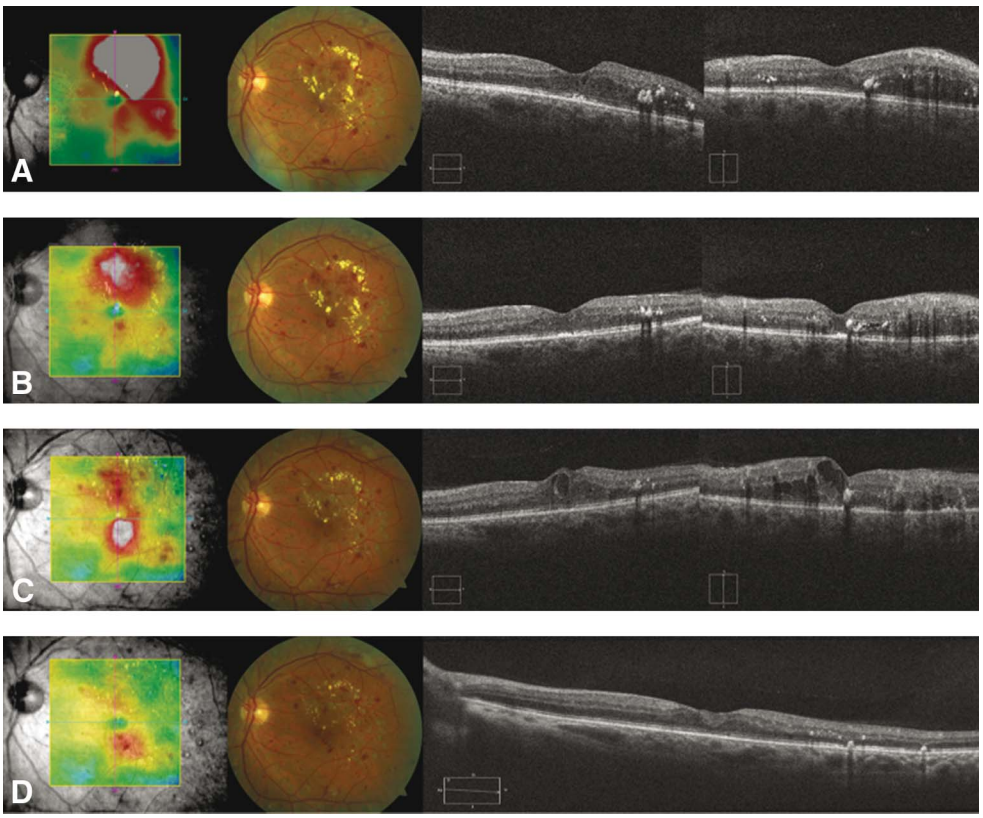

Fig. 4. A 56-year-old man was diagnosed with Type 2 diabetes 11 years before presentation. He had excellent metabolic control with oral hypoglycemiants and was previously treated with panretinal photocoagulation and macular focal laser 1 year ago. From left to right, color-coded OCT map, color fundus photograph, and horizontal and vertical OCT scans over the fovea. **A.** At baseline visit, there was a diffuse macular edema with relative foveal sparing. His BCVA was 20/25. Intravitreal bevacizumab (1.25 mg/0.5 mL) was injected at baseline and at Month 2 (BCVA 20/25) and Month 4 (combined with macular grid photocoagulation). **B.** At 6 months, there was a significant decrease of macular thickening (BCVA 20/32). Intravitreal bevacizumab was repeated at Month 6. **C.** At 12 months, there was a focal macular edema with foveal involvement (BCVA 20/25). Intravitreal bevacizumab was repeated at Month 12 and Month 18 (BCVA 20/32). **D.** At Month 24, there was a decrease in macular thickness with a marked regression of the lipid exudation (BCVA 20/25).

at baseline to 378 ± 134 μm ($P < 0.001$) in the IVB group, whereas it decreased to a lesser extent in the laser group from 481 ± 121 μm to 413 ± 135 μm ($P = 0.02$).[32]

The present study compares favorably with previous studies. Our results indicate that primary IVB at doses of 1.25 mg or 2.5 mg with or without GLP seems to provide fast stability and improvement in BCVA at 24 months. Groups A and C had a statistically significant difference from baseline BCVA at all time points of follow-up ($P < 0.0001$). Conversely, in Group B, there was no statistically significant difference from baseline BCVA until the first 6 months of treatment. We found that 6 months after GLP, BCVA improved significantly (<0.05) from baseline and these changes were maintained throughout the 24 months of follow-up. The improvement rate in Group A was statistically significantly better than in Group B (ANOVA, $P = 0.013$). However, the improvement rate was not statistically significantly different between Groups A and C (ANOVA, $P = 0.167$) or between Groups B and C (ANOVA, $P = 0.092$).

In addition, we observed that CMT improved in all treatment groups at 24 months of follow-up, although primary IVB alone produced greater decrease in CMT than treatments in Groups B and C. The comparison among 3 groups showed higher CMT decrease in Group A than in Groups B and C (ANOVA, $P < 0.001$). In addition, the number of eyes that experienced a significant improvement of visual acuity (2 ETDRS lines or more) was higher (52.5%) in Group A (IVB therapy).

About the rate of visual improvement and the absence of a statistically significant difference between Group B (GLP) and Group C (primary IVB plus GLP) at the end of follow-up, we observed that both our results and those obtained in the READ-2 study[29,30] differ surprisingly with the results obtained by the DRCR.net.[20] We believe that as ours is a retrospective study, selection of patients for combined treatment (Group C) might have happened because of increased metabolic, functional, and anatomical impairment. Table 1 demonstrates that 75% of patients in Group C were insulin dependent (with a statistically significant difference with the other groups; ANOVA, $P = 0.012$), which generally denotes a poor glycemic control at some point in therapy. In addition, in Group A, baseline BCVA was worse and CMT was higher that gives primary IVB alone more room for improvement to better functional and anatomical outcomes during

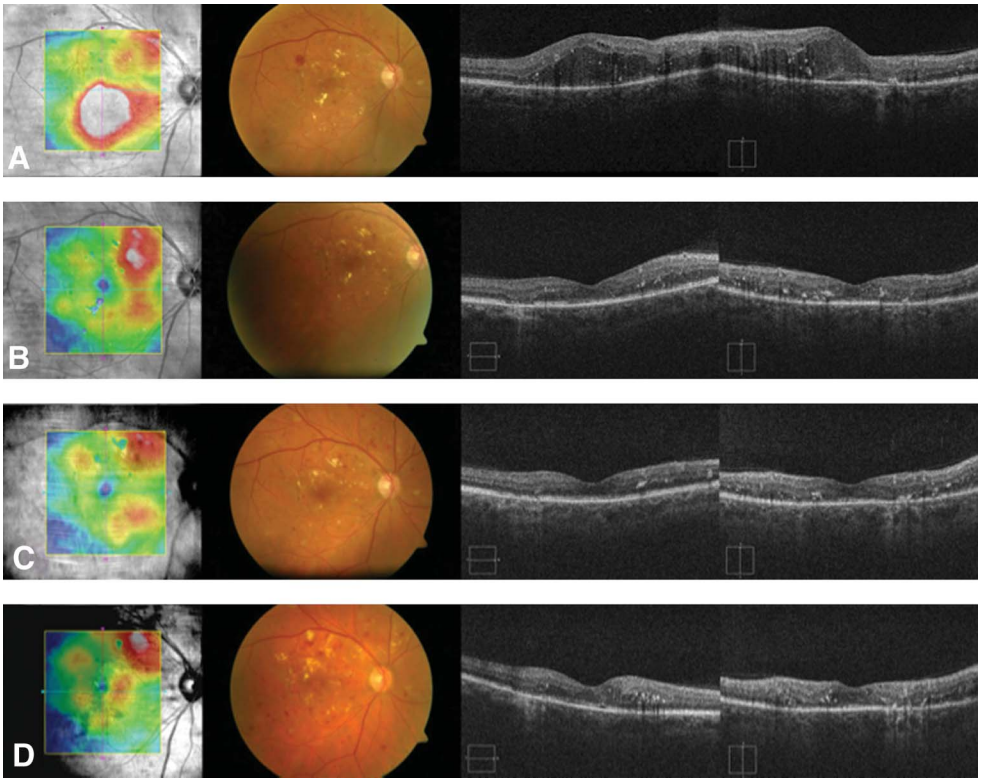

Fig. 5. A 59-year-old man was diagnosed with Type 2 diabetes 17 years before presentation. Difficult metabolic control with insulin. He experienced arterial hypertension secondary to diabetic nephropathy. The fellow eye showed a severe macular ischemia with visual acuity of counting fingers. From left to right, color-coded OCT map, color fundus photograph, and horizontal and vertical OCT scans over the fovea. **A.** At baseline visit, there was a moderate nonproliferative diabetic retinopathy with diffuse macular edema with foveal involvement and central lipid exudation. His BCVA was 20/125. Intravitreal bevacizumab (1.25 mg/0.05 mL) was injected at baseline and at Month 2 and Month 4 (combined with macular grid photocoagulation). **B.** At 6 months, there was an extrafoveal macular thickening with foveal sparing (BCVA 20/32). Intravitreal bevacizumab was repeated at Month 10 (BCVA 20/40). **C.** At 12 months, there was a persistent extrafoveal macular edema with foveal sparing (BCVA 20/32). Intravitreal bevacizumab was administered at Month 14 (BCVA 20/50) and Month 20 (BCVA 20/40). **D.** At 24 months, there was a persistent extrafoveal macular thickening with foveal sparing (BCVA 20/32).

follow-up. Other important limitation of our retrospective study is the lack of HbA_{1c} data on nearly half of the patients in all groups. This fact prevents us from knowing the real metabolic status of each group. These baseline characteristics could bias our results and could explain why the combination of anti-VEGF therapy and GLP did not result in fewer injections in our cohort.

Additional limitations of our study include that it is nonrandomized and retrospective in nature, that there is lack of a control group, and that there was no independent grading for OCT and FA. Finally, as in the READ-2 study,[29] our sample size may have been insufficient to determine whether combined treatment is superior to GLP or to anti-VEGF injections alone.

In summary, our study provides evidence to support the use of primary IVB with or without GLP as treatment for DDME. These results indicate that primary IVB had a statistically significant difference from baseline BCVA at all time points of 24 months of follow-up, and we observed that primary IVB alone produced greater decrease in CMT than GLP therapy or IVB plus GLP in patients with DDME. The effect of primary IVB with or without GLP was faster than GLP alone. Primary IVB without GLP seems to be superior to GLP alone to provide stability or improvement in BCVA in patients with DDME at 24 months. In addition, the number of eyes that experienced a significant improvement of visual acuity was higher in the IVB group.

Key words: Avastin, diffuse diabetic macular edema, grid laser photocoagulation, intravitreal bevacizumab, intravitreal injections, OCT.

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Appendix 1

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Chapter 6: Intravitreal Bevacizumab for Diabetic Macular Edema at 5 years of follow up

Arevalo JF, Lasave AF, Wu L, Acon D, Farah ME, Gallego-Pinazo R, Alezzandrini AA, Fortuna V, Quiroz-Mercado H, Salcedo-Villanueva G, Maia M, Serrano M, Rojas S; Pan-American Collaborative Retina Study Group (PACORES). Intravitreal bevacizumab for diabetic macular oedema: 5-year results of the Pan-American Collaborative Retina Study group. Br J Ophthalmol. 2016 Dec;100(12):1605-1610. doi: 10.1136/bjophthalmol-2015-307950. Epub 2016 Feb 24.

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BACKGROUND/AIMS: To report the long-term anatomical and functional outcomes of patients with centre-involved diabetic macular oedema (DME) treated with intravitreal bevacizumab (IVB).

METHODS: Retrospective case series. Patients diagnosed with centre-involved DME that were treated with at least one injection of 1.25 mg IVB and had a minimum follow-up of 60 months. Patients underwent measurement of best-corrected visual acuity (BCVA), ophthalmoscopy, optical coherence tomography and fluorescein angiography at baseline, 6-month, 12-month, 24-month, 36-month, 48-month and 60-month visits. The paired samples t test was used to compare the central macular thickness (CMT) and BCVA with baseline values. Statistical significance was indicated by $p<0.05$.

RESULTS: Two hundred and one consecutive patients (296 eyes) were included. The mean number of IVB injections per eye was 8.4±7.1 (range: 1-47 injections). At 5 years, the BCVA remained stable at 20/100 (logarithm of the minimum angle of resolution=0.7±0.4). Eighty-six (29%) eyes improved ≥2 lines of BCVA, 129 (43.6%) eyes remained stable and 81 (27.4%) eyes lost ≥2 lines of BCVA at 60 months. Mean CMT decreased from 403.5±142.2 µm at baseline to 313.7±117.7 µm over 5 years follow-up ($p\leq0.0001$).

CONCLUSIONS: The early visual gains due to IVB were not maintained 5 years after treatment.

Intravitreal bevacizumab for diabetic macular oedema: 5-year results of the Pan-American Collaborative Retina Study group

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ABSTRACT

Background/aims To report the long-term anatomical and functional outcomes of patients with centre-involved diabetic macular oedema (DME) treated with intravitreal bevacizumab (IVB).

Methods Retrospective case series. Patients diagnosed with centre-involved DME that were treated with at least one injection of 1.25 mg IVB and had a minimum follow-up of 60 months. Patients underwent measurement of best-corrected visual acuity (BCVA), ophthalmoscopy, optical coherence tomography and fluorescein angiography at baseline, 6-month, 12-month, 24-month, 36-month, 48-month and 60-month visits. The paired samples t test was used to compare the central macular thickness (CMT) and BCVA with baseline values. Statistical significance was indicated by $p<0.05$.

Results Two hundred and one consecutive patients (296 eyes) were included. The mean number of IVB injections per eye was 8.4±7.1 (range: 1–47 injections). At 5 years, the BCVA remained stable at 20/100 (logarithm of the minimum angle of resolution=0.7 ±0.4). Eighty-six (29%) eyes improved ≥2 lines of BCVA, 129 (43.6%) eyes remained stable and 81 (27.4%) eyes lost ≥2 lines of BCVA at 60 months. Mean CMT decreased from 403.5±142.2 µm at baseline to 313.7±117.7 µm over 5 years follow-up ($p\leq0.0001$).

Conclusions The early visual gains due to IVB were not maintained 5 years after treatment.

INTRODUCTION

Diabetic retinopathy (DR) is a major cause of blindness in low-income and middle-income countries.[1] Diabetic macular oedema (DME) is the most common cause of visual loss from DR.[1] Up to 26% of patients with DR present with DME.[2] Vascular endothelial growth factor (VEGF) plays a key role in the pathogenesis of DME.[3]

Ranibizumab and aflibercept for the treatment of DME are approved by several regulatory agencies. However, intravitreal bevacizumab (IVB) has been widely used off-label despite approved therapies. The lower cost of bevacizumab, perceived effectiveness and relative safety makes it a popular choice particularly in the developing world.[4 5] Numerous reports have presented beneficial visual and anatomical results.[6–8] To the best of our knowledge, none of these studies have reported long-term outcomes.

This study reports the 5-year anatomical and functional outcomes after primary IVB (1.25 mg) in patients with centre-involved DME. This report comprises a series of 296 eyes, including 58 eyes from a previously reported series with shorter follow-up.[8]

METHODS

This multi-centre retrospective study evaluated eyes with centre-involved DME that had received an initial injection of off-label IVB between September 2006 and July 2009 at 12 institutions in Latin America and Spain. Clinical records were reviewed of 201 consecutive patients (296 eyes) with centre-involved DME treated with at least one injection of 1.25 mg IVB. Each participating centre's institutional ethics committee approved this study. Informed consent was obtained for this study. This study adheres to the tenets of the Declaration of Helsinki. The off-label use of the drug and potential risks and benefits were discussed with all patients.

Patient eligibility

Patients (18 years or older) with type 1 or 2 diabetes mellitus and DME were included if they had decreased visual acuity and met the following criteria: (1) no other possible causes of decreased vision; (2) no prior treatment for DME such as macular laser photocoagulation, intravitreal triamcinolone, micropulse laser or pars plana vitrectomy; (3) no evidence of vitreomacular traction on optical coherence tomography (OCT); (4) no evidence of macular ischaemia on fluorescein angiography (FA); (5) at least 5 years follow-up and (6) no evidence of intraocular inflammation, uncontrolled intraocular pressure or cataract surgery within the previous 6 months.

Examination and treatment procedures

At baseline, ophthalmic examination included, best-corrected visual acuity (BCVA) with Snellen charts expressed as the number of lines read, and slit lamp biomicroscopy. Baseline central retinal characteristics were analysed with time domain OCT (TD-OCT) using six diagonal slow 6 mm radial line scans through a dilated pupil. The retinal thickness of the 1 mm central retina was obtained using the

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macular thickness map for calculations in all cases. Once spectral domain OCT (SD-OCT) was available, a volume scan centred on the fovea was performed. The scans were reviewed and manual measurements were used in cases of segmentation errors.

A 0.10 mL aliquot of commercially available bevacizumab was prepared for each patient and placed in a tuberculin syringe using aseptic techniques. Topical anaesthetic was instilled in the eye. The eye was prepared in a standard fashion using 5% povidone/iodine. An eyelid speculum was inserted. An injection of 1.25 mg (0.05 mL) bevacizumab was performed 3.5–4 mm posterior to the limbus, through the pars plana with a 30-gauge needle. After the injection, retinal artery perfusion was assessed with indirect ophthalmoscopy.

Patients were examined and treated on an as-needed regimen based on BCVA and OCT at the discretion of the treating physician. Therapy was initiated at the diagnosis of DME. Patients were examined and treated monthly until the oedema stabilised. Subsequently, patients returned every 3–4 months at the treating physician's discretion. All return visits included OCT. FA was performed at the examiners discretion.

Statistical analysis

Data were collected in an Excel 2007 spreadsheet (Microsoft, Redmond, Washington, USA). Statistical analysis was performed using MedCalc Software for Windows V.8.2.0.3 (MedCalc, Mariakerke, Belgium). The paired samples t test was performed to compare the central macular thickness (CMT) and BCVA with baseline values. Correlation was considered significant when $p<0.05$.

Data were analysed at 1-month, 6-month, 12-month, 24-month, 36-month, 48-month and 60-month follow-up visits. Snellen BCVA was converted to the logarithm of the minimum angle of resolution (logMAR) for statistical analysis. An increase or decrease in BCVA was defined as a change of ≥2 lines of Snellen vision prior to logMAR conversion. The main outcome measures included changes in BCVA and CMT at the 60-month follow-up visit.

RESULTS

Charts of 201 consecutive patients (296 eyes) were reviewed who fit the inclusion criteria. The mean follow-up was 66.7 ±12.1 months (range, 60 to 114 months). The mean age was 62.3±9.6 years. There were 101 (50.2%) females. The mean duration of diabetes was 15.8±8.1 years. The mean glycosylated haemoglobin was 8.6±2.4%. In this cohort, 220 (74.4%) eyes were phakic, and 76 (25.6%) were pseudophakic. Eleven (3.7%) eyes had mild DR, 80 (27.1%) had moderate DR, 92 (31.1%) had severe DR and 113 (38.1%) had proliferative diabetic retinopathy (PDR). All eyes with PDR had undergone prior scatter panretinal photocoagulation (PRP) at least 6 months prior to receiving IVB. Baseline characteristics are summarised in table 1.

Figure 1 presents the BCVA at baseline, and each follow-up visit after IVB. The mean BCVA was 0.7±0.4 logMAR (range, 0.2 to 1.6 logMAR; Snellen equivalent 20/100) at baseline, 0.5 ±0.4 logMAR (range, 0.1 to 1.4 logMAR; Snellen equivalent 20/60) ($p=0.002$) at 1 month and 0.4±0.5 logMAR (range 0 to 1.3 logMAR; Snellen equivalent 20/50) ($p=0.003$) at 6 months. At the 12-month, 24-month, 36-month, 48-month and 60-month follow-up visits the respective BCVAs were, 0.4±0.5 logMAR (range 0 to 1.3 logMAR; Snellen equivalent 20/50) ($p=0.0032$), 0.5±0.5 logMAR (range, 0.1 to 1.6 logMAR; Snellen equivalent 20/60) ($p=0.006$), 0.5±0.5 logMAR (range, 0.1 to 1.4 logMAR; Snellen equivalent 20/60) ($p=0.03$), 0.6 ±0.3 logMAR (range, 0 to 2 logMAR; Snellen equivalent 20/80) ($p=0.10$) and 0.7±0.3 logMAR (range, 0 to 2 logMAR; Snellen equivalent 20/100) ($p=0.387$).

Table 1 Baseline characteristics of patients with diabetic macular oedema*

Demographic	Number
Number of patients (n)	201
Number of eyes (n)	296
Gender n (%)	
Male	100 (48.8)
Female	101 (50.2)
Age (mean±SD) (years)	62.3±9.6
Baseline Snellen chart BCVA (mean±SD)	20/100
Baseline logMAR (mean±SD)	0.7±0.3
Baseline OCT (μm) (mean±SD)	403.5±142.2
Hypertension, n (%)	104 (51.7)
Systemic glycaemic control, n (%)	
Insulin	18 (9.1)
Oral hypoglycaemic	70 (34.7)
Combined therapy	108 (53.7)
None, n (%)	5 (2.5)
Baseline HbA1c, %	8.6±2.4
Grade of DR, n (%)	
Mild NPDR	11 (3.7)
Moderate NPDR	80 (27.1)
Severe NPDR	92 (31.1)
PDR	

*Values represent number and percentages.
BCVA, best-corrected visual acuity; DME, diffuse diabetic macular oedema; DR, diabetic retinopathy; HbA1c, glycosylated haemoglobin; logMAR, logarithm of the minimum angle of resolution; n, number of patients; NPDR, no proliferative diabetic retinopathy; OCT, optical coherence tomography; PDR, proliferative diabetic retinopathy.

Subgroup analysis of the 60-month BCVA indicated that 86 (29%) eyes improved two or more lines of BCVA, and BCVA remained stable in 129 (43.6%) eyes at 60 months. BCVA

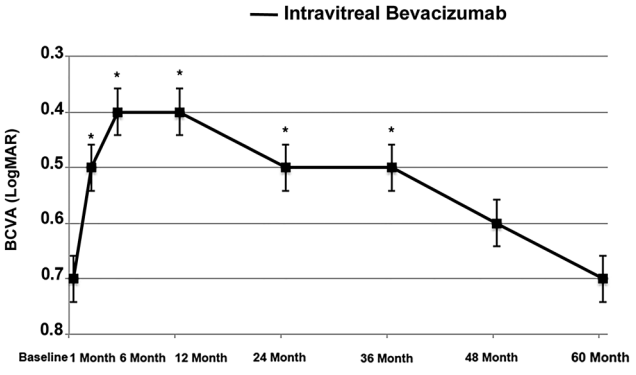

Figure 1 Graph showing changes in best-corrected visual acuity (BCVA) after intravitreal bevacizumab over 60 months of follow-up. The BCVA mean improved at 1 month from baseline BCVA 20/100, logMAR=0.7±0.4 to 20/60, logMAR=0.5±0.4 ($p=0.002$). At 6 months, the mean of BCVA was 20/50, logMAR= 0.4±0.5 ($p=0.003$). At 12 months, the BCVA mean was 20/50, logMAR=0.4±0.5 ($p=0.0032$). Mean 24-month BCVA was 20/60, logMAR=0.5±0.5 ($p=0.006$). At 36 months, the mean of BCVA was 20/60, logMAR= 0.5±0.5 ($p=0.03$). At 48 months, the mean of BCVA decreased to 20/80, logMAR=0.6 ±0.3 ($p=0.10$). At 60 months, the mean of BCVA was 20/100, logMAR=0.7±0.3 ($p=0.387$). * denotes statistically significant change ($p<0.05$) compared with baseline. logMAR, logarithm of the minimum angle of resolution.

decreased by two or more lines in 81 (27.4%) eyes at the end of follow-up (table 2).

The mean CMT decreased statistically significantly from 403.5±142.2 μm at baseline to 336.8±147 μm (p<0.001) at 6 months follow-up, and this overall improvement continued during the 60-month follow-up. At 12-month, 24-month, 36-month, 48-month and 60-month follow-up visits, the mean CMT was 297.7±82.9 μm, 314.7±118.7 μm, 315±146.1 μm, 295.7±97.8 μm and 313.7±117.7 μm, respectively, which were all significantly lower than baseline (p≤0.0001, all comparisons) (figures 2–4).

Five years after IVB, 29.4% (87/296) of eyes had a CMT≤260 μm, 21.6% (64/296) of eyes had a CMT between 261 and 300 μm, 19.9% (59/296) of eyes had a CMT between 300 and 400 μm and 13.9% (41/296) of eyes had a CMT≥401 μm. Five-year OCT data were not available for 15.2% (45/296) of eyes.

A subgroup comparison was performed of the response to IVB treatment for DME between patients with PDR and previous PRP and those with non-proliferative DR. A total of 113 (38.1%) eyes had been diagnosed with PDR and treated with PRP at least 6 months before undergoing IVB for DME. In this subgroup of eyes the mean BCVA improved from 0.83 logMAR at baseline to 0.75 logMAR at 5 years after IVB (p=0.114). In eyes with non-proliferative DR and DME treated with IVB, the mean BCVA improved statistically significantly from 0.69 logMAR at baseline to 0.58 logMAR at the end of follow-up (p=0.004). In eyes with PDR, the mean CMT decreased statistically significantly from 411.1±140.6 μm at baseline to 328.8 ±120.6 μm at 5 years (p=0.0002). In non-PDR eyes, the mean CMT decreased statistically significantly from 399.1±145.1 μm at baseline to 309.4±104.1 μm (p<0.0001) at 5 years. The repeated measures analysis of variance to compare the mean retinal thickness and the logMAR BCVA, adjusting for the grade of DR as a covariate, indicated that there was no statistical significance (p=0.718 for BCVA and p=0.164 for CMT).

A total of 2390 injections of IVB were performed during 60 months of follow-up. In the first year of follow-up a mean of 3.3±2.1 (range, 1–10) IVB injections per eye were performed. A mean of 2.1±2 (range, 1–9) IVB injections were administered during the second year. During the third, fourth and fifth year of follow-up, a mean of 1.5±2 (range, 1–10), 1.3±1.9 (range, 1–10) and 1.2±2.1 (range, 1–9) IVB was administered, respectively. The mean number of IVB injections per eye was 8.1±7.1 (range, 1–47 injections) at 5 years. Table 3 presents an analysis of injections per year of follow-up. Table 4 presents the total number of injections during 60 months of follow-up.

Ocular complications included tractional retinal detachment (TRD) in eight (2.7%) eyes, glaucoma in six (2%) eyes, uveitis in four (1.4%) eyes, rhegmatogenous retinal detachment (RRD) in three (1%) eyes, vitreous haemorrhage in two (0.7%) eyes

Table 2 Variation of best-corrected visual acuity at 60 months of intravitreal bevacizumab therapy for diabetic macular oedema in 296 eyes*

BCVA (logMAR) results	Number of eyes	Per cent
Improved two or more lines	86	29.0
Remained stable	129	43.6
Decreased two or more lines	81	27.4

*Values represent number and percentages.
BCVA, best-corrected visual acuity; logMAR, logarithm of the minimal angle of resolution.

