Evaluation of Diabetic Macular Edema response to the treatment using Optical Coherence Tomography

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Abril 2013

ABSTRACT

Objective: To demonstrate the benefits of Ranibizumab after 6 month in patients with diabetic macular edema (DME), by analyzing best corrected visual acuity (BCVA) and central subfield retinal thickness (CRT) and to investigate the correlation between DME morphological patterns seen by optical coherence tomography (OCT) and the response to Ranibizumab therapy.

Design: Single-center, retrospective, observational, longitudinal 6-month study.

Participants: Were reviewed the clinical records of 80 patients (95 eyes) with DME treated with intravitreal injection of Ranibizumab. Were included to analysis 59 eyes of 51 patients with a minimum follow-up of 6 month.

Methods: All subjects had received an initial loading dose of 3 monthly injections of Ranibizumab (months 0-1-2), followed by further treatment according to protocol-defined retreatment criteria. All underwent BCVA testing (following ETDRS protocol) and OCT (CIRRUS HD-OCT) at baseline and follow-up visits (3 and 6 month) in order to correlate the response to the treatment with the different patterns of OCT.

Main Outcome Measures: Mean average change of BCVA, CRT and OCT patterns from Baseline to month 3 and 6.

Results: An increase of 4.78 letters of BCVA after 3 months of treatment and of 5.52 letters after 6 months were found analyzing all patients. From the 51 patients, 18.64% increased BCVA after the loading doses, in more than 5, but less than 10 letters, considering them the "responders" group, 20.34% increased 10 letters or more, being the "good responders" group

and 61.02% decreased or increased less than 5 letters considering them the “poor-responders” group.

CRT decreased 80.25µm after 3 month and 106.12µm after 6 month, in comparison with the baseline mean and a moderate correlation between BCVA and CRT was found before the treatment for all the patients ($r= -0.439$; $p<0.001$) as well as for the 3 subgroups. After starting the treatment, this correlation is only maintained in the “poor responders” group ($r= -0.435$, $p=0.008$; $r= -0.585$, $p<0.001$). Type 3 (external Cystoid Macular Edema (CME)), 4 (overall CME) and serous retinal detachment respond well to the treatment. The number of OCT scans with Type 1 (diffuse DME) and type 2 (internal CME) diminished between Baseline and Month 6 but the difference was not statistically significant. Epiretinal membrane and traction were the only patterns studied that didn’t decrease after 6 month of follow-up, resisting to this treatment.

Conclusions: Eyes treated with intravitreal Ranibizumab injections show a great improvement of BCVA and reduction of CRT in a 6 month follow-up time. Assessment of patterns of DME by OCT gives useful information to the prognosis. All DME morphological patterns disappear in a large percentage of patients after the intravitreal treatment of Ranibizumab. Vitreomacular traction and epiretinal membrane, however, don’t respond to this treatment and were the only linked to a poor response.

Keywords: Diabetic Retinopathy; Optical Coherence Tomography; Macular Edema; Cystoid Macular Edema; Ranibizumab; Visual Acuity; Treatment Outcome

RESUMO

Objectivos: Demonstrar o benefício do tratamento com Ranibizumab após 6 meses em doentes com edema macular diabético (DME), analisando a melhor acuidade visual corrigida (MAVC) e a espessura da retina no campo central (ERC) e investigar a correlação entre os padrões morfológicos do EMD identificados por tomografia de coerência óptica (OCT) e a resposta ao tratamento com Ranibizumab.

Tipo de estudo: Estudo retrospectivo, observacional com a duração de 6 meses, num único centro.

Participantes: Foram revistos os registos clínicos de 80 doentes (95 olhos) com EMD, tratados previamente com injecções intravítreas de Ranibizumab. Para análise, foram incluídos 59 olhos de 51 doentes com um tempo de mínimo de seguimento de 6 meses.

Métodos: Todos os participantes tinham recebido uma dose inicial de 3 injecções mensais de Ranibizumab (meses 0-1-2), seguida de tratamento adicional de acordo com critérios de re-tratamento definidos em protocolo. Em todos os casos foi avaliada a MAVC (de acordo com o protocolo ETDRS) e realizado OCT (CIRRUS HD-OCT) no início do estudo (antes do tratamento) e em visitas de acompanhamento (3 e 6 meses), a fim de correlacionar a resposta ao tratamento com os diferentes padrões de OCT.

Principais variantes estudadas: Variação média da MAVC, ERC e padrões morfológicos de OCT no início do estudo (antes da primeira injecção), mês 3 e 6.

Resultados: Da análise de todos os 51 doentes, verificou-se que houve um aumento de 4.78 letras da MAVC aos 3 meses após o tratamento e de 5,52 letras aos 6 meses. Desses doentes, 18,64% aumentaram a MAVC, após a dose inicial, em mais de cinco, mas menos de 10 letras,

classificando-os no grupo de “respondedores" (“responders”), 20,34% aumentaram 10 letras ou mais, sendo classificados num grupo de "bons respondedores" (“good responders”) e 61,02% tiveram um aumento inferior a 5 letras ou diminuição da MAVC, sendo classificados num grupo de "maus-respondedores" (“poor-responders”).

A ERC diminuiu 80.25μm após 3 meses e, 106.12μm, após 6 meses, em comparação com a média inicial. Foi encontrado, antes do tratamento, em todos os casos, uma correlação moderada entre ERC e a MAVC (r = -0,439, p <0,001), bem como nos três subgrupos. Depois de iniciar o tratamento, essa correlação só se manteve no grupo dos "maus-respondedores" (r = -0,435, p = 0,008, r = -0,585, p <0,001). Quanto aos padrões do OCT, verificou-se uma boa resposta ao tratamento, nos casos com Edema Macular Cistóide (EMC), externo (tipo 3), EMC “total” (tipo 4) e Descolamento Seroso da Retina (tipo 5). O tipo 1 (EMD difuso) e tipo 2 (EMC interno) diminuíram a sua frequência entre o inicio do estudo e o 6 º mês, mas a diferença não foi estatisticamente significativa. Os casos com Membrana Epirretiniana e Tracção foram os únicos que não diminuíram após 6 meses de seguimento, mostrando-se resistentes ao tratamento.

Conclusões: Olhos tratados com injecções intravítreas de Ranibizumab mostraram uma melhoria da acuidade visual e redução da CRT em seis meses de seguimento. A avaliação dos padrões de EMD com base no OCT fornece informações úteis para o prognóstico. Todos os padrões morfológicos de EMD melhoraram numa grande percentagem de pacientes após o tratamento intravítreo com Ranibizumab. Os casos com Tracção vítreo-retiniana e Membrana Epirretiniana, porém, não responderam a este tratamento e foram os únicos correlacionados com uma má resposta.

Palavras-chave: Retinopatia diabética; Tomografia de Coerência Óptica; Edema Macular; Edema Macular Cistóide; Ranibizumab; Acuidade Visual; Resposta ao Tratamento.

