https://doi.org/10.5114/ko.2019.89727

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Choroidal Thickness Changes in Patients with Wet Age-related Macular Degeneration over One Year of Aflibercept Treatment

Zmiany grubości naczyniówki u chorych na wysiękową postać zwyrodnienia plamki związanego z wiekiem leczonych afliberceptem w obserwacji rocznej

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Abstract: Aim: The aim of the study was to analyze choroidal thickness changes in treatment-naive patients with exudative age-related macular degeneration treated with aflibercept in daily clinical practice.

Materials and Methods: Thirty-six patients (36 eyes), aged 78.3±8.7 years, were enrolled in a prospective, non-randomized, one-year study. Aflibercept was administered monthly for three months and then bimonthly in a fixed regimen. Retinal and choroidal thickness were measured manually on optical coherence tomography scans.

Results: Patients gained, on average, 8.5 letters ($p<0.001$). Central retinal thickness decreased significantly from 337.7±126.5 μm to 272.1±123.1 μm ($p<0.05$). Subfoveal choroidal thickness decreased significantly after a loading phase: from 190.2 ± 97.7 μm to 177.4 ± 90.0 μm ($p<0.05$), but the reduction was not significant at 12 months ($p=0.078$). Choroidal thickness measured nasally from the fovea decreased significantly at each point of follow-up ($p<0.05$). Subfoveal choroidal thickness decreased significantly from 182.80 ± 91.43 μm to 164.0 ± 86.3 μm ($p>0.05$) only in patients with visual acuity improvement of > 5 letters on the Early Treatment Diabetic Retinopathy Study chart.

Conclusions: Subfoveal choroidal thickness in patients with neovascular age-related macular degeneration decreased after the loading phase of aflibercept treatment. At 12 months, aflibercept treatment reduced subfoveal choroidal thickness only among patients with visual acuity improvement.

Key words: exudative age-related macular degeneration, choroid, choroidal thickness, aflibercept.

Abstrakt: Cel pracy: Celem pracy była analiza zmian grubości naczyniówki u chorych z wcześniej nieleczoną wysiękową postacią zwyrodnienia plamki związanego z wiekiem, leczonych afliberceptem w codziennej praktyce.

Materiał i metody: Trzydziestu sześciu chorych (36 oczu) w średnim wieku 78.3 ± 8.7 lat zostało zakwalifikowanych do prospektywnego, nierandomizowanego, rocznego badania. Aflibercept podawany był co miesiąc przez pierwsze trzy miesiące i następnie co dwa miesiące w schemacie stałym. Pomiary grubości siatkówki i naczyniówki wykonywane były na skanach optycznej kohorentnej tomografii.

Wyniki: Chorzy uzyskali poprawę o średnio 8,5 litery na tablicy Early Treatment Diabetic Retinopathy Study ($p<0.001$). Centralna grubość siatkówki zmniejszyła się istotnie z 337.7±126.5 μm do 272.1±123.1 μm ($p<0.05$). Poddołkowa grubość naczyniówki zmniejszyła się istotnie po fazie nasycenia afliberceptem z 190.2 ± 97.7 μm do 177.4 ± 90.0 μm ($p<0.05$), ale redukcja nie była istotna w 12 miesięcznej obserwacji ($p=0.078$). Grubość naczyniówki zmniejszyła się istotnie w pomiarach wykonywanych nosowo od dołeczka we wszystkich pomiarach. Poddołkowa grubość naczyniówki zmniejszyła się istotnie ze 182.80 ± 91.43 μm do 164.0 ± 86.3 μm ($p>0.05$) tylko u chorych z poprawą ostrości wzroku powyżej 5 liter na tablicach Early Treatment Diabetic Retinopathy Study.

Wnioski: Poddołkowa grubość naczyniówki ulega redukcji u chorych na wysiękową postać zwyrodnienia plamki związanego z wiekiem po fazie nasycenia afliberceptem. W 12 miesiącu leczenia afliberceptem poddołkowa grubość naczyniówki zmniejszyła się tylko w grupie chorych z poprawą ostrości wzroku.

Słowa kluczowe: zwyrodnienie plamki związane z wiekiem postać wysiękowa, naczyniówka, grubość naczyniówki, aflibercept.

The authors declare no conflict of interest/ Autorzy zgłaszają brak konfliktu interesów w związku z publikowaną pracą

Introduction

Age-related macular degeneration (AMD) is one of the main causes of visual loss in developed countries among people aged 65 years or older. AMD is a significant epidemiological problem due to population aging (1). The pathogenesis of AMD is multifactorial. AMD is associated with changes in the morphological-functional complex composed of the retinal pigment epithelium (RPE), Bruch's membrane, and choriocapillaris. Blood flow disturbances in the choriocapillaris cause hypoxia and subsequent secretion of vascular endothelial growth factor (VEGF), which induces choroidal neovascularization (CNV) and wet AMD (wAMD). These changes lead to the degradation of

the retinal layer structure, retinal exudates, and bleeding from neovascularization lesions, which worsens central vision.

