

Microperimetry in the Wet Form of Age-Related Macular Degeneration (ARMD)

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SUMMARY

Aim of the paper is to introduce and evaluate the microperimetry as a support technique in the evaluation of the ARMD wet form bevacizumab (Avastin) treatment efficacy.

Methods: Twenty-one patients (21 eyes) with ARMD wet form were included in the study; they were examined by means of OCT/SLO (optical coherence tomography / scanning laser ophthalmoscope) machine whose part is the microperimetric test. In each patient, there were two microperimetric examinations performed – the first one was done one day before the application of the treatment and the second one one month after the application. The values of retinal sensitivity and possible absolute scotomas were recorded by the microperimeter. The best-corrected visual acuity measurement was recorded as well.

Results: The measurements were performed in the group of 21 eyes of 8 men and 13 women (age 50 – 86 years). There were the differences between the genders evaluated (at the selected level of significance of 0.05) in the following categories: age, retinal sensitivity, absolute scotoma extent, and visual acuity. Differences among men and women were found in retinal sensitivity, before the treatment and after it as well. The influence of the gender we may exclude, except the category of retinal sensitivity. In the categories age, visual acuity, and even absolute scotoma extent, no differences between genders were found. The retinal sensitivity (in case of fixation up to 4 degrees) improved in the men group from 14.49 dB ± 2.44 dB to 15.65 dB ± 2.61 dB, in the women group from 10.47 dB ± 3.11 dB to 12.05 dB ± 3.10 dB. The visual acuity in the whole group (men + women) increased from 0.48 ± 0.17 to 0.60 ± 0.18, so improved by 12 %. Statistically significant is also the result of the treatment in the absolute scotoma.

Conclusion: Microperimetry is a suitable method to evaluate the function of macular region of the retina. The study confirms, that the treatment of wet form ARMD by means of intravitreal bevacizumab injections significantly increases the retinal sensitivity (in dB), decreases of the extent of absolute scotoma and improves the visual acuity by 12 %.

Key words: microperimetry, ARMD, Avastin, retinal sensitivity, visual acuity, absolute scotoma

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INTRODUCTION

Age-related macular degeneration is the most common cause of serious malfunction of central visual acuity in one or both eyes in people aged over 50 (1). ARMD wet form represents only 10% of the total prevalence of this disease, but is responsible for 90% of cases of severe loss of sight (3). It is characterised by appearance of newly formed vessels in the choroid, and of choroidal neovascularisations, which is accompanied by serious clinical symptoms such as deformation of image, development of a “central scotoma” and mostly by a rapid and pronounced reduction of visual acuity up to practical blindness (1). Since 2004, the anti-VEGF preparation bevacizumab (Avastin) has been

registered in the Czech Republic. Bevacizumab is a humanised monoclonal anti-VEGF antibody, which is approved in the Czech Republic as a cytostatic for intravenous application in a dose of 5 mg/kg of weight in the case of metastatic colorectal carcinoma (2). In ophthalmology it is applied off-label in intravitreal injections in a dose of 1.25 mg. A range of authors recommend 2-3 applications at monthly intervals and a further application only upon repetition according to FA and OCT. After the first application, others recommend a further application only after repeated increase of activity or insufficient effect of therapy (1).

A range of studies have been published on therapy with bevacizumab, one of which is the study by Michels and Rosenfeld from 2005, in which, as in many other studies, they demonstra-

ted the safety and efficacy of Avastin in the case of wet-form ARMD (5).

Microperimetry is a diagnostic method which enables observation of the effect of therapy over time. It is a combination of an objective display of the retina (digital fundus camera, analysis of fixation) and subjective computer perimetry (6). Thanks to a microperimetric test, we can evaluate sensitivity of the retina (in dB) in the macular area, absolute scotomas in individual areas, false positive and false negative values of measurement and the fixation stability of the patient. Microperimetry can be used in several maculopathies, as well as in wet-form ARMD, which we deal with in the research study.

The aim of the work is to present and evaluate the method of microperimetry as a tool in the evaluation of the

effectiveness of therapy of wet-form ARMD using bevacizumab (Avastin), to compare the measured variables on a microperimeter (retinal sensitivity, absolute scotoma) before therapy and one month after the therapy, and visual acuity on Snellen's optotype before therapy and one month after the therapy.

