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NON-RESPONSIVENESS AND TACHYPHYLAXIS TO ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR TREATMENT IN NAIVE PATIENTS WITH EXUDATIVE AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is a leading cause of central visual loss in people aged over 50 years in well developed countries. Although the anti-vascular endothelial growth factor (VEGF) therapy has become a standard treatment for exudative AMD, its effectiveness may be limited in some cases. We aimed to assess the prevalence of non-responsiveness and tachyphylaxis to anti-VEGF drugs in patients with exudative AMD. The study included 63 initially treatment-naïve AMD patients who were analyzed for non-responsiveness and tachyphylaxis to intravitreal injections (IVI) of ranibizumab and aflibercept. The participants were enrolled in a National Healthcare Fund (NHF) Therapeutic Program for the Treatment of Exudative AMD. Best-corrected visual acuity (BCVA) and morphological features of a disease activity assessed in optical coherence tomography (OCT) were evaluated during a 12-month follow-up. The percentage of non-responders achieved 22.2% (14 eyes). No significant correlation was found between the type of VEGF inhibitor and a negative response to therapy. Eight patients (12.7%) developed early tachyphylaxis, which was more common in eyes treated with aflibercept (P = 0.04). The presence of serous pigment epithelium detachment (sPED) at baseline was associated with non-responsiveness as determined by both BCVA (OR 18.2, 95% CI 2.86 – 248; P = 0.021) and OCT features (OR 23.0, 95% CI 1.80 – 321; P = 0.030). Eyes treated with aflibercept showed statistically significant greater BCVA improvement (P = 0.0034) and central retinal thickness (CRT) reduction (P = 0.0129) as compared to ranibizumab group during a loading phase of therapy. In a maintain phase of treatment the differences in BCVA and CRT between these two groups were not statistically significant, however eyes treated with aflibercept still showed better functional and anatomical results. Anti-VEGF therapy is an effective method of treatment for exudative AMD, however some patients may show week or no positive reaction or may develop tachyphylaxis. Awareness of these possible negative effects is an important clinical problem in the long-term management of AMD patients with VEGF inhibitors.

K e y   w o r d s : *age-related macular degeneration, central retinal thickness, best corrected visual acuity, anti-vascular endothelial growth factor therapy, tachyphylaxis, non-responsiveness*

INTRODUCTION

Age-related macular degeneration (AMD) is still the leading cause of severe, irreversible loss of central vision in people aged over 50 years in developed countries, significantly impacting the daily functioning of patients and their relatives (1). Worldwide estimates approximated that 30 – 50 million people are affected by AMD, and these numbers are expected to increase over time because of the aging population (2). There are two types of AMD: 'dry' and 'wet' (exudative, neovascular) (1, 3). Exudative AMD is characterized by the presence of choroidal neovascularization (CNV) and represent only 10 – 15% of all patients with this disease, although this form accounts for as many as 90% of all cases involving acute and permanent loss of central vision (2, 3).

In the last few years several hypotheses have been put forward to explain the pathogenesis of AMD. However, despite extensive clinical and experimental research, the pathogenesis underlying the disease remain unclear. It appears to be a complex disease with many factors being involved, *i.e.* increased oxidative stress, excessive complement activation and inflammatory reactions in the subretinal space, as well as genetic and environmental factors (4-6).

Vascular endothelial growth factor (VEGF) that is a naturally occurring glycoprotein in the body, regulates angiogenesis, enhances vascular permeability, and plays a major role in exudative AMD. The consistent association between CNV the clinical hallmark of exudative AMD and increased VEGF expression provides a strong reason for exploring the therapeutic potential of anti-VEGF agents in the treatment of this disorder (7, 8). Blockade of VEGF activity is currently the most effective strategy for arresting choroidal angiogenesis and reducing vascular permeability, which is frequently the main cause of visual acuity deterioration (9).

