(34)

Evaluation of treatment efficacy of intravitreal ranibizumab injections in patients with wet type of AMD

Ocena skuteczności leczenia zwyrodnienia plamki związanego z wiekiem (AMD) za pomocą doszklistkowego podawania ranibizumabu

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Streszczenie: Cel: ocena funkcji bioelektrycznej siatkówki w rejonie dołka, krążenia siatkówkowego oraz grubości siatkówki w rejonie dołka przed doszklistkowym podaniem ranibizumabu i po jego podaniu w oczach z wysiękową postacią zwyrodnienia plamki związanego z wiekiem (age-related macular degeneration – AMD).

Materiał i metody: grupę badaną stanowiło 21 oczu (20 pacjentów) z neowaskularyzacją naczyniówkową (choroidal neovascularisation – CNV) w przebiegu AMD. Podstawą do ustalenia kryteriów włączenia do badania były wyniki angiografii fluoresceinowej (fluorescein angiography – FA) oraz najlepiej skorygowanej ostrości wzroku do dali (distance best corrected visual acuity – DBCVA) – tablice logMAR. Do każdego oka podano 3 iniekcje doszklistkowe w odstępach czterotygodniowych. Reiniekcje były wykonywane w indywidualnych przypadkach w zależności od DBCVA oraz wyniku optycznej tomografii komputerowej (optical coherence tomography – OCT) przez 12 miesięcy obserwacji. Przed rozpoczęciem leczenia, w 3., 6. i 12. miesiącu obserwacji przeprowadzono następujące badania: DBCVA, elektroretinogram wieloogniskowy (multifocal electroretinogram – mfERG), OCT, a także FA przed leczeniem oraz w 3. i 12. miesiącu obserwacji.

Wyniki: przed rozpoczęciem leczenia badanie FA ujawniło głównie ukrytą CNV – 57% oczu (12/21). W 3. miesiącu obserwacji nie wykazano powiększenia średnicy CNV. Brak przecieku z aktywnej CNV obserwowano w 76% przypadków (16/21 oczu). W 12. miesiącu obserwacji powiększenie średnicy CNV zaobserwowano w 43% oczu (9/21), natomiast brak przecieku z aktywnej CNV – w 57% oczu (12/21). Średnia DBCVA statystycznie istotnie poprawiła się tylko w 3. miesiącu obserwacji ($p<0,02$). Statystyczną redukcję średniej grubości dołka obserwowano w każdym okresie obserwacji ($p<0,01$). Wyniki mfEREG z okolicy plamki pozostały niezmienione lub poprawiły się nieznacznie w niektórych przypadkach.

Wnioski: w badanej grupie pacjentów z wysiękową postacią AMD po doszklistkowym podaniu ranibizumabu w 12-miesięcznym okresie obserwacji wykazano redukcję średniej grubości dołka, podczas gdy DBCVA oraz funkcja bioelektryczna siatkówki z regionu plamki mierzona za pomocą mfERG pozostały niezmienione.

Słowa kluczowe: wysiękowe zwyrodnienie plamki związane z wiekiem (AMD), ranibizumab, krążenie siatkówkowe, funkcja plamki, grubość dołka.

Summary: Purpose: To evaluate foveal function, retinal circulation and foveal thickness before and after intravitreal ranibizumab injections in eyes with wet type of age-related macular degeneration (AMD).

Material and methods: The study group consisted of 21 eyes (20 patients) with choroidal neovascularisation (CNV) due to AMD. Inclusion criteria were based on fluorescein angiography (FA) and distance best corrected visual acuity (DBCVA) – log MAR scale. In each eye, 3 consecutive injections of ranibizumab every 4 weeks were administered and then individual course for re-injections according to DBCVA and optical coherence tomography (OCT) up to 12 months was applied. At baseline, 3, 6 and 12 months follow-up, the following tests were performed: DBCVA, multifocal electroretinogram (mfERG) and OCT. Additionally, FA was carried out before the treatment, 3 and 12 months from the baseline.

Results: At baseline, FA revealed mainly minimally occult choroidal neovascularisation – 57% (12/21) of eyes. At 3 months choroidal neovascularisation diameter was stable; no leakage from active choroidal neovascularisation was seen in 76% (16/21) of eyes. After 12 months follow-up, increase in choroidal neovascularisation diameter was seen in 43% (9/21) of eyes and no leakage in 57% (12/21) of cases. The mean DBCVA significantly improved only after 3 months ($p<0.02$). Significant decrease of mean foveal thickness was observed in each follow-ups ($p<0.01$). The mfERG data from the macular region remained stable or improved slightly in some cases.

Conclusions: In our series of patients with the wet type of AMD after intravitreal injections of ranibizumab in 12 months follow-up, the reduction of foveal thickness was noted while DBCVA and the bioelectrical function from the macular region measured by the mfERG remained stable.

Key words: Wet type of age-related macular degeneration (AMD), ranibizumab, retinal circulation, macular function, foveal thickness.