In the last two decades, repeated intravitreal injections of VEGF inhibitors have been used to treat patients with wAMD. These medications slow down the progression of AMD and decrease CNV activity. Aflibercept, one of the VEGF inhibitors, is a fusion protein, in which extracellular portions of the human VEGF receptors 1 and 2 are fused to the Fc fragment of human IgG1. Aflibercept has a high affinity to all forms of VEGF-A and VEGF-B as well as to placental growth factor (PlGF) (2). Chronic VEGF inhibition, which is the basis for wAMD therapy, affects not only the pathological neovascular membrane but also the chorioretinal complex. In wAMD, there are RPE changes that can be due to both anti-VEGF agents and spontaneous degeneration (3). Moreover, choroidal changes have been described in patients with wAMD during anti-VEGF treatment (4, 5). Because the choroid has an important place in the etiopathogenesis of wAMD, it has been examined with different methods, such as indocyanine angiography, ultrasonography, or histology. However, these methods are invasive (indocyanine angiography, histopathology) or have low repeatability (ultrasonography). In the last two decades, the development of optical coherence tomography (OCT) has enabled non-invasive and repeatable choroidal imaging *in vivo*.

No uniform method to assess choroidal thickness (CT) by OCT has been established, and there are differences in the interpretation of choroidal boundaries. Thus, it is difficult to compare the results from different studies (6, 7). Previous studies, primarily retrospective, have reported both a significantly reduced CT in patients with wAMD compared to healthy people (8, 9) and a CT reduction in patients on different treatments (4, 5, 8, 9). It seems that CT reduction, by limiting blood supply to the CNV, reduces disease activity and improves visual acuity in patients with wAMD. However, because the choroid is important for retinal nutrition, its excessive thinning may impair retinal function.

Aim

The aim of this 12-month, prospective, non-randomized study was to analyze CT in treatment-naive patients with wAMD treated in daily clinical practice with intravitreal injections of aflibercept. Visual acuity and central retinal thickness were assessed at the same time.

Materials and Methods

The study was approved by the Bioethics Committee of the Military Institute of Medicine (No. 73/WIM/2016) and was conducted in accordance with the Declaration of Helsinki. The observation was carried out at the Ophthalmology Clinic of the Warsaw Military Institute of Medicine between January 2016 and December 2017. The eligibility criteria included: 1. active CNV affecting over 50% of AMD lesions – confirmed by optical coherence tomography (OCT) and fluorescein angiography (FA) or by optical coherence tomography angiography (angio-OCT); 2. size of exudative lesions $<$ 12 times the size of the optic disc; 3. no dominant geographic atrophy or dominant hemorrhage. The exclusion criteria from the study were as follows: 1. hypersensitivity to aflibercept or to any of the excipients; 2. active ocular or periocular infection; 3. history of side effects associated with the drug, which prevented its further use; 4. rhegmatogenous retinal detachment or stage 3 or 4 macular holes; 5. corneal transparency loss or clouding of the lens that prevented OCT. 6. stroke or myocardial infraction in the last six months; 7. uncontrolled hypertension; 8. ischemic heart disease; and 9. uncontrolled diabetes.

The study included 36 patients (36 eyes), Caucasians, with untreated wAMD. The average age $\pm$ standard deviation (SD) was 78.3 $\pm$ 8.7 years. Most patients were women 19/36 (53%). At baseline, the mean best corrected visual acuity (BCVA) was 60.1 $\pm$ 14.6 letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) chart. The average spherical equivalent was 0.74 $\pm$ 2 D, and the mean axial length of the eyeball was 23.17 $\pm$ 0.86 mm.

At baseline, the patients underwent an ophthalmologic examination, which included the BCVA on the ETDRS chart, biomicroscopy of the anterior and posterior segment, and fluorescein angiography (Heidelberg Retinal Angiogram-2, HRA-2, Heidelberg Engineering GmBH, Dossenheim, Germany). The macula was evaluated using a Topcon DRI OCT Triton camera (Topcon, Inc., Tokyo, Japan) with a 1050-nm wavelength light source and a scanning speed of 100,000 A-scans/sec.

Intravitreal aflibercept (2 mg) was administered according to a schedule consistent with the VIEV protocol and product characteristics. The treatment was started with a loading phase, i.e. three monthly injections. Then, aflibercept was administered intravitreally every two months.