METHODOLOGY

21 patients (21 eyes) with wet-form ARMD with all types of CNV were included in a study which took place from November 2008 to June 2012, in which treatment was commenced using the bevacizumab formula (Avastin).

All the patients were instructed and the options for therapy of this disease were explained to them, including sending to central facilities, but following consultation the patients decided in favour of application of the off-label therapy using Avastin. The pharmaceutical was applied in a single initial intravitreal dose of 1.25 mg. The sample included patients with occult CNV, predominantly classic and minimally classic CNV with vision better than 6/60. Patients who had vision worse than 6/60 did not respond to microperimetric testing. All the patients also had an examination on OCT, the average value of CRT before therapy was 246 μ m.

Measurement of individual patients was conducted at an inpatient department of the Department of Ophthalmology at the St. Anne University Hospital using an appliance OCT/SLO (Fig. 1) distributed by EriLens, s.r.o. (Ltd.), Prague. The manufacturer of the appliance is the company OTI – Ophthalmic Technologies Inc. from Toronto. The microperimetric module is an optional component of this system OCT/SLO.

COURSE OF EXAMINATION

Two measurements were conducted on each patient – the first testing was conducted one day before the application of the pharmaceutical, the second at a time interval of one month after application of the treatment. Using the microperimeter I measured overall sensitivity in the macular area (in 56 points – Humphrey model), absolute scotoma was evaluated (point with zero value in decibels). On the day of measurement on the micrometer, the patients' value of vision was de-

termined on Snellen's optotype from a distance of 5 or 6 metres (according to examining room), by the Snellen fraction converted to a decimal value, again before and after application of the treatment. In all patients, vision was examined with the best corrected visual acuity (BCVA) by the full-row method.



Fig. 1 OCT/SLO with microperimetric module (internal photo archive)

Criteria for inclusion in the study

- Patients with wet-form ARMD with all types of CNV.
- Patients who are receiving anti-VEGF therapy for the first time (anti-VEGF therapy naïve patients) and to whom only the first injection was applied as the off-label therapy with the Avastin product.
- Vision of patients with best corrected visual acuity was not worse than 6/60.
- Patients capable of understanding the principle of testing on a microperimeter.
- Patients did not have visual acuity influenced by any other ocular diseases.

Parameters of the model of the microperimetric module:

- Humphrey model (testing in 56 points)
- Duration of display of stimulus (Duration) – 200 ms.
- Time interval between imaging of individual stimuli (Interval) – 2s.
- Size of stimulus on retina (Size) – Goldmann IV.
- Value in decibels, in which search will take place (Step size) – 2 dB.

The resulting record of measurement on the microperimeter (fig. 2) contains in its heading basic information about the patient, i.e. first name and surname, date of birth, national identification number and date of testing. In further columns the parameters of the measurement model are entered, and in the next row the results of the measurement, including the time of

the performed test and the evaluation of false negative and false positive values of measurement. The most important is the resulting value of the Test score, which evaluates the number of points which the patient saw out of the maximum number (56 x 20 – 1120). The proportion of the seen points, with the value in decibels, to the maximum number of points according to the selected model gives the resulting value of retinal sensitivity. The 56 tested points with the resulting value in decibels are displayed on a photograph of the precise area on the retina where the measurement was conducted. Each point, according to the key on the right, is displayed in a given colour, thus from 0 to 20 dB (from red to dark green colour).

At the same time the system displays the patient's fixation, which was central up to 4 degrees in all 21 patients.

RESULTS

The measurement was conducted in a sample of 21 eyes of 8 men and 13 women in the age range of 50 – 86 years (Graph 1). The men (indicated as 1) are aged 50 to 83 years; the average age of the men in the entire group is 67.38 years. The lowest age of the women (indicated as 2) in the sample is 66 years, and the oldest patient is aged 86 years, the average age in the entire group of women is 73.85 years.

The right eye was examined in 8 patients, the left eye in 13 patients.

