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João Pedro Vieira Fonseca

Vascular endothelial growth factor plasma concentrations before and after intravitreal injection of bevacizumab and ranibizumab

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Professora Doutora Ângela Maria Veloso Guimarães Carneiro

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Vascular endothelial growth factor plasma concentrations before and after intravitreal injection of bevacizumab and ranibizumab

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Abstract:

Purpose: **To evaluate the vascular endothelial growth factor (VEGF) plasma concentrations before and one, three and four weeks after intravitreal injection of bevacizumab or ranibizumab in patients with wet age-related macular degeneration (AMD).**
Methods: **12 patients with wet AMD and 18 controls were studied. 6 patients were treated with bevacizumab 1.25 mg and another 6 with ranibizumab 0.5 mg. Consecutive blood samples were taken before and one, three and four weeks after drug administration. Concentrations of VEGF in the plasma were measured by ELISA.**
Results: **At baseline there were no significant differences regarding age or sex between controls, the bevacizumab and ranibizumab groups. The median VEGF concentrations were lower (p=0.004) in controls (180.0 pg/ml) than in the wet AMD group (281.2 pg/ml). Consecutive VEGF concentrations decreased significantly at one (84,7 pg/ml; p<0.028), three (140,8 pg/ml p<0.028) and four (193,7 pg/ml p<0.028) weeks in the bevacizumab group. The same was verified in the ranibizumab group only after three (206,1 pg/ml p<0.028) and four (291,2 pg/ml p<0.046) weeks.**
Conclusions: **Both bevacizumab and ranibizumab significantly decrease the systemic VEGF concentrations in the first four weeks after the start of the treatment. Bevacizumab seems to precipitate a more pronounced VEGF drop in the first week. On the contrary, ranibizumab has a more progressive reduction along the four weeks.**

Key words: VEGF; Bevacizumab; Ranibizumab; AMD; wet AMD; anti-VEGF therapy;

Introduction:

Age-related macular degeneration (AMD) is the major cause of severe and irreversible vision loss in developed countries (Congdon 2004; Resnikoff et al. 2004). The exudative form of the disease (wet AMD) is characterized by choroidal neovascularization (CNV) that invades the subretinal space, and of its sequelae, with disrupting of the outer retinal layers resulting in scarring and vision loss (Bird et al. 1995).

CNV is created, in response to metabolic distress, by the retinal pigment epithelium and the retina. Multiple molecular factors have been implicated in angiogenesis (Carmeliet 2003). These factors act through a variety of mechanisms to cause CNV. Vascular endothelial growth factor (VEGF)-A (VEGF hereafter) in particular has been extensively studied and plays a major role in mediating the development of eye diseases characterized by neovascularization (Aiello et al. 1994; Ferrara & Kerbel 2005; Ng & Adamis 2005). Therefore, a new class of antiangiogenic drugs that suppress VEGF has been introduced for the treatment of wet AMD (Gragoudas et al. 2004; Brown et al. 2006; Rosenfeld et al. 2006).

Not long ago this condition was believed to be largely untreatable, but developments in the past few years have challenged this outlook. At present, the two pharmacologic therapies for wet AMD approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) are: pegaptanib sodium (Macugen®; Pfizer/Eyetech Pharmaceuticals, New York, NY, USA), a 28-base ribonucleic acid aptamer, wich binds and blocks the activity of extracellular VEGF-A, specifically the 165-amino-acid isoform ($VEGF_{165}$) (Ruckman et al. 1998), and ranibizumab (Lucentis®; Novartis Pharma AG, Basel, Switzerland and Genentech Inc, South San Francisco, CA, USA), a recombinant humanized monoclonal antibody fragment that neutralizes all isoforms of VEGF and their biologically active degradation products (Ferrara et al. 2006).

A third agent, bevacizumab (Avastin®; Genentech Inc), a recombinant humanized monoclonal neutralizing antibody against all isoforms of VEGF, approved for the systemic treatment of

many types of malignancy including colorectal cancer, non–small cell lung cancer, kidney cancer, breast cancer (only by the EMEA) and glioblastoma (only by the FDA), is now widely used off-label for the treatment of wet AMD (Carneiro et al. 2009; Brechner et al. 2011).

Although they differ in size, affinity for VEGF, intravitreal clearance speed (Bakri et al. 2007a; Bakri et al. 2007b), and cost, both ranibizumab and bevacizumab bind the VEGF molecule at the same position. Ranibizumab, the approved treatment for wet AMD, costs approximately $1950 per dose, whereas bevacizumab, the off label treatment, costs approximately $30 (Jager et al. 2008). Because of the difference in cost, along with the similar perceived efficacy between these two drugs, bevacizumab has been broadly adopted (Rosenfeld 2006; Brechner et al. 2011).

Whilst these new treatments are appropriately seen as important advances in wet AMD therapy, the long-term systemic safety of intravitreal anti-VEGF drugs remains uncertain. These drugs are administered by injection through the sclera into the vitreous cavity and despite the presence of the blood-retinal barrier, which shields the retina from the systemic blood circulation, systemic absorption occurs. Pharmacokinetic studies performed in rabbits and monkeys have showed that bevacizumab, and at a minor scale ranibizumab, can be detected in the serum after intravitreal injection (Gaudreault et al. 2005; Bakri et al. 2007a; Bakri et al. 2007b; Heiduschka et al. 2007). This is especially significant regarding the case of bevacizumab, which has been found to be associated with the development of hypertension and with an increased risk of venous thromboembolism, cardiac ischemia and arterial thromboembolic events in patients being treated for cancer (Kabbinavar et al. 2005; Nalluri et al. 2008; Ranpura et al. 2010). As for the ranibizumab treatment, no significant increase of nonocular adverse effects was found, although these studies were not designed or powered enough to detect small differences and only take in account a short period of time (Brown et al. 2006; Fung et al. 2006; Group 2011). Because intravitreal anti-VEGF treatment for wet AMD is given monthly (Brown et al. 2006; Rosenfeld et al. 2006) and is potentially required

for years, chronic VEGF inhibition may cause systemic adverse effects that are not promptly recognized.

