ORIGINAL ARTICLE

UDC: 617.736-08:617.751
https://doi.org/10.2298/VSP150709149R

Correlation between visual acuity, external limiting membrane and photoreceptor status in patients with neovascular age-related macular degeneration treated with bevacizumab

Korelacija između vidne oštrine, spoljašnje granične membrane i fotoreceptora kod bolesnika sa neovaskularnom senilnom degeneracijom žute mrlje lečenih bevacizumabom

Dragana Ristić*†, Miroslav Vukosavljević*†, Marko Kontić*†, Petar Ristić*‡, Dubravko Bokonjić*§, Mirjana Janićijević-Petrović‖¶, Antoaneta Adžić Zečević††, Katarina Janićijević‖¶**

University of Defence, *Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; Military Medical Academy, †Clinic for Ophthalmology, ‡Endocrinology Clinic, §National Poison Control Centre, Belgrade, Serbia; Clinical Centre Kragujevac, ‖Clinic for Ophthalmology, Kragujevac, Serbia; University of Kragujevac, ¶Faculty of Medical Sciences, Kragujevac, Serbia; Clinical Centre of Montenegro, **Clinic for Ophthalmology, Podgorica, Montenegro; University of Montenegro, ††Faculty of Medicine, Podgorica, Montenegro

Abstract

Background/Aim. The integrity of outer retinal structures, primarily the photoreceptor layer, is important because of its direct correlation with visual acuity. The aim of this study was to investigate the correlation between best-corrected visual acuity (BCVA), the foveal photoreceptor-inner segment/outer segment (IS/OS) junction and external limiting membrane (ELM) in patients with neovascular age-related macular degeneration (NVAMD) after the treatment with bevacizumab, as well as the correlation between the above-mentioned parameters and different types of neovascular membrane, classified by fluorescein angiography (FA). **Methods.** The study included 82 patients with NVAMD, treated with intravitreal bevacizumab. All patients underwent a basic ophthalmological examination, FA and optical coherence tomography (OCT). Based on the results of FA, all the patients were divided into two main groups – type I (the occult and minimally classic) and type II (classic and predominantly classic) of the choroidal neovascular membrane (CNV). The OCT images revealed either the presence or the absence of IS/OS and ELM. **Results.** After the treatment, the mean best corrected visual acuity improved significantly in both groups ($p < 0.01$). Preserved IS/OS and ELM were registered in a smaller number of patients as compared to the condition before the treatment ($p < 0.01$). After the treatment, the mean BCVA was significantly better in patients with preserved IS/OS and ELM ($p < 0.01$). In addition, we registered a higher number of patients with preserved ELM in the first group than in the second group ($p < 0.01$), whereas there was no significant difference in the integrity of IS/OS between the groups ($p > 0.05$). **Conclusion.** The patients with preserved IS/OS and ELM achieved better final visual acuity as compared to the patients without preserved IS/OS and ELM. In our patients, the absence of IS/OS and ELM were more frequent in type II (classic and predominantly classic) CNV than in type I (the occult and minimally classic) CNV.

Key words:
retina; macular degeneration; neovascularization, pathologic; bevacizumab; tomography, optical coherence; fluorescein angiography.

Apstrakt

Uvod/Cilj. Integritet spoljašnjih struktura mrežnjače, primarno sloja fotoreceptora, važan je zbog njihove direktne povezanosti sa oštrinom vida. Cilj rada bio je da se ispita povezanost između najbolje korigovane vidne oštrine, fovealnih fotoreceptora i spoljašnje granične membrane kod bolesnika sa neovaskularnom senilnom degeneracijom žute mrlje nakon lečenja bevacizumabom, kao i odnos navedenih parametara sa različitim tipovima neovaskularne membrane klasifikovane metodom fluoresceinske angiografije. **Metode.** Istraživanjem su obuhvaćena 82 bolesnika sa neovaskular-