INTRODUCTION

Diabetic retinopathy (DR) is the most frequent cause of blindness in Europe and North America in people between 20 and 74 years old (1). Associated with change in eating habits and increasingly of sedentary lifestyle the number of diabetic patients has been growing. More than 220 million people worldwide have diabetes (90% type 2) and 10% of them develop significant changes in vision after 15 years of disease (data taken from WHO. Diabetes Fact Sheet Nº 312 Jan 2011. Available from: http://www.who.int/whr/en/). Between the beginning of proliferation and retinal hemorrhage are sometimes only weeks or months. The diagnosis and prompt treatment are crucial to avoid dramatic loss of vision. Therefore, the regular prevention and control of diabetic patients is very important.

Diabetic macular edema (DME) is the most frequent cause of loss of central vision in the course of DR. DME within 1 disc diameter of the fovea is present in 29% of the diabetic population which duration was 20 or more years. (2,3) Chronic DME can be associated with cystic degeneration of the macular retina called cystoid macular edema (CME).(4)

The cause of DR is considered to be a microangiopathy of the small vessels of the retina. It leads to loss of pericytes, basal membrane thickening and damage to the capillary endothelium with consequent blockage of capillaries and ischemia. The resultant hypoxia stimulates the production by the retina of growth factors such the "vascular endothelial growth factor" (VEGF), also known as permeability factor, because it stimulates vascular leakage and neovascular proliferation. The breakdown on the blood-retinal barrier and increase of the vascular permeability causes the accumulation of fluid and proteins on the macula, causing DME.(3) Studies made on primates also proved that the injection of VEGF into normal eyes induced microaneurysm formation and increased vascular permeability, similar to what happens in the development of DR.(5) Because of this, and as an alternative to

focal laser photocoagulation (which reduces the risk of moderate visual acuity (VA) loss by 50% but shows 12% of visual deterioration at the 3 year follow-up interval on the treated eyes, with only 25% of maintenance of retinal thickening and 3% gain of 3 lines of vision)(6), it was proposed the use of antibodies against VEGF for treating DME, such as Ranibizumab. Recent approval of Ranibizumab by the European Medicines Agency to treat visual impairment caused by DME fulfils the previously unmet medical need for a treatment that can improve visual acuity (VA) in these patients.

Ranibizumab is a recombinant antigen-binding fragment of a humanized anti-VEGF monoclonal antibody derived from mouse antibody. It binds to all biologically active and active proteolytic fragments of VEGF-A isoforms. It has been approved for the treatment of neovascular age-related macular degeneration (AMD), and visual impairment due to DME and retinal vein occlusions (RVO).(7) Many studies showed an improvement of BCVA and reduction of central retinal thickness (CRT), with significantly superior benefit over standard-of-care photocoagulation in patients with visual impairment due to DME (even if recurrent and persistent). These results were sustained for at least 2 years and are generally well tolerated and with minimally clinical relevant ocular or systemic adverse events.(8–15)

However, the observation that not all the patients respond to anti-VEGF treatment points the need of a study analyzing the factors that may be associated with a good or poor response to treatment.

Optical coherence tomography (OCT) introduced in 1991, it is a powerful imaging technology because it performs “optical biopsy” of the retina in real time, allowing in situ visualization of tissue microstructure, without the need to remove and process specimens(16). OCT provides cross-sectional images derived from rapidly acquired A-scans using low-

coherence infrared light and interferometry. Tissue pathology can be imaged with resolutions of 1–15 μm, one to two orders of magnitude finer than conventional ultrasound.(17)

OCT provides images of retinal structure that cannot be obtained by any other noninvasive diagnostic technique. In DME, it allows for a precise evaluation of retinal thickness (RT) i.e., edema, vitreomacular interface, subretinal fluid and foveal microstructural changes.(18)

In this work, we will explore the response of DME to Ranibizumab in an effort to identify characteristics of the retina seen by OCT, which may be associated with differences in the response to anti-VEGF intravitreal therapy.

METHODS

Patients and Study Design

We conducted a retrospective study of eyes with DME treated with intravitreal injections of Ranibizumab (Lucentis®, Novartis Pharma AG, Basel, Switzerland and Genentech Inc., 115 South San Francisco, CA, USA) between 2009 and 2011 at AIBILI (Association for Innovation and Biomedical Research on Light and Image) and CHUC (Centro Hospitalar e Universitário de Coimbra) in Coimbra, Portugal. We reviewed the clinical records of 80 consecutive (95 eyes) with Clinically Significant Macular Edema (CSME) as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS) (6), treated with a loading dose of three monthly intravitreal injections of Ranibizumab (0.5 mg). Institutional review board/ethics committee approval and patients' informed consent were obtained for this study (Clinical Trial Number: NCT00797134). The use of the drug and its potential risks and benefits were discussed extensively with all patients. Inclusion criteria was the presence of CSME, in patients with *Diabetes Mellitus* type 1 or 2 and the previous treatment with Ranibizumab. Exclusion criteria were: intravitreal injections of steroids within a period of 18 months, and/or focal or pan photocoagulation of the retina less than 6 month before the first injection of Ranibizumab, previous injection of any anti-VEGF drug, macular edema unrelated to DR, a history of ocular hypertension or glaucoma in the study eye with concomitant retinal or choroidal disorder other than DR, study eye with significant central lens opacities and/or conditions that limit the view of the fundus, decreased vision due to other causes (that not DME), in the investigator's opinion (at visit 1), poor general condition and patients who were unwilling to adhere to visit examination schedules. 30 eyes were excluded for these reasons. From the 65 eyes, 6 were excluded for insufficient number of OCTs or follow-ups. The final number of studied patients was 51 (59 eyes). (fig.1)