Introduction

Nowadays age-related macular degeneration (AMD) is a leading cause of legal blindness in population over 50 years of age (1). Decrease in quality of life noted in the severe AMD reaches 63% of cases and is comparable to those observed in a severe stroke or a cancer with uncontrollable pain (2). Early diagnosis of wet type of AMD is essential because appropriate treatment may slow down the progression of the disease and in some cases can slightly improve the visual acuity (VA). Currently available pharmacological therapies for CNV are associated with injections of antivascular endothelial growth factor (anti-VEGF) agents like pegaptanib sodium, bevacizumab (as off-label use) and ranibizumab. Bevacizumab, which is not justified in ophthalmic clinical practice, may even improve or stabilise the VA in wet type of AMD (3,4). Ranibizumab, like pegaptanib sodium, is the only registered anti-VEGF drug and is commonly used for the wet type of AMD treatment. Intravitreal injections of ranibizumab not only prevented visual loss (5-8) but also improved mean VA even in 24 months of follow-up (8,9).

So far, in most published study results, the evaluation of ranibizumab treatment effectiveness was mainly based on VA, FA and OCT tests (8-11). The VA examination allows only to estimate retinal function of about 1 angle degree. Hence, VA examination is not precise enough to estimate retinal function from retinal regions covering CNV, which usually surpasses 1 angle degree. In comparison with VA, foveal function measured by mfERG test describes larger retinal area. The mfERG test therefore, provides more details about retinal function than VA. This technique is thought to reflect the functional mapping of the retina, especially primarily ON and OFF bipolar cells and cone photoreceptors (12). Thus, mfERG test can give additional objective information about the level of functional deterioration and it may be helpful for estimation of the efficacy of anti-VEGF intravitreal drugs in the treatment of wet type of AMD.

Results of pegaptanib sodium or bevacizumab treatment in wet type of AMD measured by the mfERG examination, revealed a decrease or stabilisation of the retinal function, respectively (7,13). In the available literature, there are only few studies in which mfERG test was used to estimate the bioelectrical function after intravitreal ranibizumab injections in treatment of wet type of AMD (5-7) and the results are inconsistent. Campa et al. (6) and Moschos et al. (7) in comparison with baseline, observed improvement of response density in the foveal region after intravitreal of ranibizumab injections. Feigl et al. (5), comparing the results with normal values and baseline but basing on smaller number of eyes ($n = 3$) as well as shorter follow-ups (3 months), indicated no improvement of central bioelectrical function after ranibizumab treatment.

The goal of our study was to estimate treatment efficacy of ranibizumab in wet type of AMD measured not only by the commonly used tests like AF, VA, OCT but also by mfERG.

Material and methods

In this prospective, uncontrolled case series 21 eyes of 20 consecutive patients with CNV due to wet type of AMD were treated with intravitreal injections of ranibizumab. There were 11 females and 9 males in the study group, aged 58–81 years (mean age 73 $\pm$ 6.8).

Inclusion criteria were as follows: normal anterior segment of the eye, normal intraocular pressure, central fixation (measured by means of direct ophthalmoscope and checked by two masked examiners), age over 50 years, active subfoveal CNV, DBCVA equal or less then 1.0 (ETDRS chart, log MAR), fibrosis or haemorrhage occupying less than 50% of the entire lesion, maximum lesion size of 12 disc areas (DA).

Patients were treated with 3 consecutive intravitreal injections of ranibizumab 0.5 mg every 4 weeks followed by additional injection – once in 33% (7/21) of eyes and twice in 24% (5/21) of eyes – up to 12 months whenever an increase of at least 100 μm in foveal thickness in OCT or decrease in DBCVA (at least 5 letters) – or new haemorrhages in macular region in fundus examination were observed.

Intravitreal injections were performed under sterile conditions of the operating theatre with conjunctival flush of 5% povidone iodine and periocular preparation with 10% povidone iodine. Injections were applied inferotemporally, 4 mm from the corneal limbus. Topical antibiotics were given from 3 days before to 7 days after the procedure. Each injection was followed by 2 control visits with routine ophthalmologic examination on the 1st and 7th day.

In each patient retinal circulation, functional examinations (DBCVA and the mfERG test), as well as foveal thickness were registered. FA was performed at baseline and at 3 and 12 months from the first injection with the total CNV diameter measurement and active CNV area evaluation. Other examinations were carried out before the first injection and then were repeated after 3, 6 and 12 months. Decision about reinjection was made upon additional DBCVA, OCT and eye fundus examinations results.

At baseline, in FA the type of CNV (predominantly classic, minimally classic and occult), as well as diameter of the entire lesion and the diameter of active (leaking) CNV were identified. Entire lesion and active CNV diameter changes were significant when there were minimum 10% difference from baseline values.

Foveal thickness was evaluated in OCT examination (Zeiss Stratus OCT, Humphrey Instruments model 3000, Carl Zeiss Inc, Dublin, California, 2007). The retinal mapping software was used calculating averaged retinal thickness of the central ring.