Correspondence to: Dragana Ristić, Military Medical Academy, Clinic for Ophtalmology, Crnotravska 17, 11 000 Belgrade, Serbia. Phone: +381 11 233 5090. E-mail: dadana25@yahoo.com

nom senilnom degeneracijom žute mrlje lečena intravitealnom primenom bevacizumaba. Svim ispitanicima urađen je osnovni oftalmološki pregled, fluoresceinska angiografija i optička koherentna tomografija. Na osnovu nalaza fluoresceinske angiografije svi bolesnici podeljeni su u dve osnovne grupe, tip I (okultna i minimalno klasična) i tip II (klasična i predominantno klasična) horoidalne neovaskularne membrane. Pomoću optičke koherentne tomografije defisano je prisustvo ili odsustvo fotoreceptora i spoljašnje granične membrane. **Rezultati.** Prosečna najbolje korigovana vidna oštrina značajno se popravila po završetku lečenja u obe grupe ($p < 0,01$). Očuvan kontinuitet fotoreceptora i spoljašnje granične membrane registrovan je kod manjeg broja ispitanika u odnosu na stanje pre tretmana ($p < 0,01$). Prosečna najbolje korigovana vidna oštrina po završetku lečenja bila je značajno bolja kod ispitanika sa očuvanim fotoreceptorima i spoljašnjom graničnom membranom ($p < 0,01$). Takođe, registrovali smo više ispitanika sa očuvanim kontinuitetom spoljašnje granične membrane u prvoj u odnosu na drugu grupu ($p < 0,01$), dok se broj ispitanika sa očuvanim kontinuitetom fotoreceptora po završetku lečenja nije značajno razlikovao između grupa ($p > 0,05$). **Zaključak.** Kod bolesnika sa očuvanim fotoreceptorima i spoljašnjom graničnom membranom vidna oštrina nakon završetka lečenja bila je značajno bolja u odnosu na ispitanike kod kojih ove strukture nisu bile prisutne. Kod naših ispitanika odsustvo fotoreceptora i spoljašnje granične membrane bilo je češće kod tipa II (klasična i predominantno klasična) u odnosu na tip I (okultna i minimalno klasična) neovaskularne membrane.

Ključne reči:
retina; žuta mrlja, degeneracija; neovaskularizacija, patološka; bevacizumab; tomografija, optička, koherentna; angiografija, fluoresceinska.

Introduction

Choroidal neovascular membrane (CNV) is the main cause of severe visual impairment in patients with wet age-related macular degeneration (AMD). Vascular endothelial growth factor (VEGF) is one of the main factors responsible for the development of CNV. The drugs that block its activity, anti-VEGF drugs, have improved considerably the course of this disease and the patients' quality of life [1–3]. Today, they represent standard therapy for the treatment of neovascular AMD (NVAMD) [4, 5].

Bevacizumab (trade name Avastin®) is a monoclonal VEGF antibody, approved for intravenous use in the management of colorectal carcinoma. In ophthalmology, it is used off-label. The first papers related to the intravitreal administration of this drug in the treatment of NVAMD were published as early as in 2006 [6–8].

Optical coherence tomography (OCT) is a useful tool in the diagnosis and monitoring of AMD. After the introduction of a spectral-domain OCT (SD-OCT), which provides image resolution of up to 5 μm, it becomes possible to see clearly all retinal structures [9, 10]. The monitoring of CNV [presence of intraretinal fluid, subretinal fluid or fluid under the retinal pigment epithelium (RPE)] is important for the assessment of its activity. After the treatment, i.e. after the fluid has retreated, a subretinal fibrosis or atrophy can occur, which consequently influences the status of inner segment/outer segment (IS/OS) and external limiting membrane [11].

The monitoring of the integrity of external retinal layers, primarily the photoreceptor layer, is important because of its direct correlation with visual acuity [12–15]. In some studies, the IS/OS line on the SD-OCT images was reportedly a good indicator for predicting best-corrected visual acuity (BCVA) in NVAMD patients treated with anti-VEGF therapy [16]. ELM status is another useful parameter during the evaluation of retinal morphology and function in patients with NVAMD [17].