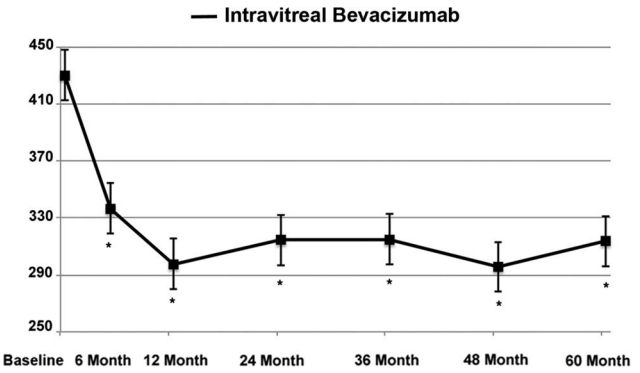

Figure 2 Changes in central macular thickness (CMT) with optical coherence tomography after intravitreal bevacizumab over 60 months of follow-up. Mean CMT decreased from 403.5±142.2 μm at baseline to 336.8±147 μm (p<0.001) at 6 months of follow-up. At 12-month, 24-month, 36-month, 48-month and 60-month follow-ups, mean CMT was 297.7±82.9 μm, 314.7±118.7 μm, 315±146.1 μm, 295.7 ±97.8 μm and 313.7±117.7 μm, respectively (p≤0.0001). * denotes statistically significant change (p<0.05) compared with baseline.

and endophthalmitis in one (0.3%) eye. Systemic adverse events included 10 (3.4%) patients who developed a stroke, nine (3%) patients died and myocardial infarction (MI) occurred in five (1.7%) patients.

The TRD incidence rate (IR) was 0.005 cases per person-year equivalent to an annual incidence of five cases per 1000 persons with DME who underwent IVB observed during 1 year of follow-up. For glaucoma the IR was 0.004 cases per person-year, and the IR for uveitis was 0.003 cases per person-year. The IR for RRD was 0.002 cases per person-year, the IR for vitreous haemorrhage was 0.001 cases per person-year and the IR for endophthalmitis was 0.0007 cases per person-year. The IR for stroke was 0.006 cases per person-year. The annual IR for death was 0.006 cases per person-year and the IR for MI was 0.003 cases per person-year (table 5).

DISCUSSION

This 5-year study shows that IVB treatment 'as needed' improves the BCVA of eyes with centre-involved DME for the first 3 years. However, these gains were not maintained over time. Several recent randomised studies have shown that anti-VEGF agents outperform macular laser photocoagulation and have become first line agents in the treatment of centre-involved DME.[9–13]

Despite the availability of approved medications for DME such as ranibizumab and aflibercept, off-label use of IVB is widespread worldwide. The lower cost of bevacizumab, perceived effectiveness and relative safety make it a popular choice particularly in low-income and middle-income countries.[4 5]

Previous studies have shown that IVB at doses up to 2.5 mg improves BCVA and CMT in eyes with DME.[8 9 14–25] The bevacizumab or laser therapy (BOLT) study prospectively compared the outcomes of eyes randomly assigned to IVB or macular laser photocoagulation. At 2 years follow-up, IVB-treated eyes had a mean gain of 8.6 letters compared with a mean loss of 0.5 letters for the laser-treated eyes. Furthermore, 32% of the IVB-treated eyes gained at least three lines of BCVA compared with only 4% of laser-treated eyes.[9] These results concur with our previous studies of clinical outcomes at 24 months of 139 eyes with centre-involved DME treated with IVB.[8]

To the best of our knowledge, there are no studies that report the 5-year results of IVB for centre-involved DME. Figure 1

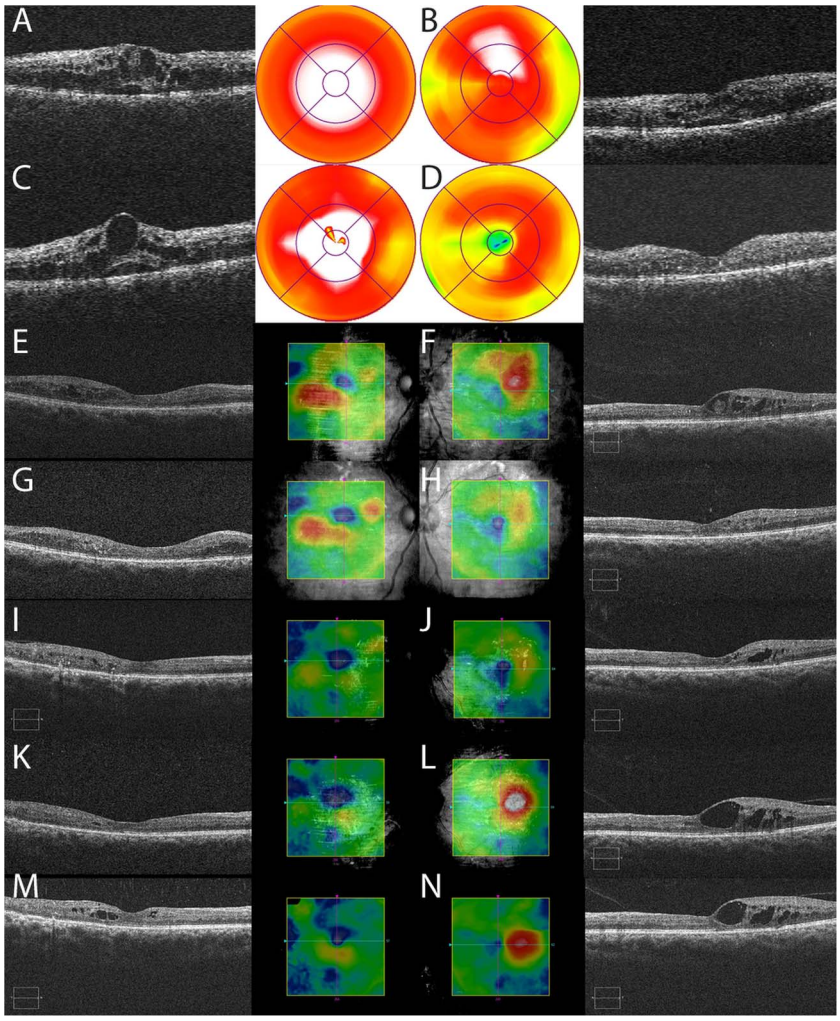

Figure 3 Illustrative case of diabetic macular oedema. A 32-year-old female with proliferative diabetic retinopathy, cystoid macular oedema and foveal detachment. Her baseline best-corrected visual acuity (BCVA) was 20/50 OD (A) and 20/30 OS (B). Initial treatment with intravitreal bevacizumab (IVB) combined with panretinal photocoagulation was performed. After 6 months of follow-up there was persistent oedema in her OD (C) with BCVA of 20/60 (after 4 IVB), and a better anatomical response in the OS (D) with BCVA of 20/40 (after 3 IVB). After 12 months of follow-up and 7 IVB the BCVA improved to 20/100 OD (E) and to 20/100 OS after 5 IVB (F) and the fovea OU showed no significant fluid. Macular grid photocoagulation was performed OU. After 24 months of follow-up and 8 IVB (G) the BCVA was 20/50 OD and 20/30 OS after 6 IVB (H) with no significant recurrence of fluid involving the fovea. After 36 months of follow-up and 10 IVB the BCVA was 20/60 OD (I) and 20/25 OS after 7 IVB (J). After 48 months of follow-up and 12 IVB the BCVA was 20/60 OD (K) and 20/25 OS after 9 IVB (L). Although OS evidenced recurrence of the oedema with foveal cysts the functional improvement was significant. After 60 months of follow-up and 13 IVB BCVA was 20/30 OD (M) and 20/30 OS after 11 IVB (N). OD, right eye; OS, left eye; OU, both eyes.

illustrates that the response to IVB was rapid and by 6 months the mean maximum gain was obtained. However by the fourth year of follow-up, these gains started to disappear. BCVA at the fifth year of follow-up was the same as the mean baseline BCVA.

IVB treatment rapidly reduced CMT achieving maximal reduction by 6. However, the mean decrease in CMT was maintained throughout the 5 years. It should be noted that at 5 years, CMT≥300 μm remained in approximately one-third of eyes which indicates persistent DME.

We can only speculate on why our patients did not maintain visual gains. One possibility is that the eyes developed tachyphylaxis to bevacizumab.[18 19] In tachyphylaxis, there is a diminished therapeutic response over time after repeated administration of a drug. This does not seem to be the case in our study as the number of injections was low, at a mean of 8.4 IVB injections per eye over 5 years.

Another possibility is that as the DME became more chronic in nature, the intravitreal cytokine profile changed. Several proinflammatory cytokines are present in the vitreous of eyes with DME.[20] Inhibition of VEGF by anti-VEGF drugs such as bevacizumab will not have an effect on other inflammatory cytokines.[21] Initially, VEGF may have played an important central role in the pathogenesis of DME. However, once VEGF is inhibited by bevacizumab, perhaps other inflammatory cytokines started to play a more prominent role. Others have suggested that chronic DME may exacerbate inflammation in diabetic retinas leading to a vicious cycle of persistent DME.[22] Of note at the 5-year follow-up, about a third of the eyes in the current study had persistent DME.

It is very likely that our patients were undertreated. In the current study, the average number of injections was 8.4 over 5 years. In contrast, patients in RIDE (A Study of Ranibizumab Injection in Subjects With Clinically Significant Macular Edema With Center Involvement Secondary to Diabetes Mellitus) and RISE (A Study of Ranibizumab Injection in Subjects With Clinically Significant Macular Edema With Center Involvement Secondary to Diabetes Mellitus) were injected monthly over 3 years.[10] In the DRCR.net protocol I study, the average number of injections over 5 years was 13 and 17 in the ranibizumab plus prompt and deferred laser groups, respectively. In the BOLT study, eyes received an average of 13 injections in 2 years.[9] This is considerably higher than our study. A key aspect in the management of patients with exudative DME is the follow-up protocol. Currently, there is no consensus on a particular follow-up protocol. In general, the best visual outcomes were obtained through fixed monthly injections followed by treatment-as-needed therapy. However, this approach does not seem to be sustainable in the medium or long term due to many barriers that differ in each country. Our data reflect the 'real world' use of anti-VEGF in Latin America and Spain, and not clinical trial data. Several studies have reported that in exudative age-related macular degeneration (AMD), there is also

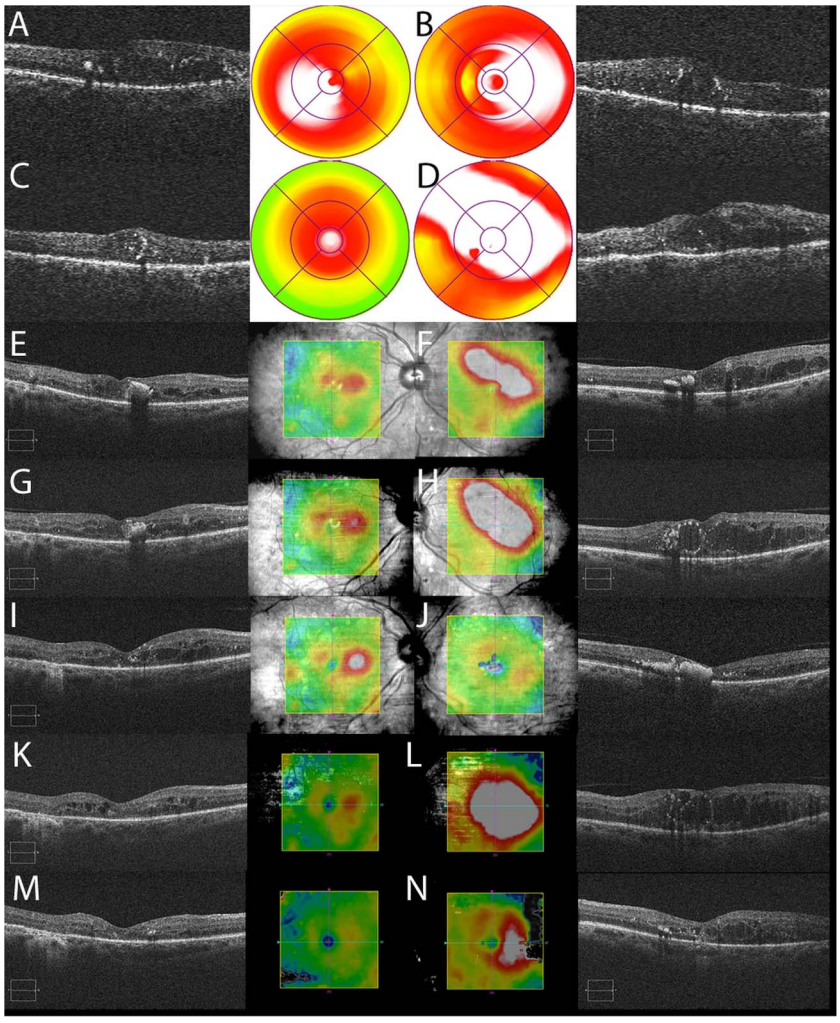

Figure 4 A case of diabetic macular oedema. A 57-year-old male with moderate-severe non-proliferative diabetic retinopathy, cystoid macular oedema and foveal detachment. His baseline best-corrected visual acuity (BCVA) was 20/60 OD (A) and 20/40 OS (B). After 6 months of follow-up and after 3 intravitreal bevacizumab (IVB) (C) there was a significant response in OD with BCVA of 20/100, and an increase of retinal thickness in the OS with BCVA of 20/400 after 1 IVB (D). After 12 months of follow-up and 5 IVB the BCVA was 20/40 OD (E) and 20/400 OS after 3 IVB (F). After 24 months of follow-up and 5 IVB the BCVA was 20/50 OD (G) and 20/400 OS after 6 IVB (H). After 36 months of follow-up and 7 IVB the BCVA was 20/30 OD (I) and 20/400 OS after 7 IVB (J). After 48 months of follow-up the BCVA was 20/30 OD after 8 IVB (L) and 20/400 OS (after 9 IVB). A severe recurrence of the oedema was observed in his OD (K). After 60 months of follow-up BCVA was 20/30 OD after 11 IVB (M) and 20/400 OS after 10 IVB (N). OD, right eye; OS, left eye.

under treatment in a 'real world' scenario.[23–25] These results call into question the sustainability of long-term anti-VEGF treatment in eyes with chronic conditions such as DME and exudative AMD. It appears that applying clinical trial protocols to daily clinical practice may not be feasible.

Table 3 Analysis of number of intravitreal bevacizumab injection required per year during 5 years of follow-up*

Number of injections	First year n (%)	Second year n (%)	Third year n (%)	Fourth year n (%)	Fifth year n (%)
1	32 (12.1)	64 (30.8)	49 (37.9)	36 (36)	34 (40.7)
2	58 (21.9)	55 (26.4)	29 (22.5)	24 (24)	11 (13.1)
3	55 (20.8)	37 (17.8)	16 (12.4)	15 (15)	10 (11.9)
4	48 (18.2)	21 (10.1)	19 (14.7)	10 (10)	9 (10.7)
5	23 (8.7)	5 (2.4)	3 (2.3)	5 (5)	3 (3.6)
6	27 (10.2)	12 (5.8)	6 (4.7)	3 (3)	8 (9.5)
7	10 (3.8)	5 (2.4)	1 (0.8)	3 (3)	3 (3.6)
8	10 (3.8)	8 (3.8)	4 (3.1)	2 (2)	3 (3.6)
9	1 (0.4)	1 (0.5)	1 (0.8)	1 (1)	3 (3.6)
10	1 (0.4)	0 (0)	1 (0.8)	1 (1)	0 (0)
Total (n^1 per year)	264	208	129	100	84
Total (n^2 per IVB)	951	574	340	268	257

*Values represent number and percentages.
IVB, intravitreal bevacizumab; n, number of eyes; n^1, number of eyes per year; n^2, number of intravitreal injection of bevacizumab per year.

Ocular adverse events in the current study included TRD, glaucoma, uveitis, RRD, vitreous haemorrhage and endophthalmitis. Systemic adverse events observed included cerebrovascular accidents, MI and death. Ocular and systemic adverse events presented in this study were consistent with the literature.[17–19] There is increasing evidence of a beneficial effect of VEGF inhibition on the severity score of DR.[10 11] Due to the retrospective nature of our study we cannot determine the change in severity of DR.

Limitations of this study are that it is non-randomised, uncontrolled and retrospective in nature. In addition, we had no standard treatment regimen, or a standard regimen for retreatment. Furthermore, the possibility that change in BCVA or CMT may be secondary to a change in systemic health cannot

Table 4 Analysis of number of injections required per eye during 60 months*

Reinjections	n	Percentage
None	27	9.1
<5	87	29.4
6–10	102	34.5
11–15	36	12.2
16–20	25	8.4
>21	19	6.4

*Values represent number and percentages.n, number of eyes.

Table 5 Person-time incidence rates of complications of IVB for DDME during 60 months of follow-up*

	Number (%)	Incidence rate	Annual incidence
Ocular complications			
TRD			5 case per 1000
Glaucoma			4 cases per 1000
Uveitis			3 case per 1000
RRD			2 cases per 1000
VH			1 case per 1000
Endophthalmitis			7 cases per 1000
Systemic complications			
Stroke			6 cases per 1000
Death			6 cases per 1000
Myocardial infarction	5 (1.7)	0.003	3 cases per 1000

*Values represent number and percentages.
DDME, diffuse diabetic macular oedema; IVB, intravitreal bevacizumab; RRD, rhegmatogenous retinal detachment; TRD, tractional retinal detachment; VH, vitreous haemorrhage.

be ruled out. Despite these limitations, the authors believe that the results of this retrospective multi-centre study may be clinically useful and can offer some valuable insight.

To the best of our knowledge, this is the first study that reports the long-term anatomical and functional results of IVB for the treatment of centre-involved DME. In summary, our 'real world' long-term visual acuity outcomes do not match the results obtained in clinical trials. Early gains in BCVA were not maintained at 5 years of follow-up.

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Collaborators For a complete listing of the participating members of Pan-American Collaborative Retina Study, see the online supplementary appendix.

Contributors All authors have given final approval of this version to be published. JFA, AFL, DA, MM and SR participated in drafting the manuscript, study design, data collection and screening, data-analysis and evidence synthesis, and revising the manuscript. LW, MEF, RG-P, AAA, VF, HQ-M, GS-V, MM and MS participated in literature search, data collection, data-analysis and evidence synthesis and drafting the manuscript. JFA, LW and AFL participated in hypothesis generation, evidence synthesis and revising the manuscript. JFA, AFL, DA, MEF, RG-P, AAA, VF, HQ-M, GS-V, MM, MS and SR participated in study design, data collection, screening and revising the manuscript.

Competing interests JFA is a consultant to Second Sight LLC, Alcon Laboratories, Engineering, DORC International BV and Bayer AG.

Ethics approval Each participating centre's institutional ethics committee approved this study.

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Chapter 7: Intravitreal Bevacizumab for Proliferative Diabetic Retinopathy at 6 months of follow up

Arevalo JF, Wu L, Sanchez JG, Maia M, Saravia MJ, Fernandez CF, Evans T.
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Hypothesis 3: IVB may decrease retinal neovascularization in patients with PDR at 6 months of follow up. However, the effect may decrease at 24 months of follow up due to tachyphylaxis, and pan-retinal photocoagulation and/or vitrectomy will be necessary.

AIMS: To study the effects of intravitreal bevacizumab (Avastin) on retinal neovascularization (RN) in patients with proliferative diabetic retinopathy (PDR).

METHODS: Retrospective study of patients with RN due to PDR who were treated with at least one intravitreal injection of 1.25 or 2.5 mg of bevacizumab. Patients underwent ETDRS best-corrected visual acuity (BCVA) testing, ophthalmoscopic examination, optical coherence tomography (OCT), and fluorescein angiography (FA) at baseline and follow-up visits.

RESULTS: Forty-four eyes of 33 patients with PDR and a mean age of 57.2-years (range: 23-82 years) participated in the study. Thirty-three eyes (75%) had previous panretinal photocoagulation (PRP). Twenty-seven eyes (61.4%) showed total regression of RN on fundus examination with absence of fluorescein leakage, 15 eyes (34.1%) demonstrated partial regression of RN on fundus examination and FA. Follow-up had a mean of 28.4 weeks (range from 24 to 40 weeks). BCVA and OCT demonstrated improvement ($P<0.0001$). Three eyes without previous PRP ('naive' eyes) and with vitreous haemorrhage have avoided vitreo-retinal surgery. One eye (2.2%) had PDR progression to tractional retinal detachment requiring vitrectomy, and one eye (2.2%) had vitreous haemorrhage with increased intraocular pressure (ghost cell glaucoma). No systemic adverse events were observed.

CONCLUSIONS: Intravitreal bevacizumab resulted in marked regression of RN in patients with PDR and previous PRP, and rapid resolution of vitreous haemorrhage in three naive eyes. Six-months results of intravitreal bevacizumab at doses of 1.25 or 2.5 mg in patients with PDR do not reveal any safety concerns.

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CLINICAL STUDY

Intravitreal bevacizumab (avastin) for proliferative diabetic retinopathy: 6-months follow-up

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Abstract

Aims **To study the effects of intravitreal bevacizumab (Avastin) on retinal neovascularization (RN) in patients with proliferative diabetic retinopathy (PDR).**
Methods **Retrospective study of patients with RN due to PDR who were treated with at least one intravitreal injection of 1.25 or 2.5 mg of bevacizumab. Patients underwent ETDRS best-corrected visual acuity (BCVA) testing, ophthalmoscopic examination, optical coherence tomography (OCT), and fluorescein angiography (FA) at baseline and follow-up visits.**
Results **Forty-four eyes of 33 patients with PDR and a mean age of 57.2-years (range: 23–82 years) participated in the study. Thirty-three eyes (75%) had previous panretinal photocoagulation (PRP). Twenty-seven eyes (61.4%) showed total regression of RN on fundus examination with absence of fluorescein leakage, 15 eyes (34.1%) demonstrated partial regression of RN on fundus examination and FA. Follow-up had a mean of 28.4 weeks (range from 24 to 40 weeks). BCVA and OCT demonstrated improvement ($P<0.0001$). Three eyes without previous PRP ('naive' eyes) and with vitreous haemorrhage have avoided vitreo-retinal surgery. One eye (2.2%) had PDR progression to tractional retinal detachment requiring vitrectomy, and one eye (2.2%) had vitreous haemorrhage with increased intraocular pressure (ghost cell glaucoma). No systemic adverse events were observed.**
Conclusions **Intravitreal bevacizumab resulted in marked regression of RN in patients with PDR and previous PRP, and rapid resolution of vitreous haemorrhage in three naive eyes. Six-months results of intravitreal bevacizumab at doses of 1.25 or 2.5 mg in patients with PDR do not reveal any safety concerns.**
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Keywords: avastin; bevacizumab; diabetic retinopathy; intravitreal injections; proliferative; retinal neovascularization

Introduction

Diabetic retinopathy remains a major threat to sight in the working age population in the developed world. Furthermore, it is increasing as a major cause of blindness in other parts of the world especially in developing countries.[1] Proliferative diabetic retinopathy (PDR) is a major cause of visual loss in diabetic patients. In PDR, the growth of new vessels from the retina or optic nerve, is thought to occur as a result of vascular endothelial grow factor (VEGF) release into the vitreous cavity as a response to ischaemia.[2–4] Because VEGF has been shown to play a major role in retinal neovascularization (RN),[2,3] although other factors may be involved as well,[5,6] anti-VEGF treatments have been hypothesized as an alternative adjunctive treatment for RN.[7,8]

Bevacizumab (Avastin™ Genentech Inc., San Francisco, CA, USA) is a complete full-length humanized antibody that binds to all subtypes of VEGF and is successfully used in tumour therapy as a systemic drug.[9] Recent studies have demonstrated the usefulness of

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an intravitreal injection of bevacizumab in the reduction of vascular permeability and fibrovascular proliferation in macular oedema secondary to central vein occlusion, RN secondary to PDR, and choroidal neovascularization secondary to age macular degeneration.[8,10–14] The amount of human retinal penetration for a complete full-length anti-VEGF antibody is not known at present. However, full thickness retinal penetration of intravitreal bevacizumab was observed in an animal model.[15,16] Additionally, intravitreal bevacizumab does not appear to be toxic to the albino rabbit retina at a concentration up to 2.5 mg.[17]

Panretinal photocoagulation (PRP) currently is the principal therapy for PDR, unless the patient already has extensive vitreous haemorrhage, which would preclude the possibility of laser photocoagulation. Neovascularization on and around the optic disc (NVD) and vitreous haemorrhage were found to be more frequently associated with severe visual loss despite PRP in the Diabetic Retinopathy Study (DRS) and Early Treatment Diabetic Retinopathy Study (ETDRS).[18,19] Long intervals between PRP sessions and the variable amount of time required for a favourable response may increase the incidence of complications due to the progression of PDR.[18,20] In fact, a single episode of PRP or shorter intervals between PRP episodes, although desirable in severe PDR and when the patient must travel long distances for treatment, are often associated with acute visual disturbances due to exudative choroidal detachment, retinal detachment, and macular oedema.[21–24]

The purpose of this retrospective study was to evaluate the effectiveness of intravitreal bevacizumab on RN in patients with PDR as a base for future studies in which bevacizumab may be used as an adjuvant treatment to PRP for PDR.

Patients and methods

We conducted a retrospective study in 44 eyes of 33 patients with RN in patients with PDR, who were treated with off-label intravitreal bevacizumab between September 2005 and August 2006 at five institutions in Venezuela, Costa Rica, Brazil, Argentina, and Peru. Institutional Review Board/Ethics Committee approval and patients' informed consent were obtained for this study at all five institutions. The off-label use of the drug and its potential risks and benefits were discussed extensively with all patients. Eyes that were previously treated with scatter photocoagulation, had prior focal/grid laser photocoagulation, and previous intravitreal triamcinolone injection were included if any of those therapies had been performed at least 6 months before intravitreal bevacizumab. An injection of 1.25 mg (0.05 ml) or 2.5 mg (0.1 ml) of bevacizumab was given according to the discretion of the treating physician.

Baseline data included age, sex, type, and duration of diabetes mellitus. Patients also underwent clinical examination including best-corrected visual acuity (BCVA) measurement with ETDRS chart, applanation tonometry, fundus examination, fluorescein angiography (FA), and optical coherence tomography (OCT). In patients with clinical significant macular oedema, baseline central retinal characteristics was observed by OCT (Stratus III OCT, Carl Zeiss, Dublin, CA, USA) using six diagonal slow 6-mm radial line scans, with software versions 3.0 and 4.0, through a dilated pupil by a retina specialist.

A 0.18-ml aliquot of commercially available bevacizumab was prepared for each patient and placed in a tuberculin syringe using aseptic techniques. Bevacizumab was stored for up to 3 weeks under refrigeration at 4°C under sterile conditions, and the syringes were capped with a needle. After the eye had been prepared in a standard manner using 5% povidone/iodine, an eyelid speculum was used to stabilize the eyelids, and the injection of 1.25 mg (0.05 ml) or 2.5 mg (0.1 ml) of bevacizumab was given 3.5–4 mm posterior to the limbus, through the infero-temporal pars plana with a 30-gauge needle under topical anaesthesia or subconjunctival lidocaine. After the injection, intraocular pressure and retinal artery perfusion were checked, and patients were instructed to administer topical antibiotics for 7 days.

Patients were examined at 1, 2 weeks, and 1 month after the first injection and monthly thereafter. One, three and six months after initial injection, patient evaluation was performed using ophthalmic examination with slit-lamp biomicroscopy, OCT, and FA. Patients were included in this consecutive series if there was a minimum of 6-months follow-up. The main outcome measure was the change in RN defined as the change in the area of vitreous leakage from NVD and new vessels elsewhere (NVE) in the late phase of FA. Patients received reinjections only if RN was not totally resolved on ophthalmic examination or FA. Data was analysed by a paired Student's *t*-test and a Fisher's exact test when appropriate.