The basic results of the measurements are presented in Tables 1, 2 and 3 (the whole sample, men, women). The tables present the numbers of measured eyes (N) in the sample, averages, 95% intervals of reliability (lower and upper), minimum and maximum and standard deviations (STD) of measured variables. RS1 indicates sensitivity of the retina before treatment, RS2 sensitivity after treatment, AS1 absolute scotoma before treatment, AS2 absolute scotoma after treatment, visus 1 is visual acuity before treatment and visus 2 is the value of visual acuity after treatment. In all groups, testing for normality was conducted for all variables (by Shapiro-Wilk test). In addition to variables AS1 and AS2, the precondition of normality could not be rejected (at the level of significance 0.05), and as a result all testing of the differences was performed by a parametric test (for AS variables by a non-parametric

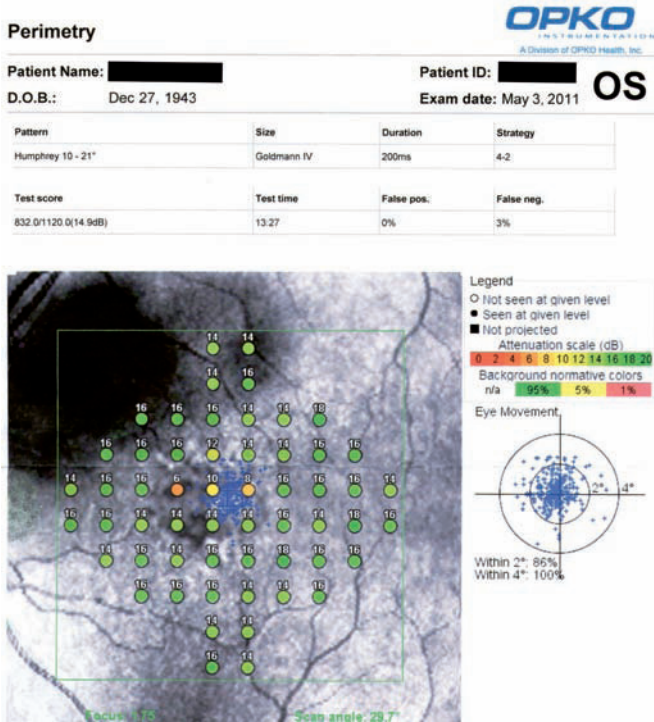


Fig. 2 Record of resulting measurement of patient on micro-meter OCT/SLO (internal photo archive).

test). In all cases of testing, the value 0.05 was selected as the level of significance.

TESTING OF DIFFERENCES BETWEEN GENDERS

Testing of the differences between men and women (Table 4) was performed for the variables Age, RS and VISUS by means of a Student t-test.

The table presents the average measured values for men (1) and for women (2), *t* – value of tested criterion, *df* – number of degrees of freedom, *p* – level of significance of test, NA (*N*₂) – number of measured eyes in the group of men (women).

Statistically significant differences between the genders for the given variable were highlighted in bold (at selected level of significance 0.05). Differences between men and women were found in the variable RS, both before and after treatment. No difference between the genders was found regarding age or visual acuity.

The test of significance of the difference between men and women for the variable AS1 and AS2 was conducted by means of a non-parametric Mann-Whitney test (AS1 and AS2 do not have a normal distribution). The results are presented in Table 5.

There is no statistically significant difference between the genders in

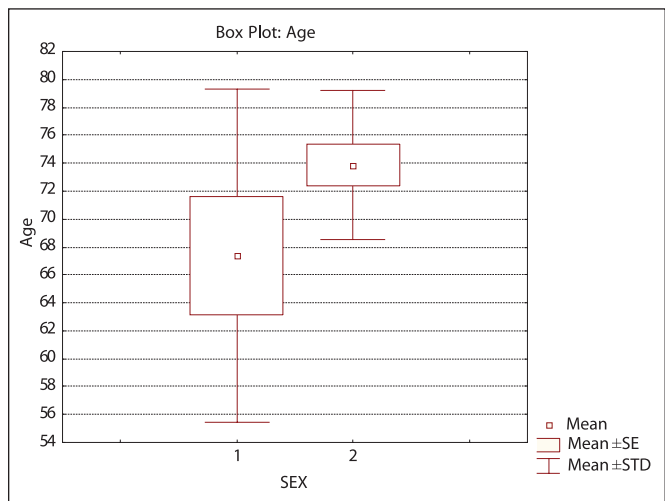


Fig. 3 Example of measurement of patient with absolute scotoma (internal photo archive).