The aim of this study was to investigate the correlation between BCVA, IS/OS and ELM in patients with NVAMD after the treatment with bevacizumab, as well as the correlation between the above-mentioned parameters and different types of neovascular membrane, classified by fluorescein angiography (FA).

Methods

This clinical, cohort, prospective, non-randomized study was conducted at the Military Medical Academy, Belgrade, Serbia between February 2013 and February 2015. The protocol of this study was approved by the Ethical Committee of the Military Medical Academy. The patients were informed about the off-label use of bevacizumab.

The study involved 105 patients in total. Out of that number, 23 patients were excluded due to insufficient documentation. Here we present 82 patients, in whom the fluid retreated after the sixth dose of the medication, i.e. the CNV activity decreased. CNV was considered active if contrast leakage was seen on FA and if OCT detected an increased and/or persistent presence of macular fluid. As there is no precise dosing protocol for this medication, based on our experience and after consulting literature, we opted for six-monthly doses.

All patients were over the age of 65 and were suffering from AMD-related subfoveolar CNV which had not been treated previously. To qualify for the study, the patients had to have the mean BCVA of 0.05 or higher (Snellen chart). The patients did not have acute or chronic eye inflammations, other fundus changes or decompensated glaucoma. The presence of early-stage/cataract or pseudophakia were not a reason for the exclusion from the study.

At the beginning, each patient underwent the following: complete ophthalmological examination, BCVA assessment, FA on Topcon Trc-NW7SF fundus camera and OCT on Topcon 1000-SD OCTTOp 1000-T3D3. Based on the FA results, the patients were divided into two main groups, depending on whether they had Type I CNV (the occult and minimally classic) or Type II CNV (classic and predominantly classic) membrane. Each group consisted of

41 patients. Using OCT scans we detected the presence or the absence of ELM and IS/OS. This analysis was conducted by two ophthalmologists independently and in the case of inconsistencies in the findings, it was supervised by the mentor. Structural changes of CNV seen on OCT were analyzed in the subfoveal area of 1 mm. The OCT findings were analyzed immediately before the administration of each dose of the medication. In cases where macular fluid disappeared completely before the sixth dose of the medication was given, we interrupted the treatment and such patients were excluded from the study.

All the patients received 1.25 mg of bevacizumab (0.05 mL of the commercial phial of Avastin®) intravitreally. The control assessments were carried out on the first, seventh and thirtieth days following the intervention. One month after administration of the first dose, the next dose was administered. A total of six doses were administered in one-month intervals (+-2).

Statistical analysis was performed before and after the therapy, using SPSS version 19.0 (SPSS., Chicago, IL, USA).

Distribution of variables was assessed by Kolmogorov-Smirnov test and it was concluded that BCVA-related data should be analyzed using non-parametric statistics. The values within a group were analyzed by Friedman's test. For the comparison of BCVAs between the groups, we used the Mann-Whitney U-test.

Results

The patients [82 patients (eyes), 41 in each group] were between 65 and 92 years old. The average age was 77.2 years in the first group and 77.8 years in the second, one. The mean initial BCVA was 0.19 in the first group, and 0.14 in the second one. After the therapy, the mean BCVA was 0.42 in the first, and 0.30 in the second group ($p < 0.01$) (Table 1).

Before the therapy, preserved ELM was registered in all patients (100%), in both groups. In the first group, after the sixth dose, ELM was preserved in 26 patients (63.4%) and in the second one in 11 (26.8%) patients. Before the treatment, the IS/OS was preserved in 39 (95.2%) patients from the first group, and in 35 (85.4%) patients in the second group. After the treatment, the number of patients with preserved IS/OS in the first group decreased to 28 (68.3%) and to 22 (53.4%) in the second group (Tables 2).