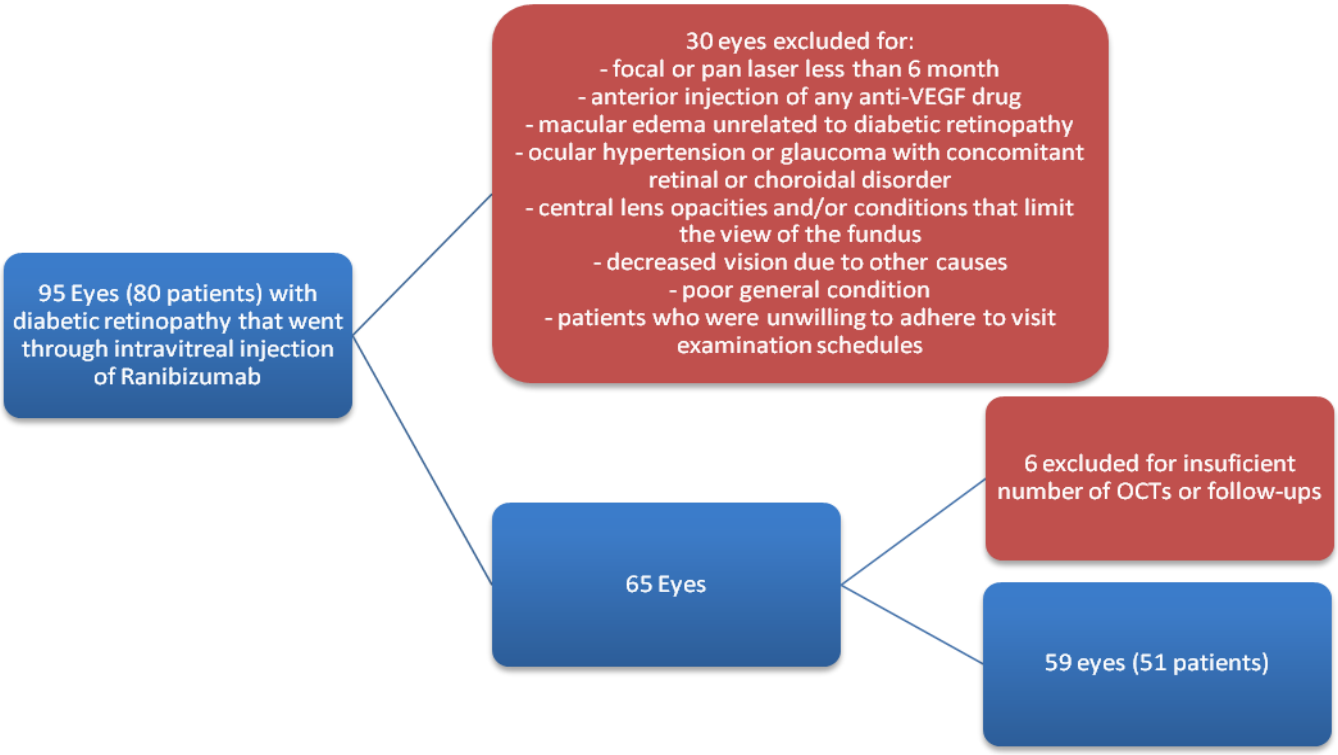


Figure 1 – Study design.

Ophthalmic examination

Patients received 3 initial consecutive monthly injections of Ranibizumab (months 0-1-2; loading phase), followed by further treatment according to protocol-defined retreatment criteria. Intravitreal Ranibizumab injections were performed using the physician usual routines; both pre- and post-injection topical antibiotics were used. As of month 3, the protocol required that 1 injection per month was to be continued if stable VA was not reached. Treatment was suspended if either of the following criteria were met: (1) if the investigator's opinion was that no (further) BCVA improvement was attributable to treatment with intravitreal injection at the last 2 consecutive visits, or (2) > 20/20 Snellen score was observed at the last 2 consecutive visits. After suspension, injections were resumed if there was a decrease in BCVA due to DME progression, confirmed by clinical evaluation and/or OCT or other anatomic and clinical assessments, in the opinion of the physician. Patients

were treated at monthly intervals until stable VA was reached again. Thus, reinitiation of intravitreal injections encompassed ≥2 successive monthly treatments. (fig.2)

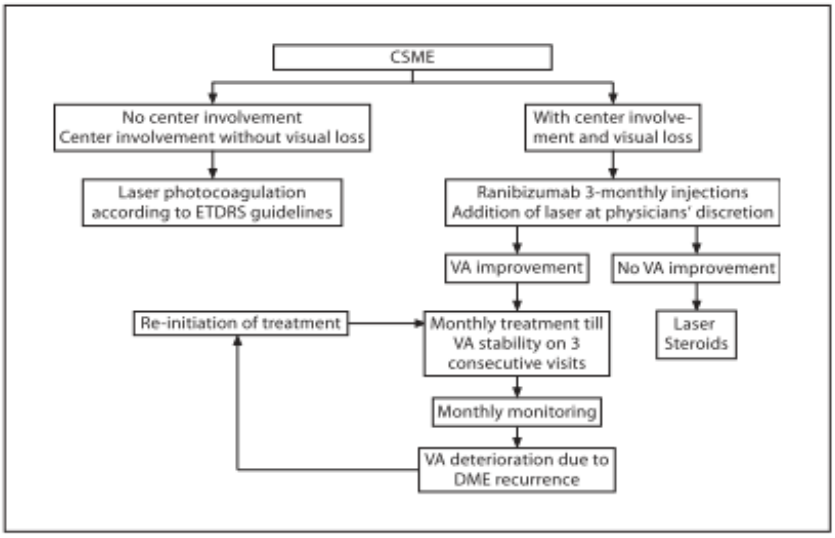


Figure 2 – Algorithm for the treatment of DME and the use of Ranibizumab in patients with visual impairment due to DME.(7)

All patients performed BCVA measurements using ETDRS protocol and Cirrus OCT at baseline, 3 and 6 months after initial injection. Baseline central retinal characteristics were analyzed by spectral domain OCT (Cirrus OCT, Carl Zeiss, Dublin, USA) using the macular cube acquisition protocol (512 × 128 scans). The retinal thickness of the 1-mm central retina (i.e., the central subfield area thickness (CRT)) was obtained from the macular thickness map and used as the OCT measurement of central area. Patients were included in this consecutive series only if there was a minimum of 6 months' follow-up.

OCT scans were graded and classified in each visit according to the following categories (Fig.3):

- Type 1 - Diffuse DME without cystoid spaces,

- Type 2 - Internal cystoid DME (meaning: macular edema with presence of cyst like empty spaces in the inner layers of the retina),
- Type 3 - External cystoid DME (meaning: macular edema with presence of cyst like empty spaces predominantly in the outer layers of the retina),
- Type 4 – Overall cystoid DME (meaning: macular edema with presence of cyst like empty spaces involving both inner and outer retina layers),
- Type 5 – presence of serous retinal detachment (SRD).
- Presence of epiretinal membrane (EPRmemb) or retina traction was also evaluated.

Each OCT scan could be classified with one or more types.

In brief, sponge-like or DME was identified as a diffuse, ill-defined hyporeflective area of retinal thickening. The cystoid DME was identified by the presence of cystoid hyporeflective empty spaces. Cystoid DME was classified depending on the location into: external cystoid edema, when the cystoid spaces were localized predominantly in the outer retinal layers, internal cystoid edema, when the cystoid spaces were situated in the inner retinal layers and the fourth group consisted of an accumulation of cystoid edema with the cystoid spaces involving all the retinal layers. The presence of SRD was diagnosed as separation of neurosensory retina from the highly reflective retinal pigment epithelium/choriocapillaris band. In cases where more than one type coexisted, all of them were registered. The EPRmemb was defined as the presence of a hyper-reflective membrane on the inner retinal surface. The vitreomacular traction was defined as a hyper-reflective band that arose from the inner retinal surface and extended peripherally or towards the optic nerve; this classification included: (1) perifoveolar posterior vitreous detachment (PVD) with foveolar attachment,

meaning the posterior hyaloid remains partially attached to the macula, or (2) incomplete PVD with residual attachment to the optic nerve meaning the posterior hyaloid looks detached from the macula but remains visible in front of it on all OCT mapping scans or (3) complete PVD. There was no distinction between the three types of traction described. The scans classification was performed by trained ophthalmologists and consensus was achieved in cases of disagreement.