Follow-up evaluations were performed on the day of injection, and they included BCVA, front and posterior biomicroscopy, and macula evaluation by OCT with the follow-up function. CT was measured at fixed times between 9.00 and 13.00.

Method of choroidal and retinal thickness measurement

Radial OCT scans, 6 x 16 mm, passing through the fovea were performed. The fovea was defined as the deepest depression in the center of the macula on a horizontal section. Central retinal thickness (CRT) in the fovea (μm) was measured manually on the scans. CT (μm) was measured in the fovea (subfoveal choroidal thickness, SFCT), 750 μm nasally and temporally from the fovea (choroidal thickness 750 μm nasally, CT750N; choroidal thickness 750 μm temporally, CT750T), and 1500 μm nasally and temporally (choroidal thickness 1500 μm nasally, CT1500N; choroidal thickness 1500 μm temporally, CT1500T).

Manual measurements of the retina and choroid were performed by one researcher using the caliper function of the Topcon software. For subsequent measurements, the follow-up function was used, which allowed repeated assessments of choroidal and retinal parameters. CRT was defined as the distance between the inner limiting membrane and the external edge of the hyperreflective RPE in the fovea; CRT was measured perpendicularly to the RPE. CT was defined as the distance between the hyperreflective line that indicated the Bruch's membrane and the chorio-scleral interface (CSI); CT was measured perpendicularly to the Bruch's membrane. CSI was defined as the external edge of the choroidal stroma, because this approach improves repeatability of choroidal measurements (6). An example measurement is shown in Figure 1 (Fig. 1).

At baseline, the mean CRT was 337.7 $\pm$126.5 μm, and the average SFCT was 190.2 $\pm$ 97.7 μm.

ISSN 0023-2157 Index 362646

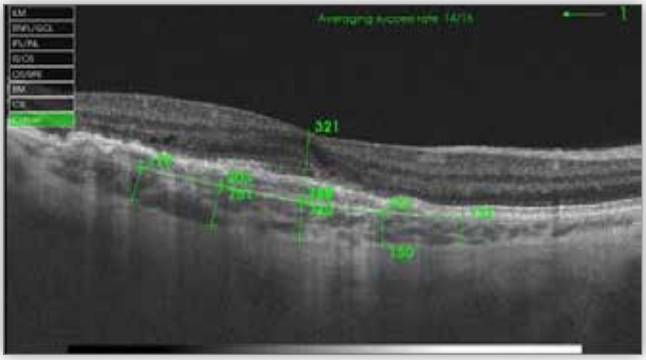

Fig. 1. Radial, horizontal optical coherence tomography B-scan passing through the fovea; CRT measured from ILM to the outer border of RPE at the center of the fovea; 321 μm. CT measured at five locations from the outer edge of the hyperreflective line corresponding to the RPE to the outer edge of choroidal stroma. SFCT – 207 μm, CT750N – 150 μm, CT1500N – 132 μm, CT750T – 203 μm, CT1500T – 170 μm.

Ryc. 1. Radialny, horyzontalny B-skan OCT przechodzący przez dołeczek; CRT mierzona od ILM do zewnętrznej granicy RPE w centrum dołeczka; 321 μm. CT mierzone w pięciu miejscach od zewnętrznej krawędzi hiperrefleksyjnej linii odpowiadającej RPE do zewnętrznej krawędzi zrębu naczyniówkowego. SFCT – 207 μm, CT750N – 150 μm, CT1500N – 132 μm, CT750T – 203 μm, CT1500T – 170 μm.

Statistical analysis

Statistical analysis was performed with the R 3.5.1 software (R Core Team (2018). For continuous variables with normal distribution, for which descriptive statistics are presented as means ± SD, p-values were calculated for the analysis of variance. For categorical variables, p-values were calculated for the chi-squared test. $P<0.05$ was considered statistically significant.

Results

There was no significant correlation between baseline SFCT, refraction ($R=0.247$, $p=0.244$), and intraocular pressure ($R=-0.613$, $p=0.101$). A significant correlation was found between SFCT, age ($R=-0.626$, $p<0.001$), and the axial length ($R=-0.340$, $p=0.043$).

The average follow-up time was 11.50±0.34 months. During this period, each patient received seven aflibercept injections. After the loading phase, the mean BCVA improved significantly to 64.8 ± 14.1 ETDRS letters ($p<0.05$). At 12 months, the mean BCVA was 68.6 ± 11.2 ETDRS letters ($p<0.05$). On average, patients improved by 8.5 ETDRS letters (Fig. 2).