Table 1 Basic statistical characteristics of measured variables in the entire sample

Variable	Male + Female						
	N	Mean	CI -95%	CI +95%	Minimum	Maximum	STD
Age	21	71.38	67.38	75.38	50.0	86.0	8.79
RS1	21	12.00	10.43	13.57	6.7	17.8	3.45
RS2	21	13.42	11.88	14.95	5.8	19.5	3.37
AS1	21	2.57	0.83	4.31	0.0	13.0	3.83
AS2	21	1.05	0.09	2.01	0.0	8.0	2.11
VISUS1	21	0.479	0.404	0.554	0.16	0.80	0.165
VISUS2	21	0.589	0.517	0.679	0.25	1.00	0.178

Table 2 Basic statistical characteristics of measured variables in the sample of men.

Variable	Male						
	N	Mean	CI -95%	CI +95%	Minimum	Maximum	STD
Age	8	67.38	57.39	77.36	50.0	83.0	11.94
RS1	8	14.19	12.45	16.53	11.2	17.8	2.44
RS2	8	15.65	13.47	17.83	10.8	19.5	2.61
AS1	8	0.75	-0.72	2.22	0.0	5.0	1.75
AS2	8	0.25	-0.34	0.84	0.0	2.0	0.71
VISUS1	8	0.556	0.436	0.676	0.33	1.00	0.144
VISUS2	8	0.650	0.515	0.805	0.50	1.00	0.174

Table 3 Basic statistical characteristics of measured variables in the sample of women.

Variable	Female						
	N	Mean	CI -95%	CI +95%	Minimum	Maximum	STD
Age	13	73.85	70.63	77.06	66.0	86.0	5.32
RS1	13	10.47	8.59	12.35	6.7	14.9	3.11
RS2	13	12.05	10.17	13.92	5.8	17.1	3.10
AS1	13	3.69	1.05	6.33	0.0	13.0	4.37
AS2	13	1.54	0.01	3.07	0.0	8.0	2.54
VISUS1	13	0.432	0.333	0.530	0.16	0.66	0.163
VISUS2	13	0.560	0.454	0.666	0.25	0.80	0.176

the variable AS before treatment (AS1) and after treatment (AS2).

On the basis of testing of the differences between the genders for the measured variables, we can exclude the influence of gender on the result of treatment, with the exception of the variable RS. As a result, for testing of the influence of therapy we can combine men and women in a single group, with the exception of the variable RS, for which we shall conduct testing separately for men and women.

Testing of influence of the treatment on sensitivity of retina, absolute scotoma and visual acuity

A parametric t-test was used for testing the influence of RS and VISUS. In the case of the variable RS, testing was conducted separately with the sample of men (Table 6) and the sample of women (Table 7). The tables present the average values of RS1 and RS2, standard deviations – STD, number of eyes (N), difference of RS values and standard deviation thereof, value of tested criterion – t, number of degrees of freedom – df, and level of significance of test – p. In the case of the variable VISUS, the test was conducted for the entire sample (men + women). A Wilcoxon non-parametric test for pair values was used for the variable AS.

Because the level of significance of the test (p) is smaller than the selected level of significance 0.05, we declare that the **influence of therapy on sensitivity of the retina in men is statistically significant**. The value of sensitivity of the retina in decibels after therapy increased from 14.49 dB ± 2.44 dB to 15.65 dB ± 2.61 – thus

Table 4 Results of testing of differences between genders in variables Age, RS and Visus (t-test).

Variable	t-tests; Group 1: Male Group 2: Female						
	Mean 1	Mean 2	T	df	p	N1	N2
Age	67.38	73.85	-1.716	19	0.102	8	13
RS1	14.49	10.47	3.101	19	0.006	8	13
RS2	15.65	12.05	2.737	19	0.013	8	13
VISUS1	0.56	0.43	1.775	19	0.092	8	13
VISUS2	0.66	0.56	1.269	19	0.220	8	13

Table 5 Results of testing of differences between genders in AS variables.