The discovery of a group of anti-VEGF agents and their application in the treatment of AMD marked a revolutionary step in the management of this sight-threatening disease (7-9). The use of intravitreal injections (IVI) of anti-VEGF made it possible to halt pathophysiological AMD processes, restore central retinal morphology and improve or at least stabilize the function of the treated eye. Intravitreal injections have become the treatment of choice for exudative AMD and they have been gradually displaced such methods as photodynamic therapy (PDT) and laser photocoagulation (9).

Currently, there are three anti-VEGF agents used in various treatment regimens for exudative AMD: aflibercept, ranibizumab and bevacizumab - the latter is used off-label (1, 10). Although intravitreal, anti-angiogenic therapy is nowadays the standard procedure for the management of exudative AMD, it is not ideal. According to the results of clinical observations, resistance to treatment may occur at any time during the course of therapy in some patients (11).

Anti-VEGF therapy may fail from the beginning or may weaken following an initial successful response to the treatment (12). Non-responders despite multiple, repeated IVI of anti-VEGF drugs, show no anatomical or functional improvement in the treated eye, and they constitutes up to 25% AMD patients (13-15). Another therapeutic problem concerns AMD patients in whom despite a positive initial response to anti-VEGF, the effect of the drug weakens with time, resulting in progression of the disease. This group consists of patients affected by tachyphylaxis and accounts 2 – 10% of cases (14, 16).

The aim of this study was to analyse the occurrence of various types of response to treatment with IVI of aflibercept or ranibizumab in patients with exuadtive AMD who were included in National Health Fund (NHF) therapeutic program: The Treatment of the Neovascular (Exudative) Form of Age-Related Macular Degeneration. We also evaluated the prevalence and characteristics of ocular and systemic predictors responsible for the development of non-responsiveness and tachyphylaxis during a 12 month-period of anti-VEGF therapy.

MATERIAL AND METHODS

From the 182 patients screened for NHF therapeutic program between November 2015 and October 2017 we included in this retrospective study 63 individuals (63 eyes) with newly diagnosed exudative AMD. The diagnosis of AMD was based on typical fundus findings documented by digital photography, optical coherence tomography (OCT, Topcon 3D OCT 2000, Japan), fluorescein angiography (FA, Heidelberg Engineering, Spectralis HRA-OCT, Germany) and in some cases on coherent optical tomography angiography (OCTA-Topcon, DRI OCT-1, Atlantis, Japan). In OCT the presence of morphological parameters indicating disease activity *i.e.* intraretinal fluid (IRF), subretinal fluid (SRF), serous pigment epithelium detachment (sPED) and central retinal thickness (CRT) were evaluated. In all patients the best corrected visual acuity (BCVA) was assessed using the Snellen charts. Follow-up examinations were performed every 4 weeks and included the same procedures as the baseline examination, except for FA and OCTA.

The inclusion criteria established for the NHF Therapeutic Program were as follows: age > 45 years, BCVA ranged from 0.2 to 0.8 on Snellen chart, the presence of active CNV involving more than 50% of the lesion due to AMD with no signs of dominant geographic atrophy (GA) and/or macular haemorrhage, with no subfoveolar fibrosis and the total lesion size < 12 disc diameter (DD). The patients were treated with one of two anti-VEGF agents approved for the therapy for exudative AMD: aflibercept (Eylea, 2 mg/0.05 ml) and ranibizumab (Lucentis,

0.5/0.05 ml). The treatment protocol consisted of a loading phase of therapy during which the patients received 3 monthly injections of aflibercept or 4 monthly injections of ranibizumab. Then, in a maintenance phase, aflibercept was injected every two months for the first year of therapy, while ranibizumab was injected on *pro re nata* regimen (PRN, as needed), based on the results of clinical examination performed every 4 weeks. The worsening of BCVA associated with clinical evidence of the disease activity on OCT - evidence of increased CRT, the presence of IRF, SRF or sPED were indications for ranibizumab reinjection.

We analysed the associations between the changes in BCVA and OCT parameters, CNV type and diameter on FA with a type of treatment response, number of injections and type of used anti-VEGF drug. The correlations between the type of reaction to anti-VEGF drug and age and gender were also assessed.