Monitored systemic conditions included myocardial infarction, stroke, systemic hypertension, thromboembolic diseases, and death. Blood pressure was measured prior to bevacizumab injection and at 1 and 2 weeks following each injection. Other systemic conditions were assessed by a thorough review of systems. If the patients were unable to attend a particular visit, a telephone interview was conducted to assess for possible systemic complications, and a new appointment was scheduled. We certify that all applicable institutional

and governmental regulations concerning the ethical use of human volunteers were followed during this research.

Results

We reviewed the clinical records of 33 consecutive patients (44 eyes) with PDR injected with intravitreal bevacizumab between September 2005 and August 2006. Patients had a mean follow-up of 28.4 weeks (range from 24 to 40 weeks). Our patients had a mean age of 57.2 years (range from 23 to 82 years), and 51.5% were female (16 men and 17 women). Twenty-three diabetic patients (69.7%) were insulin-dependent. The mean duration of diabetes was 17 years (range from 1 to 30 years). Thirty-five eyes (79.5%) were treated with an intravitreal injection of 2.5 mg of bevacizumab, and nine eyes (20.5%) with 1.25 mg of bevacizumab. Of the 33 eyes (75%) that were previously treated with scatter photocoagulation (Figure 1), 19 had prior focal/grid laser photocoagulation (Figure 2), and two patients had a previous intravitreal triamcinolone injection (Table 1). Seventeen eyes had clinical significant macular oedema (CSME) at biomicroscopic non-contact fundus examination with a 66- or a 78-D lens.

The mean baseline BCVA was log MAR = 1.21 and the final mean BCVA was log MAR = 0.70 ($P < 0.0001$). Final BCVA analysis by subgroups demonstrated that 12 eyes (27.3%) remained stable, 29 eyes (65.9%) improved two or more ETDRS lines of BCVA, and three eyes (6.8%) decreased two or more ETDRS lines of BCVA. OCT results were available for all 18 patients with CSME, the mean central macular thickness was 487.4 μm (range from 284 to 1082 μm), and decreased to a mean of 260.6 μm (range from 178 to 475 μm) at the end of follow-up ($P < 0.0001$). Final BCVA analysis by subgroups of patients with CSME demonstrated that 14 eyes (82.4%) improved two or more ETDRS lines of BCVA (Table 2).

Twenty-seven eyes (61.4%) showed total regression of RN on fundus examination with absence of fluorescein leakage (Figures 1 and 2), 15 eyes (34.1%) demonstrated partial regression of RN on fundus examination and FA, and two eyes (4.5%) of two patients showed no regression of RN. The first of those two patients who did not respond was treated with 1.25 mg of bevacizumab and had PDR progression to tractional retinal detachment requiring vitrectomy resulting in a poor final visual acuity (VA) (counting fingers) due to ischaemic optic neuropathy. The second patient was treated with 2.5 mg of bevacizumab and developed vitreous haemorrhage with increased intraocular pressure (ghost cell glaucoma). In addition, these two patients had previous PRP (Table 3).

Twenty-one eyes (47.7%) needed a second injection due to recurrence of neovascularization at a mean of

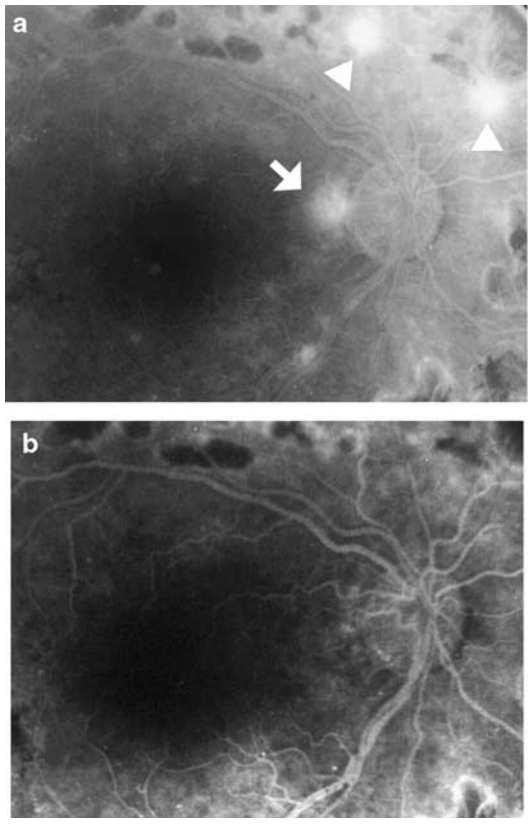

Figure 1 A 53-year-old man had a 2-month history of visual loss to 20/60 in his right eye. We had performed panretinal photocoagulation in his right eye 2 years previously. Fundus examination revealed a mild vitreous haemorrhage. (a) Fluorescein leakage from neovascularization of the disc (NVD) at baseline (arrow) between retinal vessels crossing the optic disc at 9 O'clock and 10 O'clock was demonstrated. In addition, FA showed magnification of retinal neovascularization elsewhere (NVE) in the superonasal retina (arrowheads). (b) At week 1 after intravitreal bevacizumab, total resolution of leakage from NVD and NVE are shown. His VA returned to 20/32 1 month later. He has not needed a reinjection at 5 months of follow-up.

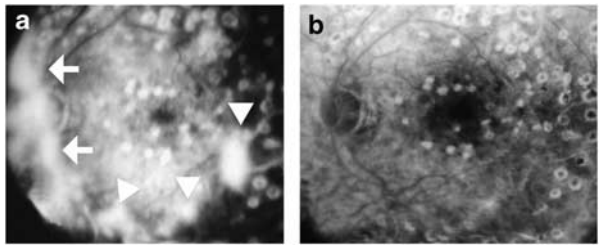

Figure 2 (a) Late-phase fluorescein angiogram demonstrating retinal neovascularization at the optic disc (NVD) (arrows) and neovascularization elsewhere (NVE) (arrowheads) in an 80-year-old man with proliferative diabetic retinopathy. He received a complete panretinal photocoagulation 2 years previously. (b) A fluorescein angiogram obtained 2 weeks after intravitreal bevacizumab injection demonstrated total regression of leakage from NVE and NVD in the late phase of the study.

12.4 weeks (range from 4 to 34 weeks), and seven eyes (15.9%) needed a third injection due to recurrence of neovascularization at a mean of 17.3 weeks (range from 11 to 22 weeks). Three eyes without previous PRP ('naive' eyes) and with vitreous haemorrhage have avoided vitreo-retinal surgery. There were no episodes of inflammation or severe decrease of vision immediately after an injection.

At 6 months, no systemic adverse events such as thromboembolic events (cerebrovascular accidents, transient ischaemic attacks, myocardial infarctions, or peripheral vascular disease) were reported.

Discussion

Although RN actually may be due to more than one cytokine, VEGF is an important, if not the most important cytokine involved.[25] Activation of the VEGF receptor pathway triggers a network of signalling processes that promotes endothelial cell growth, migration, survival from pre-existing vessels, differentiation, and mobilization of endothelial progenitor cells from the bone marrow into the peripheral circulation.[9,26,27] Furthermore, VEGF increases vessel permeability leading to deposition of proteins in the interstitium that facilitate the process of angiogenesis.[28] There are several reports published on the intravitreal administration of anti-VEGF compounds for RN in diabetic retinopathy.[7,13] In addition, there are five case reports on the use of intravitreal bevacizumab in RN in diabetic retinopathy demonstrating regression of RN in PDR.[14,29–32]

Our study demonstrated that intravitreal bevacizumab resulted in marked regression of RN on fundus examination and FA in patients with PDR and previous PRP. Furthermore, a rapid resolution of vitreous haemorrhage in three naive eyes was also seen. In addition, intravitreal bevacizumab demonstrated a similar beneficial response on macular thickness in eyes with PDR, and probably bevacizumab prevents exacerbation of macular oedema in patients with concomitant CSME and PDR. To determine the effect of an intravitreal injection of bevacizumab on actively growing new vessels, we chose the change in vitreous leakage from RN as our primary outcome. The detection of NVD and NVE on FA allowed the use of a systematic anatomical approach to monitor the area of leaking new vessels over time. Finally, to determine the effect of an intravitreal injection of bevacizumab on macular oedema, we measured the change of retinal thickening with OCT.

Regression of neovascularization and decrease of retinal thickening occurred in some injected eyes as soon as 7–15 days after the intravitreal injection of bevacizumab. Twenty-one eyes (47.7%) needed a second injection due to recurrence of neovascularization at a mean of 12.4 weeks, and seven eyes (15.9%) needed a

Table 1 Distribution of eyes according to prior treatment

Prior treatment	*2.5 mg IVT bevacizumab*	*1.25 mg IVT bevacizumab*	*Total of eyes*
PRP	12	2	14
PRP + grid	11	4	15
PRP + focal	2	2	4
Total of eyes	25	8	33

IVT, intravitreal; PRP, panretinal photocoagulation.

Table 2 Characteristics of patients with macular oedema associated to RN

Patient no.	*Prior treatment*	*Baseline VA log Mar*	*Baseline macular thickness by OCT (μm)*	*Final VA Log Mar*	*Final macular thickness by OCT (μm)*
1	PRP	0.3	284	0.1	244
4	No	1.0	1082	0.5	357
5	No	2.0	404	1.3	369
6	No	2.0	267	0.7	178
7	PRP + focal	1.3	559	1.2	178
9	PRP + grid	1.0	471	1.9	475
10	PRP	1.0	589	0.3	215
11	No	2.0	381	2.0	219
12	PRP + grid	1.8	615	0.2	209
13	PRP + grid	1.0	481	0.2	269
14	PRP + grid	1.0	481	0.3	263
15	PRP + grid	1.8	383	0.5	192
16	PRP + grid	1.3	355	0.5	213
17	PRP + grid	1.3	862	0.6	324
18	PRP + grid	1.0	362	0.5	203
19 (RE)	PRP	1.8	367	1.3	270
19 (LE)	No	2.0	343	1.3	252

CF, counting fingers; LE, left eye; OCT, optical coherence tomography; PRP, panretinal photocoagulation; RE, right eye; RN, retinal neovascularization; VA, visual acuity.

Table 3 Comparison between 2.5 and 1.25 mg of IVT bevacizumab for PDR

RN regression	*Neovascularization with previous PRP*		*Naive neovascularization*		*Total eyes*
	2.5 mg	*1.25 mg*	*2.5 mg*	*1.25 mg*	
Total	16	2	9	0	27
Partial	9	4	0	2	15
No	1	1	0	0	2
Total eyes	26	7	9	2	44

IVT, intravitreal; naive, no previous PRP; PDR, proliferative diabetic retinopathy; PRP, panretinal photocoagulation; RN, retinal neovascularization.

third injection due to recurrence of neovascularization at a mean of 17.3 weeks. Interestingly, we found that the 2.5 mg seems to be more effective than the 1.25 mg dose to induce complete regression of RN in naive eyes ($P = 0.01$; Table 3). The reason for this dose-dependant response on RN in naive eyes in unknown. In addition, the optimum dose and dosing sequence for intravitreal bevacizumab is still undetermined. We elected to defer reinjection only when there was a recurrence. Our clinical impression is that the effect of intravitreal bevacizumab on RN may be more lasting than in eyes with other pathologies such as choroidal neovascularization or macular oedema; however, the cause is not known.

Our results suggest an overall VA gain as well as a reduced risk of VA loss in eyes with diabetic macular oedema (as recognized on OCT) treated with intravitreal bevacizumab. We did not find any differences in the effectiveness between the doses of 1.25 and 2.5 mg for CSME, both of them demonstrated improvement with respect to VA and decrease in retinal thickness. Avery *et al*[13] reported similar results to the present study in 45 eyes of 32 patients with retinal and/or iris neovascularization secondary to diabetes mellitus who had received intravitreal injections of 6.2 μg–1.25 mg of bevacizumab. They demonstrated that all patients with neovascularization had complete or at least partial reduction in leakage of the neovascularization within 1 week after the injection. Additionally, they found in two cases, a subtle decrease in leakage of retinal or iris neovascularization in the fellow uninjected eye. We could not confirm their observation as in our study, utilizing higher doses (1.25–2.5 mg) of bevacizumab, all of our patients with bilateral RN underwent bilateral intravitreal injections.

Panretinal photocoagulation has been the mainstay for the treatment of PDR, and its suppressive effect on RN has been well documented.[20,21,33,34] However, substantial regression of new vessels may take weeks after completion of PRP, and in up to one-third of cases, new vessels continue to grow despite initial PRP.[21,34] In these cases, vitreous haemorrhage may induce visual loss and prevent complete laser. Moreover, macular oedema may increase after PRP and cause transient or persistent visual loss.[35,36] Our study demonstrates multiple benefits of intravitreal bevacizumab on PDR and in the future this new option could be an adjuvant agent to PRP so that more selective therapy may be applied. In addition, bevacizumab may allow long intervals between PRP sessions to avoid the development of macular oedema and other complications.[21–24]

The current study has several limitations, including a relatively small sample size and a relatively short duration of follow-up. In addition, this study included patients from five different centres and patients were treated according to the discretion of the treating physician. However, the large difference in the quantitative morphologic outcomes and the trend towards improvement in BCVA in injected eyes found at 6 months confirms our hypothesis that at least some eyes with PDR, such as those with pre-existing macular oedema or rapidly growing new vessels, may truly benefit from intravitreal bevacizumab. In addition, we can safely assume with a 95% confidence, that the true rate of systemic complications is $<9\%$ in our study.[37]

In summary, intravitreal bevacizumab seems to be a promising treatment for PDR, minimizing the risk for exudative complications, progression of RN, vitreous haemorrhage, and decreased vision caused by macular oedema. Intravitreal bevacizumab may potentially be used as an adjuvant agent to PRP for PDR. Although no serious complications of intravitreal injection of bevacizumab occurred in our series, further studies are needed to assess the efficacy and safety of intravitreal bevacizumab in the management of PDR.

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Chapter 8: Intravitreal Bevacizumab for Proliferative Diabetic Retinopathy at 2 years of follow up

Arevalo JF, Lasave AF, Wu L, Maia M, Diaz-Llopis M, Alezzandrini AA, Brito M; Pan-American Collaborative Retina Study Group (PACORES).
INTRAVITREAL BEVACIZUMAB FOR PROLIFERATIVE DIABETIC RETINOPATHY: Results From the Pan-American Collaborative Retina Study Group (PACORES) at 24 Months of Follow-up.
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Hypothesis 3: IVB may decrease retinal neovascularization in patients with PDR at 6 months of follow up. However, the effect may decrease at 24 months of follow up due to tachyphylaxis, and pan-retinal photocoagulation and/or vitrectomy will be necessary.

PURPOSE: To evaluate the effects of intravitreal bevacizumab (IVB) on retinal neovascularization in patients with proliferative diabetic retinopathy (PDR).

METHODS: Retrospective multicenter interventional case series. A chart review was performed of 81 consecutive patients (97 eyes) with retinal neovascularization due to PDR, who received at least 1 IVB injection.

RESULTS: The mean age of the patients was 55.6 ± 11.6 years. The mean number of IVB injections was 4 ± 2.5 injections (range, 1-8 injections) per eye. The mean interval between IVB applications was 3 ± 7 months. The mean duration of follow-up was 29.6 ± 2 months (range, 24-30 months). Best-corrected visual acuity and optical coherence tomography improved statistically significantly ($P < 0.0001$, both comparisons). Three eyes without previous pan-retinal photocoagulation ("naive" eyes) and with vitreous hemorrhage did not require vitreoretinal surgery. Five (5.2%) eyes with PDR progressed to tractional retinal detachment requiring vitrectomy. No systemic adverse events were noted.

CONCLUSION: Intravitreal bevacizumab resulted in marked regression of retinal neovascularization in patients with PDR and previous pan-retinal photocoagulation. Intravitreal bevacizumab in naive eyes resulted in control or regression of 42.1% of eyes without adjunctive laser or vitrectomy during 24 months of follow-up. There were no safety concerns during the 2 years of follow-up of IVB for PDR.

INTRAVITREAL BEVACIZUMAB FOR PROLIFERATIVE DIABETIC RETINOPATHY

Results From the Pan-American Collaborative Retina Study Group (PACORES) at 24 Months of Follow-up

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Purpose: To evaluate the effects of intravitreal bevacizumab (IVB) on retinal neovascularization in patients with proliferative diabetic retinopathy (PDR).

Methods: Retrospective multicenter interventional case series. A chart review was performed of 81 consecutive patients (97 eyes) with retinal neovascularization due to PDR, who received at least 1 IVB injection.

Results: The mean age of the patients was 55.6 ± 11.6 years. The mean number of IVB injections was 4 ± 2.5 injections (range, 1–8 injections) per eye. The mean interval between IVB applications was 3 ± 7 months. The mean duration of follow-up was 29.6 ± 2 months (range, 24–30 months). Best-corrected visual acuity and optical coherence tomography improved statistically significantly ($P < 0.0001$, both comparisons). Three eyes without previous panretinal photocoagulation ("naive" eyes) and with vitreous hemorrhage did not require vitreoretinal surgery. Five (5.2%) eyes with PDR progressed to tractional retinal detachment requiring vitrectomy. No systemic adverse events were noted.

Conclusion: Intravitreal bevacizumab resulted in marked regression of retinal neovascularization in patients with PDR and previous panretinal photocoagulation. Intravitreal bevacizumab in naive eyes resulted in control or regression of 42.1% of eyes without adjunctive laser or vitrectomy during 24 months of follow-up. There were no safety concerns during the 2 years of follow-up of IVB for PDR.

RETINA 37:334–343, 2017

Diabetic retinopathy remains a major sight-threatening disease of the working population in developed and developing countries.[1] Proliferative diabetic retinopathy (PDR) is a major cause of visual loss in patients with diabetes. Proliferative diabetic retinopathy is characterized by retinal neovascularization (RN), retinal capillary leakage, hemorrhage, and fibrovascular proliferation in the vitreous retinal interface, which results in vitreous hemorrhage (VH) and tractional retinal detachment (TRD).[2] Proliferative diabetic retinopathy is rapidly becoming a major cause of blindness in many countries. Approximately, 1.5% of adults with diabetes have PDR.[3] Current evidence indicates that the growth of new vessels from the retina or optic nerve is likely a result of the release of vascular endothelial growth factor (VEGF) into the vitreous cavity as a response to ischemia.[4–6] Studies have demonstrated a correlation of VEGF levels to the severity of PDR and a reduction in VEGF levels after successful laser treatment of PDR.[3] Currently, panretinal photocoagulation (PRP) is the only successful evidence-based treatment for PDR. Panretinal photocoagulation reduces the risk of severe visual loss by 50% to 60% with regression of the majority of new vessels over a period of 3 months.[7] Despite this evidence, several attempts have been made to modify PRP laser techniques to reduce side effects such as decreased visual acuity, peripheral field loss, and macular edema.[8] Despite adequate PRP, many patients still require supplemental laser treatment and nearly 4.5% show disease progression that eventually requires pars

plana vitrectomy.[9] Additionally, most patients require at least 2 PRP treatments and several patients return for multiple additional sessions for persistent neovascularization. Consequently, long intervals between PRP sessions and the variable amount of time required for a favorable response may increase the incidence of complications due to the progression of PDR.[10,11] A single episode of PRP or shorter intervals between PRP episodes is desirable for severe PDR or when the patient must travel long distances for treatment. However, shorter intervals between PRP sessions are often associated with acute visual disturbances due to exudative choroidal detachment, retinal detachment, and macular edema.[12–14]

These difficulties and complications have spurred a search of new therapies for treating PDR, such as anti-VEGF compounds. Our group previously reported on the efficacy of intravitreal bevacizumab (IVB) for RN in patients with PDR at 6 months of follow-up.[15] This previous study indicated a marked regression of RN, especially in patients with PDR and with a history of PRP.[15]

Several randomized non–placebo-controlled trials of IVB for the treatment of PDR have been recently published.[16–18] This retrospective study evaluated the effectiveness of IVB on RN in patients with PDR at 24 months of follow-up as a basis for future studies of bevacizumab, as an adjuvant treatment to PRP for PDR.

Patients and Methods

A retrospective study was performed of 97 eyes (81 patients) with RN secondary to PDR, who were treated with off-label IVB between September 2009 and August 2011 at 5 institutions in Venezuela, Costa Rica, Brazil, Argentina, and Spain. Institutional review board/ethics committee approval and informed consent was obtained for this study at all 5 institutions. The off-label use of the drug and its potential risks and benefits were discussed extensively with all patients. This study adhered to the 1964 Declaration of Helsinki for research involving human subjects. Eyes that were previously treated with scatter photocoagulation, had prior focal/grid laser photocoagulation, and had previous intravitreal triamcinolone injection were included if any of those therapies had been performed at least 6 months before IVB. Eyes treated previously with any anti-VEGF drugs were excluded from this study. An injection of 1.25 mg of bevacizumab was delivered at the discretion of the treating physician. Baseline data were collected on age, sex, type, and duration of diabetes mellitus.

Patients underwent clinical examination including best-corrected Snellen visual acuity testing, ophthalmoscopic examination, applanation tonometry, fluorescein angiography (FA), and optical coherence tomography (OCT) at baseline and follow-up visits. In patients with clinically significant macular edema, baseline central retinal characteristics were observed by time-domain OCT using 6 diagonal slow 6-mm radial line scans through a dilated pupil. The retinal thickness of the 1-mm central retina was obtained using the macular thickness map for the calculations in all cases. When spectral-domain OCT became available, a volume scan centered on the fovea was performed. The equivalence between the Stratus OCT (Zeiss GmBh, Jena, Germany) and Cirrus spectral-domain OCT (Zeiss GmBh) values was resolved by adding 50 microns to each central macular thickness (CMT) measured by Stratus OCT. The scans were reviewed, and manual measurements were used in cases of segmentation errors.

A 0.10-mL aliquot of commercially available bevacizumab was prepared for each patient and placed in a tuberculin syringe using aseptic techniques. After the eye had been prepared in a standard manner using 5% povidone/iodine, an eyelid speculum was used to stabilize the eyelids, and the injection of 1.25 mg (0.05 mL) of bevacizumab was delivered 3.5 mm to 4 mm posterior to the limbus, through the pars plana, with a 30-gauge needle under topical anesthesia. After the injection, intraocular pressure and retinal artery perfusion were checked.

To determine the effect of an intravitreal injection of bevacizumab on actively growing new vessels, we chose the change in vitreous leakage from RN on FA as our primary outcome. Retinal neovascularization was divided into two groups based on location: new vessels on the disk (NVD) and new vessels elsewhere.

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For a complete listing of participating members of PACORES see Appendix 1.

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Fluorescein angiography allowed the use of a systematic anatomical approach to monitor the area of leaking new vessels over time.

Patients were examined at 1 week, 2 weeks, and 1 month after the first injection and monthly thereafter. One, 3, 6, 12, and 24 months after the initial injection, ophthalmic examination included OCT and FA. Fluorescein angiography was performed at the discretion of the examiner and not at every post-injection evaluation, usually every 6 weeks. An assessment for retreatment with IVB, retreatment with laser, or initiation of laser treatment occurred at each visit. Patients received reinjections if RN was not totally resolved on ophthalmic examination or FA. In addition, patients received retreatment whenever there was a recurrence of neovascular activity. Recurrence was defined as an increased size of the neovascular area associated with increased and diffuse fluorescein leakage on FA, after complete or partial resolution in previous follow-up visits.

In this study, the term "immediate PRP" denoted a case with PDR treated with primary IVB who was subsequently treated with PRP within 3 months after the first IVB therapy. The term "deferred PRP" denoted a case where PRP was performed 3 months after the beginning IVB therapy. Laser augmentation was defined as "additional laser" performed due to either active residual neovascularization or new large ischemic areas in eyes previously treated with PRP (6 months or more before IVB therapy). The treating physician decided if laser augmentation was required.

Best-corrected visual acuity (BCVA) was converted from Snellen chart values to logarithm of minimum angle of resolution (logMAR) equivalent units for statistical calculations. All data were collected in a Microsoft Office Excel 2007 spreadsheet (Microsoft Corporation, Redmond, WA) and were statistically analyzed by MedCalc Software for Windows 8.2.0.3 (MedCalc, Mariakerke, Belgium). The paired *t*-test was used to compare the mean values to analyze mean logMAR visual acuity. A P value <0.05 was considered statistically significant.

Interval data were analyzed at the 1-, 3-, 6-, 12-, and 24-month follow-up time points. Repeated measures of the analysis of variance were used to compare mean values of retinal thickness and logMAR visual acuity. A significant increase or decrease in BCVA was defined as a change of two or more Snellen lines. The main outcome measures included change in RN defined as the change in the area size of vitreous leakage from new vessels (NV) in the late phase of FA and changes in BCVA with assessment of CMT measured by OCT. Patients were included in this consecutive series if there was a minimum of 24 months of follow-up.

Monitored systemic conditions included myocardial infarction, stroke, systemic hypertension, thromboembolic diseases, and death. Other systemic conditions were assessed by a thorough review of systems. If the patients were unable to attend a particular visit, a telephone interview was conducted to assess for possible systemic complications, and a new appointment was scheduled.

Results

A review of clinical records was performed of 81 consecutive patients (97 eyes) with PDR who underwent at least 1 injection of IVB. Sixty-eight (84%) patients were Hispanic, 11 (13.6%) were white, and 2 (2.5%) were African American. Patients had a mean follow-up of 29.6 ± 2 months (range: 24–30 months). The mean age of patients was 54.9 ± 11 years (range: 25–79 years). Forty-four (54.3%) patients were male. Thirty (30.9%) patients were insulin dependent, 23 (23.7%) patients were being treated with oral hypoglycemic agents, and 44 (45.3%) patients were being treated with combined therapy (insulin dependent plus oral hypoglycemic agents) at the time of PDR diagnosis. The mean duration of diabetes was 19.1 ± 9 years (range: 1–40 years). The mean glycosylated hemoglobin (HbA1c) was 9 ± 2% (range: 5.9–13.6%). Demographic data of all patients are presented in Table 1.

There was a statistically significant change in mean BCVA from 20/125 (0.8 ± 0.4 logMAR) at baseline to 20/60 (0.5 ± 0.4 logMAR) at last follow-up visit

Table 1. Demographics Data of 81 patients (97 eyes) With PDR Treated With IVB*

Variable	Patients, n (%)
Eyes	97
Age, years	54.9
Mean ± 1 SD	11
Gender	
Male	44 (54.3)
Female	37 (45.7)
Race	
Hispanic	68 (84)
White	11 (13.6)
African American	2 (2.5)
Duration of diabetes, years	
Mean	19.1
±1 SD	9
Systemic glycemic control	
OHA	23 (23.7)
Insulin	30 (30.9)
Insulin plus OHA	44 (45.4)
HbA1c, % mean	9
±1 SD	2

*Values represent number and percentages.

HbA1c, glycosylated hemoglobin; n, number of eyes; OHA, oral hypoglycemic agents; SD, standard deviation.