The detection of anti-VEGF molecules in the serum presents itself as challenging as there are no commercially available antibodies against bevacizumab or ranibizumab. For this reason we decided to measure the VEGF plasma concentrations using an ELISA technique. The purpose of this study is to determine the VEGF plasma concentrations in patients before and one, three and four weeks after an intravitreal injection of an anti-VEGF, either bevacizumab or ranibizumab.

Material and methods:

Patients

In the Department of Ophthalmology of Hospital São João, Porto, Portugal, treatment of wet AMD includes intravitreal injections of either ranibizumab or bevacizumab with follow-up visits and treatments in 4-weekly intervals.

Ranibizumab (0.5mg) (Lucentis®) was approved in Portugal by INFARMED (http://www.infarmed.pt) for the treatment of wet AMD in 2008 and became available in Hospital São João in July of that year. It was subsequently used in all patients eligible for treatment. In 2011, the administration board of the hospital decided that all patients proposed to initiate treatment for wet AMD were to be asked to receive bevacizumab instead of ranibizumab. Patients that would accept this would be required to sign an informed consent. This change in the treatment protocol was introduced because of budget restrictions. Every other patient would be treated with ranibizumab.

The study comparing the efficacy and safety of intravitreal anti-VEGF treatments in wet AMD, including VEGF blood level determination, was approved by the Ethics Committee of the Hospital São João, Portugal. Each patient signed an informed consent. The study was carried out in accordance with the tenets of the Declaration of Helsinki.

12 patients were successfully selected for this study, 6 for the bevacizumab group and another 6 for the ranibizumab group.

Visual acuity of the patients included in this study was determined before the intravitreal administration of both anti-VEGF agents. Best-corrected visual acuity (BCVA) was established using Early Treatment Diabetic Retinopathy Study (ETDRS) charts.

In total, four blood samples were collected: during preparation for the first intravitreal injection of an anti-VEGF (S0); one week ±1 day after the intravitreal injection (S1); three weeks ±1 day after the intravitreal injection (S2); and finally during preparation for the second intravitreal injection (S3), four weeks after the first one.

The first and the last blood samples (S0 and S3) were taken in the operating theatre before the intravitreal injection procedure. These injections were performed following a 3-day cycle of topical prophylactic ofloxacin. Cutaneous and conjuntival disinfection with iodopovidone was performed 5 minutes and immediately before the procedure. Ofloxacin was used again, topically, during 4 days after the procedure. Bevacizumab (Avastin® 100mg/4ml) was administered at a dose of 1.25mg (0.05 ml) by injection into the vitreous using a 30-gauge needle that was inserted 3.5-4.0 mm posterior to the limbus. Ranibizumab (Lucentis®) was administered at a dose of 0.5 mg (0.05 ml), using the same intravitreal injection technique.

The second and third samples (S1 and S2) were collected at the outpatient clinic, in fasting patients, between 8 and 9 am.

Wet AMD patients with concomitant chorioretinal diseases were excluded.

Controls for this study were established from age and sex-matched patients from the Ophthalmology department that were scheduled for cataract surgery. Blood samples were obtained only once before the surgery. Their medical records were analysed looking for systemic and other ocular pathologies so that patients with AMD, diabetes, hypertension, vasoproliferative disorders or chorioretinal abnormalities were excluded.

Blood samples preparation

Blood samples were collected, consequently prepared by centrifugation at 2000*g* for 15 minutes, the plasma isolated and then stored at -80ºC before use.

ELISA assay

The VEGF concentrations in the plasma were measured using a commercially available ELISA kit (Quantkine® Human VEGF ELISA Kit, R&D Systems Inc., Minneapolis, Minnesota, USA) in accordance with manufacturer's instructions. Optical densities were measured at 450-570 nm with a microplate reader, and VEGF concentrations were calculated as protein-adjusted amount of VEGF, according to the standards used.

Statistical analysis

Statistics were calculated using a commercially available software package (IBM SPSS Statistics 19; SPSS Inc, Chicago, Illinois, USA). In this study, data was considered not-normally distributed, and was presented as median [interquartile range]. When comparing three groups, a Kruskal-Wallis test was performed, and in the case of comparing two groups, the Mann-Whitney U-test (for un-paired test) or the Wilcoxon matched pairs signed-rank test (for paired test) was employed. A p value <0.05 was accepted as significant.

Results:

VEGF plasma concentrations were determined in 30 patients. Of these, 12 were patients with wet AMD (6 treated with ranibizumab and 6 treated with bevacizumab) and 18 were controls. Regarding VEGF plasma concentrations the 12 wet-AMD patients had significant (p=0.004 un-paired test) higher values (281,2[197,6-594,9] pg/ml, median [interquartile range]) than the control group (181.0[67.0-219.0] pg/ml), see Table 1 and Fig. 1.

At baseline (S0) there were no significant differences between the patients in the bevacizumab, ranibizumab and control groups with respect to age (p=0.462), and gender distribution (p=0.765), see Table 1 and Fig. 2.