After the loading phase, the mean CRT decreased significantly to 256.5±105.5 μm ($p<0.05$). At 12 months, the mean CRT was 272.1±123.1 μm ($p<0.05$; Fig. 3).

At baseline, the thickest mean CT was observed in the fovea (190.2 ± 97.7μm), and it decreased nasally and temporally. The thinnest CT was measured 1500 μm nasally from the fovea ($p<0.05$). There was a significant reduction in the mean SFCT (172.3±86 μm) after the second dose of aflibercept (the lowest value in the whole study) and after the third dose (loading phase; 177.4±90 μm, $p<0.05$). In the remaining months of follow-up, the reduction of SFCT was not statistically significant. At 12 months, SFCT was 178.3±101.9 μm ($p>0.05$), and it did not differ significantly from the value at baseline (Fig. 4).

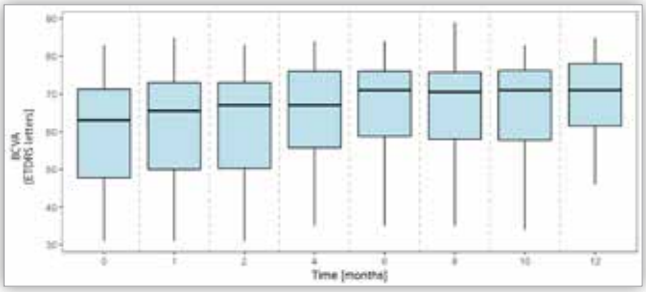

Fig. 2. Change in the average BCVA in the whole group. *BCVA – Best-corrected visual acuity. **t – Time. 0 – baseline BCVA on the day of the first injection. 1 –BCVA after 1 month, on the day of the second injection. 2 –BCVA after 2 months, on the day of the third injection. 4 –BCVA after 4 months, on the day of the fourth injection. 6 –BCVA after 6 months, on the day of the fifth injection. 8 – BCVA after 8 months, on the day of the sixth injection. 10 –BCVA after 10 months, on the day of the seventh injection. 12 – BCVA after 12 months of treatment.

Ryc. 2. Zmiana średniej BCVA w całej grupie. * BCVA – najlepsza skorygowana ostrość wzroku. ** t – czas. 0 – wyjściowa wartość BCVA w dniu pierwszej iniekcji. 1 –BCVA po 1 miesiącu, w dniu drugiej iniekcji. 2 –BCVA po 2 miesiącach, w dniu trzeciej iniekcji. 4 –BCVA po 4 miesiącach, w dniu czwartej iniekcji. 6 –BCVA po 6 miesiącach, w dniu piątej iniekcji. 8 – BCVA po 8 miesiącach, w dniu szóstej iniekcji. 10 –BCVA po 10 miesiącach, w dniu siódmej iniekcji. 12 – BCVA po 12 miesiącach leczenia.

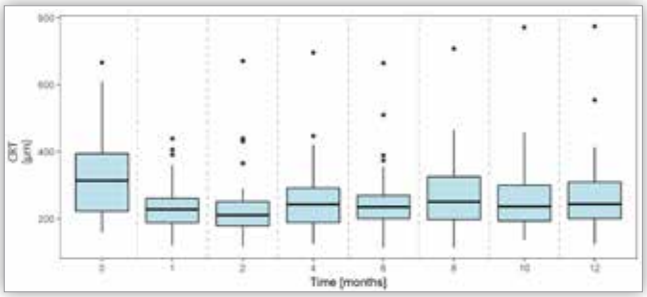

Fig. 3. Change in the average CRT in the whole group. *CRT – Central Retinal Thickness. **t – Time. 0 – baseline CRT, in the day of first injection. 1 –CRT after 1 month, on the day of the second injection. 2 – CRT after 2 months, on the day of the third injection. 4 –CRT after 4 months, on the day of the fourth injection. 6 – CRT after 6 months, on the day of the fifth injection. 8 – CRT after 8 months, on the day of the sixth injection. 10 –CRT after 10 months, on the day of the seventh injection. 12 – CRT after 12 months of treatment.

Ryc. 3. Zmiana średniej CRT w całej grupie. * CRT – centralna grubość siatkówki. ** t – czas. 0 – wyjściowa CRT, w dniu pierwszej iniekcji. 1 –CRT po 1 miesiącu, w dniu drugiej iniekcji. 2 – CRT po 2 miesiącach, w dniu trzeciej iniekcji. 4 CRT po 4 miesiącach, w dniu czwartej iniekcji. 6 – CRT po 6 miesiącach, w dniu piątej iniekcji. 8 – CRT po 8 miesiącach, w dniu szóstej iniekcji. 10 –CRT po 10 miesiącach, w dniu siódmej iniekcji. 12 – CRT po 12 miesiącach leczenia.