Variable	Mann-Whitney U test Grouping variable: SEX						
	Rank Sum	Rank Sum	Z	N1	N2	p	N2
AS1	62.5	168.5	-1958	8	13	0.064	13
AS2	73.0	158.0	-1.362	8	13	0.301	13

Key to Table 5: N1 (N2) – number of tested eyes in men (women), Z – value of test criterion, p – level of significance of the test.

Table 6 Results of testing of the treatment effect on RS in men.

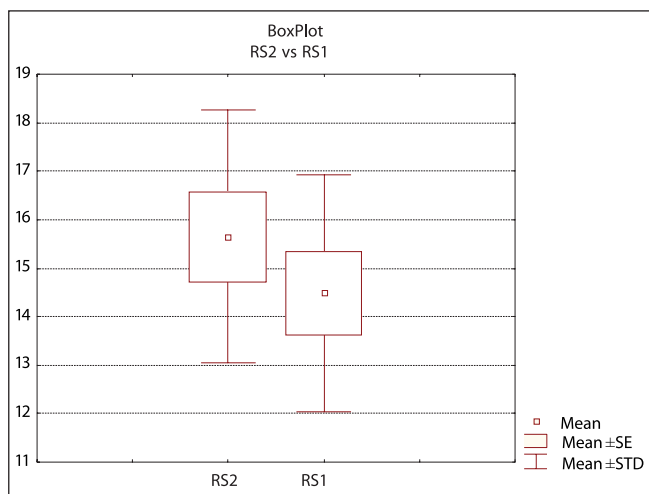
Variable	Paired t-test SEX = Male							
	Mean	STD	N	Difference	STD of Difference	t	df	p
RS2	15.65	2.61						
RS1	14.49	2.44	8	1.16	1.11	2.960	7	0.021

an improvement of 1.16 dB ± 1.11 dB. The graphical position of the averages of RS in men before and after therapy is illustrated in Graph 2.

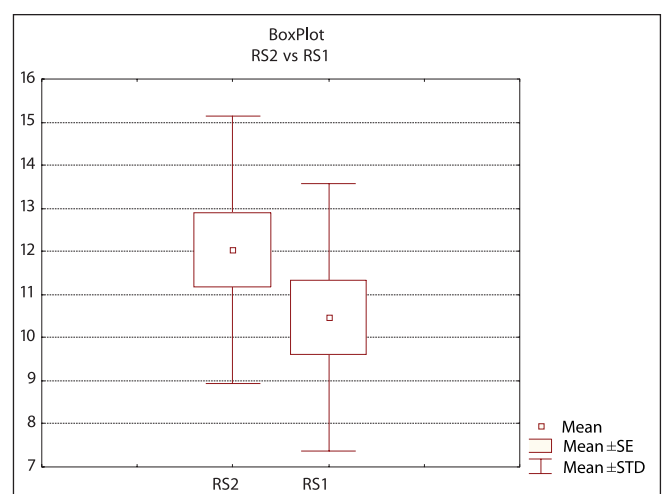
In the group of women, the value of sensitivity of the retina in decibels after therapy increased from 10.47 dB ± 3.11 dB to 12.05 dB ± 3.10 dB – thus with a difference of 1.58 dB ± 2.31 dB. Similarly to the men, **the influence of therapy on sensitivity of the retina is statistically significant in women**.

The graphical position of the averages of RS in women before and after therapy is illustrated in Graph 3.

The test of influence of the therapy on visual acuity was conducted with the entire sample. The results are presented in Table 8, where we find the average values of visual acuity before and after treatment, standard deviations – STD, number of eyes (N), difference of values of visus and standard deviation of the difference, value of



Graph 2 Box graph of RS (in decibels) before and after treatment in men



Graph 3 Box graph of RS (in decibels) before and after treatment in women

tested criterion – t, number of degrees of freedom – df, and level of significance of test – p.

From Table 8 it is evident that **the influence of therapy on visual acuity is statistically significant** ($p < 0.05$). Visual acuity showed an average improvement in all patients by 0.12 ± 0.13 (by 12%), from 0.48 ± 0.17 to 0.60 ± 0.18 (from 48% to 60%). The results are presented graphically in Graph 4.