In the first group, in patients in whom ELM was preserved, the mean BCVA was 0.19. After the treatment, ELM was preserved in 26 patients and their mean BCVA was 0.53 ($p < 0.01$). After the treatment, in 15 patients in whom ELM was not preserved, the mean BCVA was 0.23 (0.19–0.23) ($p < 0.05$). In the first group, IS/OS were preserved in 39 patients before the treatment, and their mean BCVA was 0.15. After the treatment, IS/OS was preserved in 28 patients, with mean BCVA of 0.39 ($p < 0.01$). In two patients without preserved IS/OS before the therapy the mean BCVA was 0.10, and after the therapy, IS/OS were absent in 13 patients with mean BCVA 0.18 ($p > 0.05$) (Table 3).

In the second group, in patients in whom ELM was preserved, the mean BCVA was 0.14. After the treatment, ELM was preserved in 11 patients and their mean BCVA was 0.46 ($p < 0.01$). In 30 patients in whom ELM was not preserved, after the treatment the mean BCVA was 0.24 ($p < 0.05$). In the second group, 35 patients had complete IS/OS before the treatment, and their mean BCVA was 0.22. After the treatment, IS/OS were preserved in 22 patients, with mean BCVA of 0.33 ($p < 0.05$). In six patients without preserved IS/OS before the therapy the mean BCVA was 0.15, and after the therapy IS/OS were absent in 19 patients with mean BCVA 0.22 ($p < 0.05$) (Table 3).

Table 1

Best-corrected visual acuity (BCVA) before and after the administration of bevacizumab

Group	BCVA		*p*-value
	pre-treatment	post-treatment	
I	0.19	0.42	< 0.01
II	0.14	0.30	

Group I – occult and minimally classic membrane; Group II – classic and predominantly classic membrane.

Table 2

Integrity of external limiting membrane (ELM) and inner segment/outer segment (IS/OS) in bevacizumab-treated patients according to groups

Parameter	Group I, n (%)		Group II, n (%)	
	pre-treatment	post-treatment	pre-treatment	post-treatment
ELM				
present	41 (100)	26 (63.4)	41 (100)	11 (26.8)
absent	–	15 (36.5)	–	30 (73.2)
IS/OS				
present	39 (95.2)	28 (68.3)	35 (85.4)	22 (53.4)
absent	2 (4.9)	13 (31.7)	6 (14.6)	19 (46.6)

Group I – occult and minimally classic membrane; Group II – classic and predominantly classic membrane.

Table 3
Best-corrected visual acuity (BCVA) according to external limiting membrane (ELM) and inner segment/outer segment (IS/OS) in the group I and the group II

Groups	BCVA	
	pre-treatment	post-treatment
Group I		
ELM		
present	0.19	0.53
absent	/	0.23
IS/OS		
present	0.15	0.39
absent	0.10	0.18
Group II		
ELM		
present	0.14	0.46
absent	/	0.24
IS/OS		
present	0.22	0.33
absent	0.15	0.22

Group I – occult and minimally classic membrane;
Group II – classic and predominantly classic membrane.

The results of our study showed that after the treatment there were 26 patients with preserved ELM in the first group (the occult and minimally classic CNV), as compared to 11 patients in the second group (classic and predominantly classic) ($p < 0.01$). After the treatment, IS/OS were preserved in 28 patients in the first group and in 22 patients in the second group ($p > 0.05$) (Table 1).

Discussion

The results of our study confirmed that the visual acuity in patients with wet AMD was significantly improved after the intravitreal administration of bevacizumab, as previous studies had demonstrated [1–3, 6–8].

There are several types of anti-VEGF medications, registered for intravitreal administration. Since the study by Martin al. [3] CATT showed that ranibizumab is not superior to bevacizumab in terms of their effects, and, as bevacizumab is significantly cheaper, we decided to use bevacizumab.

The protocol for the administration of anti-VEGF drugs has not been precisely established yet. In the studies such as ones by Brown et al. [1] (ANCHOR) and Rosenfeld et al. [2] (Marina), this medication was administered monthly. Some studies dealt with treat-and-extend dosing regimens, whereas in other studies, the dosing regimen was *pro re nata* (PRN) – treat and observe [5]. Based on our previous experience, we decided to apply a monthly dosing regimen.