CT1500T did not change significantly in any month of follow-up ($p>0.05$). CT750T decreased significantly after the first injection (168±86.5 μm, $p<0.05$) and the last injection (168.7±84.7 μm, $p<0.05$). CT1500N decreased significantly at each time point ($p<0.05$), and at 12 months it was 132.8±82.8 μm ($p<0.05$). CT750N decreased significantly in each treatment month, except for month 8. At 12 months, CT750N was 165.1±85.4 μm ($p<0.05$; Tab. I, Fig. 5).

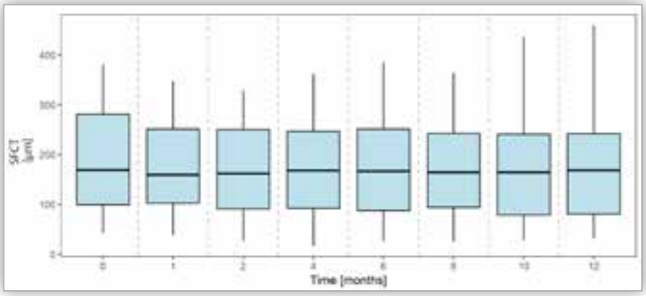

Fig. 4. Change in the average SFCT in whole group. *SFCT – Subfoveal Choroidal Thickness. **t – Time. 0 – baseline SFCT on the day of the first injection. 1 –SFCT after 1 month, on the day of the second injection. 2 – SFCT after 2 months, on the day of the third injection. 4 –SFCT after 4 months, on the day of the fourth injection. 6 – after 6 months, on the day of the fifth injection. 8 – SFCT after 8 months, on the day of the sixth injection 10 –SFCT after 10 months, on the day of the seventh injection. 12 – SFCT after 12 months of treatment.

Ryc. 4. Zmiana średniej SFCT w całej grupie. * SFCT – Poddołkowa grubość naczyniówki. ** t – czas. 0 – wyjściowy SFCT w dniu pierwszej iniekcji. 1 – SFCT po 1 miesiącu, w dniu drugiej iniekcji. 2 – SFCT po 2 miesiącach, w dniu trzeciej iniekcji. 4 SFCT po 4 miesiącach, w dniu czwartej iniekcji. 6 – po 6 miesiącach, w dniu piątej iniekcji. 8 – SFCT po 8 miesiącach, w dniu szóstej iniekcji 10 – SFCT po 10 miesiącach, w dniu siódmej iniekcji. 12 – SFCT po 12 miesiącach leczenia.

In further analyses, the patients were classified into three groups depending on the BCVA change at the end of follow-up. An improvement in BCVA, defined as an increase of > 5 ETDRS letters, was found in 21 patients (58%; at baseline, 55.8 ± 14.3; at 12 months, 70.3 ± 10.2 ETDRS letters). In this group, SFCT decreased significantly from 182.8 ± 91.43 μm to 164.0 ± 86.3 μm ($p<0.05$). BCVA stabilization, defined as any improvement or worsening by ≤ 5 ETDRS letters, was found in 13 patients (at baseline, 64.2 ±12.7; at 12 months, 63.5 ± 13.1 ETDRS letters). In this group, SFCT did not decrease significantly (at ba-