After one week ± 1 day (S1), VEGF levels were significantly lower in the bevacizumab group 84,7[55,3-100,8] pg/ml comparing to the baseline (S0) samples 277,2[220,3-480,4] (p=0.028, paired test); they also decreased in the case of the ranibizumab patients group 334,7[163,3-556,1] pg/ml when comparing with the respective S0 levels 412,2[155,7-627,6], however the difference was not significant (p=0.075). Three weeks ± 1 day after the intravitreal injection (S2) VEGF concentrations were again significantly decreased (p=0.028) in the bevacizumab group 140,8[72,8-330,4] pg/ml when comparing baseline (S0). The same happened with the ranibizumab group 206,1[72,0-342,2] pg/ml when relating to the respective S0 levels (p=0.028). Finally, 4 weeks after treatment (S3), VEGF sustained a significantly lower plasma level both in the bevacizumab group with 193,7[71,7-336,9] pg/ml (p=0.028) and in the ranibizumab group of patients with 291,2[142,2-393,5] pg/ml (p=0.046) when looking at the respective baseline (S0) levels. See Table 2 and Fig. 3.

The concentration of serum VEGF was also analyzed between the two drugs at S1: bevacizumab 84,72[55,3-100,8] pg/ml and ranibizumab 334,7[163,3-556,1] pg/ml, wich are significantly different (p=0.025, un-paired test); S2: bevacizumab 140,8[72,8-330,4] pg/ml and ranibizumab 206,1[72,0-342,2] pg/ml, wich are not statistically different (p=0.631); and

S3: bevacizumab 193,7[71,7-336,9] pg/ml and ranibizumab 291,2[142,2-393,5] pg/ml, wich are also not significantly different (p=0.337). See Table 1.

A reduction of VEGF plasma concentration was not verified in all patients: 1 of the 6 patients treated with ranibizumab did not present a lower VEGF level at S3 than at baseline (S0).

Regarding the patients BCVA at baseline (S0), both the bevacizumab and ranibizumab showed no statistically significant differences (p=0.432): bevacizumab 64,5[22,2-73,0] ETDRS letters; and ranibizumab 43,5[29,2-62,7] ETDRS letters. See Fig. 4.

Details on demographics, BCVA and VEGF concentrations are summarized in Table 1 and 2.

Discussion:

Our study has shown that VEGF plasma concentrations decrease in the first weeks after intravitreal injection of anti-VEGF drugs. In bevacizumab treated patients the lowest levels are achieved in the first week (S1) and, in ranibizumab treated patients, in the second week (S2). At the end of 4 weeks (S3) the VEGF plasma concentrations were significantly reduced comparing to the baseline values. There were no statistically significant differences in the anti-VEGF treated group comparing with the control group at baseline (S0) regarding age, sex and visual acuity, though baseline VEGF serum levels were higher in the wet-AMD group than in controls.

Normal vascular growth is a complex process involving sequential events and many ligand–receptor interactions (Ferrara et al. 2003). VEGF activity often corresponds to a rate-limiting step in normal vascular growth and also takes on a critical role in pathological angiogenesis, which is required for tumor growth and metastatic spread (Ferrara & Alitalo 1999; Rugo 2004). In Ophthalmology, the importance of VEGF originates from the fact that this molecule is a major mediator of neovascularization related to various intraocular disorders, including ischemic retinal diseases and wet AMD (Adamis & Shima 2005). Because of this, it becomes essential to analyze the systemic effects of both bevacizumab and ranibizumab anti-VEGF intravitreal therapies.

Several pharmacokinetic studies performed in rabbits and monkeys have showed that bevacizumab, and at a minor scale ranibizumab, can be detected in the serum after intravitreal injection (Gaudreault et al. 2005; Bakri et al. 2007a; Bakri et al. 2007b; Heiduschka et al. 2007). In particular, Nomoto et. al showed in a rabbit model, that intravitreal injections of bevacizumab resulted in high concentrations of this drug in the serum, and that bevacizumab was distributed into the intraocular tissues in fellow eyes via systemic circulation at a level which may be effective in blocking VEGF activity (Nomoto et al. 2009).

Matsuyama et al. showed decreased plasma levels of VEGF in patients with proliferative diabetic retinopathy being treated with intravitreal bevacizumab, and hinted that this antibody enters the general circulation (Matsuyama et al. 2010). Another study executed in infants with retinopathy of prematurity revealed results that indicate that bevacizumab can escape from the eye into the systemic circulation (Sato et al. 2012). However, in these two pathologies, which are characterized by retinal neovascularization, the new vessels are in close contact with the vitreous cavity, this way more readily facilitating diffusion of the drugs to the systemic circulation. In the particular case of wet AMD the newly formed blood vessels are located in the sub-retinal space or below the retinal pigment epithelium, which may make it harder to let the anti-VEGF drugs travel to the general circulation through the incompetent walls of the new vessels.

Carneiro et al. found that intravitreal bevacizumab significantly reduced plasma VEGF, 28 days after three initial consecutive intravitreal injections, in patients with wet AMD, though it could not confirm the same findings with ranibizumab (Carneiro et al. 2012).

To the best of our knowledge data have not been published to determine and compare the blood concentrations of VEGF after intravitreal injection of either bevacizumab or ranibizumab in a series of patients with wet AMD, specifically until the fourth week after treatment initiation. Hence, this paper aims to analyze the systemic levels of VEGF in wet AMD patients treated with intravitreal bevacizumab or ranibizumab. The sample collection took place in the first, second, and fourth weeks after therapy start, and data was then compared between the two drugs.