Testing of influence of the treatment on absolute scotoma

Absolute scotoma is considered to cover a point with a zero value in decibels – indicated by an empty red circle (Fig. 3), in this case the patient has one absolute scotoma.

A non-parametric Wilcoxon test was used for testing influence of the treatment on absolute scotoma. The entire sample was tested. The results are presented in Table 9 and Graph 5. Because $p < 0.05$, **the influence of therapy on AS is statistically significant**, at the level of significance of 0.05. Absolute scotoma appeared in 11 eyes (of 21 eyes). The number of all absolute scotomas before treatment in all 11 eyes is 54, after the treatment only 22. The average number of absolute scotomas, converted for all patients, including those who did not have an absolute scotoma, is 2.57 in the entire group and 1.04 after treatment.

DISCUSSION

Microperimetry is a valuable tool for assessing the effect of therapy in the case of macular diseases; using this method we determine functionality of the macular area of the retina. Improvement or at least stability of the sen-

Table 7 Results of testing of the treatment effect on RS in women.

Variable	Paired t-test SEX = Female							
	Mean	STD	N	Difference	STD of Difference	t	df	p
RS2	12.05	3.10						
RS1	10.47	3.11	13	1.58	2.31	2.462	12	0.030

Table 8 Results of testing of the treatment effect on visus.

Variable	Paired t-test SEX = Female							
	Mean	STD	N	Difference	STD of Difference	t	df	p
VISUS2	0.598	0.178						
VISUS1	0.479	0.165	21	0.119	0.126	4.325	20	0.000

Table 9 Results of testing of the treatment effect on absolute scotoma (AS)

Variable	Wilcoxon Signed+Rank Test		
	N	Z	P
AS2 & AS1	21	2.803	0.005

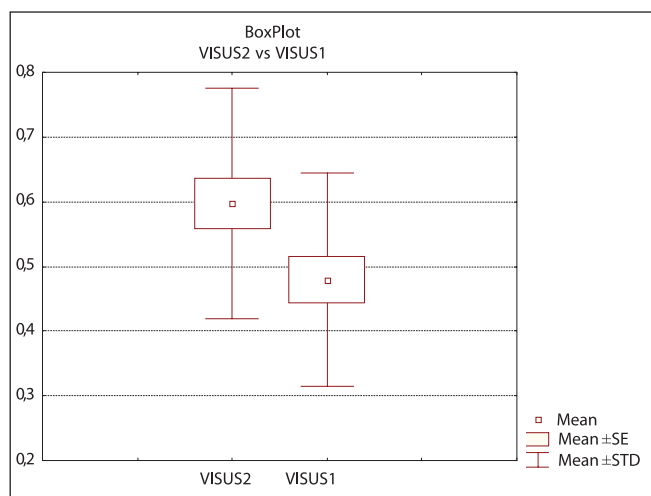
Key to Table 9: N – number of tested eyes, Z – value of test criterion, p – level of significance of the test.

sitivity of the retina and the visual field are important indicators as to whether the therapy is effective. Evaluation of retinal sensitivity and macular functions using a microperimeter provides more information than measurement of visual acuity, because microperimetry represents an objective method of evaluation of the effect of therapy, unlike a subjective evaluation of visual acuity on Snellen's or ETDRS optotypes.

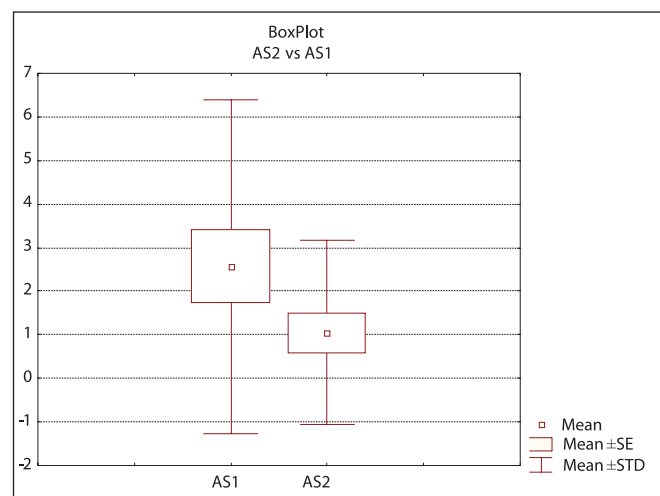
Sensitivity of the retina in our patients increased in the group of men from $14.49 \text{ dB} \pm 2.44 \text{ dB}$ to $15.65 \text{ dB} \pm 2.61 \text{ dB}$, in the group of women from $10.47 \text{ dB} \pm 3.11 \text{ dB}$ to $12.05 \text{ dB} \pm 3.10 \text{ dB}$. Visual acuity in the entire sample (men + women) increased from 0.48 ± 0.17