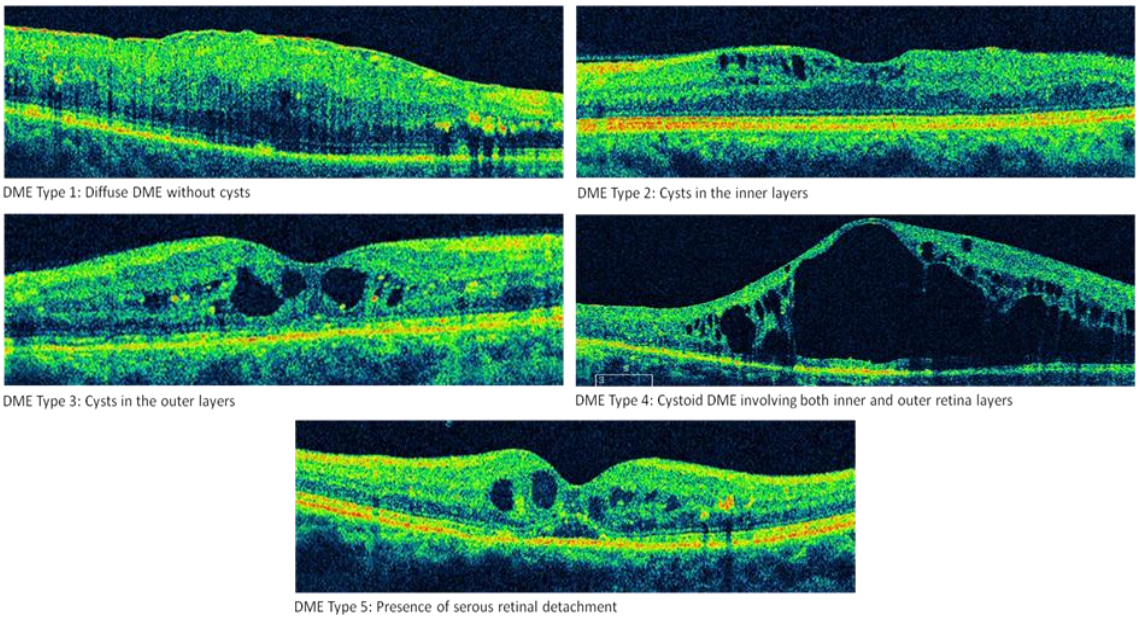


Figure 3 - Patterns of macular edema. DME Type 1 - Diffuse macular edema; DME type 2 - internal cystoid edema; DME type 3 - external cystoid edema; DME type - 4 cysts in both layers; DME type 5 - serous retinal detachment (and/or epiretinal membrane and vitreomacular traction, not present in this figure).

The OCT morphological patterns were correlated with BCVA changes between visits.

According to BCVA response, after 3 month, patients were classified in 3 groups(19–21):

- Poor-responders: BCVA decreased or increase less than 5 letters (< 5),
- Responders: BCVA increased more than 5 letters, but less than 10 letters (≥ 5 or < 10),
- Good responders: BCVA increased more than 10 letters (≥ 10).

Statistical analysis

Main outcome measures included changes in BCVA and OCT. Interval data were analyzed at 3-, and 6-month follow-up time points. Correlations between BCVA and OCT were tested using spearman correlation coefficient. Statically significant differences between groups of responders were tested using the Mann-Whitney test when considering OCT values and using the chi-square test when considering the OCT grading. Statistically significant differences between visits were tested using the Friedman and the Wilcoxon tests for OCT and BCVA values.

To identify predictive morphological patterns for response treatment an ordinal regression analysis was performed.

Statistical analyses were performed using STATA software version 12.1. Statistically significant results were considered for P values < 0.05.

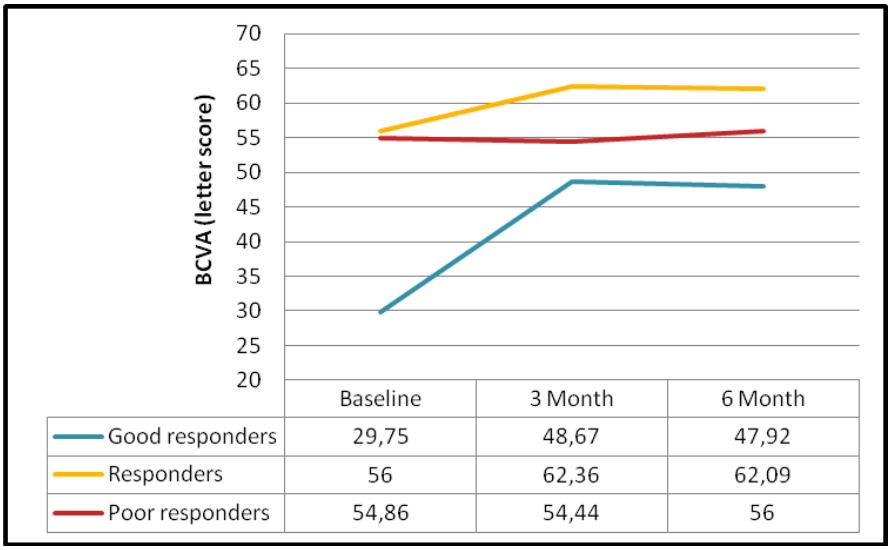
RESULTS

Fifty-one patients (59 eyes) with a minimum of 6 months' follow-up were included for analysis with a mean age of 69.02 ± 7.75 years. Thirty-three were male (64.71%) and eighteen female (35.29%).

Analysis of BCVA

At baseline visit, the mean BCVA for the 59 eyes was 49.97±20.88 letters, increasing in the 3rd month follow-up to 54.75±17.61 letters, a difference that was statistically significant (P<0.001). BCVA continued to increase in the following 3 month, from 54.75±17.61 to 55.49±19.84 letters (P=0.668). The difference between the BCVA at the baseline visit and the 6-month follow-up is statistically significant (P<0.001).

BCVA analysis by subgroups (fig. 4) demonstrated that 12 (20.34%) eyes improved ≥10 letters (2 ETDRS lines) being "good responders", 11 (18.64%) increased more than 5 letters, but less than 10 letters being "responders", and 36 (61.02%) decreased BCVA or had an increase of less than 5 letters, being "poor-responders".



	Baseline	3 Month	6 Month
Good responders	29,75	48,67	47,92
Responders	56	62,36	62,09
Poor responders	54,86	54,44	56

Figure 4 – Mean BCVA letter score by responders' subgroups ("good-responders", "responders" or "poor-responders") at baseline, 3- and 6 –month follow up.

All the subgroups showed an increasing of BCVA from baseline to the 6th month follow-up. The baseline values of BCVA differ statistically between responders subgroups (P=0.013). The group of the "good responders" had an increase of BCVA of 18.17 letters (from 29.75±20.03 to 47.92±19.54) at the 6th month follow-up, a difference that was statistically significant (P=0.002) and the group of the "responders" had an increase of 6.09 letters (from 56.0±10.75 to 62.09±13.88), which was also statistically significant (p=0.029).

When compared with the first 3 months, it was observed in both "good responder" and "responders" group, a slight decrease of BCVA score in the last 3 months of follow up (+18,92 letters (P=0.002) in the first trimester, -0,75 letters (P=0.652) in the last for the "good responders" and +6,36 letters (P=0.004) in the first trimester, -0,27 letters (P=0.893) in the last, for the "responders"). However these differences were not statistically significant.

The "poor responders" group showed no statistically significant change of BCVA from baseline to month 3 or 6, (from 54.86±19.68 to 54.44±19.05 letters; p=0.806 at 3 months and from 54.86±19.68 letters to 56±21.06 letters, p=0.844 at 6 months).