We established the following criteria to define 'non-responsiveness' to anti-VEGF therapy: no changes or reduction in CRT $\leq$ 10% (anatomical 'non-responders') or no improvement or a deterioration in BCVA by $\geq$ 1 line by Snellen charts (functional 'non-responders'). Also the presence of morphological parameters such as: increased IRF, SRF and enlarged sPED in OCT were considered as 'non-responsiveness'.

The tachyphylaxis was defined as evidence of an initial positive reaction to the treatment (reduction in CRT by more than 10% and an improvement in BCVA by at least 1 line on Snellen chart), followed by an eventual poor, weakening response to therapy (increase in CRT by at least 10% and/or a deterioration in BCVA by at least 1 line) despite at least 2 more monthly injections after the loading phase.

Statistical analysis

The statistical analysis was performed using STATISTICA 10.0 software. The analysis of variance models (ANOVA) was used for repeatable parameters. The BCVA Snellen results after conversion into BCVA logMAR scale did not show normal distribution and Friedmann test was used for statistical analysis. For independent variables, the Mann-Whitney U test was used. The relationship between the qualitative variables was examined on the basis of contingency tables using the Chi-square test. Adjusted ORs and 95% CIs for non-responders were estimated with logistic regression models to examine the effects of the confounding factors on the unadjusted results. To determine the strength of the relationship between quotient and order (rank) type variables, the Spearman's rank correlation coefficient was used. P values < 0.05 were considered statistically significant.

RESULTS

Of the 182 patients qualified for the NHF therapeutic programme, 63 had been newly diagnosed for exudative AMD and had received no prior treatment. There were 35 women (55.6%) and 28 men (44.4%). The patients age ranged from 63 to 87 years (average age: 72.8 years). A total of 47 patients were treated with aflibercept and 16 with ranibizumab.

At baseline, 24 of 63 eyes (38.1%) had IRF with or without SRF and 26 (41.3%) eyes had SRF alone. Of 23 eyes (36.5%) that had sPED, 10 had associated IRF, and 21 had additional SRF. The baseline average BCVA was 0.34 Snellen; 0.46 logMAR (ranging from 0.2 to 0.8 Snellen; 0.7 – 0.1 logMAR) and was significantly worse in eyes with IRF (0.61 Snellen; 0.28 logMAR; 95% CI, 0.48 – 0.80 Snellen; 0.38 – 0.1 logMAR) compared with eyes with SRF alone (0.3 Snellen; 0.48 logMAR; 95% CI, 0.23 – 0.51 Snellen; 0.65 – 0.29 logMAR, P = 0.006).
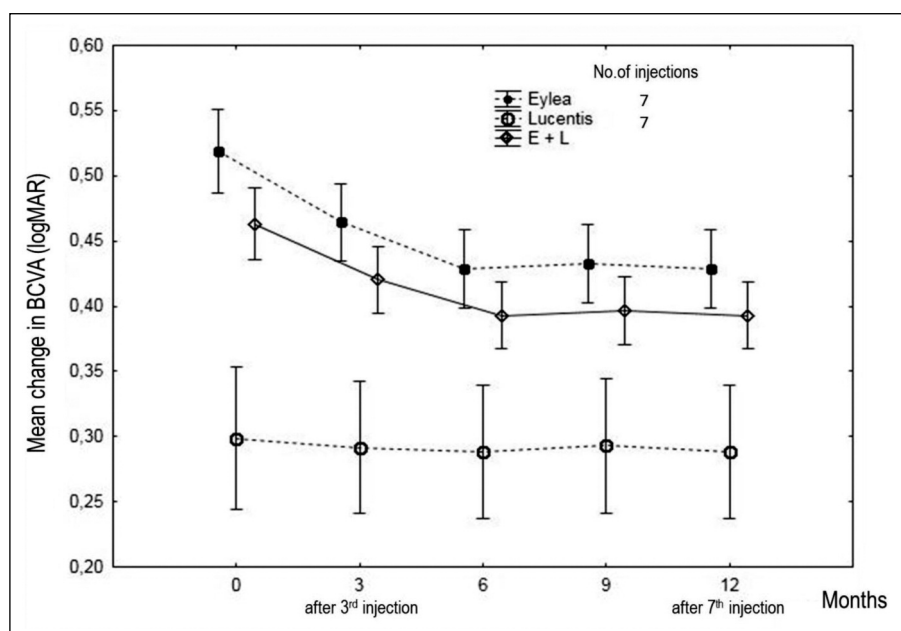
Fig. 1. Mean change in best corrected visual acuity (BCVA) in patients with age-related macular degeneration (AMD) treated with intravitreal injections of aflibercept and ranibizumab during a 12 month follow-up.