This study found statistically significant higher plasma levels of VEGF in patients with wet AMD as compared with controls. This goes accordingly with the data reported by Tsai et al. in a series of 42 patients with active AMD/CNV (Tsai et al. 2006) but contrary to what Carneiro et al. published in a study with 43 wet AMD patients (Carneiro et al. 2012). Our data is supported by the fact that wet-AMD is a disease characterized by neovascular proliferation,

for which VEGF is a key agent (Jager et al. 2008). This could explain why baseline VEGF would be higher in this group. On the other hand, the population chosen for this study approaches that one used by Carneiro et al., so our small sample size could be in the origin of this difference (Carneiro et al. 2012).

Regarding the first week after the intravitreal injection (S1), VEGF concentrations are only substantially decreased comparing to the baseline (S0) in the group of patients treated with bevacizumab.

Bevacizumab, has been found in prior studies about diabetic retinopathy (Matsuyama et al. 2010) and retinopathy of prematurity (Sato et al. 2012) to decrease systemic VEGF 1 week after administration, which is line with our results. This is further reinforced by bevacizumab pharmacokinetic studies in rabbits (Bakri et al. 2007b; Nomoto et al. 2009) and monkeys (Heiduschka et al. 2007; Miyake et al. 2010) that found that bevacizumab is detected in the general circulation one week after intraocular administration. Miyake et al., in particular, reported a serum concentration of 1430 ± 186 ng/ml (Miyake et al. 2010). Results in in vitro studies have suggested that a molar ratio of 2.6 to 1 of bevacizumab to $VEGF_{165}$ is necessary for maximum inhibition of endothelial proliferation. The half-maximum inhibitory concentration of bevacizumab is 22 ng/ml, and the minimum concentration that completely blocks VEGF activity including endothelial cell growth, migration, and hyperpermeability is 500 ng/ml (Wang et al. 2004). This could raise the conclusion that, by its pharmacokinetics, bevacizumab is indeed present in the general circulation at concentrations well above the necessary to effectively suppress VEGF activity.

On the other hand, studies in rabbits (Bakri et al. 2007a) and in monkeys (Gaudreault et al. 2005) have reported that ranibizumab is, respectively, either undetectable or at very low concentrations in the serum after intravitreal administration. So, we would expect that VEGF levels would not drop significantly. Notwithstanding the concentration drop we witnessed was mild, and to our knowledge there are no studies published with VEGF serum levels after

ranibizumab intraocular administration in the first week.

Three weeks after the intravitreal administration of the anti-VEGF drugs (S2), serum VEGF concentrations were decreased significantly. This was the case in both drugs. Again, like for the measurements made at 1 week (S1), no studies have been reported for the VEGF serum concentrations three weeks after an intravitreal treatment (S2).

Finally, at 4 weeks after intravitreal administration (S3), both bevacizumab and ranibizumab group have a significantly decreased level of VEGF in the serum comparing to baseline (S0). Minding previous studies, this decrease was expected in the bevacizumab group (Matsuyama et al. 2010). It seems that bevacizumab can still effectively inhibit systemic VEGF levels one month after an intraocular 1.25 mg (0.05ml) injection. In the ranibizumab group we could not compare our values with any particular study, as there are no published papers with this specific information. One could, though, relate to the results from various rabbit and monkey models. These state that at 4 weeks after administration no ranibizumab can be detected in the serum, and therefore no significant VEGF inhibition would be expected. Carneiro et al., analyzed VEGF systemic levels 28 days after a loading dose of ranibizumab, which put the patients under an even more sustained VEGF suppression than in this work, also did not see a statistically significant decrease in VEGF (Carneiro et al. 2012). In our study we saw a substantial drop in the ranibizumab group. This could be consequence from some limitations of our study, like the low number of patients that were included in the assay. A larger sample would give this study more statistical power.

There has been some debate as to which level does bevacizumab and ranibizumab surpass the blood-retinal barrier when injected into the vitreous cavity.

Various studies have approached the systemic repercussions of intravitreal anti-VEGF treatments in humans (Sawada et al. 2007; Matsuyama et al. 2009; Carneiro et al. 2012; Sato et al. 2012).

Matsuyama et al. showed decreased plasma levels of VEGF in patients with proliferative diabetic retinopathy being treated with intravitreal bevacizumab, and hinted that bevacizumab enters the general circulation (Matsuyama et al. 2010). Another study executed in infants with retinopathy of prematurity revealed that the bevacizumab molecule can escape from the eye into the systemic circulation, as its presence was confirmed and measured in the serum (Sato et al. 2012).

Carneiro et al. found that intravitreal bevacizumab significantly reduced plasma VEGF 28 days after an intravitreal injection in patients with wet AMD, though it could not confirm the same findings with ranibizumab. (Carneiro et al. 2012)

Since the inner ocular tissues in the blood-ocular barrier is separated from the blood system (Cunha-vaz 1997) one would not expect to detect a full-length antibody in the blood system only a short time after intravitreous injection. However, as explored previously, recent pharmacokinetics data from monkeys and humans all indicate that intravitreous bevacizumab appears in the blood within hours after intravitreous injection. It has been demonstrated that the neonatal Fc receptor (FcRn) is present in several different ocular tissues including the blood-retinal barrier in the rodent eye (Kim et al. 2008). The expression of FcRn receptor at the blood-ocular barrier suggests transportation of IgG across complex tight junctions in the eye may occur via FcRn-mediated transcytosis. This could help to explain why bevacizumab is so readily transported to the general circulation, implicating a decrease of systemic VEGF activity.