MfERG test was performed with the RetiScan (Roland Consult, Germany) system, according to the International Society for Clinical Electrophysiology of Vision (ISCEV) (guidelines 2007) (14). Protocol of the mfERG test was implemented in the science version of the software.

Patient's pupils were dilated (1% Tropicamide, 10% Neosynephrine), monocular stimulation was used. Refraction correction was applied with respect to the eye-screen distance to see clearly the small fixation spot in the center of the stimulus matrix. Fifteen minutes preadaptation was used to the illumination conditions in the examination room. Monitoring with a TV camera (to stop the stimulations when frequent blinking or fixation loss) was carried out, any segment associated with blinks, eye movements, brakes of fixation were eliminated and recorded again. Fixation was directly observed by an experienced electrophysiology technician. Twenty-one inch (CRT) monitor with a frame rate equal to 75 frames per second (fps) was used. Black & white matrix of 61 scaled hexagons (distortion factor equal to 4) was displayed in the 30° field of vision (FOV) – (center to edge). Luminance for white elements was 100 cd/m^2 and contrast was equal 97%.

Electrodes: thread DTL electrode was used as active and gold disc placed at the ipsilateral outer canthi as reference, with ground (gold disc) electrode placed on the forehead (Fpz).

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Parameters of the recording system were as follows: amplifiers range: ±100 μV, filters: 10–300 Hz. Notch filters: off. Plots time: 83 ms. Artifact reject threshold: 8%. Averaging: six cycles. Off-line processing: digital smoothing (2x) and software reduction of line interference.

Results analysis: response density (the response amplitude divided by the retinal area, in nV/deg^2) and implicit time of the P1-wave were analyzed in the ring 1 (R1) and ring 2 (R2), manual correction, if necessary, was applied to the automatic cursors placement. The mfERG stimuli location and anatomic area of R1 (0.0–2.3°) corresponded roughly to the fovea and of R2 (2.3–7.4°) to the parafovea and partially to the perifovea (15).

Values of all parameters of our patients were also compared with the age, sex and refractive error-matched normal values (30 eyes from 15 healthy subjects).

After 3, 6 and 12 months from the baseline, the individual intersessional variabilities were determined by calculating the coefficients of variation (CV) in the recordings for P1-response density as well as for P1-implicit time in R1.

Faith et al. (14) reported that the individual inter-sessional variabilities in healthy subjects for P1-response density and P1-implicit time were less than 36.0% and 8.1%, respectively. According to the data mentioned above, using the same machine and procedure of mfERG recording, we assumed that improvement for individual eye after treatment was significant, when CV for P1-response density increase more than 36.0% and P1-implicit time decrease more than 8.1%.

The Shapiro-Wilk test was used to evaluate the normality of distribution of analyzed parameters. If the data were normally distributed, Student t-test was used. If the data failed the normality, Wilcoxon signed-rank test was performed. Furthermore, for statistical analysis each eye of patients with wet type of AMD was compared 3, 6 and 12 months after the treatment with the baseline as well as with responses of the healthy age-matched control group. To assess the changes in the tests (VA, OCT, mfERG) after intravitreal ranibizumab injections, the differences between the examination periods (second-baseline, third-baseline, fourth-baseline), were divided by the value in the baseline (preinjection) value. In order to estimate whether a correlation exists between VA, OCT and mfERG results, the Pearson coefficient (r) for normally distributed data or Spearman's rank coefficient (rS) for not normal distributed data were adopted. The p value < 0.05 was considered significant.

All the patients signed a written consent form in the regard of the mfERG examination (not necessary in regular follow-up ranibizumab therapy). The study was approved by the Pomeranian Medical University Research Ethics Committee.

Results

During 12 months follow-up time, 43% (9/21) of eyes needed only 3 consecutive ranibizumab injections. In 57% (12/21) of cases the additional reinjections were required: 33% (7/21) of eyes once and 24% (5/21) of eyes twice.

Fluorescein angiography

At baseline in FA 57% (12/21) of eyes presented occult CNV, 33.5% (7/21) of eyes had minimally classic CNV and 9.5% (2/21) of eyes showed predominantly classic CNV secondary to AMD.

After 3 consecutive injections total CNV diameter remained stable in 81% (17/21) of eyes, decreased in 14% (3/21) of eyes from 3217 ± 732 mm to 2850 ± 876 mm and increased in 5% (1/21) of eyes from 2100 mm to 2250 mm (Fig. 1.). The inactive scar was present in 48% (10/21) of eyes. In other cases area of active CNV remained unchanged in 38% (8/21), decreased in 9% (2/21) and increased in 5% (1/21) of the eyes.

In 12 months follow-up total CNV diameter remained stable in 43% (9/21) of eyes, decreased in 14% (3/21; from 3275 ± 1025 mm to 2925 ± 1167 mm) of eyes and increased in 43% (9/21; from 3000 ± 1445 mm to 4375 ± 2598 mm) of eyes (Fig. 1.). Inactive scar was seen in 57% (12/21) of cases. Active CNV was still present in 43% (9/21) of eyes – being decreased in 38% (8/21) and stable in 5% (1/21) of cases as compared to the baseline.