($P < 0.0005$). Best-corrected visual acuity improved from 20/125 (0.8 ± 0.4 logMAR) at baseline to 20/80 (0.6 ± 0.4 logMAR) ($P = 0.018$) at 1 month and remained stable during the 3 months and 6 months of follow-up, with a BCVA mean of 20/80 (0.6 ± 0.4 logMAR) ($P = 0.038$) and 20/80 (0.6 ± 0.4 logMAR) ($P = 0.022$), respectively. The mean BCVA was 20/60, (0.5 ± 0.4 logMAR) ($P = 0.001$) at 12 months of follow-up. At month 24, the mean BCVA was 20/60 (0.5 ± 0.4 logMAR) ($P < 0.0005$) (Figure 1).

Final BCVA remained stable in 49 (50.5%) eyes, improved by 2 or more Snellen lines in 42 (43.3%) eyes, and decreased by 2 or more Snellen lines in 6 (6.2%) eyes (Table 2).

Optical coherence tomography results were available for all cases at 1, 3, 6, 12, and 24 months. At 1 month, the mean CMT decreased from 359.9 ± 152.5 μm to 348.4 ± 120.5 μm ($P = 0.441$). At 3 months and 6 months, the mean CMT measurements were 359.7 ± 159.9 μm ($P = 0.821$) and 351.5 ± 103.9 μm ($P = 0.322$), respectively. At 12 months and 24 months, the mean CMT measurements were 325.2 ± 102.5 μm ($P = 0.016$) and 311.7 ± 93.7 μm ($P < 0.0001$), respectively (Figure 2).

The mean number of IVB injections per eye was 4 ± 2.5 (range: 1–8 injections) (Figure 3). The mean interval between IVB applications was 3 ± 7 months (range 1–12 months) (Table 3). Regression of neovascularization occurred in some eyes within 7 days to 15 days after the intravitreal injection.

Retinal neovascularization lesions were divided into 2 groups according to the area affected by NV at presentation. We observed 54 (55.6%) eyes with NVD. Of these eyes, complete regression of neovascularization occurred in 22 (40.7%) eyes, 14 (25.4%) eyes had partial regression, and 18 (32.6%) eyes had no regression after IVB therapy. New vessels elsewhere on the retina occurred in 71 (73.1%) eyes, which had total neovascular regression in 42 (59.1%) eyes, partial regression in 22 (30.9%) eyes, and no regression in 7 (9.8%) eyes. Including all RN cases, complete NV regression occurred in 58 (59.7%) eyes at the end of follow-up, partial resolution in 17 (17.7%) eyes, and no regression in 22 (22.6%) eyes (Table 4).

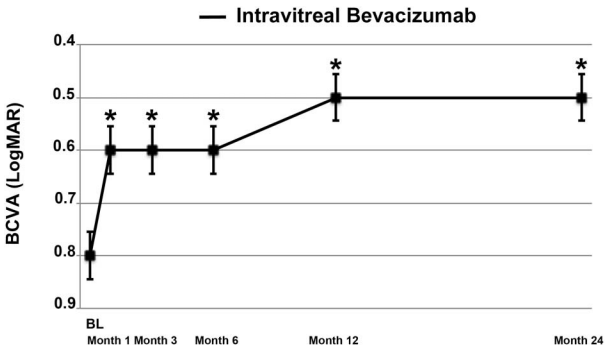

Fig. 1. The changes in BCVA in eyes with RN after IVB are plotted versus time. The mean BCVA improved at 1 month from the baseline (BL) value of logMAR = 0.8 ± 0.4 (Snellen equivalent 20/125) to logMAR = 0.6 ± 0.4 (Snellen equivalent 20/80, $P = 0.018$). At 3 months, the mean BCVA was stable at logMAR = 0.6 ± 0.8 (Snellen equivalent 20/80, $P = 0.038$). At 6 months, the average BCVA was logMAR = 0.6 ± 0.4 (Snellen equivalent 20/80, $P = 0.022$). At 12 months, the mean BCVA decreased to logMAR = 0.5 ± 0.4 (Snellen equivalent 20/60, $P = 0.001$). The mean BCVA at 24 months was logMAR = 0.5 ± 0.4 (Snellen equivalent 20/60, ($P < 0.0005$)). *Statistically significant changes ($P < 0.05$) with respect to the baseline mean BCVA with baseline.

Data were subdivided into eyes treated with PRP before IVB therapy and untreated eyes (naive eyes) before IVB therapy. All cases treated previously with PRP were performed at least 6 months before the IVB treatment. Sixty (61.9%) eyes had been treated previously with PRP. Of these, 41 (68.3%) eyes showed good response to IVB therapy alone, 10 (16.6%) eyes underwent laser augmentation immediately after the first IVB due to the presence of large ischemic areas on FA, and 9 (15%) eyes needed at least one session of laser augmentation during follow-up (Table 5). This subgroup of 60 eyes with prior PRP showed a complete regression of NV in 44 (73.3%) eyes at the end of follow-up, partial resolution (with reduction of NV present) in 9 (15.1%) eyes, and no regression in 7 (11.6%) eyes at 2 years of follow-up (Table 4). Of the 37 (38.1%) eyes that had no history of previous PRP, 18 (48.6%) eyes were treated with IVB plus immediate PRP after IVB and 19 (51.4%) eyes were treated with IVB therapy only. However, of these 19 (100%) eyes, 11 (57.8%) eyes required either PRP therapy or surgery to improve the diabetic retinopathy during follow-up, whereas the other 8 (42.1%) eyes that underwent only IVB achieved a complete regression of PDR during the 24 months of follow-up. This subgroup of 37 naive eyes with PDR treated firstly with IVB showed a complete regression of NV in 14 (37.9%) eyes at the end of follow-up, partial resolution (with reduction of NV present) in 8 (21.6%) eyes, and no regression in 15 (40.5%) eyes at 2 years of follow-up (Table 4).

Ocular complications associated with either progression or the lack of response to treatment were observed in 12 (12.3%) eyes including 1 (1.0%) case of epiretinal membrane, 2 (2.1%) cases of neovascular glaucoma, 4 (4.1%) cases of VH, and 5 (5.2%) cases with TRD. Out of four VH cases, only two resolved spontaneously. One case of neovascular glaucoma was controlled with medical treatment. All cases of ocular complications are presented in Table 6.

Over the study period, 9 (11.1%) patients (eyes) underwent surgery. A total of 9 surgical procedures

Table 2. Analysis of BCVA by Subgroups in 97 Eyes With PDR Treated With IVB Therapy*

	Month 1	Month 3	Month 6	Month 12	Month 24
Improved 2 or more Snellen lines of BCVA	29 (29.9)	33 (34.1)	30 (30.9)	38 (39.2)	41 (42.2)
Remained stable	63 (64.5)	55 (56.7)	56 (57.7)	48 (49.5)	49 (50.5)
Decreased 2 or more Snellen lines of BCVA	5 (5.2)	9 (9.3)	11 (11.3)	11 (11.3)	7 (7.2)

*Values represent number and percentages.
n, number of eyes.

were performed, of which 8 (8.2%) eyes underwent pars plana vitrectomy and 1 (1.0%) eye underwent trabeculectomy and a valve implant due to neovascular glaucoma. Pars plana vitrectomy was performed because 1 (1.0%) eye had an epiretinal membrane, 2 (2.1%) eyes presented with VH, and 5 (5.2%) eyes developed TRD.

Local adverse events that may be associated with IVB therapy included 1 (1.0%) eye with anterior uveitis, 1 (1.0%) eye with ocular hypertension which was controlled with antiglaucoma medications, and 1 (1.0%) eye with VH only 2 days after IVB injection, which resolved a few days later. Systemic adverse events included 1 (1.0%) case of stroke, and 1 (1.0%) patient died during follow-up.

Discussion

This retrospective multicenter study included 81 patients (97 eyes) with PDR who were treated with at least 1 primary IVB injection or treated with IVB for reactivated PDR after PRP over a 2-year period. We observed that 58 (59.7%) eyes had complete regression of RN on fundus examination with the absence of fluorescein leakage, 17 (17.7%) eyes demonstrated partial regression of RN, and 22 (22.6%) eyes had active NV on fundus examination and FA at the end of follow-up.

Panretinal photocoagulation has been the mainstay for the treatment of PDR, and its suppressive effect on RN has been well documented.[11,19–21] However, substantial regression of new vessels may take weeks after completion of PRP, and in up to one third of cases, new vessels continue to grow despite initial PRP.[14,20] In these cases, VH may induce visual loss and prevent complete laser.[22] Sinawat et al[23] evaluated the efficacy and safety of IVB for the treatment of PDR with new dense VH after previous full panretinal photocoagulation. Out of 18 eyes, VH cleared completely in 7 (38.8%), 9 (50%), and 13 (72.2%) eyes after 6 weeks, 6 months, and 12 months, respectively. We observed that 60 (61.9%) eyes had been treated previously with photocoagulation. Of these, 41 (68.3%) eyes showed good response to IVB therapy alone, including 4 (9.1%) eyes that presented with VH. In addition, we observed 3 eyes without previous PRP ("naive" eyes) and with VH to have avoided vitreoretinal surgery.

Many studies have evaluated the efficacy of combined IVB injection and PRP for treatment of high-risk PDR but most were not large-scale trials.[15–18,24,25]

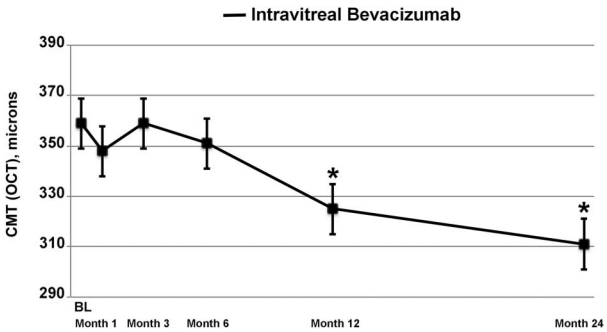

Fig. 2. Changes in CMT with OCT after IVB in eyes with RN along 24 months of follow-up. CMT at baseline had a mean of 359.9 ± 152.5 μm which was reduced to a mean of 348.4 ± 120.5 μm ($P = 0.441$) at the first month of follow-up. At 3 and 6 months, the mean CMT measurements were 359.7 ± 159.9 μm ($P = 0.821$) and 351.5 ± 103.9 μm ($P = 0.322$), respectively. At 12 months and 24 months, the mean CMT was 325.2 ± 102.5 μm ($P = 0.016$) and 311.7 ± 93.7 μm ($P < 0.0001$), respectively. *Statistically significant change ($P < 0.05$) compared with baseline.

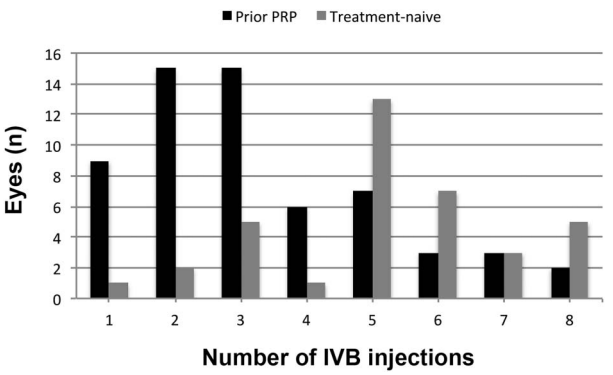

Fig. 3. The number of intravitreal injections of bevacizumab in 97 eyes with PDR divided by prior PRP versus treatment naive eyes. The mean number of intravitreal injections of bevacizumab (IVB) per eye was 4 ± 2.5 (range: 1–8 injections). The mean interval between IVB applications was 3 ± 7 months (range 1–12 months).

Table 3. Mean Number of IVB Injections in 97 Eyes With PDR*

Number IVB	Eyes, n (%)	Mean Interval, weeks	Range, weeks	Prior PRP	TN
1	10 (10.3)	—	—	9 (90)	1 (10)
2	17 (17.5)	22.7 ± 15.1	4–54	15 (88)	2 (12%)
3	20 (18.5)	17.5 ± 13.9	4–50	15 (75)	5 (25)
4	7 (7.2)	16.4 ± 10.6	4–42	6 (86)	1 (14)
5	20 (20.6)	7.5 ± 6.3	4–28	7 (35)	13 (65)
6	10 (10.3)	9.3 ± 6.2	5–20	3 (30)	7 (70)
7	6 (6.2)	12 ± 5.6	8–16	3 (50)	3 (50)
8	7 (7.2)	5 ± 2.0	4–6	2 (29)	5 (71)

*Values represent number and percentages.
n, number of eyes; prior PRP, prior PRP; TN, treatment naive.

Tonello et al[17] evaluated the effects of PRP compared with PRP plus IVB on BCVA and total area of fluorescein leakage from active new vessels in 30 eyes with high-risk PDR.

The authors[17] suggested that in the short term (16 weeks), the combination of IVB with PRP may be associated with a higher rate of regression of active leaking NV than PRP alone in patients with high-risk PDR. Additionally, Jorge et al[26] evaluated the effect of IVB injection in eyes with persistent, active PDR in a noncomparative study. The authors[26] administered 1 injection of bevacizumab to 15 eyes at 12 weeks of follow-up. As a result, BCVA improved significantly from baseline at all time points (1, 6, and 12 weeks), from 20/160 at baseline to 20/125 at 12 weeks.[23] The mean area of fluorescein leakage also improved significantly at all time points.[23]

Recently, investigators from the Diabetic Retinopathy Clinical Research Network[27] (DRCR.net) presented the results of a clinical trial comparing PRP and intravitreal ranibizumab (IVR) among patients with PDR. Patients in the IVR group (n = 191 eyes) received IVR (0.5 mg) (and PRP if treatment failed) and received ranibizumab as needed for diabetic macular edema, whereas patients in the PRP group (n = 203 eyes) received PRP and ranibizumab as needed for diabetic macular edema. In this randomized clinical trial, IVR met the primary noninferiority outcome of visual acuity change at 2 years. There was no statistically significant difference in visual acuity between the ranibizumab and PRP groups at 2 years; however, notably, 53% of the PRP group received ranibizumab injections for diabetic macular edema (DME). Greater peripheral visual field loss occurred (95% confidence interval for difference, 213–531 dB) and more vitrectomies were performed in the PRP group than in the ranibizumab group (95% confidence interval for difference, 4–15%). Among eyes without center-involved DME at baseline, development of DME with vision impairment was substantially more frequent in the PRP group (95% confidence interval for difference, 10–28%). Only 12 eyes (6%) in the ranibizumab group received PRP; more than half of the eyes in the PRP group received ranibizumab for DME; thus, the protocol essentially tested ranibizumab for PDR versus PRP plus ranibizumab as needed for DME treatment.

Table 4. Retinal Neovascularization Regression in 97 Eyes With IVB Therapy for PDR at 2 Years Follow-up*

NV Localization	RN	Prior PRP	TN
RN Regression			
Complete	58 (59.7)	44 (73.3)	14 (37.9)
Partial	17 (17.7)	9 (15.1)	8 (21.6)
No	22 (22.6)	7 (11.6)	15 (40.5)
Total	97 (100)	60 (100)	37 (100)

*Values represent number and percentages.
n, Number of eyes; prior PRP, prior PRP; TN, treatment naive.

In another study, Mirshahi et al[16] evaluated 40 patients with Type 2 diabetes and bilateral PDR with a high-risk profile who underwent scatter laser treatment after the Early Treatment Diabetic Retinopathy Study protocol and a single bevacizumab injection in 1 eye, and sham injection in the contralateral eye. They[16] demonstrated that at Week 6, 87.5% of the eyes treated with bevacizumab presented with complete regression of neovascularization versus 25% in the sham-treated group ($P < 0.005$).[16]

Alternately, small nonrandomized pilot studies have reported successful short-term results with IVB in iris neovascularization and RN demonstrating regression of RN in PDR.[28–30] An interventional consecutive retrospective case series[28] reported that 100% of 44 eyes (32 patients) demonstrated some degree of regression of leakage on FA secondary to PDR within 1 week of IVB, even with doses as low as 6.2 μg. The authors[28] also noted a complete resolution of new vessels elsewhere in 59% of eyes, NVD in 73% of eyes, and iris neo-vascularization in 82% of eyes. In the current study, evaluating the topographic location of RN, we found a complete resolution of new vessels

Table 5. Eyes With PDR Treated With IVB Therapy According to the Presence of PRP Before Intravitreal Therapy*

	Prior PRP	TN	Total	*P*
Eyes	60 (61.8)	37 (38.1)	97 (100)	
Modality therapy				
IVB Alone	41 (68.3)	8 (32.4)	52 (53.6)	0.0002†
IVB + Immediate LA or PRP	10 (16.6)	18 (48.6)	25 (25.7)	0.0001†
IVB + Deferred LA or PRP	9 (15)	11 (18.9)	20 (20.6)	0.825

*Values represent number and percentages.
†Statistically significant.
LA, laser augmentation; n, number of eyes; TN, treatment naive.

elsewhere in 42 (59.1%) eyes and in 22 (40.7%) eyes with NVD. Additionally, we observed complete regression of RN in 58 (59.7%) eyes at 2 years of follow-up, of which 44 (73.3%) eyes were previously treated with PRP 6 months or more before IVB therapy and 14 (37.9%) eyes were treatment-naive eyes. Avery et al[28] also noted, however, that recurrence of RN began as early as 2 weeks after intravitreal injections of bevacizumab, which could be its major shortcoming compared with PRP. In contrast to these results, in our study, the mean interval between IVB applications was 3 ± 7 months. This observation indicates that the mean recurrence of NV in our patients was longer than those described by Avery et al.[24] However, a retrospective analysis by Adamis et al[31] demonstrated a persistent beneficial effect of intravitreal pegaptanib in patients with PDR, with 62% of the treated eyes showing regression or absence of neovascularization 6 months after injection. In addition, Mendrinos et al[21] reported complete regression of neovascularization 1 year after a single injection of pegaptanib in a patient with previous PRP. In concordance with our study, Minnella et al[32] reported that the effects of bevacizumab were maintained at 3 months in 15 treated eyes with PDR.[32] Additionally, Schmidinger et al[33] showed that 62% (8 of 13) of the treated eyes required retreatment with bevacizumab 3 months after baseline injection because of the appearance of new vessels. Therefore, a major limitation of anti-VEGF therapy for PDR seems to be the recurrence of RN from 2 weeks[28] to 12 months after injection.[21,34,35]

Table 6. Ocular Complications to PDR Observed in 97 (100%) Eyes Treated With IVB Therapy During 24 Months of Follow-up*

Ocular Complications	Eyes
ERM	1 (1.0)
VH	4 (4.1)
TRD	5 (5.2)
NVG	2 (2.1)
Total OC	12 (12.3)

*Values represent number and percentages.
ERM, epirretinal membrane; n, number of eyes; NVG, neovascular glaucoma; OC, ocular complications.

Our study concurs with Chung et al[36] who suggest that repeated injections, panretinal photocoagulation, and/or pars plana vitrectomy may be necessary after IVB to reinforce the anti-VEGF effect of the drug. The authors[36] demonstrated that a single IVB injection might not be sufficient in inducing complete blockage of VEGF and pathologic neovascularization in active patients with PDR. In our series, the mean number of IVB injections per eye was 4 ± 2.5 (range: 1–8 injections) at 24 months of follow-up, with a mean interval of 3 ± 7 months. We found that out of 37 (38.1%) eyes that had not received previously PRP (naive eyes), 18 (48.6%) eyes were treated with PRP immediately (within the third month) after the first IVB, and 19 (51.4%) eyes were treated with IVB therapy only. However, 11 (57.8%) of the eyes treated only with IVB underwent PRP therapy or surgery during follow-up.

Alternately, we observed that >60% of the eyes included in the current study had been previously treated with PRP. Therefore, we compared the general results based on the presence or absence of previous PRP before IVB therapy. We found that almost 75% of eyes that had been treated with prior PRP had a good response to IVB therapy alone. These findings suggest that most patients (73%) with active PDR who had previously undergone PRP may be treated with IVB alone. However, in patients with PDR treated with IVB therapy only (19 eyes) (PRP naive), a complete regression of the NV was seen in 42.1% of the treated eyes. The remaining 11 eyes needed additional laser therapy to improve the retinopathy during the monitoring period. DRCR.net reported that there were 58% of eyes in the ranibizumab group, and 54% in the PRP group without signs of PDR, with complete regression of neovascularization at the disk or elsewhere (on fundus photographs) at 2 years. The difference in favorable responses in our IVB-only group (42.1%) and the ranibizumab group (58%) from DRCR.net is likely due to the lower frequency of IVB in our study. In our study, patients treated only with IVB were treated

at a mean interval of 3 months between doses, with a mean of 4 injections with 2 years of follow-up, whereas most patients in the ranibizumab group from DRCR.net received 6 monthly injections initially and many had additional visits and injections throughout the 2 years of follow-up. Another important difference was that our sample size was smaller than the DRCR.net sample (19 eyes in our IVB-only therapy group versus 142 eyes in the ranibizumab group from DRCR.net). Hence, the administration of a loading series of injections to completely suppress the effects of VEGF in eyes with PDR could be more effective than the application of IVB every 3 months. Moradian et al[37] reported the use of IVB for cases with PDR that were not responsive to PRP therapy. The authors[37] observed 2 cases of TRD (5.3% of study eyes) at the end of follow-up. We have previously reported 11 eyes (patients) out of 211 eyes that development or had progression of TRD with decreased BCVA after IVB before vitrectomy for the management of PDR for an incidence of 5.2%.[38] Sinawat et al[23] published a case series of 18 eyes with PDR and VH treated with IVB. The authors[23] observed that 2 (11%) eyes had severe visual loss because of the TRD. In a previous study, we found 25 eyes (patients) (3.5%) out of 698 patients who underwent IVB and developed or had progression of TRD after the injection.[39] Risk factors for TRD after IVB identified in our previous study were long-standing diabetes mellitus of more than 15 years, greater than 13 days from injection to vitrectomy, and the use of a higher dose (2.5 mg) of bevacizumab.[39] In the current study, we included eyes treated with a dose of 1.25 mg/mL of IVB. We observed that 5 (5.2%) cases presented with TRD after IVB therapy. Our study demonstrates that IVB can generate marked regression of RN on fundus examination and FA in patients with PDR, especially in patients with previous PRP. In addition, IVB could be preventing exacerbation of macular edema in patients with concomitant clinically significant macular edema and PDR.

The mean interval between IVB applications was 3 ± 7 months. Therefore, bevacizumab may allow long intervals between PRP sessions to avoid the development of macular edema and other complications.[12–14,40] In addition, bevacizumab could subsequently play a role in the treatment of actively leaking new vessels elsewhere or NVD refractory to laser treatment in patients with PDR.[41] Our findings suggest that IVB might be the first line of treatment in patients with previous PRP and recurrence of neovascular activity. Intravitreal bevacizumab therapy seems to have some advantages over PRP, including better average BCVA, due to either a lower rate of development of DME or a more controlled clinically significant diabetic macular edema, preservation of peripheral visual field sensitivity, and lower rates of vitrectomy.[27] However, regular follow-up is critical to managing patients treated with intravitreal anti-VEGF therapy. Treatment-naive patients who have undergone several IVB treatments but do not regularly present for follow-up may develop recurrent neovascularization leading to vision loss. Similarly, the DRCR.net[27] concluded that among eyes with PDR, treatment with IVR resulted in visual acuity that was noninferior to (not worse than) PRP at 2 years of follow-up.

Limitations of our study include that it is nonrandomized, uncontrolled, and retrospective. In addition, this study included patients from five different centers, and patients were treated according to the discretion of the treating physician. However, the large difference in the quantitative morphologic outcomes and the trend toward improvement in BCVA in injected eyes found at 24 months confirms our hypothesis that at least some eyes with PDR, such as those with previous PRP or preexisting macular edema, may truly benefit from IVB. We also observed that in 42.1% of naive eyes, PDR was controlled or PDR regressed with IVB injections during 24 months of follow-up without requiring additional laser or vitrectomy. This percentage is lower than the results of the IVR group from DRCR.net which reported that 58% of the treated eyes had no signs of PDR or the neovascularization regressed at the end of follow-up. However, in the same study, the percentage of eyes with active neovascularization was 42% in the ranibizumab group and 46% in the PRP group at the end of follow-up, which is similar to the 15 (40.5%) naive eyes treated only with IVB in our study.

In summary, IVB seems to be a promising treatment for PDR, minimizing the risk for exudative complications, progression of RN, VH, and decreased vision caused by macular edema. Intravitreal bevacizumab is a potential adjuvant to PRP for PDR. Although no serious complications of intravitreal injection of bevacizumab occurred in our series, further studies are needed to assess the efficacy and safety of IVB in the management of PDR.

Key words: bevacizumab, diabetic retinopathy, intravitreal injections, proliferative, retinal neovascularization.

Acknowledgments

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Africa. This article is part of his PhD thesis on "Intravitreal Bevacizumab as Anti-Vascular Endothelial Growth Factor in the Management of Complications of Diabetic Retinopathy."

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Appendix 1.

The following investigators belong to the Pan-American Collaborative Retina Study Group (PACORES):

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Chapter 9: Intravitreal Bevacizumab as an Adjuvant to Vitrectomy in Tractional Retinal Detachment at 2 years of follow up

Arevalo JF, Serrano MA, Arias JD. Perfluorocarbon in vitreoretinal surgery and preoperative bevacizumab in diabetic tractional retinal detachment. World J Diabetes. 2014 Oct 15;5(5):724-9. doi: 10.4239/wjd.v5.i5.724.

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Hypothesis 4: Preoperative IVB may be beneficial for membrane dissection in diabetic tractional retinal detachment with minimally invasive vitreoretinal surgery (23-gauge transconjunctival sutureless vitrectomy [TSV]). In addition, post-operative rebleeding may be decreased.

AIM: To describe the en bloc perfluorodissection (EBPD) technique and to demonstrate the applicability of using preoperative intravitreal bevacizumab during small-gauge vitreoretinal surgery (23-gauge transconjunctival sutureless vitrectomy) in eyes with advanced proliferative diabetic retinopathy (PDR) with tractional retinal detachment (TRD).

METHODS: This is a prospective, interventional case series. Participants included 114 (eyes) with advanced proliferative diabetic retinopathy and TRD. EBPD was performed in 114 eyes (consecutive patients) during 23-gauge vitrectomy with the utilization of preoperative bevacizumab (1.25 mg/0.05 mL). Patients mean age was 45 years (range, 21-85 years). Surgical time had a mean of 55 min (Range, 25-85 min). Mean follow up of this group of patients was 24 mo (range, 12-32 mo). Main outcome measures included best-corrected visual acuity (BCVA), retinal reattachment, and complications.

RESULTS: Anatomic success occurred in 100% (114/114) of eyes. Significant visual improvement [≥ 2 Early Treatment Diabetic Retinopathy Study (ETDRS) lines] was obtained in 69.2% (79/114), in 26 eyes (22.8%) BCVA remained stable, and in 8 eyes (7%) BCVA decreased (≥ 2 ETDRS lines). Final BCVA was 20/50 or better in 24% of eyes, between 20/60 and 20/400 in 46% of eyes, and worse than 20/400 in 30% of eyes. Complications included cataract in 32 (28%) eyes, iatrogenic retinal breaks in 9 (7.8%) eyes, vitreous hemorrhage requiring another procedure in 7 (6.1%) eyes, and phthisis bulbi in 1 (0.9%) eye.

CONCLUSION: This study demonstrates the usefulness of using preoperative intravitreal bevacizumab and EBPD during small-gauge vitreoretinal surgery in eyes with TRD in PDR.