A comparative study of bevacizumab and ranibizumab using human microvascular endothelial cells showed that although both agents inhibited angiogenesis, a more solid down-regulation of VEGFR2 was obtained with bevacizumab comparing to ranibizumab (Costa et al. 2009). Regardless both agents presented anti-angiogenic actions, distinct effects were exerted by the two molecules resulting in different cell behaviors. Ranibizumab, due to the absence of an Fc portion, is much more rapidly degraded than bevacizumab. Conversely, the

full antibody characteristics of latter render this molecule a much more stable half-life, which probably results in longer clinical effects.

Systemic intravenous bevacizumab therapy has been associated with increased risk of hypertension, venous thromboembolism, cardiac ischemia and arterial thromboembolic events in patients being treated for cancer (Kabbinavar et al. 2005; Nalluri et al. 2008; Ranpura et al. 2010). The dose used for intravitreal administration of anti-VEGF therapy is much less than that used intravenously, and the injection target is an intraocular site rather than blood vessels. Therefore, systemic effects caused by intravitreal treatments are considered to be much less significant. However, not only the potential ocular adverse events (Avery et al. 2006; Fung et al. 2006; Meyer et al. 2006; Wu et al. 2008) but also the possible drug related systemic effects have been reported recently (Fung et al. 2006; Shima et al. 2008; Wu et al. 2008; Carneiro et al. 2011). The sustained reduction in systemic VEGF concentrations in an older population group, such as the wet AMD patients, with all its inherent co-morbidities, raises concerns about the long-term effects o this kind of therapy. This seems to be of heavier preponderance when talking about bevacizumab, as it has been found to decrease more sustainably the serum VEGF levels, than with the case of ranibizumab (Carneiro et al. 2012).

In conclusion, with this study we found that both bevacizumab and ranibizumab significantly constrain the systemic VEGF in the first four weeks after the start of the treatment, and also that bevacizumab seems to precipitate a more pronounced VEGF drop in the first week, contrary to ranibizumab that has a more progressive reduction along the four weeks.

Tables:

Table 1. Demographic, best corrected visual acuity (BCVA) and vascular endothelial growth factor (VEGF) data obtained from the three groups.

	Bevacizumab	Ranibizumab	Control	p-value
Age (years)	70.5[63.0-79.5]	78.0[68.8-89.0]	74.0[68.8-86.8]	0.463
Gender (F/M)	2:4	3:3	9:9	0.765
BCVA (S0) (letters)	64,5[22,2-73,0]	43,5[29,2-62,7]	-	0.432
VEGF S0 (pg/ml)	281,2[197,6-594,9]		180.0[65.5-224.0]	0.004
VEGF S0 (pg/ml)	277,2[220,3-480,4]	412,2[155,7-627,6]	-	-
VEGF S1 (pg/ml)	84,7[55,3-100,8]	334,7[163,3-556,1]	-	0.025
VEGF S2 (pg/ml)	140,8[72,8-330,4]	206,1[72,0-342,2]	-	0.631
VEGF S3 (pg/ml)	193,7[71,9-336,9]	291,2[142,2-393,5]	-	0.337

The groups are comparable with respect to age and gender. The S0 (baseline) plasma levels of VEGF are significantly increased in the wet age-related macular degeneration group, treated with either bevacizumab or ranibizumab, when comparing to the control group. VEGF levels decrease achieving their lowest concentrations at S1 (one week) in the case of bevacizumab and S2 (three weeks) in the case of ranibizumab. There are only significant VEGF differences between bevacizumab and ranibizumab groups at S1. At S2 and S3 (four weeks) the differences are not substantial.

Not-normally distributed values are presented as median and the interquartile range (median[interquartile range]). F, female. M, male.

Table 2. Consecutive vascular endothelial growth factor (VEGF) plasma concentrations.

	VEGF S0 (pg/ml)		VEGF (pg/ml)	p-value
Bevacizumab	277,22[220,25-480,43]	S1:	84,72[55,25-100,79]	0.028
		S2:	140,79[72,75-330,43]	0.028
		S3:	193,65[71,68-336,86]	0.028
Ranibizumab	412,20[155,68-627,58]	S1:	334,72[163,28-556,10]	0.075
		S2:	206,14[72,04-342,21]	0.028
		S3:	291,15[142,23-393,47]	0.046

All the bevacizumab VEGF values in the following one (S1), three (S2) and four (S3) weeks are statistically lower than the baseline (S0). In the ranibizumab group we only start to see a substantial decrease of VEGF concentrations at S2 and S3.

Not-normally distributed values are presented as median and the interquartile range (median[interquartile range]).

Ilustrations and graphics:

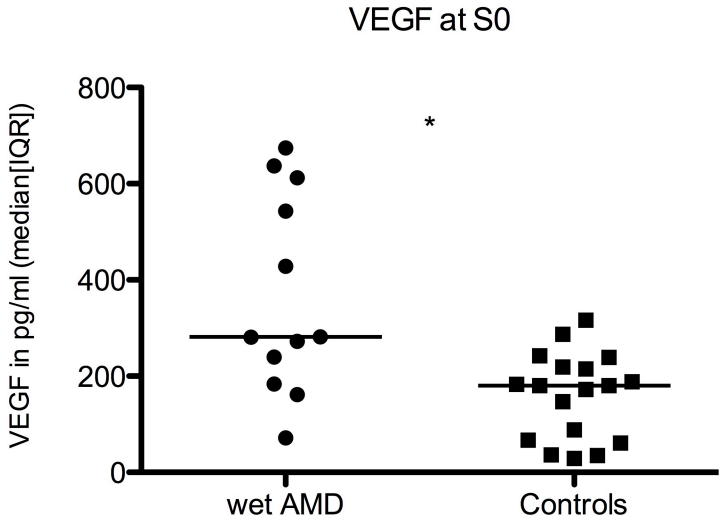

Fig. 1. Vascular endothelial growth factor (VEGF) plasma levels at baseline (S0). Wet age-related macular degeneration (AMD) patients have substantially higher VEGF plasma concentrations. The horizontal bars represent the interquartile range. * = $p<0.05$.