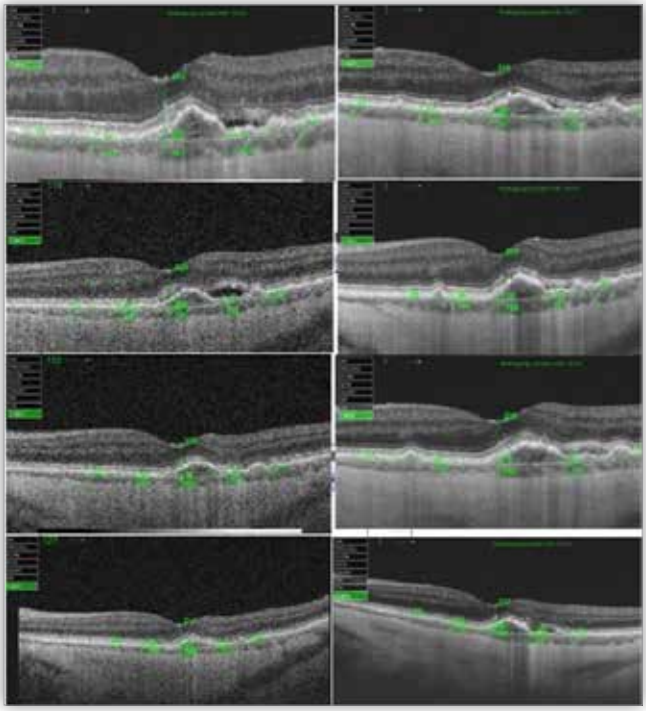

Fig. 5. Radial, horizontal optical coherence tomography B-scan series passing through the fovea of a 77-year-old female. Initial CRT – 223 μm. CRT after loading dose of aflibercept – 211 μm. CRT after 12-month follow-up – 235 μm. Initial SFCT – 76 μm. SFCT after loading dose of aflibercept – 69 μm. SFCT after 12-month follow-up – 74 μm. BCVA improved from 80 to 83 ETDRS letters.

Ryc. 5. Radialny, horyzontalny B-skan OCT przechodzący przez dołeczek 77-letniej kobiety. Początkowa CRT – 223 μm. CRT po fazie nasycenia afliberceptem – 211 μm. CRT po 12-miesięcznym okresie obserwacji – 235 μm. Początkowy SFCT – 76 μm. SFCT po fazie nasycenia afliberceptem – 69 μm. SFCT po 12-miesięcznym okresie obserwacji – 74 μm. U chorej uzyskano poprawę BCVA z 80 do 83 liter ETDRS.

Months	mSFCT	CT750N	CT1500N	CT750S	CT1500S	PANOVA
0	190.2 ± 97.7	178.1±101.2	158.0±92.6	179.6±92.1	172.2±85.1	0.036[1]
1	179.2 ± 90.9	164.1*±90.4	140.2*±88.6	168.0*±86.5	170.8±87.3	0.419
2	172.3* ±86.0	159.9*±86.7	147.4*±90.7	175.0±89.6	171.8±89.3	0.212
4	177.4* ± 90.0	161.2*±82.4	138.8*±79.3	174.2±82.4	173.2±82.5	0.173
6	180.4 ± 91.9	164.5*±93.0	141.1*±85.8	174.6±90.1	172.4±84.5	0.180
8	178.4 ± 90.5	167.6±96.9	139.5*±92.6	168.2±82.0	163.9±74.4	0.091
10	178.7 ± 102.2	158.8*±91.4	134.6*±90.5	173.1±88.9	172.6±80.5	0.044[2]
12	178.3 ± 101.9	165.1*±95.4	132.8*±82.8	168.7*±84.7	166.1±83.6	0.082

* Statistically significant difference in regard to 0 point
[1] statistically significant difference between mSFCT and CT1500N
[2] statistically significant difference between mSFCT and CT1500N
**CT – choroidal thickness, CT750N – CT 750 μm nasal from the fovea; CT750T – CT 750 μm temporal from the fovea, CT1500N – CT 1500 μm nasal from the fovea, CT1500T – CT 1500 μm temporal from the fovea.
***0 –baseline SFCT in the day of first injection. 1 –SFCT after 1 month, in the day of second injection. 2 – SFCT after 2 months, in the day of third injection 4 – SFCT after 4 months, in the day of fourth injection. 6 – SFCT after 6 months, in the day of fifth injection. 8 – SFCT after 8 months, in the day of sixth injection. 10 –SFCT after 10 months, in the day of seventh injection. 12 – SFCT after 12 months treatment./
* Statystycznie istotna różnica w odniesieniu do punktu 0
[1] statystycznie istotna różnica między mSFCT i CT1500N
[2] statystycznie istotna różnica między mSFCT i CT1500N
** CT – grubość naczyniówki, CT750N – CT 750 μm nosowo od dołeczka; CT750T – CT 750 μm skroniowo od dołeczka, CT1500N – CT 1500 μm nosowo od dołeczka, CT1500T – CT 1500 μm skroniowo od dołeczka.
*** 0 – wyjściowa SFCT w dniu pierwszej iniekcji. 1 –SFCT po 1 miesiącu, w dniu drugiej iniekcji. 2 – SFCT po 2 miesiącach, w dniu trzeciej iniekcji 4 – SFCT po 4 miesiącach, w dniu czwartej iniekcji 6 – SFCT po 6 miesiącach, w dniu piątej iniekcji. 8 – SFCT po 8 miesiącach, w dniu szóstej iniekcji. 10 –SFCT po 10 miesiącach, w dniu siódmej iniekcji. 12 – SFCT po 12 miesiącach leczenia.

Tab. I. CT changes during 12 months observation in 5 measuring points.

Tab. I. Zmiany CT w 12 miesięcznej obserwacji w 5 punktach pomiarowych.