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PROSPECTIVE STUDY

Perfluorocarbon in vitreoretinal surgery and preoperative bevacizumab in diabetic tractional retinal detachment

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Abstract

AIM: To describe the en bloc perfluorodissection (EBPD) technique and to demonstrate the applicability of using preoperative intravitreal bevacizumab during small-gauge vitreoretinal surgery (23-gauge transconjunctival sutureless vitrectomy) in eyes with advanced proliferative diabetic retinopathy (PDR) with tractional retinal detachment (TRD).

METHODS: This is a prospective, interventional case series. Participants included 114 (eyes) with advanced proliferative diabetic retinopathy and TRD. EBPD was performed in 114 eyes (consecutive patients) during 23-gauge vitrectomy with the utilization of preoperative bevacizumab (1.25 mg/0.05 mL). Patients mean age was 45 years (range, 21-85 years). Surgical time had a mean of 55 min (Range, 25-85 min). Mean follow up of this group of patients was 24 mo (range, 12-32 mo). Main outcome measures included best-corrected visual acuity (BCVA), retinal reattachment, and complications.

RESULTS: Anatomic success occurred in 100% (114/114) of eyes. Significant visual improvement [≥ 2 Early Treatment Diabetic Retinopathy Study (ETDRS) lines] was obtained in 69.2% (79/114), in 26 eyes (22.8%) BCVA remained stable, and in 8 eyes (7%) BCVA decreased (≥ 2 ETDRS lines). Final BCVA was 20/50 or better in 24% of eyes, between 20/60 and 20/400 in 46% of eyes, and worse than 20/400 in 30% of eyes. Complications included cataract in 32 (28%) eyes, iatrogenic retinal breaks in 9 (7.8%) eyes, vitreous hemorrhage requiring another procedure in 7 (6.1%) eyes, and phthisis bulbi in 1 (0.9%) eye.

CONCLUSION: This study demonstrates the usefulness of using preoperative intravitreal bevacizumab and EBPD during small-gauge vitreoretinal surgery in eyes with TRD in PDR.

Key words: Avastin; Intravitreal bevacizumab; Intravitreal injections; Proliferative diabetic retinopathy; Tractional retinal detachment; Perfluorodissection; Minimally invasive vitreoretinal surgery; Vitrectomy

Core tip: *En bloc* perfluorodissection and preoperative intravitreal bevacizumab use for small-gauge vitrectomy in patients with proliferative diabetic retinopathy and tractional retinal detachment are very useful, the combination reduces complications and operative time. *En bloc* perfluorodissection and preoperative intravitreal bevacizumab use seems to have many advantages including that the retina remains stable during vitrectomy, better visibility of the ocular structures in the vitreous cavity, immediate reattachment of the retina,

bleeding control, subretinal fluid reabsorbsion and drainage, bleeding sites' tamponade, and easier dissection of epiretinal tissues.

Arevalo JF, Serrano MA, Arias JD. Perfluorocarbon in vitreoretinal surgery and preoperative bevacizumab in diabetic tractional retinal detachment. *World J Diabetes* 2014; 5(5): 724-729 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/724.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.724

INTRODUCTION

Pars plana vitrectomy is a successful surgical technique for the complications of proliferative diabetic retinopathy (PDR)[1,2]. It is usually necessary within one year in up to 10% of patients presenting with PDR[3]. The commonest indication for surgery is non-clearing vitreous hemorrhage. Unfortunately[1,2], postoperative vitreous hemorrhage is a significant complication occurring in about 20% to 30% of cases[4-10].

Some advances in surgical techniques and instrumentation, such as; *en bloc* dissection, delamination, segmentation, and bimanual surgical techniques, have allowed better results in the treatment of severe PDR[11-13]. Viscodissection, described by Stenkula and Tornquist[12], and the use of perfluorocarbon liquids (PFCL), introduced as a surgical adjuvant in vitrectomy in 1987 by Chang *et al*[14], facilitate removal of epiretinal membranes, the management of proliferative vitreoretinopathy (PVR) with retinal detachment, tractional retinal detachments in diabetics, and control of intraoperative hemorrhage.

Quiroz-Mercado *et al*[15,16] published a technique called perfluorocarbon-perfused vitrectomy (PCPV). In their technique, PFCL is used in the infusion in a continuous way during vitrectomy. In selected cases PFCL may offer several advantages over saline solution, because of their properties including gravitational forces, immiscibility with fluids, and ability to transport oxygen[15,16]. Regardless of PFCL's advantages, the use of PCPV has not extended worldwide. In addition, PCPV utilizes a considerable amount of PFCL, and membranes may be pushed against the retina during PCPV.

We have previously described "*En bloc* perfluorodissection" (EBPD), which combines the advantages of viscodissection and PCPV. EBPD helps the surgeon during removal of membranes over the retina and to create a posterior vitreous detachment by injecting PFCL between the retina and the posterior hyaloid separating tissues over the retina[17,18]. In addition, identification and removal of all posterior vitreoretinal traction is very important. Furthermore, vitreoschisis can also occur in patients with PDR, it is important to identify this feature and to perform dissection in the true vitreoretinal plane, to avoid recurrent traction and postoperative bleeding from retinal neovascularization[19].

Postoperative vitreous cavity hemorrhage is a significant complication following vitrectomy for the treatment of PDR. It has two main forms, "early" when hemorrhage (bleeding) is present in the first few postoperative days and "late", when hemorrhage occurs a number of months after surgery. The presence of postoperative vitreous hemorrhage delays visual recovery can lead to elevated pressure within the eye and can make further treatment for diabetic retinopathy difficult. Revision surgery is required in 10% of patients, which has significant implications for resources, time and cost. The use of anti-vascular endothelial growth factor (anti-VEGF) before surgery (preoperatively) has been proposed as an intervention to reduce the incidence of postoperative vitreous hemorrhage[20].

Recently, it has been reported that intravitreal bevacizumab in patients with vitreous hemorrhage and PDR resulted in regression of retinal neovascularization and resolution of vitreous hemorrhage[21]. Chen *et al*[22] and Avery *et al*[23], have reported that preoperative intravitreal bevacizumab (Avastin®, Genentech Inc., San Francisco, CA) reduce the risk of bleeding during vitrectomy facilitating the removal of fibrovascular tissues.

The aim of this article is to describe the surgical technique and demonstrate the usefulness of combining *en bloc* perfluorodissection and preoperative intravitreal bevacizumab use for membrane peeling in tractional retinal detachment in advanced diabetic retinopathy with small-gauge vitreoretinal surgery (23-gauge transconjunctival sutureless vitrectomy).

MATERIALS AND METHODS

This is a prospective, interventional case series. One hundred fourteen (eyes) with tractional retinal detachment (TRD) in PDR participated. The authors performed EBPD in 114 eyes (consecutive patients) during 23-gauge transconjunctival sutureless vitrectomy for tractional retinal detachment in severe PDR with the utilization of preoperative bevacizumab (1.25 mg/0.05 mL). Main outcome measures were best-corrected visual acuity (BCVA), retinal status, and complications. This study has been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki and it was approved by the Institution's Ethics Committee.

An aliquot of commercially available bevacizumab was prepared for each patient and placed in a tuberculin syringe using aseptic techniques. Four days before vitrectomy, after preparation of the eye using 5% povidone/iodine, an eyelid speculum was used to open the eyelids, and the injection of 1.25 mg (0.05 mL) of bevacizumab was performed 4 mm posterior to the limbus, through the superotemporal or inferotemporal pars plana with a 30-gauge needle under topical anesthesia. After the injection, retinal artery perfusion was checked with the indirect ophthalmoscope. In none of our cases an anterior chamber paracenthesis was necessary. No topical antibiotics were administered preoperatively.

A 23-gauge transconjunctival sutureless vitrectomy was performed in all cases. A core vitrectomy is done first to clear any vitreous hemorrhage present. A hole is then

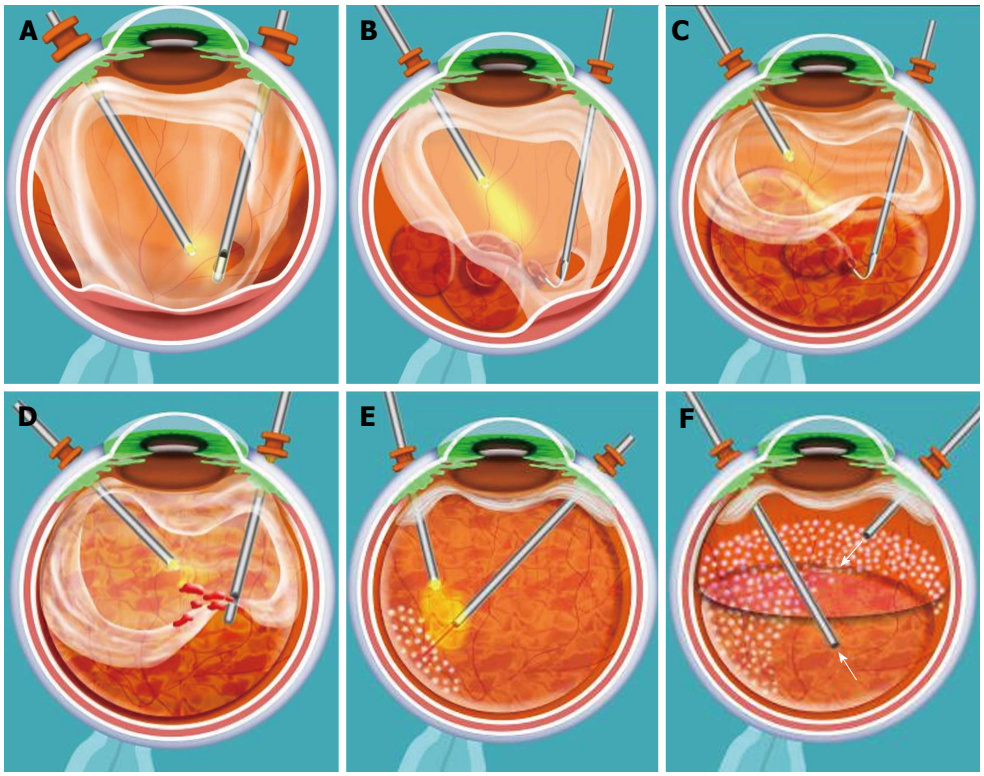

Figure 1 Artist's representation of surgical technique. A: An opening is made with the vitrector in the mid-periphery of the posterior hyaloid; B and C: Perfluorocarbon liquid (PFCL) is injected to separate the posterior hyaloid from the retina. A dual bore cannula (for 23-gauge cases) attached to a 5 cc syringe filled with PFCL is used to separate membranes and posterior hyaloid from the underlying retina; D: Once all the tissues have been separated from the retina, vitrectomy can be continued up to the periphery; E: Endolaser is applied under PFCL; F: An air-fluid and an air-gas (C3F8) exchange exchange are performed to end the case.

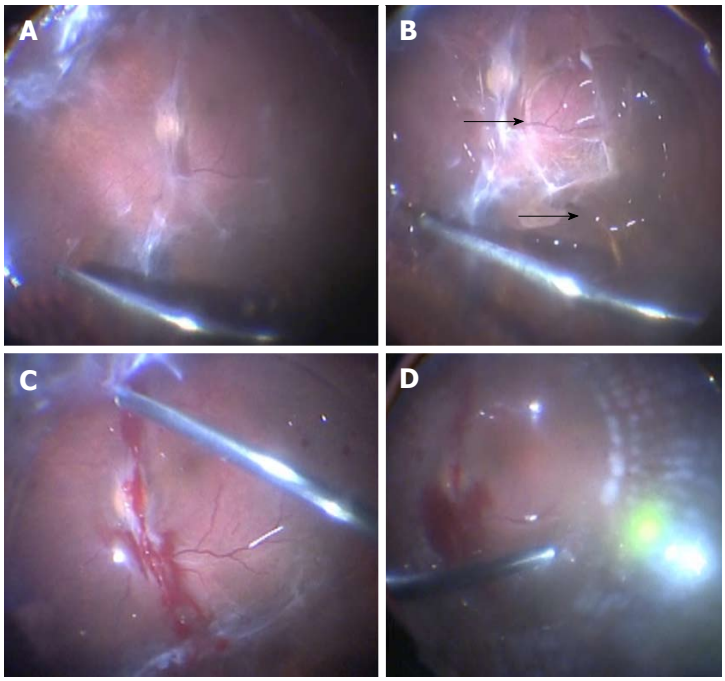

Figure 2 En bloc perfluorodissection performed in a case of tractional retinal detachment in proliferative diabetic retinopathy. A: An opening is made with the vitrector in the mid-periphery of the posterior hyaloid; B: Perfluorocarbon liquid (PFCL) is injected to separate the posterior hyaloid from the retina (arrows). A dual bore cannula (for 23-gauge cases) attached to a 5 cc syringe filled with PFCL is used to separate membranes and posterior hyaloid from the underlying retina; C: Once all the tissues have been separated from the retina, vitrectomy can be continued up to the periphery; D: Endolaser is applied under PFCL (shown). An air-fluid and an air-gas (C3F8) exchange are performed to end the case (not shown).

made in the mid-peripheral posterior hyaloid (Figures 1A and 2A) to inject the perfluorocarbon liquid (PFCL) [Perfluorooctane (C_8F_{18})] and mechanically detach the posterior hyaloid from the retina (Figures 1B, 1C and 2B). We

use a 23-gauge Dual Bore cannula (Dual Bore cannula 0.6 mm, MedOne, Sarasota, FL) attached to a 5 cc syringe filled with PFCL to separate the posterior hyaloid and membranes from the retina. After all the membranes and posterior hyaloid have been separated from the retina, vitrectomy is completed up to the periphery (Figures 1D and 2C), endolaser is applied (Figures 1E and 2D), an air-fluid and air-gas [Perfluoropropane (C_3F_8), Escalon Medical Corporation, New Berlin, WI] exchange is performed to finish the case (Figure 1F).

Non-illuminated instrumentation was usually used in our cases[7] combined with a non-contact wide-angle viewing system (BIOM, Oculus, Wetzlar, Germany). An illuminated cannula was utilized (25ga, Awh chandelier, Synergetics Inc., O'Fallon, MO) in some cases for bimanual surgery.

RESULTS

Patients were prospectively enrolled from January 2006 to January 2010 at Clinica Oftalmologica Centro Caracas in Caracas, Venezuela. Inclusion criteria included patients with TRD in advanced PDR and macular involvement or impending macular involvement with or without vitreous hemorrhage. EBPD was performed in 114 consecutive eyes (patients) during small-gauge vitrectomy for severe PDR with TRD. The mean age of the patients was 45 years (range, 21-85 years). Surgical time had a mean of 55 min (Range, 25-85 min). Mean follow up of our patients was 24 mo (range: 12-32 mo).

Each patient underwent BCVA measurement with ETDRS. Patients were followed postoperatively on day 1, at one week, at three weeks, at 7 wk, and every 3 mo with complete eye examination at each visit, including BCVA, anterior segment examination, IOP determination, and fundus biomicroscopy. Patients were included only with a minimum 12 mo of follow-up. An increase or decrease in BCVA was considered to have occurred if there was a change of two or more Early Treatment Diabetic Retinopathy Study (ETDRS) lines. Main outcome measures were changes in BCVA, and retinal reattachment.

En bloc perfluorodissection was performed using a mean volume of PFCL of 4 mL (range: 3 to 8 mL). No patients in our series have shown ocular hypertension or inflammation. Anatomic success occurred in 100% (114/114) of eyes. Significant visual improvement ($\geq$ 2 ETDRS lines) was seen in 69.2% (79/114), in 26 eyes (22.8%) BCVA remained stable, and in 8 eyes (7%) BCVA decreased ($\geq$ 2 ETDRS lines). Final BCVA was 20/50 or better in 24%, between 20/60 and 20/400 in 46%, and worse than 20/400 in 30%. Complications included cataract in 32 (28%) eyes, iatrogenic retinal breaks in 9 (7.8%) eyes, vitreous hemorrhage requiring another procedure in 7 (6.1%) eyes, and phthisis bulbi in 1 (0.9%) eye.

DISCUSSION

In selected cases *en bloc* perfluorodissection during vitrectomy in eyes with TRD in PDR and preoperative use of intravitreal bevacizumab, we can obtained an anatomic (100%) and functional success (69.2%). Other benefits of this technique include that the retina remains stable during vitrectomy, less blood in the vitreous cavity, rapid retinal reattachment, better visualization of vitreous and intraocular structures, blood confinement, and easier dissection of epiretinal membranes.

In our study, the authors have not seen any difficulties with the technique. However, in one case PFCL was injected within a vitreous schisis. After a short amount of instillation (1 mL) that situation was apparent, and PFCL was aspirated and a new hole in the posterior hyaloid was made at another location making sure that the proper plane was found between the posterior hyaloid and the retina this time. No complications rose from this event. In addition, there were 2 eyes (1.7%) with subretinal PCL that were solved with a peripheral retinotomy, aspiration with an extrusion cannulae, and the injection of additional PCL in the posterior pole. In our study the prevalence of postoperative vitreous hemorrhage was lower (6.1%) than that reported in other studies (20% to 30%)[4-10] which can be explained by the use of intravitreal bevacizumab 4 d preoperatively.

Surgeons with extensive experience can manage complex retinal detachments in patients with TRD using either viscodissection or conventional techniques with pick and scissors. Thus, surgeons should deal with these cases selectively according to their level of experience. An ideal case for EBPD might be one in which there is a TRD with no tears, with limited posterior vitreous detachment, and relatively loose attachment of the posterior hyaloid to the retina. We use a combination of several techniques in our cases including EBPFD, and the use of picks and forceps with bimanual surgery. Currently, the use of small-gauge vitreoretinal surgery (23-gauge transconjunctival sutureless vitrectomy) and preoperative intravitreal bevacizumab for TRD in diabetics have improved our surgical time and results.

In the future, MIVS with 23-gauge transconjunctival sutureless vitrectomy techniques will be increasingly performed in diabetic patients due to the increased incidence of diabetes and its complications. In the coming years we will use techniques that are less invasive in vitreoretinal surgery such as 25+, and 27-gauge. We will have available other anti-VEGF antibodies capable of blocking all types of VEGF isoforms before and after surgery, reducing intraoperative bleeding, and postoperative inflammation. It is likely that the use of preoperative agents that promote the detachment of the posterior hyaloid and facilitate the removal of membranes will become routine. They will facilitate surgery of complex cases such as PDR cases. Optical coherence tomography equipment will be available in the operating room and that will facilitate intraoperative tissue differentiation, and help us get better functional results. The advent of new lasers will permit us faster retinal photocoagulation, and will minimize collateral damage of the retina.

In summary, EBPD and preoperative intravitreal bevacizumab use for vitrectomy in eyes with TRD in PDR it is very useful. *En bloc* perfluorodissection and preoperative intravitreal bevacizumab use seems to have many advantages including that the retina remains stable during vitrectomy, better visibility of intraocular structures, immediate reattachment of the retina, bleeding control, reabsorbsion and drainage of subretinal fluid, bleeding sites' tamponade, and easier dissection of epiretinal tissues.

ACKNOWLEDGMENTS

Dr. Arevalo is a PhD student at Faculty of Health Sciences, Stellenbosch University, Stellenbosch, South Africa. This article is part of his PhD thesis on "Intravitreal Bevacizumab as Anti-Vascular Endothelial Growth Factor in the Management of Complications of Diabetic Retinopathy".

COMMENTS

Background

Authors have previously described a new surgical dissection technique, namely "*En bloc* perfluorodissection" (EBPD), which combines the advantages of viscodissection and perfluorocarbon-perfused vitrectomy. EBPD helps the surgeon during removal of epiretinal membranes and to detach the posterior hyaloid by injecting perfluorocarbon liquid between the retina and the posterior hyaloid to separate the epiretinal tissues from the retina.

Research frontiers

The objective of this article is to describe the surgical technique and demonstrate the usefulness of combining *en bloc* perfluorodissection and preoperative intravitreal bevacizumab use for membrane peeling in tractional retinal detachment in advanced diabetic retinopathy with small-gauge vitreoretinal surgery (23-gauge transconjunctival sutureless vitrectomy).

Innovations and breakthroughs

En bloc perfluorodissection and preoperative intravitreal bevacizumab use seems to have many advantages including that the retina remains stable during vitrectomy, better visibility of vitreous and intraocular structures, immediate retinal reattachment, bleeding control in the vitreous cavity, subretinal fluid reabsorbsion and drainage, bleeding sites' tamponade, and easier dissection of epiretinal tissues.

Applications

En bloc perfluorodissection and preoperative intravitreal bevacizumab use for vitrectomy in eyes with tractional retinal detachment in advanced proliferative diabetic retinopathy it is very useful technique, reduces complication and operative time.

Peer review

The report is interesting, well documented, and the paper should be published.

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Chapter 10: Tractional Retinal Detachment Following Intravitreal Bevacizumab in patients with Severe Proliferative Diabetic Retinopathy

Arevalo JF, Maia M, Flynn HW Jr, Saravia M, Avery RL, Wu L, Eid Farah M, Pieramici DJ, Berrocal MH, Sanchez JG. Tractional retinal detachment following intravitreal bevacizumab (Avastin) in patients with severe proliferative diabetic retinopathy. Br J Ophthalmol. 2008 Feb;92(2):213-6. Epub 2007 Oct 26.

DOI: 10.1136/bjo.2007.127142

Hypothesis 5: Tractional retinal detachment (TRD) may occur following IVB as an adjuvant to vitrectomy for the management of severe PDR.

AIMS: The aim of this study was to report the development or progression of tractional retinal detachment (TRD) after the injection of intravitreal bevacizumab (Avastin) used as an adjuvant to vitrectomy for the management of severe proliferative diabetic retinopathy (PDR).

METHODS: The clinical charts of patients who experienced the development or progression of TRD after an intravitreal injection of 1.25 mg bevacizumab before vitrectomy for the management of PDR were reviewed.

RESULTS: Eleven eyes (patients) out of 211 intravitreal injections (5.2%) that developed or had progression of TRD were identified. All eyes had PDR refractory to panretinal photocoagulation (PRP). Nine patients had type 1 diabetes mellitus (DM), and two patients had type 2 DM. Patients had a mean age of 39.5 years (range 22-62 years). In the current study, all patients used insulin administration and had poor glycaemic control (mean HbA(1c) 10.6%). Time from injection to TRD was a mean of 13 days (range 3-31 days). Mean best correct visual acuity (BCVA) at TRD development or progression was logarithm of the minimal angle of resolution (LogMAR) 2.2 (range 1.0-2.6) (mean Snellen equivalent hand motions; range 20/200 to light perception), a statistically significant worsening compared with baseline BCVA ($p<0.0001$). Eight eyes underwent vitrectomy and three patients refused or were unable to undergo surgery. The final mean BCVA after surgery was LogMAR 0.9 (range 0.2-2.0) (mean Snellen equivalent 20/160; range 20/32 to counting fingers), a statistically significant improvement compared with TRD BCVA ($p = 0.002$).

CONCLUSIONS: TRD may occur or progress shortly following administration of intravitreal bevacizumab in patients with severe PDR.

Tractional retinal detachment following intravitreal bevacizumab (Avastin) in patients with severe proliferative diabetic retinopathy

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ABSTRACT

Aims: The aim of this study was to report the development or progression of tractional retinal detachment (TRD) after the injection of intravitreal bevacizumab (Avastin) used as an adjuvant to vitrectomy for the management of severe proliferative diabetic retinopathy (PDR).

Methods: The clinical charts of patients who experienced the development or progression of TRD after an intravitreal injection of 1.25 mg bevacizumab before vitrectomy for the management of PDR were reviewed.

Results: Eleven eyes (patients) out of 211 intravitreal injections (5.2%) that developed or had progression of TRD were identified. All eyes had PDR refractory to panretinal photocoagulation (PRP). Nine patients had type 1 diabetes mellitus (DM), and two patients had type 2 DM. Patients had a mean age of 39.5 years (range 22–62 years). In the current study, all patients used insulin administration and had poor glycaemic control (mean HbA_{1c} 10.6%). Time from injection to TRD was a mean of 13 days (range 3–31 days). Mean best correct visual acuity (BCVA) at TRD development or progression was logarithm of the minimal angle of resolution (LogMAR) 2.2 (range 1.0–2.6) (mean Snellen equivalent hand motions; range 20/200 to light perception), a statistically significant worsening compared with baseline BCVA ($p<0.0001$). Eight eyes underwent vitrectomy and three patients refused or were unable to undergo surgery. The final mean BCVA after surgery was LogMAR 0.9 (range 0.2–2.0) (mean Snellen equivalent 20/160; range 20/32 to counting fingers), a statistically significant improvement compared with TRD BCVA ($p = 0.002$).

Conclusions: TRD may occur or progress shortly following administration of intravitreal bevacizumab in patients with severe PDR.

Proliferative diabetic retinopathy (PDR) is a major cause of visual loss in patients with diabetes mellitus.[1–3] Studies have demonstrated not only a correlation of vascular endothelial growth factor (VEGF) levels with the severity of PDR, but also a reduction in levels after successful laser treatment of PDR.[4 5] Thus a rational approach to treating neovascularisation in these patients would include the use of anti-VEGF agents.[6–8]

Bevacizumab (Avastin; Genentech Inc., San Francisco, CA, USA) is a full length humanised antibody that binds to all subtypes of VEGF and has been approved by the US Food and Drug Administration for the treatment of metastatic colorectal cancer.[9] Recent reports have suggested that bevacizumab may be useful in the treatment of choroidal neovascularisation, diabetic macular oedema, PDR and macular oedema associated with retinal venous occlusive disease.[7 10–15]

Panretinal photocoagulation (PRP) is currently the mainstay of therapy for PDR, significantly reducing the risk of blindness in these patients.[16 17] However, there are instances when it is difficult or impossible to administer PRP because of media opacity, such as vitreous haemorrhage. There are also cases that do not respond with complete regression even after extensive PRP: in such cases intravitreal bevacizumab could be considered as an alternative salvage therapy.

Recently, it has been reported that intravitreal injection of bevacizumab may be also useful for early vitreous haemorrhage in PDR in order to decrease the risk of new haemorrhages while clearing occurs and to minimise the indications of vitrectomy.[18] In addition, Chen and Park[19] and Avery *et al*[20] have suggested that preoperative intravitreal bevacizumab might be helpful to facilitate vitrectomy in severe PDR cases. In such cases, the preoperative use of bevacizumab might reduce the risk of intraoperative bleeding facilitating the removal of fibrovascular membranes, particularly when preoperative PRP cannot be placed.

The purpose of this retrospective case series is to report the development or progression of tractional retinal detachment (TRD) following intravitreal bevacizumab as an adjuvant to vitrectomy for the management of severe PDR.

PATIENTS AND METHODS

We reviewed the medical records and obtained follow-up information on all patients in our files with TRD who had undergone intravitreal injection of 1.25 mg bevacizumab before vitrectomy for the management of PDR from September 2005 to November 2006 at seven centres from Brazil, Argentina, the USA, Costa Rica, Puerto Rico and Venezuela. The ''off-label'' status of this medication, and possible systemic and ocular complications, were discussed in detail and informed consent was obtained from all patients. Institutional review board/ethics committee approval was obtained for this study at all seven institutions.

Pre-injection examination included Snellen or Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity measurement, slit-lamp biomicroscopy and dilated fundus examination. Although not a formal exclusion criterion, patients

with a history of uncontrolled hypertension and recent thromboembolic events were not usually injected with bevacizumab; however, this decision was taken at the discretion of the treating physician. Inclusion criteria for the use of intravitreal bevacizumab before vitrectomy for the management of PDR included TRD, persistent vitreous haemorrhage and incomplete regression even after extensive PRP.