The correlation between the type of neovascular membrane (identified by FA), and its structural characteristics (identified by OCT) was described by Freund et al [18]. The final visual acuity in patients with NVAMD is influenced by various changes in the macula (subretinal, intraretinal or fluid under the RPE) [11, 19]. The drug itself has no effect either on ELM or on IS/OS, but after the treatment is over, and after the fluid retreats, the integrity of these parameters influences the final visual acuity, which was described by many authors [14, 15, 20].

A correlation between visual acuity, ELM and IS/OS integrity in the treatment of neovascular AMD with photodynamic therapy was described as early as in 2010 by Oishi et al. [15], and was also mentioned by Sayanagi et al. [16], but subsequent to anti-VEGF therapy. The importance of these structures in the preservation of vision in patients with uveitic macular oedema was described by Tortorela et al. [14].

Sayanagi et al. [16] confirm the importance of IS/OS status in patients who were given anti-VEGF therapy and conclude that IS/OS is a good indicator in terms of prognosis for visual acuity following an anti-VEGF therapy.

Shin et al. [20] conclude that the integrity of foveal photoreceptors is strongly correlated with final visual acuity after the treatment in patients with NVAMD.

Kwon et al. [13] conclude that IS/OS and ELM can be good predictors of visual acuity after anti-VEGF therapy.

Upon the analysis of our data, we noted that the presence of ELM and IS/OS correlate with better visual acuity, both before and after the therapy. A significant role of ELM and IS/OS, as important prognostic factors for final BCVA, was also confirmed by Mathew et al [19].

Our study showed the significant improvement of BCVA in patients with preserved ELM and IS/OS in both groups, whereas in patients with not preserved ELM and IS/OS, the BCVA was lower (there was an improvement, but the difference was not statistically significant).

The number of patients with preserved IS/OS and ELM decreased after the treatment, especially in the second group, where the number of patients with preserved ELM after the therapy was considerably lower. This means that there were more patients with preserved ELM and IS/OS in the first group (the occult and minimally classic CNV) than in the second group (classic and predominantly classic CNV). The literature offers diverse findings regarding this issue. Bloch et al. [21] in their study obtained the findings similar to ours, while Freund et al. [18] in their study actually argue that the response to anti-VEGF therapy is better in patients from the second group (classic and predominantly classic CNV).

The studies, such as ANCHOR [1] and MARINA [2], showed that, regardless of the type of neovascular membra-

ne, classified by FA method, the vision would improve after the anti-VEGF therapy. We confirmed such findings, as well as that visual acuity is considerably better if IS/OS and ELM are preserved at the end of the treatment.

Conclusion

The presence of ELM and IS/OS is an important prognostic factor for final visual acuity in patients with NVAMD. The absence of ELM and IS/OS is more frequent in type II CNV (classic and predominantly classic) than in type I CNV (the occult and minimally classic) and this is the reason why these patients can be expected to have somewhat lower final visual acuity after the anti-VEGF therapy.

The development of modern technology provides an even more precise insight into the structure of the neovascular membrane, so future analyses will be able to define even more precisely its various structural parameters and open new approaches to treating these patients.