ISSN 0023-2157 Index 362646

seline, 186.5 ± 109.9 μm; at 12 months, 176.9 ± 114.6 μm; p>0.05). BCVA worsening, defined as a loss of ≥ 5 ETDRS letters, was found in two patients (at baseline, 79 ± 5.7; at 12 months, 70 ± 7.1 ETDRS letters). In these patients, SFCT did not increase significantly (at baseline, 291.5 ± 10.6 μm; at 12 months, 337.5 ± 47.4 μm, p>0.05).

Discussion

Patients with wAMD have lower CT values than those observed in healthy people. On average, SFCT ranges from 203.6±105.9 μm to 264.5±109.8 μm in healthy adults (4, 5, 8). Reduced CT could be due to degenerative choroid changes, which decrease blood flow, particularly in the choriocapillaris. In patients with AMD, the expression of nitric oxide synthase isoforms is reduced in the retina and choroid, which suggests a decreased production of nitric oxide, which likely leads to vasospasm and hypoxia (10). In our study, the average SFCT at baseline was 190.2±97.7 μm, lower than in similar studies, which could be due to the advanced age of patients (78.3±8.7 years). It has been demonstrated that CT decreases, on average, by 4 μm per year in people aged 65 years or older (11). In our study, patients were older than in the study by Kang et al., in which the average SFCT was 216.7±100.4 μm and the average age was 72.1±8.1. Koizumi et al. reported an average SFCT of 264.5±109.8 μm in patents with an average age of 73.6±9.1 years (4, 12). Wei et al. described a relationship between CT and the axial length, refraction, lens thickness, and anterior chamber depth (11). In our study, there was no significant correlation between baseline SFCT and refraction or intraocular pressure. A significant correlation was found between SFCT, the axial length, and age. Kang et al. did not find any significant correlation between CT and age (12).

In our study, additional choroidal measurements (CT750N, CT1500N, CT750T, CT1500T) allowed for a more accurate assessment. A significant difference was found between the baseline CT values at individual measurement sites. SFCT and CT measured 1500 um from the fovea differed significantly at baseline and at month 10; this difference was not significant at the remaining time points. In healthy people, the shape of the choroid-scleral border is regular. Brachini et al. described it as "bowl shaped", with the highest CT values in the fovea (13). The curve showing CT values was steeper in the nasal part. In the diseases of the posterior pole, CT has an irregular distribution. In patients with severe myopia, the greatest CT was described temporally from the fovea (14).

Because we used a comprehensive method to measure CT, we could thoroughly examine the effect of aflibercept therapy on CT. Nasally from the fovea, CT was significantly reduced in nearly every treatment month. Temporally from the fovea (750 μm), a significant reduction was noticed only in the first and last months. Among patients with wAMD, who received aflibercept for over three months, Mazaraki et al. reported a reduction in subfoveal CT and in CT measured 300 μm and 2500 μm nasally and temporally from the fovea. Among untreated patients, the greatest CT reduction was found 300 μm nasally from the fovea (5). The cause of a greater CT reduction in the nasal part of the macula is not known. Smaller CT changes in the temporal part of the macula may be due the anatomical structure of the eye, e.g. the presence of the inferior oblique muscle attachment in the lower, posterior, and temporal quadrant at the level of the macula (15).