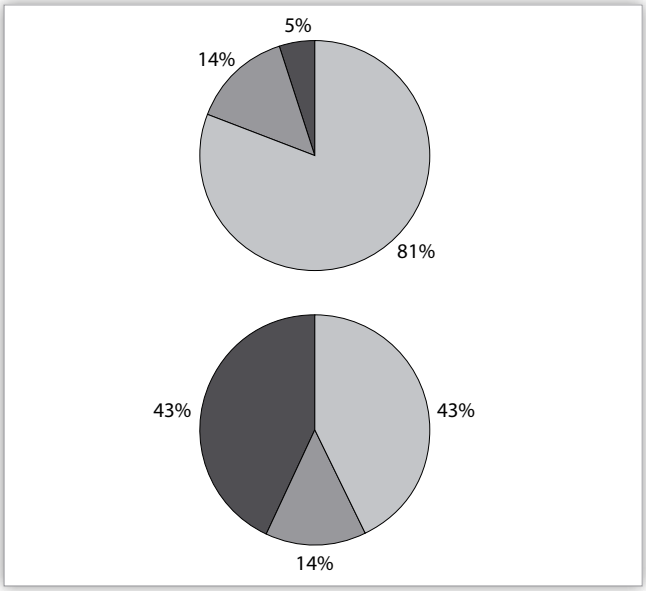

Fig. 1. Wet type of AMD – CNV diameter 3 months after (above) and 12 months after (below) inravitreal ranibizumab injectons (n = 21).
■ – stable CNV diameter, ■ – decreased of CNV diameter, ■ – increased of CNV diameter

Ryc. 1. Wysiękowa postać AMD – średnica CNV w 3. miesiącu obserwacji (powyżej) i w 12. miesiącu obserwacji (poniżej) doszklistkowymi iniekcjami ranibizumabu (n = 21).
■ – niezmieniona średnica CNV, ■ – zmniejszenie średnicy CNV, ■ – zwiększenie średnicy

Distance best corrected visual acuity

The mean of the DBCVA improved significantly from 0.58 ± 0.21 (log MAR scale) at baseline to 0.43 ± 0.24 ($p<0.02$) only after 3 months (Fig. 2.). After 3 months, improvement of VA was noted in 66.7% (14/21) of the treated eyes. Comparing the mean of the DBCVA at baseline and 3, 6 and 12 months follow-up in patients with wet type of AMD and the mean of the DBCVA of the healthy age-matched group (0.04 ± 0.02), the significant increase of VA was never observed ($p<0.001$) (Fig. 2.). During the treatment, the mean of the DBCVA of patients with wet type of AMD never reached the mean normal values. Decrease of VA was observed in 33.3 % (7/21), 57.2% (12/21) and 38.1% (8/21) of eyes after 3, 6 and 12 months follow-up, respectively.

Optical coherence tomography

There was a significant decrease of the mean foveal thickness from 328.2 ± 117.8 μm to 226.2 ± 61.7 μm ($p<0.001$) after 3 months, to 246.4 ± 61.8 μm ($p<0.007$) after 6 months and to 283.0 ± 108.9 μm ($p<0.01$) after 12 months follow-up from baseline (Fig. 3.). The comparison of the mean foveal thickness in

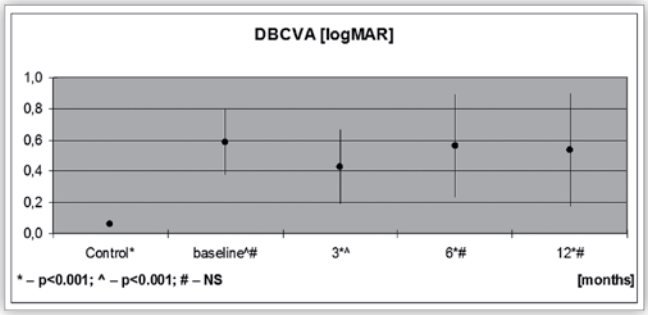

Fig. 2. Wet type of AMD – average of DBCVA 3, 6, 12 months follow-up after intravitreal injections of ranibizumab in comparison with baseline and control (n = 21).
(* – comparison between the results of control group and results at baseline and 3, 6, 12 months follow up; ^ –comparison between the results at baseline and results after 3 months follow-up; # – comparison between the results at baseline and results after 6 and 12 months follow-up, NS – not significant)

Ryc. 2. Wysiękowa postać AMD – średnia DBCVA w 3., 6., 12. miesiącach obserwacji po doszklistkowych iniekcjach ranibizumabu w porównaniu z wynikami wyjściowymi i wynikami uzyskanymi w grupie kontrolnej (n = 21).
(* – porównanie wyników w grupie kontrolnej z wynikami wyjściowymi oraz wynikami uzyskanymi w 3., 6., 12. miesiącu obserwacji; ^ – porównanie wyjściowych wyników z wynikami uzyskanymi w 3., 6., 12. miesiącu obserwacji; # – porównanie wyjściowych wyników z wynikami uzyskanymi w 6. i 12. miesiącu obserwacji; NS – wynik nieistotny statystycznie)