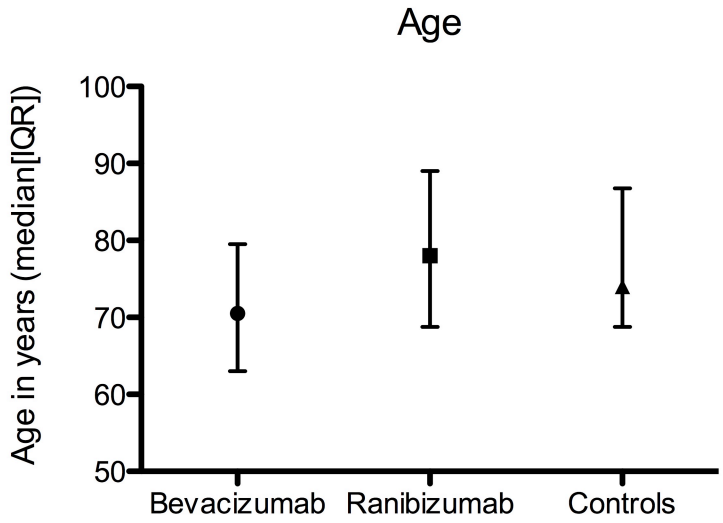

Fig. 2. The distribution of age among the three groups. The circle and the square represent the median; the bars represent the interquartile range. There are no statistically significant differences between groups.

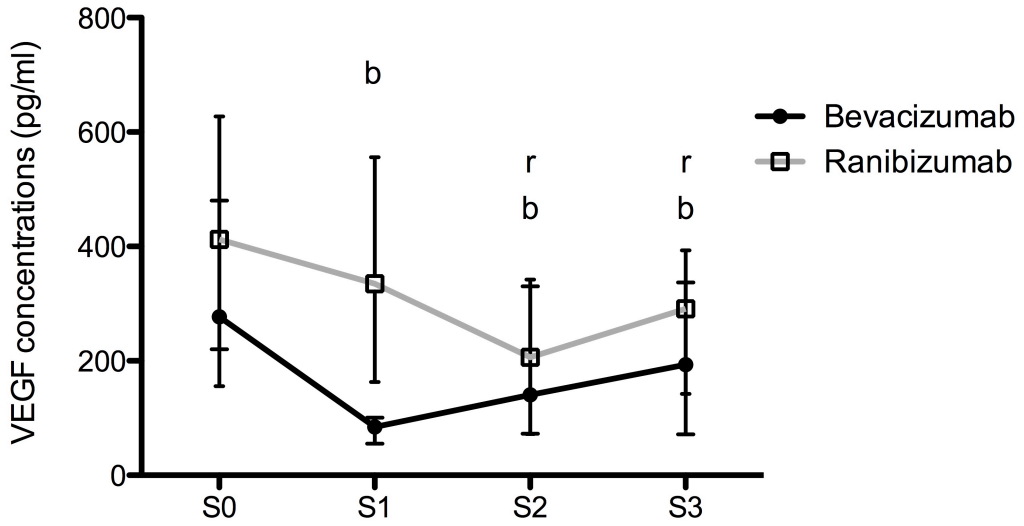

Fig. 3. Plasma vascular endothelial growth factor (VEGF) concentrations at baseline (S0), one (S1), three (S2) and four (S3) weeks after the intravitreal administration of both bevacizumab and ranibizumab groups. In the bevacizumab group S1, S2 and S3 consecutive VEGF levels are significantly decreased comparing to S0. In the ranibizumab group only at S2 and S3 we found a substantial reduction of VEGF concentrations comparing to baseline. The circle and the square represent the median; the bars represent the interquartile range; b = $p<0.05$ in the bevacizumab group; r = $p<0.05$ in the ranibizumab group.

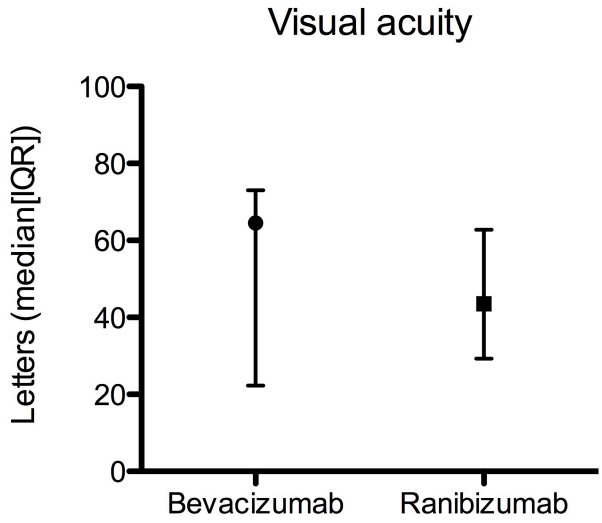

Fig. 4. The visual acuities at baseline for the bevacizumab and ranibizumab group. The circule and the square represent the median; the bars represent the interquartile range. There are no statistically significant differences between groups.