Despite the higher improvement of the "Good responders" group in the BCVA score after 6 months follow-up, the final BCVA score of the "Responders" and "Poor responders" is still better (see figure 4) due to a better baseline BCVA score.

Analysis of CRT

The mean retinal thickness in central area (1mm diameter) for all patients was 507.61 ± 147.36 µm at Baseline. By the 3rd month follow-up, mean CRT, decreased to 427.36±154.33 µm, a difference that was statistically significant (P<0.001). CRT continued to decrease in the following 3 month, to 401.49±153.20 µm (p=0.190), being statistically different from baseline (P<0.001).

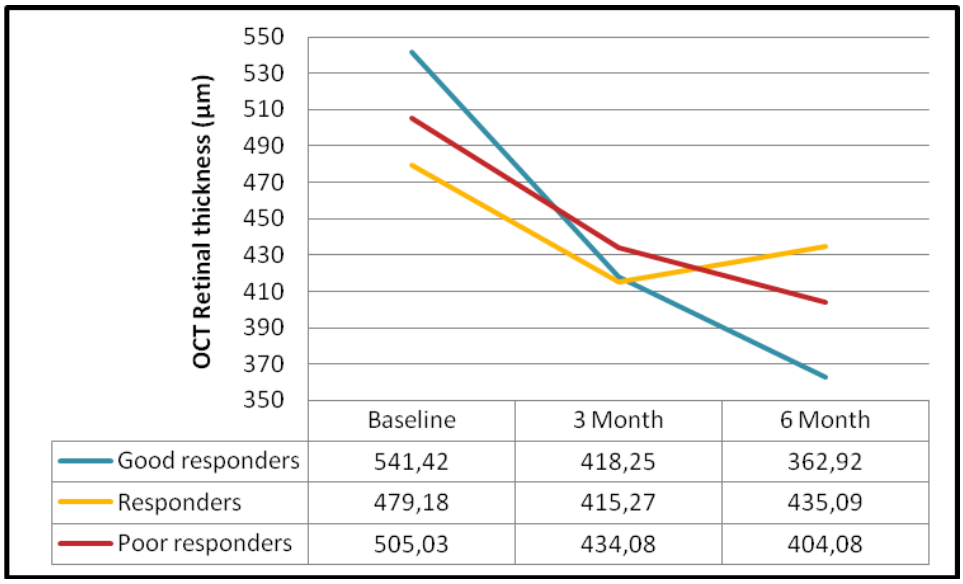


Figure 5 - Mean central retinal thickness (CRT) (µm) by responders' subgroups ("good-responders", "responders" or "poor-responders") at baseline, 3- and 6 –month follow up.

All groups showed a reduction of the central retinal thickness (fig. 5). The baseline values of CRT do not differ statistically between responders subgroups (P=0.364). The "good responders" group showed the largest decrease of retinal thickness from baseline to 6th month follow-up (from 541.4±132.70 µm to 369.92±166.79 µm), a difference that was statistically significant (P=0.003), in comparison with "responders" group that had only a decrease from 479.18±115.68 µm to 435.09±111.09 µm, not statistically significant (P=0.182). "Poor responders" group showed a big statistically significant decrease from 505.03±161.16 µm to 404.08±160.08 µm (p<0.001).

In all groups, the decrease of CRT was higher in the first three months than in the last three. The "good responders" reduced a mean of 123.17 µm of CRT from baseline to the 3rd month follow-up (P=0.023), followed by a reduction of only 55.33 µm in the last 3 months (P=0.308). Similarly, the "poor responders" reduced a mean of 70.95 µm of CRT from baseline to the 3rd month follow-up (P<0.001), followed by a reduction of only 30.0 µm in the second trimester (P=0.126).

In the "responders" group, a slight increase of CRT was observed in the last 3 months, comparing with the first 3 months of the treatment (- 63.91 µm (P=0.168) in the first trimester, and + 19.82 µm (P=0.657) in the last). However these differences were not statistically significant.

Correlation between BCVA and CRT

There is a moderate correlation between BCVA and CRT at baseline considering all patients (r= -0.439; p<0.001) and the 3 groups separately: "poor responders" (r= -0.473; p=0.004), "responders" (r= -0.456; p=0.159) and "good responders" (r= -0.330, p=0.295) showing that the higher the mean CRT, the lower the BCVA.

After starting the treatment, this correlation is only maintained in the group of patients that did not respond to the treatment ("poor responders", at month 3 r= -0.435, p=0.008 and month 6 r= -0.585, p<0.001).

In the groups that respond to treatment, BCVA is not correlated with CRT after the treatment began ("good responders": r= 0.274, p= 0.389 at month 3 and r= -0.390, p= 0.210 at month 6; "responders": r= -0.193, p= 0.570 at month 3 and r=0.196, p= 0.564 at month 6).

Patterns of DME in OCT and response to treatment

Considering the different retinal morphological patterns (Table 1) we classified, at baseline, 28 eyes (47.5%) with Diffuse Macular Edema (type 1) pattern in OCT scan, 36 eyes (61.0%) with internal cystoid macular edema (type 2) pattern in OCT scan, 52 eyes (88.1%) with external cystoid macular edema (type 3) pattern in OCT scan, 31 eyes (52.5%) with overall cystoid macular edema (type 4) pattern in OCT scan and 11 eyes (18.6%) with serous retinal detachment (type 5). Presence of epiretinal membrane was observed in 20 eyes (33.9%) and 10 eyes (16.9%) presented vitreomacular traction.

Morphological Patterns								
		Dif DME	Int CME	Ext CME	Overall CME	SRD	EPRMemb	Traction
Baseline	Good resp.	6 (50%)	5 (41,7%)	11 (91,7%)	8 (66,7%)	3 (25%)	5 (41,7%)	1 (8,3%)
	Responders	7 (63,6%)	8 (72,7%)	9 (81,8%)	5 (45,5%)	4 (36,4%)	4 (36,4%)	2 (18,2%)
	Poor resp.	15 (41,7%)	23 (63,9%)	32 (88,9%)	18 (50%)	4 (11,1%)	11 (30,6%)	7 (19,4%)
3M	Good resp.	4 (33,3%)	6 (50%)	10 (83,3%)	3 (25%)	1 (8,3%)	4 (33,3%)	1 (8,3%)
	Responders	6 (54,5%)	6 (54,5%)	8 (72,7%)	2 (18,2%)	1 (9,1%)	4 (36,4%)	2 (18,2%)
	Poor resp.	18 (50%)	18 (50%)	28 (77,8%)	10 (27,8%)	2 (5,6%)	14 (38,9%)	8 (22,2%)
6M	Good resp.	4 (33,3%)	3 (25%)	8 (66,7%)	2 (16,7%)	0	4 (33,3%)	1 (8,3%)
	Responders	6 (54,5%)	8 (72,7%)	7 (63,6%)	7 (63,6%)	1 (9,1%)	4 (36,4%)	2 (18,2%)
	Poor resp.	14 (38,9%)	19 (52,8%)	23 (63,9%)	10 (27,8%)	1 (2,8%)	12 (33,3%)	7 (19,4%)

Table 1 – Number and percentage of the different morphological patterns and features observed in OCT for each group and in each follow-up visit (baseline, 3 and 6 month). Dif DME (diffuse DME), Int CME (internal cystoid macular edema), Ext CME (external cystoid macular edema), Overall CME, SRD (serous retinal detachment), EPRmemb (epiretinal membrane), Traction (vitreomacular traction).