to 0.60 ± 0.18 , showing an improvement of 12%. The result of therapy on absolute scotoma is also statistically significant; this was reduced from 54 to 22. The average value of the results from OCT-CRT before treatment was $246 \mu\text{m}$, following a month therapy it decreased to $236 \mu\text{m}$, CRT therefore improved on average by $10 \mu\text{m}$.

In a range of studies the quality effect of treatment with Avastin has already been demonstrated, although we cannot compare their results since they included patients in various stages of the disease, patients following previous unsuccessful therapy using PDT, another type of anti-VEGF etc., but from the perspective of visual acuity the average improvement in these



Graph 4 Box graph of visual acuity before and after treatment



Graph 5 Box graph of AS before and after treatment

studies was from 6/60 to 6/24 (1, 4). A similar study on the theme of microperimetry in wet-form ARMD was conducted by Ozdemir with a team of doctors from the Istanbul Retina Institute, with an identical number of patients as in our study (21). For measurement he used a different type of microperimeter, MP-1 Nidek, for evaluation of visual acuity he used an ETDRS table. He assessed the effect of bevacizumab on macular functions in wet-form ARMD with the help of retinal sensitivity, absolute scotoma and visual acuity. In his study, retinal sensitivity increased from $3.69 \text{ dB} \pm 3.44 \text{ dB}$ to $6.09 \text{ dB} \pm 3.37 \text{ dB}$ after one month of treatment. The value of absolute scotoma was reduced from 12 to 7 absolute scotomas after the first month. Visus improved from $0.59 \pm 0.25 \text{ logMAR}$ to $0.36 \pm 0.18 \text{ logMAR}$. As in our case, the study also demonstrated a significant increase in retinal sensitivity, a reduction of absolute scotoma and an improvement of visual acuity before and after treatment (7). Prager et al., in a study with Avastin in 14 patients, determined a pronounced increase in retinal sensitivity and a re-

duction of absolute scotomas (8). A negative aspect of microperimetry is its time factor; an average test takes approximately 20 minutes, depending on the selected model (the more displayed points, the longer the measurement). Co-operation of the patient is necessary, which is not always possible in the case of older patients.

CONCLUSION

The aim of the study was to evaluate and verify the benefit of the method of microperimetry as a tool in the evaluation of the efficacy of therapy of wet form age-related macular degeneration using bevacizumab (Avastin). In the study we measured and evaluated retinal sensitivity, the number of absolute scotomas in patients with wet for ARMD before treatment and one month after treatment, and also visual acuity on Snellen's optotype over time. On the basis of the microperimetric results and measurement of visual acuity, treatment with Avastin demonstrates statistical significance in the treated sample of 21 patients,

retinal sensitivity increased significantly and visual acuity improved by an average of 12% in comparison with the results before and after treatment. Microperimetry demonstrated that it is a beneficial method in the ophthalmological practice, which enabled us to evaluate the function of the macular area of the retina in patients with wet-form ARMD. We see the value of microperimetry especially in the fact that this represents a combination of subjective and objective methods, can inform doctors effectively regarding the function of the macular area of the retina in direct visualisation, and serves as an effective tool for monitoring of the set therapeutic strategy. On the basis of the results of this study, we can recommend microperimetry as a suitable and beneficial method for evaluating the effect of therapy not only for wet-form ARMD but also for other macular diseases.

Note: Technical data on the perimeter is adopted from the operation manual of the appliance OCT/SLO supplied by the company EriLens s.r.o. Prague.

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