REFERENCES

1. *Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T.* ANCHOR Study Group. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. Ophthalmology 2009; 116(1): e57−65.
2. *Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY.* MARINA Study Group. Ranibizumab for neovascular age-related macular degeneration. N Eng J Med 2006; 355(14): 1419−31.
3. *Martin DF, Maguire MG, Fine SL, Ying GS, Jaffe GJ, Grunwald JE*, et al. Comparison of Age-related Macular Degeneration Treatments Trials (CATT) Research Group. Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. Ophthalmology 2012; 119(7): 1388−98.
4. *American Academy of Ophthalmology.* Age-related macular degeneration. Prefferd practice pattern guideline. San Francisco, CA: American Academy of Ophthalmology; 2014.
5. *Agarwal A, Rhoades WR, Hanout M, Soliman MK, Sarwar S, Sadiq MA*, et al. Management of neovascular age-related macular degeneration: Current state-of-the-art care for optimizing visual outcomes and therapies in development. Clin Ophthalmol 2015; 9: 1001−15.
6. *Rosenfeld PJ, Moshfeghi AA, Puliafito CA.* Optical coherence tomography findings after an intravitreal injection of bevacizumab (avastin) for neovascular age-related macular degeneration. Ophthalmic Surg Lasers Imaging 2005; 36(4): 331−5.
7. *Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ.* Intravitreal bevacizumab (Avastin) for age-related macular degeneration. Ophthalmology 2006; 113: 363−72. e5.
8. *Bashshur ZF, Bazarbachi A, Schakal A, Haddad ZA, El Haibi CP, Noureddin BN.* Intravitreal bevacizumab for the management of choroidal neovascularization in age-related macular degeneration. Am J Ophthalmol 2006; 142(1): 1−9.
9. *Keane PA, Liakopoulos S, Chang KT, Wang M, Dustin L, Walsh AC,* et al. Relationship between optical coherence tomography retinal parameters and visual acuity in neovascular age-related macular degeneration. Ophthalmology 2008; 115(12): 2206−14.
10. *Kashani AH, Keane PA, Dustin L, Walsh AC, Sadda SR.* Quantitative subanalysis of cystoid spaces and outer nuclear layer using optical coherence tomography in age-related macular degeneration. Invest Ophthalmol Vis Sci 2009; 50(7): 3366−73.
11. *Jaffe GJ, Martin DF, Toth CA, Daniel E, Maguire MG, Ying GS,* et al. Comparison of age-related macular degeneration treatments trials research group. Macular morphology and visual acuity in the comparison of age-related macular degeneration treatments trials. Ophthalmology 2013; 120(9): 1860−70.
12. *Wong IY, Iu LP, Koizumi H, Lai WW.* The inner segment/outer segment junction: What have we learnt so far? Curr Opin Ophthalmol 2012; 23(3): 210−8.
13. *Kwon YH, Lee DK, Kim HE, Kwon O*W. Predictive findings of visual outcome in spectral domain optical coherence tomography after ranibizumab treatment in age-related macular degeneration. Korean J Ophthalmol 2014; 28(5): 386−92.
14. *Tortotella P, Ambrosio E, Iannetti L, de Marco F, la Cava M.* Correlation betwen visual acuity, inner segment/outer segment junction, and cone outer segment tips line integrity in uveitic macular edema. Biomed Res Int 2015: 853728.
15. *Oishi A, Hata M, Shimozono M, Mandai M, Nishida A, Kurimoto Y.* The significance of external limiting membrane status for visual acuity in age-related macular degeneration. Am J Ophthalmol 2010; 150(1): 27−32.e1.
16. *Sayanagi K, Sharma S, Kaiser PK.* Photoreceptor status after antivascular endothelial growth factor therapy in exudative age-related macular degeneration. Br J Ophthalmol 2009; 93(5): 622−6.
17. *Hagan MJ, Alvarado JA, Weddell JE.* Histology of the human eye: an atlas and textbook. Philadelphia: Saunders; 1971. p. 393−522.
18. *Freund KB, Zweifel SA, Retina EM.* Do we need a new classification for choroidal neovascularization in age-related macular degeneration? Retina 2010; 30(9): 1333−49.
19. *Mathew R, Richardson M, Sivaprasad S.* Predictive value of spectral-domain optical coherence tomography features in assessment of visual prognosis in eyes with neovascular age-related macular degeneration treated with ranibizumab. Am J Ophthalmology 2013; 155(4): 720−6, 726.e1.
20. *Shin HJ, Chung H, Kim HC.* Association between foveal microstructure and visual outcome in age-related macular degeneration. Retina 2011; 31(8): 1627−36.
21. *Bloch SB, Lund-Andersen H, Sander B, Larsen M.* Subfoveal fibrosis in eyes with neovascular age-related macular degeneration treated with intravitreal ranibizumab. Am J Ophthalmol 2013; 156(1): 116−24.

Received on July 9, 2015.
Revised on March 10, 2016.
Accepted on May 18, 2016.
Online First June, 2016.