Despite no statistically significant differences were found between groups of responders, we can see a larger number of cases of vitreomacular traction in the "poor responders" group at baseline (19,4%) comparing with the other subgroups, which is maintained during the 6 months of follow-up.

From the ordinal regression analysis no specific pattern seems to be predictive of a good response to treatment.

Analysis of the evolution of each parameter along the 6 month

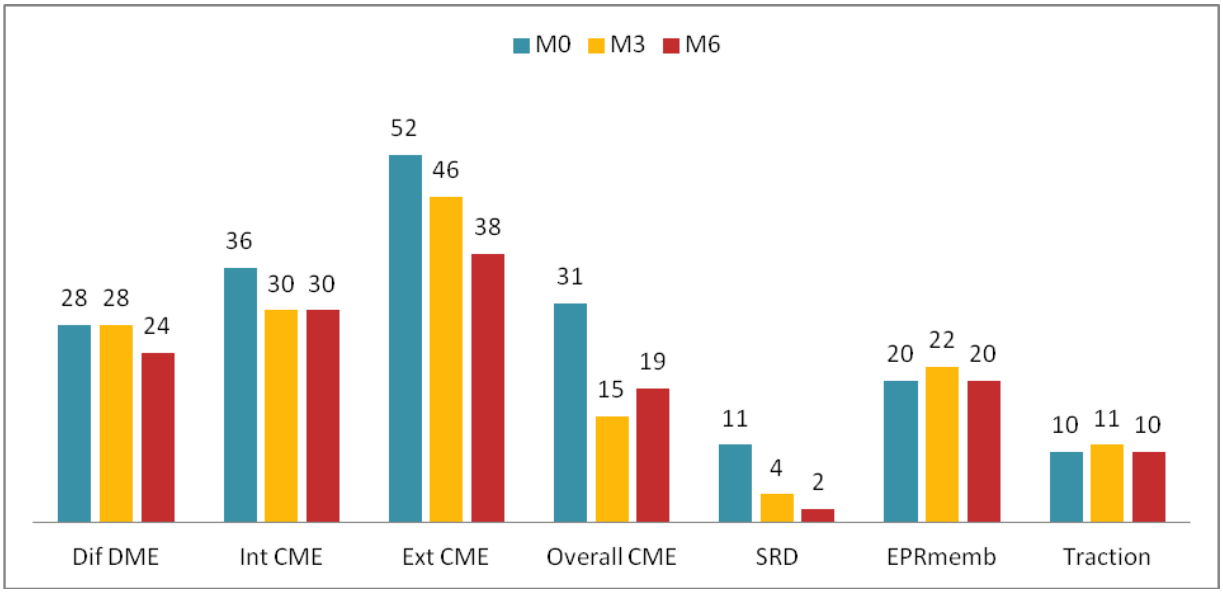


Figure 6 – Graphic comparing the frequency of the different studied patterns of all the studied patients (without division per subgroups) at baseline (M0), 3- (M3) and 6-month (M6) follow-ups.

From the analysis of all the patients, without the division per subgroups (Fig. 6), we observed a decreasing of all the patterns from baseline to the 6th month follow-up, except for EPR membrane and vitreomacular traction (that maintained their values along the 6 month, P=1.000 for both). However, at month 6 only the diminishing of external CME (P<0.001), overall CME (P=0.036) and SRD (p=0.004) are statistically different from Baseline, this may be explained with the reduced number of OCTs [Diffuse DME (p=0.481) and Internal CME (P=0.238)].

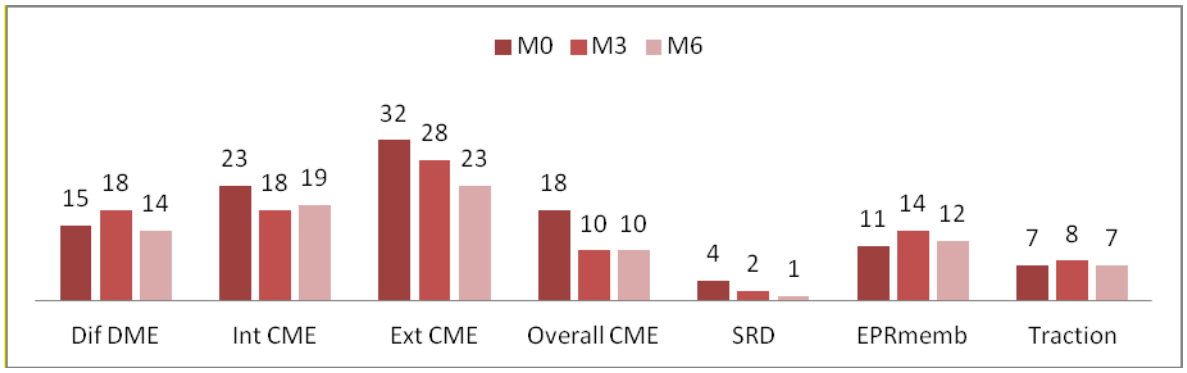


Figure 7 - Graphic comparing the frequency of the different studied patterns (as before) in the subgroup of the "poor-responders" at baseline, 3- and 6-month follow-ups.

In the "poor-responders" group (Fig. 7) we see that there is no statistically difference after the 6 months of follow-up, in the number of eyes with: Diffuse DME , EPR membrane and Traction (P=1.000).

However, there was a decrease of the presence of all the other patterns but only statistically significant for External CME (-9 cases (p=0.004))

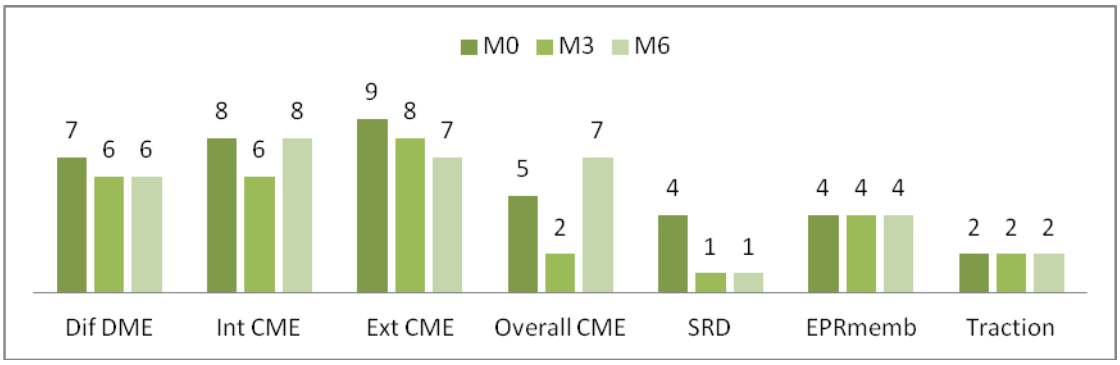


Figure 8 - Graphic comparing the frequency of the different studied patterns (as before) in the subgroup of the "responders" at baseline, 3- and 6-month follow-ups.