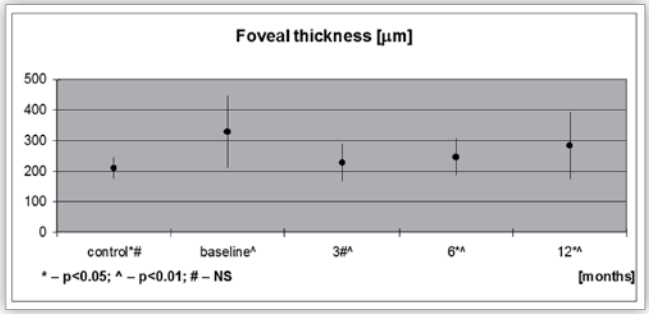

Fig. 3. Wet type of AMD – average of foveal thickness 3, 6, 12 months follow-up after intravitreal injections of ranibizumab in comparison with baseline and control (n = 21).
(* – comparison between the results of control group and results at baseline and 6, 12 months follow up; ^ – comparison between the results at baseline and results 3, 6 and 12 months follow-up; # – comparison between the results of control group and 3 months follow-up, NS – not significant)

Ryc. 3. Wysiękowa postać AMD – średnia grubość siatkówki w rejonie dołka mierzona w 3., 6., 12. miesiącach obserwacji po doszklistkowych iniekcjach ranibizumabu w porównaniu z wynikami wyjściowymi i wynikami uzyskanymi w grupie kontrolnej (n = 21).
(* – porównanie wyników uzyskanych w grupie kontrolnej z wynikami wyjściowymi oraz wynikami w 6., 12. miesiącu obserwacji; ^ – porównanie wyjściowych wyników z wynikami uzyskanymi w 3., 6., 12. miesiącu obserwacji; # – porównanie wyników uzyskanych w grupie kontrolnej z wynikami uzyskanymi w 3. miesiącu obserwacji; NS – wynik nieistotny statystycznie)

patients with wet type of AMD before the treatment and 3, 6, 12 months follow-up with the mean foveal thickness of the healthy age-matched group (204.7 ± 19.7 μm) is shown in figure 3. After 3 months follow-up, the mean retinal thickness did not differ significantly in comparison with the normal values but after 6 and 12 months follow-ups the significant increase of foveal thickness were observed.

Multifocal electroretinogram

After 3, 6 and 12 months follow-up, the mean P1-response density and the mean P1-implicit time (R1 and R2) did not differ significantly in comparison with baseline. Averages and standard deviations of P1-response density and P1-implicit time from controls as well as from patients with wet type of AMD at baseline and 3, 6 and 12 months follow-up are shown in figure 4. In comparison with the healthy control group, the normal values were never achieved.