In our observation, the average CRT decreased significantly after the first dose of aflibercept, and it was maintained reduced in each month of follow-up. Previous studies reported a different pattern of CRT changes. However, previous studies on CT were mainly short-term (3, 6 months) (5, 16) and rarely had a follow-up of 12 months (4, 17). Moreover, few studies evaluated the effect of intravitreal aflibercept on CT (4, 17). In our study, SFCT decreased significantly after the loading phase of aflibercept treatment (by 12.8 μm, 6.7%, p<0.05). Similar CT changes were shown by Ono et al. and Koizumi et al. (16, 17). In our study, the SFCT change at 12 months of treatment was not significant (p>0.05). This result contrasts with other reports among similar groups of patients treated with aflibercept. Koizumi et al., in a retrospective one-year study of 58 previously untreated patients with wAMD, found a significant reduction of SFCT from 264.5±109.8 μm to 228.7±98.5 μm (13%, p<0.05) (4). Similarly, Ono et al., in a prospective study of 48 patients, reported a significant reduction of SFCT (16% after one year) (17). In both studies, baseline SFCTs were higher than in our study (Koizumi et al., 268.1±101.3 μm; Ono et al., 247.9 ±96.7 μm). In addition, in the study by Ono el al., only 11 of 48 patients (22.9%) were diagnosed with wAMD. The remaining patients had wAMD phenotypes: Polypoidal Choroidal Vasculopathy (PCV) (64.6%) and Retinal Angiomatous Proliferation (RAP) (12.5%). Our group of patients was homogeneous, because all patients had wAMD. The lack of a statistically significant change in SFCT at the end of the study can be explained by a low but significant difference in SFCT after the loading phase. This trend did not persist until the end of follow-up. In addition, previous studies suggested that changes in SFCT of ≥ 50 μm in healthy people and of ≥ 70 μm in people with macular exudates may be considered clinically significant (7). CT changes were also observed in patients with wAMD who received ranibizumab. Yamazaki et al. reported a significant SFCT reduction in a one--year study among 23 patients with wAMD, who received, on average, 5.8±2.9 injections of ranibizumab. In that study, SFCT decreased from 235±65 to 215±73 μm after the loading phase (p<0.001), and it decreased by 7% at the end of follow-up compared to baseline (p=0.002) (18). On the other hand, insignificant reduction of SFCT (1.4% during nine months of ranibizumab treatment) was observed by Ellabban et al. (19). Previous reports suggest that intravitreal ranibizumab may have a smaller effect on the choroid than does aflibercept, which could be because aflibercept has a larger spectrum of activity, greater ability to bind to VEGF, and a longer duration of action compared to ranibizumab. In the study by Jung et al., an average SFCT reduction over three months was significantly higher in the aflibercept group than in the ranibizumab group (23 vs. 31 patients; -35 ± 34 μm vs. -9 ± 22 μm; p=0.013), but the reduction was statistically significant in both groups (20). Fein et al., in a retrospective study, showed an SFCT reduction over six months in patients with wAMD on different therapies, including bevacizumab, pegaptanib, triamcinolone, and photodynamic therapy (9).

In our study, a significant reduction in SFCT was accompanied by improved visual acuity (> 5 ETDRS letters). In a one-year aflibercept study, Koizumi et al. did not show any

correlation between SFCT reduction and BCVA improvement in patients with wAMD. However, SFCT reduction correlated with BCVA improvement in patients with PCV ($p=0.008$). In addition, those investigators showed that baseline SFCT did not predict VA improvement (4). Similar analyses were performed in patients with wAMD treated with ranibizumab. Kang et al. showed that baseline SFCT may be predictive of VA improvement. In patients with a morphological improvement (reduction in thickness of the subretinal fluid layer by 100 μm or retraction), SFCT was greater at the beginning of treatment and at months 3 and 6 than in patients without the morphological improvement (12).

The strong affinity of aflibercept to the growth factors carries a potential risk of vascular-retinal atrophy (3). In this study, we observed a significant SFCT reduction among patients with BCVA improvement of $\geq$ 5 letters. This finding indicates that aflibercept therapy in wAMD enables a sufficient blood flow that maintains the nutrition and oxygen supply to ensure proper retinal function, although aflibercept reduces blood flow through CNV, which decreases CNV activity.

Limitations

The study has several limitations. The examinations were performed by one experienced researcher. Although there are no significant differences in measurements when they are taken by experienced investigators, measurements made by one person may be less reliable (4, 9). The measurements were performed manually with a caliper. Examinations are time-consuming and more attention is paid to the use of automatic binarization methods to better contrast and automatically separate individual retinal layers. However, subretinal fluid and degradation of the retinal structure that occurs in wAMD could impair the repeatability of automatic measurements. CT is subject to daily variations of up to several dozen micrometers. In this study, measurements were made at fixed times of the day to minimize this variability, but such variability cannot be ruled out. Moreover, we did not assess whether CT depended on disease activity, and a previous short-term study reported that a significant reduction in SFCT was associated with the reduction in disease activity.

In our study, one patient had diabetes, but the disease was well controlled (HbA1c $<$7.5%). Five patients were treated because of hypertension, but it was well controlled in all of them. Multivariate analysis performed by Wei et al. did not show any relationship between SFCT and blood pressure (11).

The current study should be continued in a larger group. Patients enrolled in this study remain on treatment and further follow-up.

Conclusions

Subfoveal choroidal thickness was reduced in patients with neovascular age-related macular degeneration after the loading phase of aflibercept. At 12 months, aflibercept treatment reduced subfoveal choroidal thickness only among patients with an improvement of visual acuity. In patients with wAMD treated with aflibercept, blood flow is reduced in the neovascular membrane, but it enables adequate nutrition and function of the retina.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: own statutory project [NO 0000000468].

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ISSN 0023-2157 Index 362646

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The paper was originally received 22.08.2019 (KO-00212-2019)/
Praca wpłynęła do Redakcji 22.08.2019 (KO-00212-2019)
Accepted for publication 12.09.2019/
Zakwalifikowano do druku 12.09.2019

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