In the responders group there was no statistically significant difference in the 6 months of follow-up for the diffuse DME and Internal CME patterns and for the presence of EPR membrane and Traction (P=1.000).

The presence of SRD was the pattern with larger reduction in this group (P= 0.250).

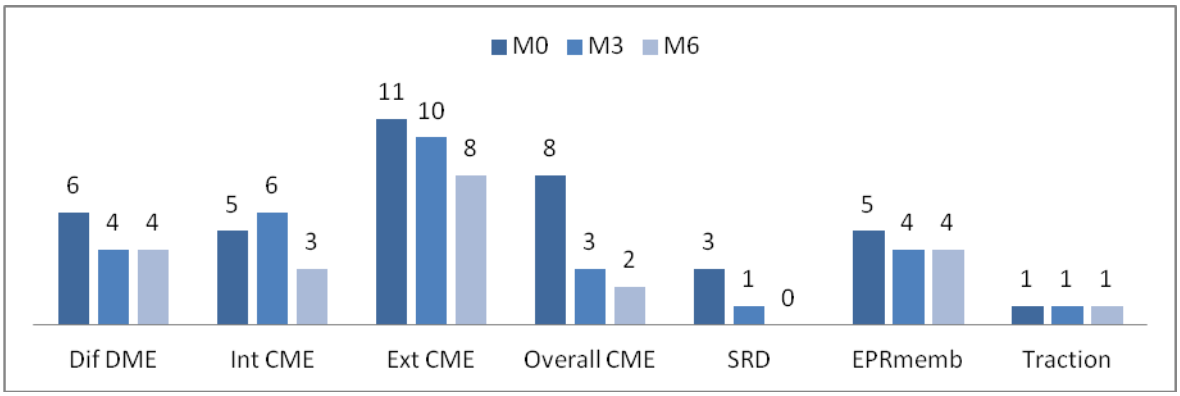


Figure 9 - Graphic comparing the frequency of the different studied patterns (as before) in the subgroup of the "good-responders" at baseline, 3- and 6-month follow-ups.

In the group of the "good responders" (Fig. 9), we see that there was a reduction of most of the OCT patterns from baseline to the 6 months of follow-up (however, presence of Traction or EPR Membrane did not change in this group during the study period, P=1.000).

The largest reduction was observed in the number of cases with Overall CME pattern (P=0.071) and SRD (P=0.250). However, the differences were not statistically significant, probably due to the small number of eyes in the "good responders" group.

DISCUSSION

The goal of treatment with laser photocoagulation was mostly VA stabilization. Although the ETDRS(6) 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. Anti-VEGF drug, Ranibizumab was approved for the treatment of visual impairment due to DME and fulfills the unmet medical need for a therapy that can improve VA in patients with visual loss. Evidence-based treatment recommendations are needed in view of this newly approved drug.(7)

Our patients increased, after 3 month, a mean of 4.78 letters, and after 6 month, 5.52 letters of BCVA in comparison with the baseline mean (49.97±20.88 letters).

The results showed a high percentage of patients (61.02%) with a decrease of BCVA or an increase of less than 5 letters after a loading doses (3 monthly injections) of Ranibizumab, making them "poor-responders", 20.34% of patients responding good to the treatment, with an increase of 10 letters or more after the loading doses and 18.64% with an increased of BCVA > 5 but < 10 letters, placing them on the group of the "responders".

These results are similar to other studies, however with a mean increase of BCVA slightly worse. Study READ-2(10) showed an improvement at month 6 of 7.4 letters for the group of patients that had Ranibizumab (0.5mg) monotherapy and, after 2 years, 7.7 letters. The improvement of BCVA was numerically higher than with combination therapy (which showed 3.8 letters at month 6 and 6.8 after 2 years) (combination of 0.5 mg Ranibizumab with focal or grid laser). The number of injections of the combination therapy was fewer than with the monotherapy, without big disadvantage in the BCVA outcome. Other study,

RESOLVE(8), used a similar method than ours, making also a loading doses of three monthly injections of Ranibizumab (0.3 or 0.5mg). At 12 months, the patients gained 10.3 letters in BCVA, in comparison with -1.4 letters for the sham group. RESTORE(9) showed an increase of BCVA, after 12 months, of 6.8 letters for Ranibizumab monotherapy and 6.4 letters for combined therapy, both significantly higher than 0.9 letters gain of patients treated with laser monotherapy.

The percentage of patients with more than 10 letter BCVA gain (correspondent to our group of "good responders") were, in RESTORE study, 37.4% for the patients with Ranibizumab monotherapy, 43.2% for the group with combination therapy and 15.5% in the group that only had laser therapy. In RESOLVE study, this percentage was 60.8% for patients treated with Ranibizumab, in comparison with only 18.4% for the sham group.

In this study, a moderate correlation was found between baseline BCVA and the mean retinal thickness ($r= -0.439$; $p<0.001$) considering all the patients and for each subgroup, showing that the higher the mean central RT, the lower the BCVA. However, this correlation is only maintained in the group of the "poor responders" after the beginning of treatment (at month 3 $r= -0.435$, $p=0.008$ and month 6 $r= -0.585$, $p<0.001$). The correlation disappear at month 3 and 6 for the groups responding to the therapy ("good responders" and "responders"), which may mean that therapy reduces initially the macular thickness but may not be accompanied by the same increase of BCVA. It is possible that BCVA may not improve with the same time course as thinning of the macula. Macular function improvement may lag behind anatomic improvement.(22)

Our patients CRT decreased a mean of 80.25µm after 3 month and 106.12µm after 6 month of follow up in comparison with the baseline (507.61 ± 147.36 µm).

There was no statistical differences in CRT between subgroups in Baseline (P=0.364) and all the studied subgroups showed a reduction of central area thickness (1mm subfield). The group of "good responders" showed a decrease of 178.5 μm, the "responders" a decrease of 44.1 μm (P=182) and the "poor responders" showed a big statistically significant decrease of 100.95 μm ($p<0.001$).

These results are similar to other studies; however the separation by subgroups was not done until now. The mean CRT reduction in the RESOLVE study was 194 μm with Ranibizumab (in comparison with only 48.4 μm with sham injections), after 12 months. In the RESTORE study, with Ranibizumab alone the reduction was 118.7 μm, and in the combination group was 128.3 μm (in comparison with only 61.3 μm with laser monotherapy). In the READ-2 the reduction of mean CRT at month 24 was 340, 286 and 258 μm, for the group treated with Ranibizumab alone, laser photocoagulation and combination, respectively.

It is interesting to note that in our study, the group of the "good responders" (which increase 18.17 letters after the 6 months of follow up), started with the lowest BCVA score (29.75±20.03 letters), while the group of the "poor responders" (which had a not statistically significant increase of 1.14 letters from baseline) started with the second best BCVA score of the study (54.86±19.68 letters). A similar result was found in the "responders" group which increased only 6,09 letters during the 6 months and registered a baseline BCVA score of 56.0±10.75 letters, very close to the "poor responders group".