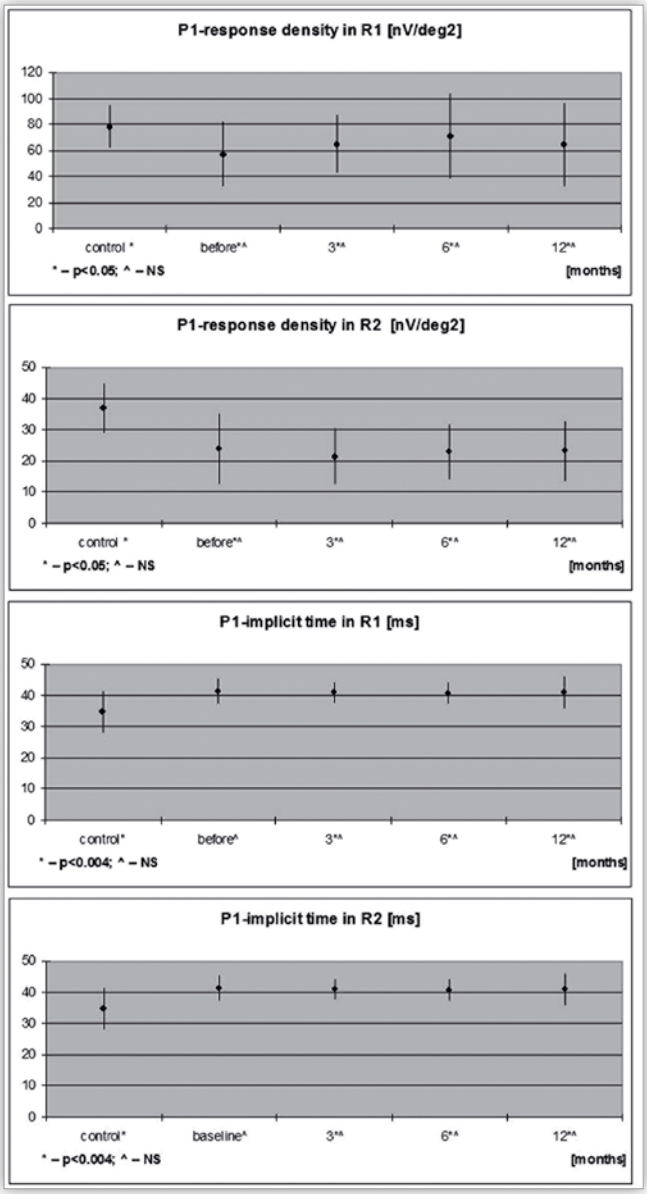

Fig. 4. Wet type of AMD – average of response density and P1-implicit time 3, 6, 12 months follow-up after intravitreal injections of ranibizumab in comparison with baseline and control (n = 21).
(* – comparison between the results of control group and results at baseline and 3, 6, 12 months follow up, ^ – comparison between the results at baseline and results 3, 6 and 12 months follow-up; NS – not significant)

Ryc. 4. Wysiękowa postać AMD – średnia gęstość i czas latencji fali P1 w 3., 6., 12. miesiącach obserwacji po doszklistkowych iniekcjach ranibizumabu w porównaniu z wynikami wyjściowymi i wynikami uzyskanymi w grupie kontrolnej (n=21).
(* – porównanie wyników uzyskanych w grupie kontrolnej z wynikami wyjściowymi oraz wynikami uzyskanymi w 3., 6., 12. miesiącu obserwacji; ^ – porównanie wyjściowych wyników z wynikami uzyskanymi w 3., 6., 12. miesiącu obserwacji; NS – wynik nieistotny statystycznie)

When we analyzed mfERG results separately from each treated eye, increase of P1-response density was seen in 6/21 (28.6%), 5/21 (23.8%) and 3/21 (14.3%) of eyes after 3, 6 and 12 months follow-up, respectively. This increase was in accordance with improvement of DBCVA in 4/6 (66.7%), 3/5 (60.0%) and 2/3 (66.7%) of eyes as well as reduction of foveal thickness in 6/6 (100.0%), 3/5 (60.0%) and 2/3 (66.7%) of treated eyes after 3, 6 and 12 months of follow-up, respectively.

Decrease of P1-implicit time in R1 was observed in 1/21 (4.8%), 1/21 (4.8%) and 0/21 (0.0%) of eyes after 3, 6 and 12 months fol-

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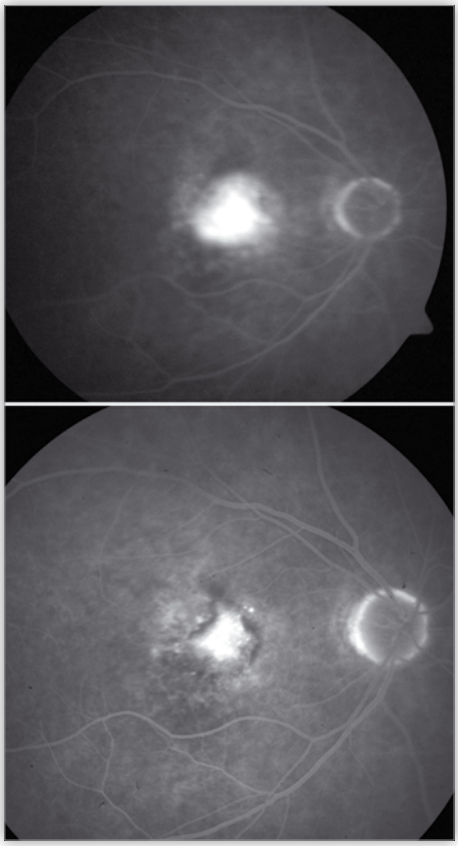

Fig. 5a. Wet type of AMD – an example of FA result at baseline (above) and 12 months follow-up (below) after intravitreal ranibizumab injections – reduction of leakage was observed.

Ryc. 5a. Wysiękowa postać AMD – przykład wyniku angiografii fluoresceinowej przed leczeniem (powyżej) i w 12. miesiącu obserwacji (poniżej) po doszklistkowych iniekcjach ranibizumabu – zmniejszenie przecieku.

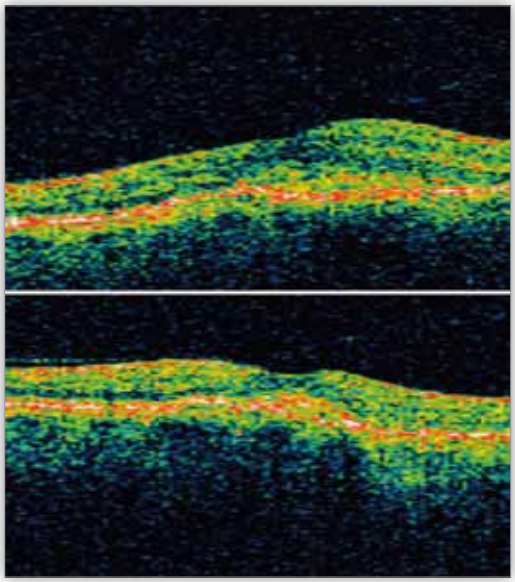

Fig. 5b. Wet type of AMD – an example of OCT result at baseline (above) and 12 months follow-up (below) after intravitreal ranibizumab injections – reduction of foveal thickness was detected and formation of the scar.