An aliquot of commercially available bevacizumab (0.18 ml) was prepared for each patient and placed in a tuberculin syringe using aseptic techniques. After the eye had been prepared in a standard fashion using 5% povidone/iodine, an eyelid speculum was used to stabilise the eyelids, and the injection of 1.25 mg (0.05 ml) bevacizumab was performed 3.5–4 mm posterior to the limbus, through the pars plana with a 30-gauge needle under topical anaesthesia or subconjunctival lidocaine. Following the injection, intraocular pressure and retinal artery perfusion were confirmed, and patients were instructed to administer topical antibiotics for 3–7 days.

Statistical analysis was performed using GraphPad Instat Version 3.0 for Mac OSX (GraphPad Software, San Diego, CA, USA). Patients' BCVAs were transferred from their records and converted to a logarithm of the minimal angle of resolution (logMAR) scale for analysis. The paired t test was used to compare mean values to analyse mean logMAR visual acuity statistically. Correlation was considered significant if the p value was $\leqslant 0.05$.

RESULTS

Eleven eyes (patients) out of 211 intravitreal injections (5.2%) that developed or had progression of TRD were identified. All patients had had a PRP at least 2 months before intravitreal bevacizumab. All eyes had PDR refractory to PRP. Eight (72.7%) patients were white and 3 (27.3%) patients were Hispanic. The mean age of the study group was 39.5 years (range 22–62 years), and 54.5% were male (six men and five women). Nine patients had type 1 diabetes mellitus (DM) with more than 15.5 years from diagnosis (range 14–30 years), and two patients had type 2 DM with 1 year from diagnosis. In the current study, all patients used insulin administration for glycaemic control and had uncontrolled diabetes associated with elevated glycosylated haemoglobin (mean HbA_{1c} 10.6%). Seven eyes had local TRD on indirect ophthalmoscopy, ultrasound, or biomicroscopic non-contact fundus examination with a 66- or a 78-diopter lens before intravitreal bevacizumab. The clinical findings of all 11 eyes with PDR and TRD after intravitreal bevacizumab are presented in table 1.

Time from injection to TRD was a mean of 13 days (range 3–31 days). The mean baseline (before intravitreal bevacizumab) BCVA was LogMAR 0.8 (range 0.3–1.6) (mean Snellen equivalent 20/125; range 20/40 to 5/200). At TRD development or progression, the mean BCVA was LogMAR 2.2 (range 1.0–2.6) (mean Snellen equivalent hand motions (HM); range 20/200 to light perception (LP)), a statistically significant worsening compared with baseline BCVA ($p<0.0001$). One patient (case 9) developed a retinal break as a result of the increased traction, and a combined total tractional-rhegmatogenous retinal detachment was apparent 3 weeks after intravitreal bevacizumab.

Eight eyes underwent vitrectomy, two patients refused or were unable to undergo surgery, and in one patient surgery was not recommended. Vitrectomy was performed within 10 days after the development or progression of TRD. Tractional retinal detachments were managed with vitrectomy, membranectomy, photocoagulation and extended intraocular tamponade with gas

Table 1 Clinical findings of patients with TRD after intravitreal bevacizumab in PDR

Patient no.	Sex	Age (years)	DM type (1 or 2)	Time with DM (years)	HbA_{1c} (%)	Previous PRP	Previous vitrectomy	Retinal status before bevacizumab	Baseline BCVA	Bevacizumab dose (mg)	[Bevacizumab-TRD] (days)	BCVA after bevacizumab	Reason for decreased BCVA after bevacizumab	[Bevacizumab-Vit] (days)	BCVA after surgery
1	M	35	1	20	11	Yes	No	Partial RD	20/200	1.25	3	HM	Progression of RD	3	CF at 1 m
2	M	37	1	22	12	Yes	No	VH/no RD	10/200	1.25	5	CF at 2 m	Development of RD	5	10/200
3	M	39	1	19	10.9	Yes	No	Partial RD	20/200	1.25	17	HM	Progression of RD	17	20/63
4	M	34	1	21	10	Yes	No	Partial RD	20/40	1.25	12	HM	Progression of RD	12	20/32
5	F	27	1	18	12	Yes	No	Partial RD	20/100	1.25	5	LP	Progression of RD	5	20/200
6	F	22	1	14	9.5	Yes	No	Unresponsive PDR/no RD	20/160	1.25	5	LP	VH/development of RD	5	20/50
7	M	45	2	1	11.4	Yes	No	Partial RD	20/80	1.25	30	HM	VH/progression of RD	30	CF at 1 m
8	F	56	1	26	10.5	Yes	No	Unresponsive PDR/no RD	20/63	1.25	21	20/200	Development of RD	Refused surgery	N/A
9	M	31	1	18	10.1	Yes	No	Partial RD	20/50	1.25	21	LP	Combined RD	Surgery not recommended	N/A
10	F	62	2	NA	NA	Yes	No	Partial RD	20/200	1.25	14	20/400	Progression of RD	Refused surgery	N/A
11	M	46	1	30	8.0	Yes	No	VH/no RD	10/400	1.25	3	HM	Development of RD	20	20/40

[Bevacizumab-TRD], time from injection to TRD; [Bevacizumab-Vit], time of surgery after ITV bevacizumab; IVT, intravitreal; F, female; M, male; N/A, not applicable; NA, not available; RD, retinal detachment; VH, persistent vitreous haemorrhage.

in all patients that underwent surgery. Final mean BCVA after surgery was LogMAR 0.9 (range 0.2–2.0) (mean Snellen equivalent 20/160; range 20/32 to counting fingers (CF)), a statistically significant improvement compared with TRD BCVA ($p = 0.002$). Sub-group analysis of final BCVA after vitrectomy demonstrated that all eight (100%) eyes improved two or more ETDRS lines of BCVA compared with TRD BCVA. However, compared with baseline BCVA, final BCVA after vitrectomy demonstrated that four (50%) eyes improved, two (25%) eyes remained stable, and two (25%) eyes lost two or more ETDRS lines of BCVA (table 1).

Selected case reports

Case 6

A 22-year-old woman with a history of poorly controlled type 1 DM since age 6 years presented with bilateral PDR. She had a history of panretinal photocoagulation in her right eye. At presentation, her BCVA was 20/200 and fundus examination revealed NVD of 6 disc diameters emanating from the optic disc into the vitreous cavity without any signs of bleeding (fig 1A). Intravitreal bevacizumab at a dose of 1.25 mg was injected into the vitreous cavity in preparation for a vitrectomy for incomplete regression even after extensive PRP. Four days later the patient returned complaining of a sudden visual loss in her right eye. A dense vitreous haemorrhage was present. Extensive fibrovascular proliferation extending from the optic nerve into the vitreous cavity was causing a partial TRD (fig 1B). Pars plana vitrectomy was performed 5 days later. Panretinal photocoagulation was completed and 20% SF6 was left as intraocular tamponade. The retina was successfully re-attached and she recovered BCVA of 20/50 at her final follow-up (20 weeks).

Case 7

A 45-year-old Hispanic man presented with a 1-month history of visual loss in his left eye. He had poorly controlled type 2 DM diagnosed 1 year earlier. His vision was 20/80 in his right eye and HM in his left eye. He had severe PDR in the right eye with a small localised TRD along the superior temporal arcade (fig 2A). The left eye had dense vitreous haemorrhage without evidence of retinal detachment by ultrasound. His right eye received an intravitreal injection of 1.25 mg bevacizumab in preparation for a vitrectomy. Three weeks later he complained of sudden loss of vision in his right eye, and was noted to have HM vision due to vitreous haemorrhage and a TRD (fig 2B). Vitrectomy was performed the next day in his right eye. His BCVA returned to 20/400 and has been stable for the past 8 months.

DISCUSSION

Recently, intravitreal bevacizumab has become popular as a preoperative coadjuvant in cases of severe PDR.[19 20] Preoperative suppression of intraocular VEGF should reduce intraoperative haemorrhaging during membrane dissection facilitating the surgery. In our retrospective review, we identified 11 eyes (patients) with development or progression of TRD with decrease BCVA after intravitreal bevacizumab prior to vitrectomy for the management of PDR for an incidence of 5.2%.

The natural course of PDR is characterised by a cycle of proliferation and regression typical of new vessels; proliferation of fibrous tissue accompanying new vessels; formation of adhesions between the fibrovascular proliferations and the posterior vitreous surface; and contraction of the posterior vitreous surface and associated proliferation. The development or progression of TRD in PDR following intravitreal bevacizumab in our patients could have happened by natural history or rapid neovascular involution with accelerated fibrosis and posterior hyaloidal contraction as a response to decreased levels of VEGF.

It could be argued that TRD may develop soon after extensive PRP in diabetes. In addition, all our patients were refractory to extensive PRP. However, all patients had had a PRP at least 2 months before intravitreal bevacizumab. The short time interval between the injection and TRD (mean 13 days; range 3–31 days) suggests a cause–effect relationship. It also suggests that in cases at risk for progression of TRD that might involve the central macular region, timely surgery should be anticipated following intravitreal bevacizumab. Nine out of 11 (81.8%) TRDs developed or progressed 5 days or more after the injection. All patients who developed or had progression of TRD in our study used insulin administration for glycaemic control, and nine patients had elevated HbA_{1c} (≥9.5%; mean 10.6%).

Avery *et al*[20] have reported that diabetic eyes may be very sensitive to intravitreal bevacizumab. In their study, several patients underwent intravitreal injection of lower doses of bevacizumab: 6.2, 12.5, 62, 125 and 625 μg. Biological effects were noted at all doses, sometimes at 24 h. The durability of this effect is unknown, and larger doses may be shown to produce a longer duration of effect, but to use lower doses seems prudent in eyes with pre-existing significant traction.

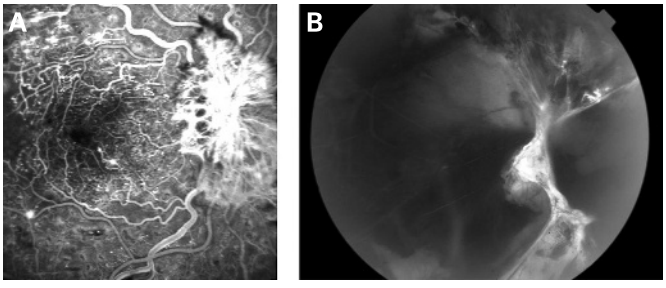

Figure 1 Case 6. (A) Fluorescein angiogram before intravitreal bevacizumab reveals marked hyperfluorescence resulting from leakage of dye from new vessels and fibrous tissue on the disc (NVD). Notice confluent scatter photocoagulation scars. The retina is attached and BCVA is 20/100. (B) Fundus photograph 4 days after 1.25 mg intravitreal bevacizumab demonstrating dense fibrous tissue contraction, vitreous haemorrhage and tractional retinal detachment. BCVA is CF at 2 m.

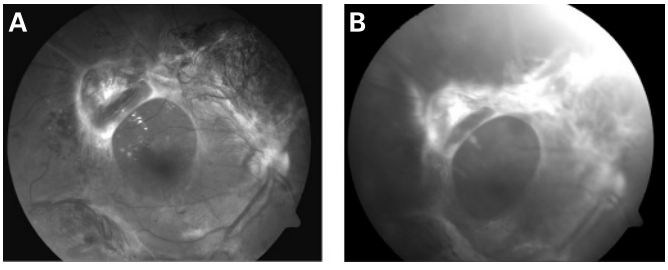

Figure 2 Case 7. Fundus photograph before (A) and after (B) intravitreal bevacizumab. (A) Fundus photograph demonstrates severe proliferative diabetic retinopathy in the right eye with a small localised TRD along the supero-temporal vascular arcade without macular involvement. (B) Fundus photograph 3 weeks after 1.25 mg intravitreal bevacizumab demonstrates progression of partial TRD along the supero-temporal vascular arcade with decrease neovascularisation.

In those eyes that underwent vitrectomy, we had the impression that there was a reduced risk of intraoperative bleeding facilitating the removal of fibrovascular membranes. A bloodless field allows for better visibility and the surgeon may be less likely to create an iatrogenic retinal break. In addition, the chances of postoperative complications such as rebleeding or fibrinoid syndrome may be decreased. All these advantages may allow us to save more eyes by utilising preoperative intravitreal bevacizumab regardless of increased traction on some severe PDR cases. In addition, in our TRD cases that underwent vitrectomy, final BCVA was significantly better than BCVA before surgery and when TRD was present.

In summary, TRD in PDR may occur or progress after intravitreal bevacizumab used as an adjuvant to vitrectomy. Most patients had poorly controlled DM associated with elevated HbA_{1c}, insulin administration, PDR refractory to panretinal photocoagulation, and a longer time interval between intravitreal bevacizumab and vitrectomy.

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Tractional retinal detachment following intravitreal bevacizumab (Avastin) in patients with severe proliferative diabetic retinopathy

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Chapter 11: Risk Factors for the Development of Tractional Retinal Detachment Following Intravitreal Bevacizumab in patients with Severe Proliferative Diabetic Retinopathy

Arevalo JF, Sanchez JG, Saldarriaga L, Berrocal MH, Fromow-Guerra J, Morales-Canton V, Wu L, Maia M, Saravia MJ, Bareño J; Pan American Collaborative Retina Study Group. Retinal detachment after bevacizumab. Ophthalmology. 2011 Nov;118(11):2304.e3-7. doi: 10.1016/j.ophtha.2011.05.015.

DOI: 10.1016/j.ophtha.2011.05.015

Hypothesis 6: Risk factors for the progression or development of TRD following IVB as an adjuvant to vitrectomy in severe PDR may include age, time from diabetes mellitus (DM) diagnosis, glycemic control, cholesterol levels, triglycerides levels, hemoglobin A1c (HbA1C), dose of bevacizumab, and time from injection to vitrectomy.

Twenty-five eyes (patients) (3.5%) out of 698 intravitreal injections (patients) developed or had progression to a TRD after intravitreal bevacizumab. Based on our data, we now believe that extreme care must be taken in using a dose of 2.5 mg or more of bevacizumab in patients with PDR. In addition, to have more than 15 years with a diagnosis of diabetes can increase the risk of TRD and that careful follow-up evaluation following injection is recommended. The timing of surgery after the injection is also important, as there are concerns that bevacizumab may cause progression of the TRD. It is important that surgery is performed once the anti-angiogenic effect of bevacizumab has fully developed, but before there is further fibrous proliferation; physicians must be prepared to perform the vitrectomy preferably before about 13 days after the application of bevacizumab and to perform a vitrectomy immediately on those patients in whom a TRD occurs.

Retinal Detachment after Bevacizumab

Dear Editor:

We have previously reported 11 eyes (patients) out of 211 intravitreal injections with development or progression of tractional retinal detachment (TRD) with decrease best-corrected visual acuity (BCVA) after intravitreal bevacizumab (Avastin; Genentech Inc., San Francisco, CA) prior to vitrectomy for the management of proliferative diabetic retinopathy (PDR) for an incidence of 5.2%. Most patients had poorly controlled diabetes mellitus associated with elevated HbA1c, insulin administration, PDR refractory to pan-retinal photocoagulation (PRP), and longer time between intravitreal bevacizumab and vitrectomy.[1] The purpose of this study was to determine risk factors for the progression or development of tractional retinal detachment following intravitreal bevacizumab (IVB) as an adjuvant to vitrectomy in severe PDR.

We reviewed the clinical records of 698 intravitreal injections and obtained follow-up information on all patients in our files with TRD who had undergone IVB before vitrectomy for the management of PDR from July 2006 to July 2009 at 6 centers. Clinical parameters of patients previously identified as potential risk factors for TRD were obtained and analyzed and compared with the clinical characteristics of those patients from the same cohort that did not developed a TRD after IVB for PDR. These potential risk factors included systemic and surgical background, age, time from diabetes mellitus (DM) diagnosis, glycemic control, cholesterol levels, triglycerides levels, hemoglobin A1c (HbA1C), dose of bevacizumab, and time from injection to vitrectomy.

Twenty-five eyes (patients) (3.5%) out of 698 intravitreal injections (patients) developed or had progression to a TRD after intravitreal bevacizumab (Figure 1 and Table 1; available at http://aaojournal.org). Of these 698 intravitreal injections, 626 applications were with 1.25 mg of bevacizumab (of which 19 patients had TRD, 3%) and 72 injections with 2.5 mg of bevacizumab (of which 6 patients had TRD, 8.3%). No systemic adverse events such as thromboembolic events were reported. Before the initial bevacizumab injection in patients with TRD, the mean baseline BCVA was logMAR 1.25 (range, 1.046–1.454), SD (0.45) – (mean Snellen equivalent 20/360; range, 20/223 – 20/570). At TRD development or progression, the mean BCVA was logMAR 2.423 (range, 1.869– 2.971) (mean Snellen equivalent Counting Fingers to light perception). Final mean BCVA after bevacizumab and vitrectomy was logMAR 1.029 (range, 0.736–1.322), SD (0.570) – (Snellen equivalent 20/109 – 20/422), a statistically significant improvement ($P<0.0001$) compared with baseline BCVA. We did not observe any statistically significant differences in changes of BCVA between doses of 1.25 and 2.5 mg of IVB. Risk factors for TRD after IVB identified in our study included, more than 15 years from the diagnosis of diabetes mellitus (DM) ($P = 0.009$), (OR = 0.30), (95% CI = 0.10–0.83), (RR = 0.35), more than 13 days from injection to vitrectomy ($P = 0.0001$), (OR = 9.9), (95% CI = 3.4–29), (RR = 6.9) and the use of a higher dose (2.5 mg) of bevacizumab ($P = 0.022$), (OR = 2.7), (95% CI = 1.05–7.18), (RR = 2.38). We did not find a statistical associations between others risk factors and TRD, such as macular thickness ($P = 0.123$), or previous use of bevacizumab ($P = 0.653$). Fibrovascular proliferation was present in most patients with TRD (95% CI = 73.9–99.8). However, no significant correlation was found between TRD and fibrovascular proliferation despite that 94.7% of the patients had this factor present ($P = 0.260$) (Table 2; available at http://aaojournal.org).

Based on our data, we now believe that extreme care must be taken in using a dose of 2.5 mg or more of bevacizumab in patients with PDR. In addition, to have more than 15 years with a diagnosis of diabetes can increase the risk of TRD and that careful follow-up evaluation following injection is recommended. The timing of surgery after the injection is also important, as there are concerns that bevacizumab may cause progression of the TRD. It is important that surgery is performed once the anti-angiogenic effect of bevacizumab has fully developed, but before there is further fibrous proliferation; physicians must be prepared to perform the vitrectomy preferably before about 13 days after the application of bevacizumab and to perform a vitrectomy immediately on those patients in whom a TRD occurs.

Limitations of our study include that it is nonrandomized, uncontrolled, and retrospective, i.e., features which preclude any estimation on the safety of bevacizumab. In addition, because no control group is present we cannot rule out the possibility that TRD is due to the natural history of disease. However, the results suggest the need for further investigation to adequately elucidate the risk factors that are associated with TRD after IVB in PDR.

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Appendix

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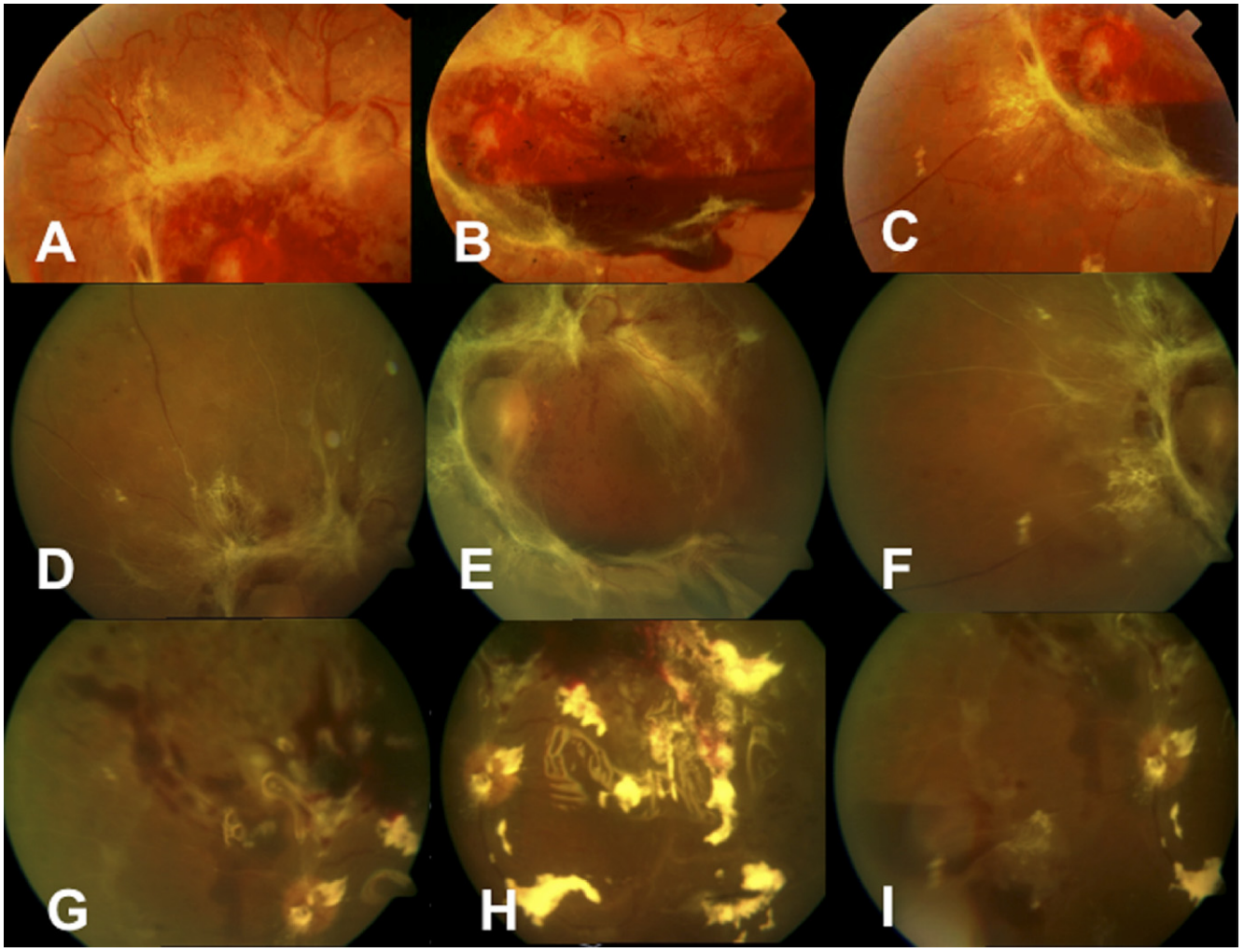

Figure 1. (**A**-**C**) Color photographs before intravitreal bevacizumab. Severe proliferative diabetic retinopathy with abundant fibrovascular tissue and subhyaloid hemorrhage. The retina is attached and best-corrected visual acuity (BCVA) is 20/80. (**D**-**F**) Color photographs 10 days after 2.5 mg of intravitreal bevacizumab demonstrating dense fibrous tissue contraction, and tractional retinal detachment with macular involvement; BCVA is hand motions at 2 meters. (**G**-**I**) Same eye, 8 days after vitrectomy. The retina is re-attached and BCVA is 20/120 with silicone oil tamponade.

Table 1. Clinical Findings of 25 Eyes (Patients) that Developed or

Eye N°	Sex	Age	DM Type	Time with DM Years	History of Smoking	History of HTA	HbA1c (%)	Previous PRP	Macular Thickness/DME (μ)	Fibrovascular Proliferations	Previous Vitrectomy	Previous TRD
1	F	24	1	10	No	Yes	9.06	Yes	450	Yes	No	No
2	M	44	1	11	No	No	8.2	Yes	560	Yes	No	No
3	F	76	2	18	No	No	7	Yes	566	Yes	No	No
4	F	46	2	10	No	No	6.7	No	462	Yes	No	No
5	M	49	2	17	No	No	9	No	670	Yes	No	No
6	F	56	2	18	No	Yes	8.2	Yes	494	Yes	No	No
7	M	65	2	15	No	No	9.28	Yes	848	Yes	No	Partial
8	M	52	2	10	No	Yes	10.21	Yes	1244	Yes	No	Partial
9	M	42	2	10	No	Yes	8.46	Yes	456	Yes	No	No
10	M	65	2	15	No	Yes	7.83	Yes	638	Yes	No	Partial
11	M	58	2	23	No	Yes	10.73	Yes	533	Yes	No	Total
12	F	45	1	30	No	Yes	8.72	Yes	406	Yes	No	Partial
13	M	60	2	15	No	Yes	9.56	Yes	600	Yes	No	No
14	F	41	1	22	No	Yes	7.7	No	512	No	No	No
15	F	67	1	15	No	Yes	7.1	Yes	435	No	No	No
16	M	65	1	19	Yes	Yes	7.8	Yes	621	No	No	Total
17	F	61	1	7	Yes	Yes	7.9	Yes	448	No	No	No
18	M	67	2	15	No	Yes	14	Yes	459	Yes	No	Total
19	F	49	2	25	No	Yes	14.3	Yes	288	No	No	No
20	F	59	2	15	No	No	13.1	Yes	675	Yes	No	Partial
21	M	63	2	5	No	Yes	6.8	Yes	551	Yes	No	Total
22	F	47	2	15	No	No	8.2	No	587	Yes	No	Total
23	F	52	2	0	No	No	13.7	Yes	532	Yes	No	No
24	F	52	1	13	No	Yes	8.03	Yes	560	Yes	No	Partial
25	M	56	2	15	No	No	10.53	No	573	No	No	No

*F = Female; M = male; DM = Diabetes Mellitus; HTA = Hypertension; HbA1c = Hemoglobin Glycosylated; PRP = Pan retinal Photocoagulation; Less Bleeding; EMP = Easier Membrane Peeling; FS = Faster Surgery.