Following the loading phase of treatment BCVA improved (average BCVA improvement 0.38 Snellen; 0.42 logMAR), and at the end of a follow-up period average BCVA was 0.40 Snellen; 0.39 logMAR. At 12th month, eyes with SRF at baseline had better BCVA (0.64 Snellen; 0.22 logMAR; 95% CI, 0.48 – 0.76 Snellen; 0.32 – 0.18 logMAR) as compared with those with IRF component (0.53 Snellen; 0.28 logMAR; 95% CI, 0.34 – 0.65 Snellen; 0.48 – 0.21 logMAR; P = 0.01). The presence of persistent fluid was associated with a reduced chance of visual improvement at 12 months (23.6% versus 41.6%; P = 0.02). Although the presence of sPED did not significantly impact on BCVA at baseline, the presence of persistent sPED was associated with a reduced chance of visual improvement at the end point of a follow-up (16.1% versus 33.5%; P = 0.04).

Fourteen of 23 eyes with sPED (60.8%) at baseline were noted to have residual sPED at 12 month with no difference in final BCVA. The average baseline BCVA was better in the ranibizumab group (average BCVA - 0.5 Snellen; 0.3 logMAR) as compared to the aflibercept group (average BCVA - 0.3 Snellen; 0.52 logMAR). After the loading phase, a significant improvement in the average BCVA was observed in both groups as compared to the baseline (P = 0.0269). Statistical analysis showed that BCVA improvement was greater in the group treated with aflibercept (P = 0.0034). At the end of the follow-up a further improvement in average BCVA was observed in the aflibercept group (P = 0.0265), while in the ranibizumab group the average BCVA remained stable (P = 0.324). The analysis of BCVA dynamic changes revealed that the greatest improvement in average BCVA was observed during the loading phase of therapy and it did not depend on the type of used VEGF inhibitor.

The changes in mean BCVA (logMAR) in the aflibercept and ranibizumab groups during the 12-month follow-up period are shown in *Fig. 1*.

The average baseline CRT was 318.8 μm (range from 145 to 602 μm). After the loading phase, there was a noticeable reduction in CRT. The average CRT decreased to 251.9 μm (range from 124 to 485 μm), and after a year of treatment decreased to 247.0 μm (range: 122 to 335 μm). The differences between mean CRT at the assesed points of the study were statistically significant (P = 0.042).

The average baseline CRT in the ranibizumab group was 286.3 μm, and in the aflibercept group reached 329.9 μm. After the loading phase, a reduction in mean CRT was observed in both groups, but was significantly greater in the aflibercept group as compared to the baseline examination (P = 0.01). At the end of the observation period, a further decline in the CRT was noted in the aflibercept group, while in the ranibizumab group the CRT increased. However the differences in the CRT changes were not statistically significant between these two groups nor between the analysed points of the study. Similarly to BCVA changes, the largest reduction in CRT was noted after the loading phase of therapy and was greater in the aflibercept group (P = 0.0129). *Fig. 2* shows the mean changes in CRT in patients treated with ranibizumab and aflibercept during the 12-month follow-up period.