Ryc. 5b. Wysiękowa postać AMD – przykład wyniku badania OCT przed leczeniem (powyżej) i w 12. miesiącu obserwacji (poniżej) po doszklistkowych iniekcjach ranibizumabu – redukcja grubości dołka oraz tworzenie się blizny.

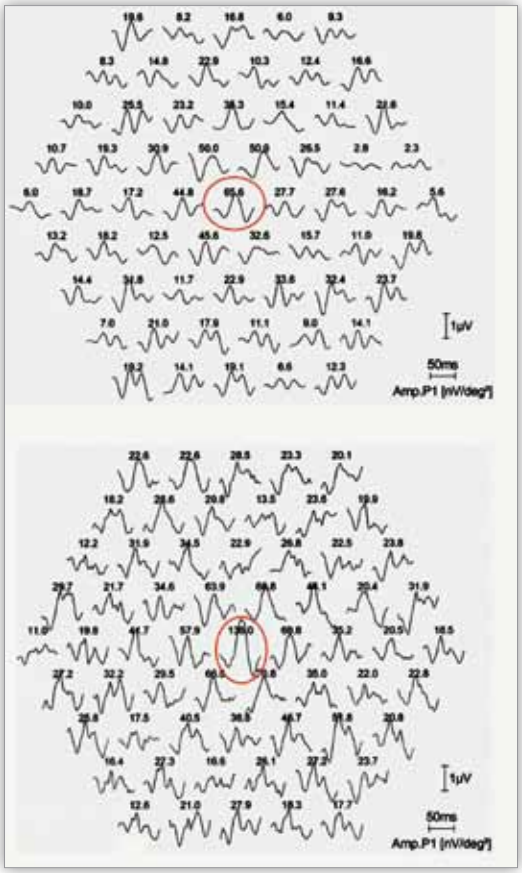

Fig. 5c. Wet type of AMD – an example of mfERG result at baseline (above) and 12 months follow-up (below) after the intravitreal ranibizumab injections – increase of P1-response density and decrease of P1-implicit time in R1 and R2 were noted.

Ryc. 5c. Wysiękowa postać AMD – przykład wyniku badania mfERG przed leczeniem (powyżej) i w 12. miesiącu obserwacji (poniżej) po doszklistkowych iniekcjach ranibizumabu – zwiększenie gęstości fali P1 oraz skrócenie czasu latencji fali P1w R1 i R2.

low-up, respectively. Shortness of P1-implicit time did not connect either with DBCVA increase or with foveal thickness reduction.

Reduction of active CNV diameter (FA) after 3 and 12 months follow-up was in accordance with increase of P1-response density in 4/6 (66.7%) and 2/3 (66.7%) of eyes, respectively but it did not connect with shortness of P1-implicit time.

In most cases, in which the improvement of mfERG, DBCVA and OCT results was obtained, the minimally classic or ocult CNV were observed and reduction of active CNV occurred. In these eyes the diameter of CNV do not cover the total area of the fovea.

Figures 5a–c show an example of patient with the wet type of AMD (12 months follow-up) treated with intravitreal ranibizumab injections with reduced CNV diameter and with improvement of DBCVA and mfERG result (P1-response density increase and P1-implicit time decrease) associated with reduction of the foveal thickness.

Correlation analysis

Correlation analysis between the morphologic and functional changes after ranibizumab intravitreal injections were performed. There were no correlations between DBCVA change and P1-response density or P1-implicit time changes (R1 and R2) as well as between foveal thickness change and response density or P1-implicit time changes (R1 and R2) – with the exception of a positive

3 months follow-up								
	P1-response density changes				**P1-implicit time changes**			
	R1		**R2**		**R1**		**R2**	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
DBCVA change	-0.006‡	NS	0.262‡	NS	-0.241†	NS	-0.262†	NS
OCT change	-0.019‡	NS	0.229‡	NS	-0.298†	NS	0.401†	NS
6 months follow-up								
	P1-response density changes				**P1-implicit time changes**			
	R1		**R2**		**R1**		**R2**	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
DBCVA change	-0.106‡	NS	0.077‡	NS	0.168‡	NS	-0.169‡	NS
OCT change	0.113‡	NS	0.068†	NS	-0.554†	0.009	0.292†	NS
12 months follow-up								
	P1-response density changes				**P1-implicit time changes**			
	R1		**R2**		**R1**		**R2**	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
DBCVA change	-0.226†	NS	-0.373†	NS	0.012†	NS	0.103†	NS
OCT change	-0.217†	NS	0.275†	NS	-0.092†	NS	0.156†	NS

Tab. I. The correlations between DBCVA / foveal thickness changes and P1-response density / P1-implicit time changes in R1and in R2 at 3, 6 and 12 months follow-ups after intravitreal ranibizumab injections.