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References:

Adamis AP & Shima DT (2005): The Role of Vascular Endothelial Growth Factor in Ocular Health and Disease. Retina **25**: 111-118.

Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, Nguyen HV, Aiello LM, Ferrara N & King GL (1994): Vascular Endothelial Growth Factor in Ocular Fluid of Patients with Diabetic Retinopathy and Other Retinal Disorders. N Engl J Med **331**: 1480-1487.

Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MaA & Giust MJ (2006): Intravitreal Bevacizumab (Avastin) for Neovascular Age-Related Macular Degeneration. Ophthalmology **113**: 363-372.e365.

Bakri SJ, Snyder MR, Reid JM, Pulido JS, Ezzat MK & Singh RJ (2007a): Pharmacokinetics of Intravitreal Ranibizumab (Lucentis). Ophthalmology **114**: 2179-2182.

Bakri SJ, Snyder MR, Reid JM, Pulido JS & Singh RJ (2007b): Pharmacokinetics of Intravitreal Bevacizumab (Avastin). Ophthalmology **114**: 855-859.

Bird AC, Bressler NM, Bressler SB, Chisholm IH, Coscas G, Davis MD, De Jong PTVM, Klaver CCW, Klein BEK, Klein R, Mitchell P, Sarks JP, Sarks SH, Soubrane G, Taylor HR & Vingerling JR (1995): An international classification and grading system for age-related maculopathy and age-related macular degeneration: The International ARM Epidemiological Study group. Surv Ophthalmol **39**: 367-374.

Brechner RJ, Rosenfeld PJ, Babish JD & Caplan S (2011): Pharmacotherapy for neovascular age-related macular degeneration: an analysis of the 100% 2008 medicare fee-for-service part B claims file. Am J Ophthalmol **151**: 887-895 e881.

Brown DM, Kaiser PK, Michels M, Soubrane G, Heier JS, Kim RY, Sy JP & Schneider S (2006): Ranibizumab versus verteporfin for neovascular age-related macular degeneration. N Engl J Med **355**: 1432-1444.

Carmeliet P (2003): Angiogenesis in health and disease. Nat Med **9**: 653-660.

Carneiro Â, Barthelmes D, Falcão MS, Mendonça LS, Fonseca SL, Gonçalves RM, Faria-Correia F & Falcão-Reis FM (2011): Arterial thromboembolic events in patients with exudative age-related macular degeneration treated with intravitreal bevacizumab or ranibizumab. Ophthalmologica **225**: 211-221.

Carneiro Â, Costa R, Falcão MS, Barthelmes D, Mendonça LS, Fonseca SL, Gonçalves R, Gonçalves C, Falcão-Reis FM & Soares R (2012): Vascular endothelial growth factor plasma levels before and after treatment of neovascular age-related macular degeneration with bevacizumab or ranibizumab. Acta Ophthalmol **90**: e25-e30.

Carneiro Â, Falcão M, Azevedo I, Falcão Reis F & Soares R (2009): Multiple effects of bevacizumab in angiogenesis: implications for its use in age-related macular degeneration. Acta Ophthalmol **87**: 517-523.

Congdon N (2004): Causes and Prevalence of Visual Impairment among Adults in the United States. Arch Ophthalmol **122**: 477-485.

Costa R, Carneiro Â, Rocha A, Pirraco A, Falcão M, Vasques L & Soares R (2009): Bevacizumab and ranibizumab on microvascular endothelial cells: A comparative study. J Cell Biochem **108**: 1410-1417.

Cunha-vaz J (1997): The blood-ocular barriers: past, present, and future. Doc Ophthalmol **93**: 149-157.

Ferrara N & Alitalo K (1999): Clinical applications of angiogenic growth factors and their inhibitors. Nat Med **5**: 1359-1364.

Ferrara N, Damico L, Shams N, Lowman H & Kim R (2006): Development of Ranibizumab, An Anti-Vascular Endothelial Growth Factor Antigen Binding Fragment, As Therapy for Neovascular Age-Related Macular Degeneration. Retina **26**.

Ferrara N, Gerber H-P & LeCouter J (2003): The biology of VEGF and its receptors. Nat Med **9**: 669.

Ferrara N & Kerbel RS (2005): Angiogenesis as a therapeutic target. Nature **438**: 967-974.

Fung AE, Rosenfeld PJ & Reichel E (2006): The International Intravitreal Bevacizumab Safety Survey: using the internet to assess drug safety worldwide. Br J Ophthalmol **90**: 1344-1349.

Gaudreault J, Fei D, Rusit J, Suboc P & Shiu V (2005): Preclinical Pharmacokinetics of Ranibizumab (rhuFabV2) after a Single Intravitreal Administration. Invest Ophthalmol Vis Sci **46**: 726-733.

Gragoudas ES, Adamis AP, Cunningham Jr ET, Feinsod M & Guyer DR (2004): Pegaptanib for neovascular age-related macular degeneration. N Engl J Med **351**: 2805-2816.

Group TCR (2011): Ranibizumab and Bevacizumab for Neovascular Age-Related Macular Degeneration. N Engl J Med **364**: 1897-1908.

Heiduschka P, Fietz H, Hofmeister S, Schultheiss S, Mack AF, Peters S, Ziemssen F, Niggemann B, Julien S, Bartz-Schmidt KU & Schraermeyer U (2007): Penetration of bevacizumab through the retina after intravitreal injection in the monkey. Invest Ophthalmol Vis Sci **48**: 2814-2823.

Jager RD, Mieler WF & Miller JW (2008): Age-related macular degeneration. N Engl J Med **358**: 2606-2617+2544.