At the end of the 6 months follow-up, "Responders" and" Poor responders group" still show a higher BCVA score than the "Good Responders" group (62.09±13.88, 56±21.06 and 47.92±19.54 letters, respectively).

This can mean, as already showed in other studies that, in general, patients with poorer BCVA may have a better BCVA benefit than the others.(23)

Even though, the "good responders" improved BCVA and the "poor responders" maintained, their initial central retinal thickness was not statistically different (541.4±132.70 μm and 505.03±161.16 μm, respectively), and the final reduction (after 6 month), was also not so different (178.5 μm and 100.95 μm, respectively). This seems to point out that there should be other factors, apart from initial BCVA, that have influence in the response to Ranibizumab.

In all the groups, the decrease of CRT was higher in the first three months than in the last. The "responders" group showed even an increase of the thickness in the second trimester comparing with the first (-63.91 μm (P=0.168) in the first 3 months and +19.82 μm (P=0.657) in the second), however not statistically significant.

Besides that, "responders" group, as well as "good responders" group had an increase of BCVA in the first three months followed by a small but not statistically significant decrease of BCVA in the last 3 months. This may suggest that Ranibizumab may be more effective to the improvement of BCVA during the administration of the "loading doses", than after it, where its role may be more the maintenance of BCVA. It would be interesting to study these patients longer than 6 month, to evaluate the BCVA and CRT behavior during continuation of therapy (if needed).

Our study shows that OCT scans should not only be analyzed quantitatively, as done before by measuring the thickness of retina, but also a qualitative analyze should be done (concerning the characterization of the reflectivity profiles and morphological properties of intraocular structures) to be integrated with clinical data, previous exams and other diagnostic tools.(24) We classified the OCTs as described in the methods based in several previous

studies (25,26) with the purpose of describing the possible different patterns of DME and analyzing the usefulness of these OCT patterns in predicting response to treatment. We hypothesized that detailed interpretation of OCT images in each follow-up visit of patients with DME may be an additional tool to determine the prognosis of DME and the response to treatment and may help to understand some of the discrepancies found in the correlation between central subfield thickness and visual acuity in these patients. The various morphological patterns of DME seen in OCT may represent different levels of severity or chronicity of the disease.

Diffuse macular edema (type 1) was seen at baseline in 47.5% of 59 eyes. The cross-sectional images showed that the swollen retina looked like a sponge and had clearly increased retinal thickness, where the area of low reflectivity had expanded. The outer retinal layers appeared to be the privileged site for tissue swelling in our study, which is consistent with previous findings, that suggest fluid and protein accumulate there, not only because they enter the extracellular space, but also because the external limiting membrane (ELM) retains albumin and other osmotically-active molecules, causing edema.(27). This seems to correspond to the beginning of the process; progressing by growing of the extracellular spaces creating cystoid spaces (cystoid edema, CME). The cystoid cavities within the neurosensory retina are separated by highly reflective septa bridging retinal layers. They can be located both in the inner or outer retinal layers (internal and external CME). Later, the cystoids spaces can occupy the whole retinal width, creating large cysts (overall CME). (24)

Eyes with Diffuse DME tend to have better visual acuity and less macular thickness than eyes with CME.(26) Sivaprasad, showed in his study about diffuse and cystoid macular edema in patients with uveitis, that a fair proportion of Diffuse ME are not detected clinically and has no short-term influence on visual acuity.(26) We found out that the group with higher

percentage of this pattern was the “responders” (63.6%), which as Baseline had the smallest CRT (479.18±115.68 µm) and the higher BCVA (56.0±10.75 letters) compared with the other two subgroups.

We also observed 11 cases (18.6%) of serous retinal detachment, which is also a cause of retinal thickening. It is the accumulation of fluid under the neurosensory retina and corresponds to an optically clear space between the retina and retinal pigment epithelium (RPE), with a distinct outer border of the detached retina.(24)

From the general patient analysis of the OCT, we can tell that type 3 (external CME), 4 (overall CME) and serous retinal detachment respond significantly to the treatment ($P<0.001$, $P=0.036$ and $P=0.004$, respectively), decreasing their occurrence along the 6 month. There was also a decrease of type 1 (Diffuse Macular Edema) and 2 (Internal CME), however it wasn’t statistically different from Baseline to month 6 ($P=0.481$ and $P=0.238$). Other studies with superior number of subjects are needed.

With OCT, we now have the possibility of observing and analyzing the vitreomacular interface and recognize in an earlier stage of the disease if there is vitreoretinal traction (even it is only slightly detached) or presence of epiretinal membrane.(24,28) Gaucher et all supported the importance of the vitreoretinal interface in the development of macular edema in diabetics (DME). Their retrospective case-control study of 35 diabetic patients with edema and 35 age-matched diabetic controls showed a high prevalence of perifoveolar PVD with persistent foveolar attachment in patients with DME.(29) The vitreomacular traction syndrome is almost invariably accompanied by epiretinal membrane,(30) which may play a role in increasing the strength of the residual vitreomacular adhesion and prolonging the duration of vitreomacular tractional stress.(31) We identified 20 cases (33.9%) of epiretinal membrane and 10 cases (16.9%) of vitreomacular traction, in all 59 eyes at baseline.

From the general patient analysis and the subgroup analysis, epiretinal membrane and traction didn't decrease after 6 month of follow-up, maintaining their values (P=1.00). These results are consistent with the previously referenced studies. The detection of traction or epiretinal membranes using OCT on DME is particularly important, since they could be easily missed by clinical examination or fluorescein angiography alone(31) and these features would benefit more from treatment with vitrectomy (28,32,33) than from intravitreal injections of anti-VEGFs, unlike the other OCT morphological patterns.

We recognize the limited power of this study; however, our findings suggest that anti-VEGF drugs are an important breakthrough in the treatment of DME. DME responds well to treatment with intravitreal Ranibizumab over 6 months. In light of the sustained improvements in BCVA and CRT over the 6- month study period, Ranibizumab appears to be a promising pharmacological agent for the management of visual impairment due to DME.

This study considered also the OCT patterns that may influence the differences of outcome between patients. From this analysis, we proved that diffuse DME and CME disappear in a large percentage of patients after the intravitreal treatment of Ranibizumab. Traction and EPRmemb, however don't respond to this treatment and were the only linked to a poor response, which may be connected with the benefits proved for these features with pars plana vitrectomy.(34)

AGRADECIMENTOS

Quero, em primeiro lugar, agradecer à minha orientadora Professora Doutora Maria da Conceição Lopes Lobo da Fonseca por toda a ajuda, paciência e ensinamentos. E ao meu co-orientador Professor Doutor José Guilherme Cunha-Vaz pela transmissão de experiência nesta área e por me ter proporcionado as condições para a realização deste trabalho.

Gostaria também de agradecer à Dr.ª Sandrina Nunes por toda a ajuda relativamente ao tratamento estatístico.

Agradeço também a Dr.ª Ana Rita Santos por toda a disponibilidade a qualquer dúvida e simpatia que sempre demonstrou.

Por último, gostaria de agradecer aos meus amigos e família, sem os quais não teria conseguido ultrapassar muitas dificuldades na realização deste trabalho e que sempre me deram forças para continuar.

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