Based on the type of response to therapy, three groups were selected. Fourty one patients (65.1%) demonstrated positive reaction to anti-VEGF treatment with improvement in both, foveolar morphology and function of the treated eye. Fourteen patients (22.2%) were non-responders as determined by both BCVA and OCT findings and they showed no anatomical nor functional positive response to therapy during a loading phase of therapy. Among these patients there were 6 treated with ranibizumab and 8 with aflibercept. We found no incidence of delayed but good response to anti-VEGF therapy up to 12 month in this group. Eight from 63 patients (12.7%) met the criteria for tachyphylaxis, that was mainly observed in eyes treated with aflibercept (7 eyes) as compared to ranibizumab (P = 0.02). Tachyphylaxis occurred after 4 – 5 doses of anti-VEGF drug. The mean number of IVI injections at month 12 was seven in both groups of patients. Therefore we can assume that in analyzed material the treatment regimens for aflibercept and ranibizumab were comparable in the first year of therapy in the course of the NHF Therapeutic Program.

There were no statistically significant differences in age, gender, OCT findings, or CNV type and diameter among these three groups at baseline. However, the presence of sPED before treatment was associated with non-responsiveness as determined by both BCVA (OR 18.2, 95% CI 2.86 – 248, P = 0.021) and OCT findings (OR 23.0, 95% CI 1.80 – 321, P = 0.030) in the study group. *Table 1* presents three determined
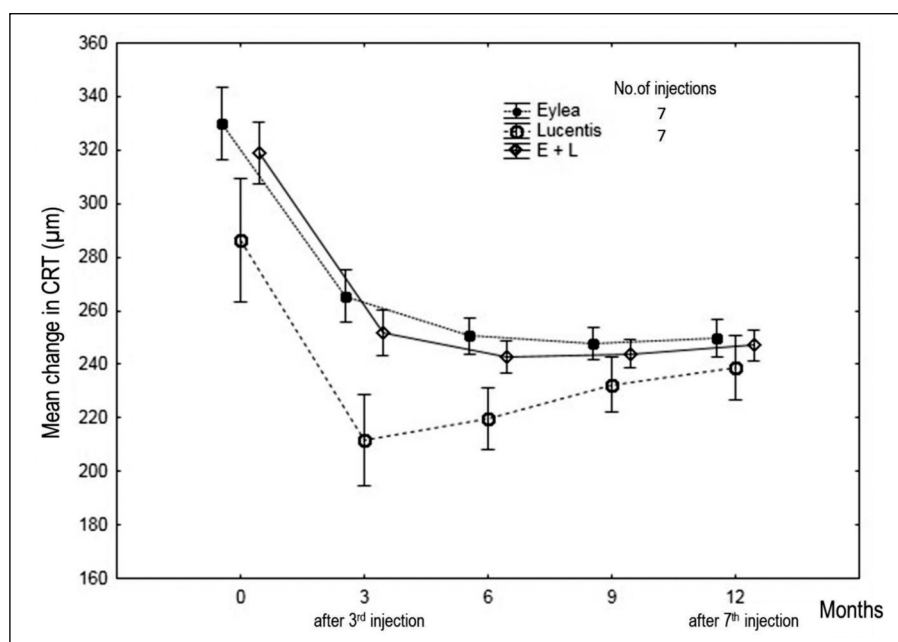
Fig. 2. Mean change in central retinal thickness (CRT) in patients with age-related macular degeneration (AMD) treated with intravitreal injections of aflibercept and ranibizumab during a 12 month follow-up.

Table 1. Baseline characteristics of three determined groups of patients with age-related macular degeneration (AMD) treated with intravitreal injections of aflibercept and ranibizumab; responders, non-responders and patients with tachyphylaxis.

Type of response to anti-VEGF therapy	**Responders**	**Non-responders**	**Tachyphylaxis**
No. of patients	41 (65.1%)	14 (22.2%)	8 (12.7%)
Mean age (years)	71.7	74.2	75.5
Gender	24 (58.5%) female 17 (41.5%) male	8 (57.1%) female 6 (42.9%) male	3 (37.5%) female 5 (62.5%) male
Number of eyes treated with aflibercept and ranibizumab	aflibercept 32 (78%) ranibizumab 9 (22%)	aflibercept 8 (57.1%) ranibizumab 6 (42.9%)	aflibercept 7 (87.5%) ranibizumab 1 (12.5%)
Mean baseline BCVA (Snellen)	0.4	0.4	0.3
Mean baseline CRT (μm)	322.6	284.3	359.8
Presence of sPED	13 (31.7%)	7 (50%)	3 (37.5%)
Presence of SRF	37 (90.2%)	14 (100%)	7 (87.5%)
Presence of IRF	17 (41.5%)	3 (21.4%)	3 (37.5%)
Type of CNV on FA	classic 14 (34.1%) occult 17 (41.5%) mixed 10 (24.4%)	classic 9 (64.3%) occult 5 (35.7%)	classic 6 (75%) occult 1 (12.5%) mixed 1 (12.5%)
Baseline CNV area on FA (mm^2)	3.60	3.31	4.38