Had Progression To a TRD after Intravitreal Bevacizumab*

Dosage of Bevacizumab	Time from Injection to Vitrect Omy (days)	Prior Use of Bevacizumab	Baseline BCVA	BCVA with TRD	BCVA after Vitrectomy for TRD	Reason for Decrease BCVA after Bevacizumab	Findings During Vitrectomy after Bevacizumab	Total Follow-up (weeks)
2.5	6	No	20/63	20/400	20/200	TRD	LB, EMP	16
2.5	31	Yes	20/32	20/320	20.63	TRD	LB, EMP	56
2.5	32	No	20/500	HM	20/1250	TRD	LB, EMP, FS	25
2.5	35	No	20/1250	HM	20/1250	TRD	FS	12
2.5	28	No	20/400	LP	20/800	TRD	LB, EMP, FS	11
2.5	6	No	20/1250		20/1250	TRD	LB	12
1.25	35	No	20/1250	HM	20/400	TRD	EMP, FS	2
1.25	37	Yes	20/400	HM	HM	TRD	EMP, FS	0
1.25	15	Yes	20/400	HM	20/100	TRD	EMP, FS	0
1.25	35	No	20/1250	HM	20/400	TRD	EMP, FS	0
1.25	21	No	20/200	20/400	20/200	TRD	EMP, FS	2
1.25	24	No	20/500	HM	20/32	TRD	EMP, FS	0
1.25	20	No	20/500	HM	20/630	TRD	LB	8
1.25	7	No	20/1250	LP	HM	TRD	LB, EMP, FS	53
1.25	7	No	20/400	HM	20/63	TRD	LB, EMP, FS	43
1.25	5	Yes	20/630	HM	20/40	TRD	LB, EMP, FS	52
1.25	5	Yes	20/400	HM	20/32	TRD	LB, EMP, FS	53
2.5	10	Yes	20/400	LP	NLP	TRD	LB, FS	170
2.5	10	No	20/40	20/100	20/80	TRD	EMP, FS	190
2.5	10	No	20/200	20/320	20/100	TRD	LB, EMP, FS	60
1.25	14	No	20/200	HM	LP	TRD	LB, EMP, FS	114
2.5	14	No	20/400	20/400	20/400	TRD	LB, EMP, FS	110
2.5	16	Yes	20/200	LP	NLP	TRD	LB, EMP, FS	136
1.25	17	No	20/400	HM	20/100	TRD	LB	63
2.5	19	Yes	20/200	20/500	20/200	TRD	FS,EMP	35

DME = Diabetic Macular Edema; RD = Retinal Detachment; TRD = Tractional Retinal Detachment; BCVA = Best Corrected Visual Acuity; LB =

Table 2. Risk factors for Tractional Retinal Detachment*

Risk Factor	P	CI 95%
More than 15 years from the diagnosis of diabetes mellitus	0.009‡	0.10–0.83
More than 13 days from injection to vitrectomy	0.0001‡	3.4–29
Use of a higher dose (2.5 mg) of bevacizumab	0.022‡	1.05–7.18
Diabetes Type	0.833	0.34–2.20
Fibrovascular proliferation	0.260	0.38–12.15
History of smoking	0.408	0.11–2.41
History of HTA	0.534	0.29–1.87
Prior Myocardial Infarction	0.10	0.088–2.73
Prior Cerebrovascular Accident	0.51	0.33–14.7
Total Cholesterol	0.895	0.16–4.53
Triglycerides	0.453	0.08–9.12
Hemoglobin A1C levels	0.796	0.07–4.40
Macular Thickness of DME	0.123	0.33–11.65
Prerretinal Hemorrhage	0.317	0.33–17.5
Vitreous Hemorrhage	0.292	0.24–1.47
Previous vitrectomy	0.632	0.49–24.97
Age > 60 years	0.521	0.30–13.7
Previous use of Bevacizumab	0.730	0.07–5.40

*DME = Diabetic Macular Edema; TRD = Tractional Retinal Detachment; HTA = Hypertension.
‡Statistically significant.

Chapter 12: Prospective Unpublished *Chapter: Pre-Operative Intravitreal Bevacizumab for Tractional Retinal Detachment Secondary to Proliferative Diabetic Retinopathy:* Prospective Randomized Clinical Trial of the Pan-American Collaborative Retina Study (PACORES) Group

***Hypothesis 7 (*prospective unpublished study):** Intravitreal injection of 1.25 mg of bevacizumab as a pre-operative adjunct to PPV in eyes with TRD secondary to PDR will be safe and effective. IVB (compared to sham) will decrease intraoperative bleeding, total surgical time, post-operative vitreous hemorrhage, and visual acuity at 12 months.

Pre-Operative Intravitreal Bevacizumab for Tractional Retinal Detachment Secondary to Proliferative Diabetic Retinopathy: Prospective Randomized Clinical Trial of the Pan-American Collaborative Retina Study (PACORES) Group*

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‡ For a complete listing of participating members of PACORES, see Appendix.

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Abstract

Purpose: To assess the effectiveness and safety of an intravitreal injection of 1.25 mg of bevacizumab (IVB) as a pre-operative adjunct to small-gauge pars plana vitrectomy (PPV) compared to PPV alone in eyes with tractional retinal detachment (TRD) secondary to proliferative diabetic retinopathy (PDR).

Design: Randomized clinical trial.

Methods: This prospective, double-masked, randomized, multicenter, active-controlled clinical trial enrolled 234 eyes among 234 patients between November 2013 and July 2015. All randomized eyes underwent a baseline exam included best corrected visual acuity (BCVA), color photos, optical coherence tomography (OCT) and fluorescein angiography (FA). Improved surgical visualization, reduced operative time, and intra-operative complications were the main outcome measures. Patients were followed for 12 months.

Results: Two hundred and fourteen (214 eyes) patients were randomized with a 1:1 ratio to PPV plus IVB ([Study group] 102 eyes) or PPV plus sham ([control] 112 eyes). Iatrogenic retinal breaks were intraoperatively noted in 35 eyes (34.3%) in the study group, and 66 eyes (58.9%) in the control group (p=0.001). In the study group 32 (31.3%) eyes had grade 2 intraoperative bleeding, and 58 (51.7 %) eyes in the control group (p=0.001). Endodiathermy applications were necessary in 28 (27.4 %) eyes in the study group, compared with 75 (66.9 %) eyes in the control group (p=0.0001). Mean surgical time was 71.3 ± 32.1 minutes in the study group and 83.6 ± 38.7 minutes in the control group (p=0.061).

Conclusion: Pre-operative IVB seems to reduce intraoperative bleeding, improving surgical field visualization, and reducing intraoperative and postoperative complications.

Introduction

Proliferative diabetic retinopathy (PDR), is an ocular disorder characterized by retinal ischemia, recurrent retinal neovascularization and fibrous proliferation, which can lead to blindness if not properly treated.[1, 2] Patients with non-clearing vitreous hemorrhage, tractional retinal detachment (TRD) or extensive fibrovascular proliferations are candidates for pars plana vitrectomy (PPV). One of the most common intraoperative complications of PPV is bleeding. Intraoperative bleeding makes surgeons' maneuvers very difficult due to poor visualization. This difficult situation could prolong the total surgical time and hinder the surgical outcomes. Moreover, massive bleeding during surgery may even be uncontrollable and lead to surgical failure. [3]

The use of intravitreal injection of bevacizumab (Avastin; Genentech, South San Francisco, CA) has been effective to decrease vascular permeability and proliferation, improving diabetic macular edema and reducing the risk of intraocular bleeding in patients with PDR. [4] In addition, in eyes with advanced PDR characterized by, ample, active neovascularization and/or extensive or multiple layers of fibrovascular proliferation, an intravitreal bevacizumab (IVB) injection before surgery may potentially further decrease intraoperative hemorrhage, facilitate fibrovascular membrane dissection,[5-14] and reduce intra- and post-operative ocular complications. [11,14-16] A meta-analysis supports the use of pre-operative bevacizumab in diabetic vitrectomies. [13] However, we have previously reported that TRD may occur or progress shortly following administration of intravitreal bevacizumab in patients with severe PDR. [17,18] In addition, other reports show that IVB may cause TRD in cases with PRD and pre-existing pre-retinal fibrosis.[19] For that reason, there is no consensus in the retina community about the usefulness and risk/benefit ratio of IVB as an adjuvant to PPV in TRD in PDR. To provide more evidence to the literature, we decided to conduct a randomized trial evaluating the effectiveness and safety of an intravitreal injection of 1.25 mg of bevacizumab as a pre-operative adjunct to small-gauge PPV in eyes with TRD secondary to PDR.

Material and Methods

This prospective, double-masked, randomized, multicenter, active-controlled clinical trial (http://clinicaltrials.gov/show/NCT01976923) was conducted by the Pan-American Collaborative Retina Study (PACORES) Group at 13 clinical sites from 9 countries. Institutional Review Board/Ethics Committee approval and patients' informed consent were obtained for this study at all institutions. The off-label use of the drug and its potential risks and benefits were discussed extensively with all patients. In addition, this study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki for research involving human subjects. Patients were randomized with a 1:1 ratio to small gauge PPV plus IVB (Study group) or small gauge PPV plus sham injection (Control group). Randomization was done by using the method of randomly permuted blocks by an internet-based randomization generator (http://www.randomization.com).

Eligibility criteria for participants

Patient eligibility

Patients (18 years or older) with type 1 or 2 diabetes mellitus if they had PDR and TRD threatening or involving the macula, with or without a rhegmatogenous component, and with or without vitreous hemorrhage (VH), were included in the analysis. Eyes with VH dense, enough to prevent visualization of the macula pre-pars plana vitrectomy (PPV), were included in the study if macular-involving TRD was seen with ultrasound. Eyes with or without DME were also eligible. Participants with both eyes enrolled had 1 eye assigned randomly to PPV with sham injection and the other eye to PPV with pre-operative IVB. The patients receiving intravitreal medication were explained the off-label use of the drug, the potential risks of thromboembolic events, endophthalmitis and uveitis. Written informed consent was obtained from all patients before the intravitreal injection as well as prior to small gauge PPV.

Ocular Exclusion Criteria

The following exclusions applied to the study eye only (i.e., they may have been present for the non-study eye): If TRD was considered to be due to a cause other than diabetes. An ocular condition such that, in the opinion of the investigator, visual acuity loss would not improve from resolution of TRD (e.g., foveal atrophy, pigment abnormalities, dense subfoveal hard exudates, non-retinal condition, optic atrophy). An ocular condition (other than diabetes) that, in the opinion of the investigator, might affect retinal status or alter visual acuity during the course of the study (e.g., retinal vein occlusion, uveitis or other ocular inflammatory disease, glaucoma, etc.). History of treatment for diabetic macular edema or diabetic retinopathy at any time in the past 4 months with anti-VEGF drugs. History of major ocular surgery (including vitrectomy, scleral buckle, any intraocular surgery [except cataract surgery], etc.) within prior 4 months of randomization. History of neodymium-doped yttrium aluminium garnet (YAG) capsulotomy performed within 2 months prior to randomization. Intraocular pressure either equal or more than 25 mmHg. Exam evidence of external ocular infection, including conjunctivitis, chalazion, or significant blepharitis.

Systemic Exclusion Criteria

Those patients who presented any of the following exclusion criteria were excluded: Significant renal disease, defined as a history of chronic renal failure requiring dialysis or kidney transplant. A condition that, in the opinion of the investigator, would preclude participation in the study (e.g., unstable medical status including hypertension, cardiovascular disease, and glycemic control). Participation in an investigational trial within 30 days of randomization that involved treatment with any drug that has not received regulatory approval at the time of study entry. Known allergy to any component of the study drug. Blood pressure > 180/110 mmHg (systolic above 180 or diastolic above 110). Major surgery within 28 days prior to randomization or major surgery planned during the following 6 months to randomization. Myocardial infarction, other cardiac event requiring hospitalization, stroke, transient ischemic attack, or treatment for acute congestive heart failure within 4 months prior to randomization. Systemic anti-VEGF treatment within 4 months prior to randomization. For women of child-bearing potential: pregnant or lactating or intending to become

pregnant within the next 12 months. Participant was expecting to move out of the area of the clinical center to an area not covered by another clinical center during the first 12 months of the study. History of blood diseases associated with abnormal coagulation. Anti-coagulant therapy.

Patients were enrolled and randomized with a 1:1 ratio to small gauge PPV plus sham (Control Arm) or small gauge PPV plus IVB (Study Arm).

Control Arm:

A sham intravitreal injection was scheduled 3-5 days before surgery. The patient was prepared for intravitreal injection in the usual manner but only pressure with the hub of the syringe without the needle was applied 3.5 mm from the limbus under sterile conditions. PPV was scheduled 3-5 days after the sham intravitreal injection.

Study Arm:

An intravitreal injection of 1.25 mg/ 0.1 ml was scheduled and performed (3 to 5 days) before surgery.

Anti-VEGF Injection Technique

The patients receiving IVB (or sham) were explained the off-label use of the drug, the potential risks of thromboembolic events, endophthalmitis and uveitis. Written informed consent was obtained from all patients before the IVB injection as well as prior to small gauge PPV.

A sterile lid speculum was used to separate the lids after topical anesthesia (proparacaine hydrochloride 4 %). A 5% povidone iodine solution is used to disinfect the entire conjunctival surface. 1.25 mg / 0.05 mL of bevacizumab was injected using a 30-gauge needle inserted through the inferotemporal pars plana 3.5 mm from the limbus. At baseline and at each follow-up visit, masked personnel measured best-corrected visual acuity (BCVA) using Early Treatment Diabetic Retinopathy Study (ETDRS) charts.

PPV Surgical Technique

Under local anesthesia and intravenous sedation or general anesthesia, the eye was prepped with a 5% povidone iodine solution and draped in the usual fashion. Standard three port 23-, 25- or 27-small gauge PPV was performed in the usual fashion. Intraoperative maneuvers to deal with fibrovascular tissue dissection and retinal re-attachment was be left to each surgeon's individual preference. Intraocular tamponade and the need for laser photocoagulation was also left to each surgeon's discretion. During surgery intraoperative bleeding degree was registered as follows; grade 0: None, grade 1: Minor bleeding stopping spontaneously or with transient bottle/pressure elevation, and grade 2: Moderate to severe bleeding requiring endodiathermy or with formation of broad sheets of clots. [20] Additionally, the total surgical time was also registered.

Examination Schedule

Participants were examined at day 1, week 2, month 1, month 3, month 6, and moth 12. At each of these visits, each patient underwent BCVA measurement with ETDRS charts and ophthalmic examination, including slit-lamp biomicroscopy and applanation tonometry. Mean change in BCVA at 12 months were registered. Baseline central retinal thickness (CRT) were measured by optical coherence tomography (OCT) and fluorescein angiography (FA) were registered before surgery. Dilated color fundus images of the posterior pole, optic nerve and macula description was registered each visit. Ultrasound in the presence of vitreous hemorrhage was also performed to certify the presence of TRD if needed.

The following variables were used for primary outcome comparisons: Intraoperative bleeding, total surgical time, early (<1 month) post-operative vitreous hemorrhage (VH), and mean change in BCVA at 12 months. Secondary outcome comparisons: Number of endodiathermy

applications, intraoperative retinal breaks, change in central macular thickness, proportion of eyes gaining at least 2 lines (10 letters) of ETDRS BCVA were considered as secondary outcomes.

Statistical analysis

Data were collected in an Excel 2011 spreadsheet (Microsoft, Redmond, Washington, USA). Statistical analysis was performed using MedCalc Software for Windows V.8.2.0.3 (MedCalc, Mariakerke, Belgium). To determine whether there is a significant difference in proportion of postoperative vitreous hemorrhage occurred between cases (6%) with injection of intravitreal bevacizumab and controls (19%) without injection as per Ahmadieh et al. [21] to achieve 80% power and 95% two-sided confidence level with equal ratio of controls to cases, at least 102 cases and 102 controls were needed to study. The initial total sample required with 10% drop out has been 224 (112 cases and 112 controls). ETDRS BCVA was converted to the logarithm of the minimum angle of resolution (logMAR) for statistical analysis. An increase or decrease in BCVA was defined as a change of $\geq$ 2 lines (10 letters) of ETDRS vision prior to logMAR conversion. The paired samples t test was performed to compare the CRT and BCVA with baseline values. Correlation was considered significant when $p<0.05$.

Results

Two hundred and thirty-four (234 eyes) patients were initially enrolled in this study. However, 20 participants (20 eyes) were not eligible for results because they did not meet the criteria for the study; 15 of them were lost before undergoing randomization, 3 patients requested to withdraw and 2 were withdrawn by the site. Two hundred and fourteen (214 eyes) patients were included and randomized with a 1:1 ratio to PPV plus sham injection (control group; 112) eyes or PPV plus IVB (Study group; 102 eyes). In the study group, 7 (6.8%) eyes underwent to 27-gauge PPV, 15 (14.7%) 25-gauge PPV, and 80 (78.4%) 23-gauge PPV. In the control group, 6 (5.3%) eyes underwent 27-gauge PPV, 12 (10.7%) 25-gauge PPV, and 94 (83.9%) eyes underwent to 23-gauge PPV. The gender distribution, age, and major systemic data between the study group and control group were not significantly different (Table 1). In the study group 96/102 eyes (94.12%) were reattached (figures 1 to 4). In the control group 98/112 eyes (87.5%) were reattached at the end of follow up with one procedure. This difference was not statistically significant (p=0.097).

Intraoperative Complications

In the study group 69 (67.6%) eyes had some degree of intraoperative bleeding, while 100 (89.2%) eyes had some intraoperative bleeding in the control group (p=0.0002). Of these eyes, 32 (31.3%) eyes had grade 2 intraoperative bleeding in the study group, while 58 (51.7 %) eyes had that level in the control group (p=0.001). We observed a mean of 1.5 ± 2.2 (range from 0 to 7) endodiathermy applications in the IVB group, and 3.6 ± 3.1 (range from 0 to 10) in those eyes involved in the sham group. This difference was not statistically significant (p=0.062). However, the use of endodiathermy applications was necessary in 28 (27.4 %) eyes in the study group, compared with 75 (66.9 %) eyes involved in the control group, and this was statistically significant (p=0.0001).

At least one iatrogenic retinal break either along the TRD, or elsewhere was intraoperatively noted in 35 eyes (34.3%) in the study group, and 66 eyes (58.9 %) in the control group (p=0.001). Mean operating time was 71.3 ± 32.1 minutes (range 35 to 150) in the study group and 83.6 ± 38.7

minutes (range 35 to 185) in the control group. The study group had a shorter mean surgical time than the control group, but we did not observe a statistically significant difference (p=0.061). On the other hand, 24 (23.5%) eyes required silicone oil tamponade at the end of surgery in the study group, while 48 (42.8%) eyes required silicone oil tamponade in the control group (p=0.003).

Visual Acuity (VA) Outcomes

The BCVA mean was comparable in both groups at baseline (Table 1). In the study group, the mean baseline BCVA improved from 20/250, logMAR 1.1 ± 0.5 to 20/80, logMAR 0.6 ± 0.48 at the end of follow up (p<0.0001), whereas in the control group, baseline BCVA improved from 20/250, logMAR 1.1 ± 1.4 to 20/100, logMAR 0.7 ± 0.31 (p=0.034). These results represent a mean gain of 4 lines of vision in the first month of follow up, and 5 lines of vision at the end of follow up in the study group. In the sham group a mean gain of 2 lines of vision was observed in the first postoperative month, and a mean of 4 lines of vision was observed at the end of follow up (Figure 5). Both groups significantly improved the mean of BCVA at the end of follow up.

Each group was divided into two sub-groups according to the presence or not of VH before surgery to calculate in these sub groups of patients demonstrated variability in terms of both BCVA and central retinal thickness during the follow up. In the study group 55 (53%) eyes had some degree of vitreous hemorrhage before surgery with a mean of preoperative BCVA of 20/400, logMAR 1.3 ± 0.4 while in the control group 63 (56%) eyes presented VH at baseline with a mean of baseline BCVA of 20/500, logMAR 1.4 ± 2.1. Both groups presented a statistically significant visual improvement at the end of the follow-up, demonstrating a mean of BCVA 20/60, logMAR 0.5 ± 2.1 (p< 0.0001) and 20/80, logMAR 0.6 ± 2.1 (p=0.032), respectively.

On the other hand, the study group included 47 patients (eyes) with TRD without VH before surgery. This subgroup had a mean of BCVA 20/100, logMAR 0.7 ± 1.9 at presentation, and the mean of BCVA improved to 20/60 logMAR 0.5 ± 2.9 (p=0.002) at 12 months of follow up. On the other hand, 49 patients (eyes) included in the control group presented TRD without VH at presentation registered a mean of BCVA 20/125, logMAR 0. 8 ± 1.3 improving to BCVA 20/60,

logMAR 0.6 ± 1.5 at the end of follow up (p=0.004). In both groups, there was a significant postoperative gain in vision but there was no significant difference between groups during the follow-up visits.

In the study group, the 12-month BCVA analysis by subgroups demonstrated that 75 (73%) eyes improved 2 or more ETDRS lines of BCVA, and that BCVA remained stable in 16 (15.6%) eyes at 12 months. In addition, 11 (10.7%) eyes decreased 2 or more ETDRS lines of BCVA at the end of follow-up. In the control group 76 (67.8%) eyes improved 2 or more ETDRS lines of BCVA, and BCVA remained stable in 20 (17.8%) eyes, and 16 (14.2%) eyes decreased 2 or more ETDRS lines of BCVA at the end of follow-up (Table 2).

Postoperative Retinal Macular Thickness

Optical coherence tomography (OCT) results were available for all patients who had no dense vitreous hemorrhage at presentation. OCT results were available for 47 (46.1%) eyes in the study group, and 49 (43.7%) eyes in the control group. In the study group, mean CRT decreased from 382.2 µm ± 116.2 µm to 334.5 µm ± 109.2 µm (p= 0.061) at the end of follow up, while in the control group the mean of CRT decreased from 409.4 ± 160.4 to 345.5 µm ±119.2 (p=0.089) at 12 months of follow up. None of the groups had a significant difference between baseline mean CRT OCT and the mean final CRT OCT at the end of follow up.

Postoperative Complications

Postoperatively, vitreous hemorrhage was observed in 29 (28.4 %) eyes in the study group, and 48 (42.8%) eyes in the control group had some grade of vitreous hemorrhage within the first month after surgery (p=0.028).

We registered 6 (5.8%) cases of tractional re-detachment in the study group. Four (66.6%) of these patients had been treated with intraocular gas (SF6 20%) as tamponade and 2 (33.3) eyes were

under silicone oil. In the control group we observed 14 (12.5%) cases of re-detachment of which 9 (64.3%) cases had an intraocular gas (SF6 20%) and 5 (35.7%) eyes had silicone oil as tamponade. All cases required a second vitrectomy within first year of follow up. Number of re-interventions due to recurrence of tractional retinal detachment was lower in the IVB group, but we did not demonstrate a statistically significant difference between both groups (p=0.097). On the other hand, we observed 8 (7.8%) cases of dense recurrent VH in the study group, of which 3 (37.5%) cases required a second vitrectomy. In the control group 26 (23.2%) cases presented with dense recurrent VH within first year of follow up, of which 14 (58.3%) patients required a second vitrectomy during the follow up period. Cases of dense recurrent VH were more frequent in the control group at one year of follow up, and the difference was statistically significant between both groups (p=0.002). Neovascular glaucoma occurred in 3 (2.9%) eyes in the study group and 7 (6.3 %) eyes in the control group (p=0.252) at the end of follow up. The only adverse event related to IVB injection was that there were 3/102 eyes (2.94%) of TRD progression 3 days after the injection. However, BCVA improved after PPV in those cases. This complication was not seen in the control group.

We found no statistical differences in our surgical results and complications among the cases operated on with 23-,25-, or 27-gauge PPV.

Discussion

This randomized study demonstrates that preoperative intravitreal injection of bevacizumab might be helpful to facilitate vitrectomy in cases of TRD secondary to advanced PDR. Intravitreal bevacizumab as a preoperative adjunct in cases of severe PDR seems to be beneficial and to aid in rapid clearing of vitreous hemorrhage and reducing the surgical time by reducing intraoperative bleeding. [22] Tractional retinal detachment in PDR is challenging because of thin ischemic retina and extensive neovascularization. Intravitreal anti-angiogenic therapy is being widely used for neovascular complications of PDR. [23-26, 13] Surgical outcomes may limit visual prognosis of the disease and it could be compromised by intra and postoperative complications. Perioperative vitreous hemorrhage resulting in formation of adherent fibrinous clots may necessitate frequent application of diathermy and exchange of instruments. Severe intraoperative hemorrhages represent the main obstacle in the surgical removal of pre-retinal tissue, and it may interfere with completion of the surgical procedure in nearly 4 % of cases.[3] Conversely, a bloodless field allows for better visibility facilitating completion of surgery.

In our study we observed that 32 (31.3%) eyes had grade 2 intraoperative bleeding in the study group, while 58 (51.7 %) eyes had that level in the control group (p=0.001). This reduced risk of intraoperative bleeding may also decrease the use of endodiathermy applications that represent less trauma in a previously damaged and ischemic retina. In our study, the use of endodiathermy applications was necessary in 28 (27.4 %) eyes treated with pre-operative IVB, compared with 75 (66.9 %) eyes involved in the control group (p=0.0001). In addition, the decrease of intraoperative bleeding may also facilitate removal of the fibrovascular membranes with less damage to subjacent retina. We also observed that the eyes in the study group had a lower incidence of intraoperatory iatrogenic retinal breaks than those eyes in the control group (sham). Previously, a lower incidence of intraoperative retinal tears had been observed in the IVB-PPV group than the conventional PPV group, possibly due to minimal intraoperative bleeding and improved retinal visualization during surgery. [27-30] Gupta et al [27] evaluated the effect of a single preoperative injection of IVB on visual

outcome in patients undergoing PPV for PDR. Intraoperatively, they described an incidence of iatrogenic retinal breaks comparable in the two groups [20 (21.3 %) vs. 15 (17.2 %) in groups A and B, respectively. However, the authors describe only a presence of TRD in 42 eyes in IVB group (44.7 %) and 31 eyes (35.6 %) in control group. Recently Dong et al [31] investigated intravitreal injection of ranibizumab (IVR) on the surgical outcome for diabetic patients who had tractional retinal detachment but in cases that did not receive any preoperative retinal photocoagulation. They described results of ninety-seven patients (97 eyes) who had diabetic retinopathy with TRD enrolled to receive 23-gauge PPV. They were assigned to an experimental group (preoperative IVR plus PPV, n=47 eyes) and a control group (VPP, n=50 eyes). They describe that iatrogenic breaks were noted in 5 eyes (11%) in the experimental group and 17 eyes (34%) in the control group; the difference was significant (p=0.006). In concordance with these results, we report at least one iatrogenic retinal break either along the TRD, or elsewhere intraoperatively in 35 eyes (34.3%) in the study group, and 66 eyes (58.9 %) in the control group (p=0.001). In addition, we observed that more than 70% of the retinal tears described in the control group were along the fibrovascular traction and that they probably occurred after fibrovascular membrane peeling. Therefore, iatrogenic retinal breaks may be strongly linked to the dissection maneuver of tractional fibrovascular tissue on the retina. This lower incidence of iatrogenic retinal breaks in study (IVB) group is an important finding that could be explained due to the significant regression of the vascular component after preoperative IVB, which could change the pathophysiology of the fibrovascular complex making it more prone to readily separate from the retina, and make it easier and faster to be dissected. These two findings, both the lower rate of intraoperative bleeding and the lower number of retinal breaks in the study group, suggest that preoperative IVB could make TRD surgery more efficacious and safe. This is concordance with the finding by Yeoh et al [28] that found that IVB was particularly useful in diabetic eyes with traction retinal detachments of short duration in which there was still active neovascularization. In our study, 72 (72.5%) eyes in the IVB group had some degree of vitreous hemorrhage before surgery which means that neovascularization was still active at the moment of IVB application. El-Sabagh et al [12] described a profibrotic switch in diabetic fibrovascular proliferation after IVB, and their results suggest that the vascular component of proliferation is

markedly reduced post-IVB. These findings are in concordance with our results, which suggest that PPV is safer and more complete when IVB is administered before surgery. Similar to our results, IVB has been previously used as a preoperative adjunct for TRD repair in severe PDR by Chen [11] and was also reported to make surgery more successful with significant regression of neovascularization in the fibrovascular proliferative membranes, consequently minimizing intraoperative bleeding during segmentation and delamination of membranes. On the other hand, we also observed that postoperative intravitreal hemorrhage was less markedly in the IVB group.

In our study, there were 3/102 eyes (2.94%) that had a progression of TRD 3 days after IVB injection. However, BCVA improved after PPV. This complication was not seen in the control group. This rate is in concordance with previous reports.[17, 18] We have previously reported that the majority of cases with progression or development of TRD after IVB occur 5 days or more after the injection. The low incidence in our study, and good visual outcomes confirm that IVB is safe and effective even in eyes that develop contraction and progression of TRD.