Kabbinavar FF, Hambleton J, Mass RD, Hurwitz HI, Bergsland E & Sarkar S (2005): Combined Analysis of Efficacy: The Addition of Bevacizumab to Fluorouracil/Leucovorin Improves Survival for Patients With Metastatic Colorectal Cancer. Journal of Clinical Oncology **23**: 3706-3712.

Kim H, Fariss RN, Zhang C, Robinson SB, Thill M & Csaky KG (2008): Mapping of the Neonatal Fc Receptor in the Rodent Eye. Invest Ophthalmol Vis Sci **49**: 2025-2029.

Matsuyama K, Ogata N, Jo N, Shima C, Matsuoka M & Matsumura M (2009): Levels of vascular endothelial growth factor and pigment epithelium-derived factor in eyes before and after intravitreal injection of bevacizumab. Jpn J Ophthalmol **53**: 243-248.

Matsuyama K, Ogata N, Matsuoka M, Wada M, Takahashi K & Nishimura T (2010): Plasma levels of vascular endothelial growth factor and pigment epithelium-derived factor

before and after intravitreal injection of bevacizumab. Br J Ophthalmol **94**: 1215-1218.

Meyer CH, Mennel S, Schmidt JC & Kroll P (2006): Acute retinal pigment epithelial tear following intravitreal bevacizumab (Avastin) injection for occult choroidal neovascularisation secondary to age related macular degeneration. Br J Ophthalmol **90**: 1207-1208.

Miyake T, Sawada O, Kakinoki M, Sawada T, Kawamura H, Ogasawara K & Ohji M (2010): Pharmacokinetics of Bevacizumab and Its Effect on Vascular Endothelial Growth Factor after Intravitreal Injection of Bevacizumab in Macaque Eyes. Invest Ophthalmol Vis Sci **51**: 1606-1608.

Nalluri SR, Chu D, Keresztes R, Zhu X & Wu S (2008): Risk of Venous Thromboembolism With the Angiogenesis Inhibitor Bevacizumab in Cancer Patients. JAMA **300**: 2277-2285.

Ng EWM & Adamis AP (2005): Targeting angiogenesis, the underlying disorder in neovascular age-related macular degeneration. Can J Ophthalmol **40**: 352-368.

Nomoto H, Shiraga F, Kuno N, Kimura E, Fujii S, Shinomiya K, Nugent AK, Hirooka K & Baba T (2009): Pharmacokinetics of Bevacizumab after Topical, Subconjunctival, and Intravitreal Administration in Rabbits. Invest Ophthalmol Vis Sci **50**: 4807-4813.

Ranpura V, Hapani S, Chuang J & Wu S (2010): Risk of cardiac ischemia and arterial thromboembolic events with the angiogenesis inhibitor bevacizumab in cancer patients: a meta-analysis of randomized controlled trials. Acta Oncol **49**: 287-297.

Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP & Mariotti SP (2004): Global data on visual impairment in the year 2002. Bull World Health Organ **82**: 844-851.

Rosenfeld PJ (2006): Intravitreal Avastin: The Low Cost Alternative to Lucentis? Am J Ophthalmol **142**: 141-143.

Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY & Kim RY (2006): Ranibizumab for neovascular age-related macular degeneration. N Engl J Med **355**: 1419-1431.

Ruckman J, Green LS, Beeson J, Waugh S, Gillette WL, Henninger DD, Claesson-Welsh L & Janjic N (1998): 2′-Fluoropyrimidine RNA-based Aptamers to the 165-Amino Acid Form of Vascular Endothelial Growth Factor (VEGF165). Journal of Biological Chemistry **273**: 20556-20567.

Rugo HS (2004): Bevacizumab in the Treatment of Breast Cancer: Rationale and Current Data. Oncologist **9**: 43-49.

Sato T, Wada K, Arahori H, Kuno N, Imoto K, Iwahashi-Shima C & Kusaka S (2012): Serum Concentrations of Bevacizumab (Avastin) and Vascular Endothelial Growth Factor in Infants With Retinopathy of Prematurity. Am J Ophthalmol **153**: 327-333.e321.

Sawada O, Kawamura H, Kakinoki M, Sawada T & Ohji M (2007): Vascular Endothelial Growth Factor in Aqueous Humor Before and After Intravitreal Injection of Bevacizumab in Eyes With Diabetic Retinopathy. Arch Ophthalmol **125**: 1363-1366.

Shima C, Sakaguchi H, Gomi F, Kamei M, Ikuno Y, Oshima Y, Sawa M, Tsujikawa M, Kusaka S & Tano Y (2008): Complications in patients after intravitreal injection of bevacizumab. Acta Ophthalmol **86**: 372-376.

Tsai DC, Charng MJ, Lee FL, Hsu WM & Chen SJ (2006): Different plasma levels of vascular endothelial growth factor and nitric oxide between patients with choroidal and retinal neovascularization. Ophthalmologica **220**: 246-251.

Wang Y, Fei D, Vanderlaan M & Song A (2004): Biological activity of bevacizumab, a humanized anti-VEGF antibody in vitro. Angiogenesis **7**: 335-345.

Wu L, Martínez-Castellanos M, Quiroz-Mercado H, Arevalo J, Berrocal M, Farah M, Maia M, Roca J, Rodriguez F & Group ftPACR (2008): Twelve-month safety of intravitreal injections of bevacizumab (Avastin®): results of the Pan-American

Collaborative Retina Study Group (PACORES). Graefes Arch Clin Exp Ophthalmol **246**: 81-87.

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