Abbreviations: BCVA, best corrected visual acuity; CRT, central retinal thickness; sPED, serous pigment epithelium detachment; SRF, subretinal fluid; IRF, intraretinal fluid; CNV, choroidal neovascularization; FA, fluorescein angiography.

groups of patients with AMD and their baseline clinical characteristics.

DISCUSSION

Among proinflammatory cytokines produced by endothelial cells, VEGF-A is the principal one, responsible for neovascularization in a course of exudative AMD. VEGF-A induces angiogenesis and increases vascular permeability and inflammation, leading to progressive degeneration of photoreceptors, RPE and fluid accumulation within retina and/or in the subretinal space. Interestingly senescent endothelial cells produce more proinflammatory cytokines and VEGF as compared to young endothelium. These observations have been noted in an experimental study by Korybalska *et al*. who measured VEGF concentrations under the condition of wound healing evoked by scratch assay *in vitro* (17). Inhibiting the neovascularization process by introducing anti-VEGF factors in ocular pharmacology at the turn of the 21st century completely revolutionized the approach to the treatment of exudative AMD. Currently, these drugs are the gold standard of treatment for this condition, which leads to central visual impairment (1, 18). However, the experiences of the last 10 years have shown that some patients do not achieve positive response to therapy (12). Based on morphological and functional analysis in 14 – 25% of AMD patients there is a poor or no response to anti-VEGF treatment (13-15). In this group of patients ('non-responders'), the disease continued to progress, CRT is observed to increase and BCVA deteriorates, leading to irreversible damage to the

central vision. The response to anti-VEGF therapy have been found to be dependent on a variety of factors including patient's age, disease duration, baseline BCVA, the presence of particular genotype risk alleles. Also some anatomical findings seem to be predictors of therapy failure, and they include: the initial lesion with subfoveal fibrosis or atrophy in retina pigment epithelium and photoreceptors, lesion in large size, type 1 choroidal neovascularization, sPED, haemorrhagic PED, fibrovascular PED, polypoidal choroidal vasculopathy (PCV), foveal scarring and vitreomacular traction (VMT), outer retinal tubulation, cystoid degeneration in outer retina (11, 12).

In this study there were 22.2% of 'non-responders' to anti-VEGF therapy. The presence of sPED was the main anatomical predictor of lack of positive reaction to anti-VEGF in analyzed group of patients. There are reports describing positive effects of anti-VEGF drugs, specially aflibercept, on sPED resolution (19-21). However, there are also data showing that sPED is associated with poor response to anti-VEGF treatment (22-25). Failure to resolve fluid from subretinal or sub-retinal pigment epithelium (RPE) space after initial treatment may be a marker of irreversible structural damage to the photoreceptors and RPE (8, 19). Similarly to our results, where eyes with persistent sPED after the loading phase still presented persistent sPED at 12 month of a follow-up, suggesting that the ability to dry the retina is determined early in the course of treatment (20, 21).

In our study 'non-responsiveness' was found with almost the same prevalence in eyes treated with aflibercept (6 eyes) and ranibizumab (8 eyes), while the results of other studies indicate that 'non-responsiveness' is more often noted in eyes treated with ranibizumab (26, 27). This could be related to aflibercept's broader suppressive effects on VEGF family proteins, including VEGF-B and placenta growth factor (PlGF), its greater affinity for VEGF-A, and the longer half-life of the drug. These differences among the results of studies may be associated with different clinical material, variants of exudative AMD *i.e.* PCV and retinal angiomatous proliferation (RAP), different AMD stage and various anti-VEGF treatment protocols (16).