In this study, we found that 75 (73%) eyes improved 2 or more ETDRS lines of BCVA in the study group, and 76 (67.8%) eyes improved 2 or more ETDRS lines of BCVA in the control group at the end of follow up. No significant difference we found between both groups. Our results show a significant improvement in BCVA in both groups. This, as others have suggested,[11] is most likely due to improved vitrectomy techniques. Similar improvement in visual acuity with the use of preoperative IVB has been reported previously in a number of series of patients after surgery. [13, 21, 28, 32]

Compared with the control group, patients who received preoperative IVB had lower use of silicone oil as internal tamponade suggesting that the surgical procedure was less complex. We reported that in the study group 24 (23.5%) eyes required silicone oil tamponade at the end of surgery while 48 (42.8%) eyes required silicone oil tamponade in the control group (0.003). Similar outcomes were showed by Dong et al who describe that silicone oil was used as internal tamponade agent in 22 eyes (47%) of IVR group and 37 eyes (74%) of control group (p=0.006). Our results also demonstrated that the incidence of neovascular glaucoma following surgery was not significantly different between the study group and the control group.

The strength of our study is that it is a prospective randomized clinical trial, however, its multicentric nature is a limitation as multiple surgeons from several centers participated with a non-standardized surgical technique including all available gauges for minimally invasive vitrectomy. However, the number of patients is significant for a surgical trial, and shows the benefits of intravitreal bevacizumab to facilitate PPV in TRD in PDR. Another limitation of our study is that we changed our original proposed randomization scheme from 3:1 to 1:1. The protocol was amended to reflect this change. Originally, we had calculated that 374 patients (187 per group) would be needed to determine whether there is a significant difference in proportion of postoperative vitreous hemorrhage occurred between cases (6%) with injection of intravitreal bevacizumab and controls (19%) without injection as per Gupta et al. [27] to achieve 80% power and 95% two-sided confidence level. We realized that with the stringent inclusion and exclusion criteria it would be very difficult to achieve those numbers. Therefore, we recalculated our sample size to determine whether there is a significant difference in proportion of postoperative vitreous hemorrhage occurred between cases (6%) with injection of intravitreal bevacizumab and controls (19%) without injection as per Ahmadieh et al. [21] to achieve 80% power and 95% two-sided confidence level with equal ratio of controls to cases, we determined that at least 102 cases and 102 controls were needed to study. The initial total sample required with 10% drop out calculated was 224 (112 cases and 112 controls). We achived those numbers.

In summary, we demonstrated that pre-operative intravitreal bevacizumab as adjuvant to small-gauge pars plana vitrectomy may be helpful and beneficial for patients with TRD secondary to severe PDR. Pre-operative IVB seems to reduce intraoperative bleeding, improving surgical visual field visualization, and reducing intraoperative and postoperative complications including iatrogenic retinal tears and postoperative bleeding. However, retinal reattachment rates were also similar between both groups. In addition, neither the postoperative BCVA nor the proportion of eyes with BCVA improvement showed significant difference between groups at the end of follow up, which means that preoperative IVB may not be a determinant factor for postoperative BCVA, as previous studies have shown.[29,31,33]

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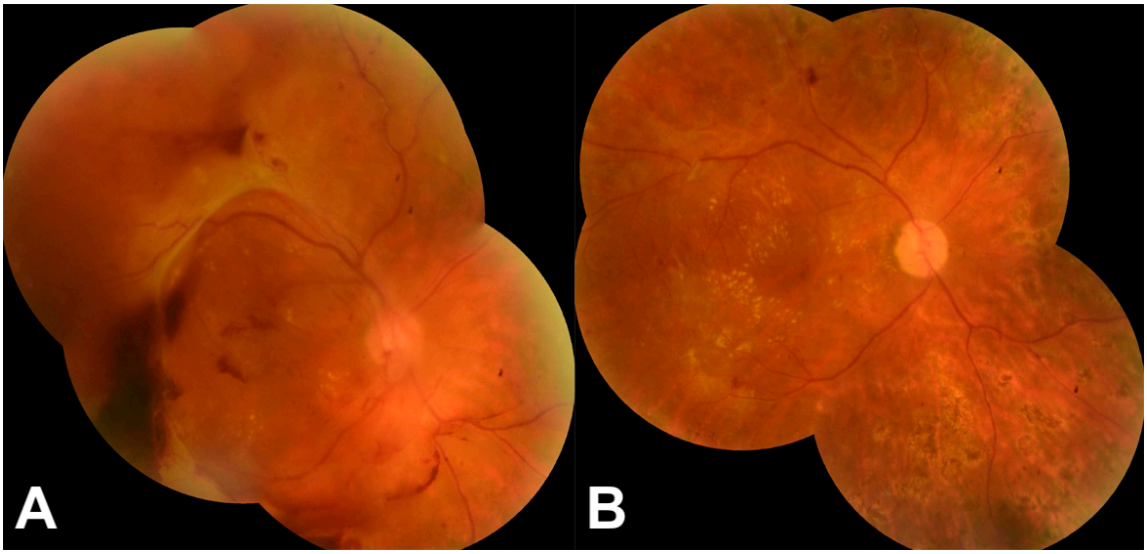

Figure 1. (A) Color photograph of proliferative diabetic retinopathy (PDR) complicated by tractional retinal detachment (TRD) temporal to the macular area in the right eye in a 43-year-old type II diabetic man. (B) At 12 months after pars plana vitrectomy with preoperative bevacizumab, visual acuity (VA) had improved from 20/50 to 20/25.

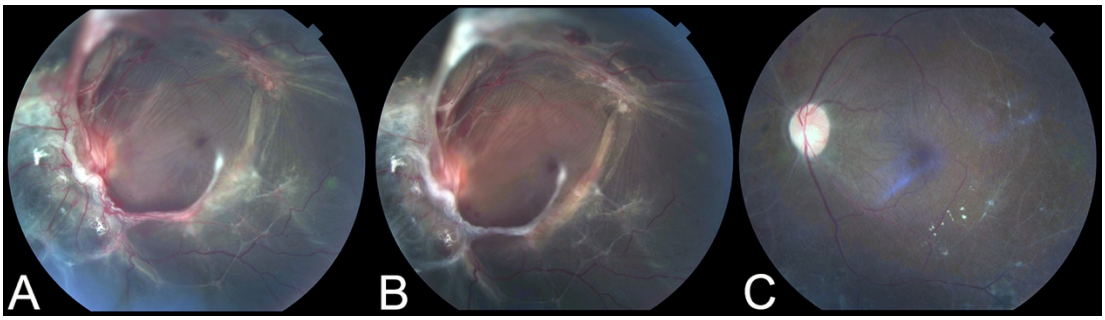

Figure 2. (A) A 39-year-old man with type II diabetes showing extensive tractional retinal detachment and fibrovascular proliferation in the left eye and visual acuity (VA) of 20/400. (B) 3 days after preoperative intravitreal bevacizumab and 1 day before surgery. Note significant vascular reabsorption on fibrovascular proliferations. (C) 12 months of follow up after 25-gauge pars plana vitrectomy with intraocular gas tamponade. His retina remained attached, and his postoperative VA was 20/70.

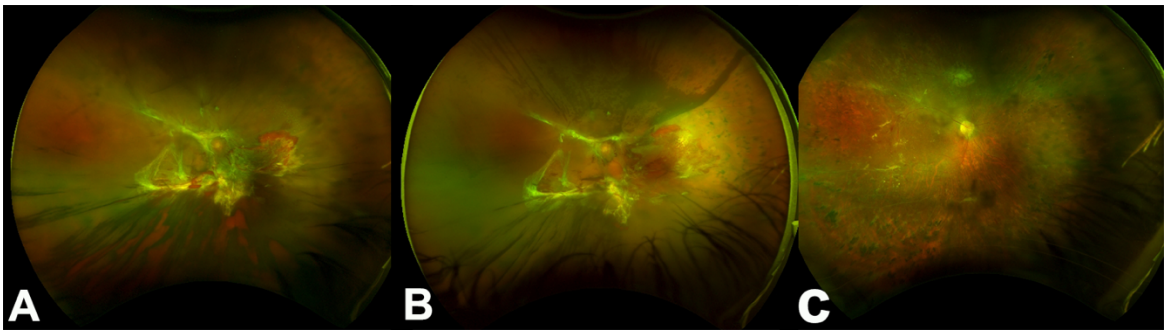

Figure 3. (A) A 54-year-old man with diagnosis of type II diabetes. His visual acuity (VA) was 20/800. Note extensive neovascularization and fibrovascular traction along the vascular arcade with foveal involvement. (B) He received intravitreal bevacizumab 4 days before surgery. (C) One year after 25-gauge pars plana vitrectomy (PPV) and C3F8 14% gas as endo-tamponade, his retina remained attached, and his VA improved to 20/100.

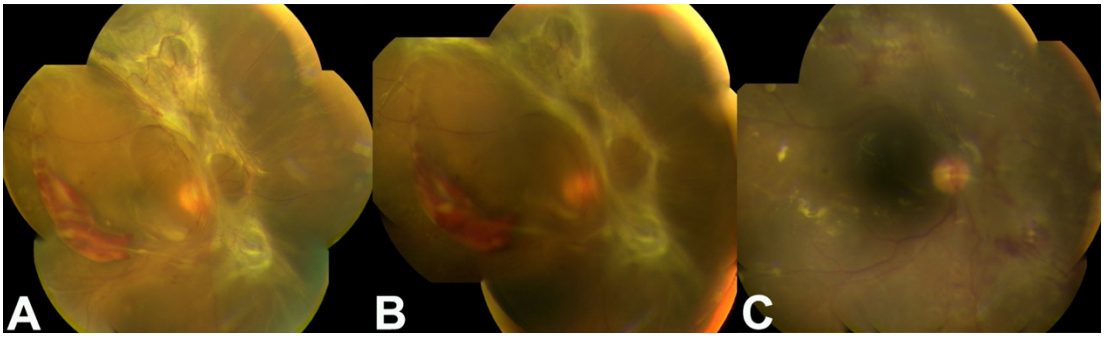

Figure 4. (A) A 27-year-old male with type II diabetes showing vitreous hemorrhage and tractional retinal detachment in the right eye and visual acuity of 20/400. (B) He received intravitreal bevacizumab 5 days before vitrectomy. (B) At 5 months of follow-up after surgery, her retina remained attached, and his visual acuity (VA) improved to 20/80.

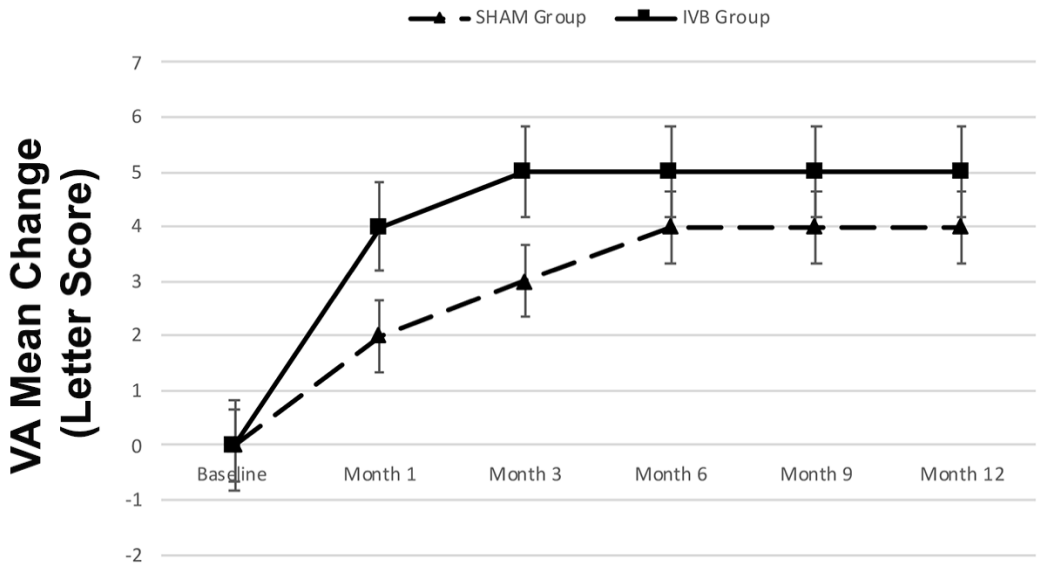

Figure 5. A mean gain of 4 lines of vision in the first month of follow up, and 5 lines of vision at the end of follow up in the study group. In the sham group, a mean gain of 2 lines of vision was observed in the first postoperative month, and a mean of 4 lines of vision was observed at the end of follow up. In the study group, the mean baseline BCVA improved from 20/250, logMAR 1.1 ± 0.5 to 20/80, logMAR 0.6 ± 0.48 at the end of follow up ($p<0.0001$), whereas in the control group, baseline BCVA improved from 20/250, logMAR 1.1 ± 1.4 to 20/100, logMAR 0.7 ± 0.31 ($p=0.034$).

Table 1. Patient's Baseline Characteristics*

	Study Group (n=102)	Control Group (n=112)
Age (mean ± SD), years	59.5 ± 11	61.3 ± 10
Gender (male/female)	62 % / 38 %	54 % / 46%
Hypertension (%)	81%	94%
Systemic glycemic control, n (%)		
Insulin	9 (9%)	21 (19%)
Oral hypoglycemic	12 (12%)	17 (15%)
Combined therapy	81 (79%)	74 (66%)
HbA1c at time of surgery	8.5 ± 2.1	8.9 ± 2.5
Previous history of PRP	79 (77.4%)	92 (82.1%)
VH at presentation (n/%)		
Dense	55 (53%)	63 (56.2%)
Moderate	4 (3.9%)	5 (4.4%)
Mild	15 (14.7%)	5 (4.4%)
No	28 (27.4%)	39 (34.8%)
Pre-operative BCVA (logMAR)		
TRD with DVH	(n=55) 1.3 ± 0.4	(n=63) 1.4 ± 2.1
TRD without DVH	(n=47) 0.7 ± 2.1	(n=49) 0. 8 ± 1.3
Total TRD cases	1.1 ± 0.5	1.1 ± 0.3
Mean OCT baseline (um)		
TRD without DVH (n/%)	47 (46.1%)	49 (43.7%)
CRT (microns)	382.2 ± 116.2	409.4 ± 160.4
Lens status		
phakic	73 (71.6%)	85 (76%)
pseudophakic	29 (28.4%)	27 (24%)

* Values represent number and percentages, IVB= Intravitreal bevacizumab; logMAR = logarithm of the minimal angle of resolution; HbA1c= Glycosylated hemoglobin; n, number of patients; PRP= Pan-retinal photocoagulation; VH=vitreous hemorrhage; TRD=tractional retinal detachment; DVH= dense vitreous hemorrhage; OCT= optical coherence tomography; CRT=central retinal thickness.

Table 2. Variation of BCVA at 12 months of Pre-operative IVB versus Sham for TRD secondary to PDR (214 eyes)*

	Study Group	Control Group	
BCVA (logMAR) Results	**(n / %)**	**(n / %)**	**p**
Improved 2 or more lines	75 (73%)	76 (67.8)	0.555
Remained Stable	16 (15.6%)	20 (17.8%)	0.672
Decreased 2 or more lines	11 (10.7%)	16 (14.2%)	0.441
Total	102 (100 %)	112 (100%)	

*Values represent number and percentages; IVB = Intravitreal bevacizumab; logMAR = logarithm of the minimal angle of resolution; BCVA = Best-corrected visual acuity; n = represents number of patients.

Appendix

The following investigators belong to the Pan-American Collaborative Retina Study Group (PACORES):

The Pan-American Collaborative Retina Study Group (PACORES)*-

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Hospital Universitario Ramón y Cajal, Departamento de Retina, and VISSUM Madrid, Madrid, Spain.

*PI = Principal Investigator.

Chapter 13: Conclusions

The advent of intravitreal anti-VEGF medications has revolutionized the treatment of diabetic eye diseases. Whereas ranibizumab and aflibercept are specifically formulated for intraocular uses, off-label use of bevacizumab remains popular and widespread in the world, especially in low-income and middle-income countries, given its lower cost, perceived effectiveness, and comparable safety profile.[23-35] Based on our experience, we recognize intravitreal bevacizumab to be an important tool in our armamentarium for treating the complications of diabetic retinopathy. For center-involving DME, our real-world data suggests that there is a roughly 70% chance of stabilized or improved vision with intravitreal bevacizumab treatments over a time course of 5 years. Nevertheless, many patients so benefit from anti-VEGF injections long-term if a more frequent regime of injections is given specially during the first 2 years. It is possible that new molecules targeting different or combined cytokines and growth factors will lead to less frequent injections and the use of reservoirs for these agents that can be implanted surgical will reduce the burden of injections making the process and access easier although cost is always going to be an issue.

For PDR, intravitreal bevacizumab is a good adjuvant therapy for patients who have already received prior PRP treatment, and unless frequent follow-up visits and treatments can be ensured, treatment-naive patients will likely have better control of retinal neovascularization with prompt PRP in conjunction with initiation of intravitreal treatments. I think we are going towards a change in treatment paradigm with combination therapy with a less aggressive PRP and anti-VEGF therapy. Lastly, preoperative intravitreal bevacizumab before vitrectomy is safe, with a low incidence (3.5%) of TRD development or progression. This risk can further be reduced by performing surgery within 4 days of injection and by avoiding the usage of a higher dose (2.5 mg) of bevacizumab. In terms of future research directions, our group recently carried out a prospective study on the utility of preoperative intravitreal bevacizumab before diabetic TRD repair, and the data demonstrates that IVB as an adjuvant to vitrectomy can decrease surgical bleeding, facilitating surgical maneuvers, and techniques. In addition, postoperative complications such as iatrogenic retinal breaks and postoperative bleeding are reduced.

Originality of the proposed research

At this point in time, many studies have looked at IVB for complications of DR. However, when we started our research (September 2005) we were one of the first groups to use it and demonstrate its efficacy in DME and PDR. Furthermore, our group was the first to describe tractional retinal detachment following IVB in patients with severe PDR. In addition, our collaborative work has changed the way research is performed in Latin-America.[37]

Contribution of the proposed research to existing knowledge

Our *central research question and purpose* of our research is to determine if intravitreal bevacizumab as anti-vascular endothelial growth factor is helpful in the management of complications of diabetic retinopathy. We have proven that in several steps described in our chapters that have been able to respond to our several hypotheses:

Hypothesis 1: Intravitreal bevacizumab (IVB) may have a beneficial anatomic (Optical Coherence Tomography [OCT]), and functional (visual acuity [VA]) effect on eyes with diffuse diabetic macular edema at 24 months of follow up. In addition, the lower dose (1.25 mg) may be as effective or more than the higher dose (2.5 mg) of IVB.

We progressively reported over the years our experienced as we followed patients with DME treated with IVB at 6 months, 12 months, and 24 months of follow up. In addition, 5 year follow up data was added later on. We found that primary IVB at doses of 1.25 to 2.5 mg seem to provide stability or improvement in BCVA, OCT, and FA in DDME at 24 months. The results show no evident difference between IVB at doses of 1.25 or 2.5 mg. However, the early visual gains due to IVB were not maintained 5 years after treatment. Undertreatment seems to be the main reason for the loss of visual acuity over the years as patients did have at the end of follow up reduction in CMT measured with OCT, nevertheless between injections the increase in CMT chronically may lead to loss of vision gains at the end of follow up in many patients.

Hypothesis 2: IVB combined with grid laser photocoagulation may have a beneficial anatomic (OCT), and functional (VA) effect on eyes with diffuse diabetic macular edema at 24 months of follow up as compared to monotherapy. In addition, IVB combined with grid laser photocoagulation (GLP) may decrease the number of injections if IVB necessary at 24 months.

Our study provides evidence to support the use of primary IVB with or without GLP as treatment of diffuse diabetic macular edema. Primary IVB without GLP seems to be superior to GLP alone to provide stability or improvement in best-corrected visual acuity in patients with diffuse diabetic macular edema at 24 months. The best evidence continues to be for monotherapy, laser could only worsen visual acuity and does not reduce the number of injections.

Hypothesis 3: IVB may decrease retinal neovascularization in patients with PDR at 6 months of follow up. However, the effect may decrease at 24 months of follow up due to tachyphylaxis, and pan-retinal photocoagulation and/or vitrectomy will be necessary.

We showed first that intravitreal bevacizumab resulted in marked regression of RN in patients with PDR and previous PRP, and rapid resolution of vitreous hemorrhage in three naive eyes. Six-months results of intravitreal bevacizumab at doses of 1.25 or 2.5 mg in patients with PDR did not reveal any safety concerns. Later, we published that IVB resulted in marked regression of retinal neo-vascularization in patients with PDR and previous pan-retinal photocoagulation at 2 years. Intravitreal bevacizumab in naive eyes resulted in control or regression of 42.1% of eyes without adjunctive laser or vitrectomy during 24 months of follow-up. Meaning that a large number of patients (almost 58%) needed PRP or vitrectomy. There were no safety concerns during the 2 years of follow-up of IVB for PDR. Again, I think we are going towards a change in treatment paradigm with combination therapy with a less aggressive PRP and anti-VEGF therapy.

Hypothesis 4: Preoperative IVB may be beneficial for membrane dissection in diabetic tractional retinal detachment with minimally invasive vitreoretinal surgery (23-gauge transconjunctival sutureless vitrectomy [TSV]). In addition, post-operative rebleeding may be decreased.

This study demonstrated the usefulness of using preoperative intravitreal bevacizumab during small-gauge vitreoretinal surgery in eyes with TRD in PDR. This is was a prospective non-comparative study and patients had anatomic success in 100% (114/114) of eyes. Significant visual improvement [≥ 2 Early Treatment Diabetic Retinopathy Study (ETDRS) lines] was obtained in 69.2% (79/114). Complications decreased compared to previous studies. This study lays the ground for the need for a prospective randomized clinical trial comparing IVB before vitrectomy for TRD in PDR vs placebo.

Hypothesis 5: Tractional retinal detachment (TRD) may occur following IVB as an adjuvant to vitrectomy for the management of severe PDR.

We reported for the first time ever that TRD may occur or progress shortly following administration of intravitreal bevacizumab in patients with severe PDR (5.2%). The reduction of VEGF after IVB may lead to a relative increase in cytokines that can cause contraction and this is known as a fibrotic switch. Surgery should be performed before this contraction occurs.

Hypothesis 6: Risk factors for the progression or development of TRD following IVB as an adjuvant to vitrectomy in severe PDR may include age, time from diabetes mellitus (DM) diagnosis, glycemic control, cholesterol levels, triglycerides levels, hemoglobin A1c (HbA1C), dose of bevacizumab, and time from injection to vitrectomy.

TRD may occur or progress shortly following administration of intravitreal bevacizumab in patients with severe PDR (3.2%) in a larger number of patients. Based on our data, we now believe that extreme care must be taken in using a dose of 2.5 mg or more of bevacizumab in patients with PDR. In addition, to have more than 15 years with a diagnosis of diabetes can increase the risk of TRD and that careful follow-up evaluation following injection is recommended. The timing of surgery after the

injection is also important, as there are concerns that bevacizumab may cause progression of the TRD. It is important that surgery is performed once the anti-angiogenic effect of bevacizumab has fully developed, but before there is further fibrous proliferation; physicians must be prepared to perform the vitrectomy preferably before about 13 days after the application of bevacizumab and to perform a vitrectomy immediately on those patients in whom a TRD occurs. We recommend less than 5 days after injection as more than 80% of the retinal detachments developed after that period of time.

***Hypothesis 7 (*prospective unpublished study):** Intravitreal injection of 1.25 mg of bevacizumab as a pre-operative adjunct to PPV in eyes with TRD secondary to PDR will be safe and effective. IVB (compared to sham) will decrease intraoperative bleeding, total surgical time, post-operative vitreous hemorrhage, and visual acuity at 12 months.

We demonstrated that pre-operative intravitreal bevacizumab as adjuvant to small-gauge pars plana vitrectomy may be helpful and beneficial for patients with TRD secondary to severe PDR. Pre-operative IVB seems to reduce intraoperative bleeding, improving surgical visual field visualization, and reducing intraoperative and postoperative complications including iatrogenic retinal tears and postoperative bleeding. However, retinal reattachment rates were also similar between both groups. In addition, neither the postoperative BCVA nor the proportion of eyes with BCVA improvement showed significant difference between groups at the end of follow up, which means that preoperative IVB may not be a determinant factor for postoperative BCVA.

In summary, given the off-label nature of intravitreal bevacizumab, its effectiveness and safety have not been as thoroughly studied as other approved similar drugs. Our results have been important to determine that DME can be treated as effectively with monotherapy IVB rather than combined IVB plus grid/focal photocoagulation. However, undertreatment will lead in the long-term to loss of the visual acuity gains of the early years of follow up. In addition, we have been able to determine that IVB should be used as an adjuvant to PRP in patients with PDR, and that PRP and/or vitrectomy seem to be necessary to control PDR in the long-run in a large number of patients. Furthermore, we have determined that IVB can be used as an adjuvant to vitrectomy in patients with severe PDR and TRD, and that it decreases the chances of re-bleeding postoperatively as demonstrated in our unpublished data. In addition, we have been able to confirm that TRD do occur or progress after IVB in severe PDR, and determine the risk factors for this occurrence, and the best time to perform surgery after IVB. Finally, our prospective study shows that pre-operative intravitreal bevacizumab therapy as adjuvant to PPV may be helpful and beneficial for patients with TRD secondary to severe PDR. Pre-operative IVB seems to reduce intraoperative bleeding, improving

surgical visual field visualization, and reducing intraoperative and postoperative complications including iatrogenic retinal breaks and postoperative hemorrhage.

Future Research Directions

DME is multifactorial, anti-VEGF therapy does not seem to be the final answer to control DME over time. Other molecules need to be targeted and combined therapies will possibly be beneficial for long term control of the disease including regression of severity of DR. Combination therapy with new molecules and even steroids need to be studied. Reservoirs with anti-angiogenic agents inserted surgically and refilled in the clinic will reduce the number of injections.

Data from our studies show that patients with PDR that had a previous PRP have a better response to anti-VEGF therapy monotherapy than treatment naïve eyes. This suggests that combined therapy may be more effective than PRP alone or anti-VEGF therapy alone. There are many advantages to both procedures and further studies should explore combination therapy.

Anti-VEGF therapy as an adjuvant before vitrectomy for TRD in PDR as demonstrated in our prospective data, may be helpful and beneficial for patients with TRD secondary to severe PDR. Pre-operative IVB seems to reduce intraoperative bleeding, improving surgical visual field visualization, and reducing intraoperative and postoperative complications including iatrogenic retinal breaks and postoperative hemorrhage. However, these complications do still occur and surgery is still a challenge to surgeons. There is a need for new pharmacologic agents to address the contraction component of the fibrovascular membranes.

Finally, other advances in the future may include minimally invasive vitreoretinal surgery (MIVS) with 23-gauge transconjunctival suture-less vitrectomy techniques will be increasingly performed in diabetic patients due to the increased incidence of diabetes and its complications. In the coming years we will use techniques that are less invasive in vitreoretinal surgery such as 25+, and 27-gauge. We will have available other anti-VEGF antibodies capable of blocking all types of VEGF isoforms before and after surgery, reducing intraoperative bleeding, and postoperative inflammation. It is likely that the use of preoperative agents that promote the detachment of the posterior hyaloid and facilitate the removal of membranes will become routine. They will facilitate surgery of complex cases such as PDR cases. Optical coherence tomography equipment will be available in the operating room as routine and that will facilitate intraoperative tissue differentiation, and help us get better functional results. The advent of new lasers will permit us faster retinal photocoagulation, and will minimize collateral damage of the retina.

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