Tab. I. Korelacja między zmianami DBCVA / grubością siatkówki w rejonie dołka oraz zmianami gęstości fali P1 / czasu latencji fali P1 w 3., 6. i 12. miesiącach obserwacji po doszklistkowych podaniach ranibizumabu.

(† – Pearson coefficient, ‡ – Spearman's rank coefficient, NS – not significant)

(† – współczynnik korelacji Pearsona, ‡ – współczynnik korelacji rang Spearmana, NS – wynik nieistotny statystycznie)

correlation between foveal thickness change and P1-implicit time change in R1 at 6 months follow-up (Tab. I).

Discussion

The results of our study indicate that the treatment of the wet type of AMD with ranibizumab injections, according to doses and frequencies described in methodology, is effective in preventing the progression of the disease (stable DBCVA, stable or decreased total CNV diameter, decreased foveal thickness, stable foveal, parafoveal and partially perifoveal bioelectrical function). After 12 months follow-up, stable or decreased total CNV was observed in 57% (12/21) of eyes. Significant improvement of the mean of the DBCVA after 3 months follow-up as well as significant reduction of subretinal fluid after 6 and 12 months follow-up were noted (Fig. 2, 3). The mean of the foveal as well as parafoveal and partially perifoveal bioelectrical function measured by the mfERG examination (R1 and R2) was stable in all analyzed eyes during observation time (Fig. 4).

Significant improvement of the mean DBCVA 3 months follow-up might be a result of reduction of the subretinal fluid observed in the OCT examination. Our data are in agreement with the results of other authors (5-7).

In our study, correlations between the morphologic and functional changes in patients with wet type of AMD after ranibizumab theraphy were not found with one exception (P1-implicit time and foveal thickness after 6 months follow-up) (Tab. I).

One of the possible explanations of the lack of correlation between DBCVA or foveal thickness changes and bioelectrical function changes is the duration and advancement of the disease. In the long-lasting wet type of AMD pre-existing photoreceptors loss is present (16), so irreversible retinal damage can be expected and applied treatment in those cases is less effective. That is why in our group of patients we did not observe the statistically significant improvement of the macular function measured by mfERG test.

During 12 months follow-up, the results of the mean DBCVA, the mean foveal thickness as well as the mean response density and the mean P1-implicit time did not reach the values from the control group (Fig. 2–4). This observation suggests that treatment of the wet type of AMD with intravitreal ranibizumab injections (according to the treatment regimen described above), does not restore normal function and structure in the macular region. However, intravitreal administration of ranibizumab in wet type of AMD is effective in preserving the DBCVA and in stabilization the bioelectrical function of the foveal, parafoveal and partially perifoveal region. The results of commonly used tests like VA, OCT and additionally mfERG, strongly suggest the usefulness of ranibizumab theraphy in wet type of AMD. Up to 90% of patients treated with ranibizumab have a chance of maintenance of vision, but in not treated patients progression of this disease is shown (8).

In some of our patients after ranibizumab therapy, there were positive relations between the improvement of DBCVA and the fo-

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veal thickness reduction as well as between the improvement of DBCVA and the improvement of mfERG results. In these cases the minimally classic or ocult CNV were observed, reduction of active CNV after treatment were noted and the area of CNV did not cover the total area of the fovea. An example of such case is shown in figure 5a–c.

Presumably, in these eyes macular dysfunction occurred not for a long time so reversible retinal changes were more expected. It is known the cellular origin of mfERG is from ON and OFF- bipollar cells as well as from the cone photoreceptors (17). The reduction of the subretinal fluid after the treatment induced a return, at least partially, of the anatomical connections between these retinal cells in the macular region and is a cause of functional improvement seen in mfERG test when compared with values before treatment. However, bioelectrical function in the foveal, parafoveal and partially perifoveal region did not reach normal values.

The correlation results of our study are in concordance with those described with Feigl et al. (5) but are not in consistent with Moschos et al. (7), and Campa et al. (6). Feigl et al. (5) observed no change in central retinal function. Moschos et al. (7) found a significant increase of the foveal response density, but no association of the foveal thickness and response density or VA whereas borderline positive association of the response density and VA. Campa et al. (6) described positive correlation of mfERG response with VA and negative with foveal thickness. The differences between studies results can be explained by various number of treated eyes, stages of the disease, type or diameter and location of CNV, treatment regimen, observation time as well as by using different mfERG equipment.

In conclusion in our group of patients intravitreal ranibizumab treatment was effective because DBCVA significantly improved after 3 months and remained stable after 6 and 12 months follow-up, significant reduction of foveal thickness during all follow-ups as well as stable macular function were observed.

MfERG brings additional objective information about macular region, so it should be performed in parallel to the other test like VA or OCT.

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