It is noteworthy that treatment response to anti-VEGF in AMD patients is also determined by genetic factors that may modulate the drug response. This observation was reported in our previous study as well as by other authors (28-31).

Another negative effect associated with the AMD treatment with intravitreal anti-VEGF injections is tachyphylaxis. According to reports, it may occur in up to 10% of patients (14, 16). This condition involves a rapid loss of sensitivity to a drug, *i.e.* after its repeated administration there is a noticeable weakening in its effect, and the symptoms of the disease reappearance despite a positive initial response to treatment (14).

Tachyphylaxis develops over a short period of time after the therapy initiation, in contrary to drug tolerance which is define as a significant decrease in response in a long-term drug application (32). The timing of onset of tachyphylaxis remains unclear and is presumably highly individualized. In our study 8 patients (12.7%) developed tachyphylaxis after the loading phase of therapy. Usually tachyphylaxis occurs in response to a drug after its frequent administration (14). Analysis of therapeutic regimens for aflibercept (3 loading doses and then every two months) and ranibizumab (4 loading doses and then PRN), showed that tachyphylaxis was mainly observed in eyes treated with aflibercept, which loading phase included only three IVI, in contrary to ranibizumab schedule with four 'loading' IVI. This observation may be suggestive that the type of therapeutic regimen does not influence the occurrence of this unfavorable phenomenon. However this result should be interpreted with caution because of a smaller number of patients treated with ranibizumab and the same number of IVI performed in both groups of patients at month 12.

The etiology of tachyphylaxis is thought to be multifactorial. Chronic VEGF blockade may alter the expression pattern of surface receptors and modify the quantity and/or sensitivity of receptors (16). Another possible cause of a decrease in the response may be an increase of the expression of VEGF or other angiogenic factors to compensate for blocked VEGF activity (16, 33). The decreasing efficacy of the drug may also be explained by the development of a systemic immune response with neutralizing antibodies. Production of VEGF may also be upregulated by macrophages within CNV tissue and the response to anti-VEGF treatment may be dependent on the composition and structural integrity of the CNV lesion (33).

Tachyphylaxis cannot be overcome by increasing the dose of the drug (12). However, the effectiveness of therapy can be improved by stopping the injections for a short period of time, or by extending the intervals between doses (12). The most effective method of preventing tachyphylaxis is to replace one drug with another from the same group (34, 35). Currently there is no effective therapeutic strategies for patients with no positive reaction to anti-VEGF therapy (12, 16, 35, 36). However there are some data indicating that the presence of sPED, one of the predictors of 'non-responsiveness' to anti-VEGF therapy, may be effectively treated with afliberept (35, 36). Aflibercept has been proven to be more effective in inhibiting VEGF with a wider spectrum of action, and has been successfully used as an effective replacement drug in eyes treated previously with bevacizumab or ranibizumab (16, 35, 36).

Study limitations

The sample size was relatively small, and genetic analysis was not performed in analyzed group. Additionally, it was a retrospective study conducted within the NHF Therapeutic Program and the 'switching' to another anti-VEGF agent, in accordance to the protocol, was not allowed during the first year of therapy. Moreover, our group of patients included significantly less patients treated with ranibizumab that may suggest the need of extending the group of patients treated with this anti-VEGF agent in the NHF Therapeutic Program which may allow in future more objective analysis and recognition which VEGF inhibitor is associated with a higher prevalence of tachyphylaxis and non-responsiveness.

In summary, the results of this study indicate that intravitreal anti-angiogenic therapy, currently considered as the 'gold standard' for treating of exudative AMD, is not ideal. Some patients do not respond positively to therapy or develop rapid decreased therapeutic response over time leading to the progression of the disease. Thus, awareness of these possible negative effects is important in the long-term anti-VEGF therapy. Serous PED was a main risk factor for 'non-responsiveness' in the treatment-naïve patients with exudative AMD.

Conflict of interests: None declared.

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