SUBRETINAL HYPERREFLECTIVE MATERIAL IN WET AGE-RELATED MACULAR DEGENERATION AS MONITORING BIOMARKER FOR DISEASE ACTIVITY AND THE RESPONSE TO ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR THERAPY

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Abstract

Background and Objective: Hyper-reflective material can be considered as a surrogate Optical Coherence Tomography (OCT) biomarker in predicting disease activity and final visual outcome in patients with neovascular age-related macular degeneration (nARMD). The aim of this study was to assess the relationship between subretinal hyperreflective material (SHRM) morphological features, volume, response to anti-vascular endothelial growth factor (VEGF) therapy for choroidal neovascularization in wet age-related macular degeneration (AMD) and the best corrected visual acuity (BCVA).

Patients and Methods: The study is a prospective cohort. The study included 80 eyes with diagnosed wet form of AMD. The conducted study lasted for 1 year. 78 of the patients had finished the treatment regime. They were all previously untreated. A complete basic ophthalmological examination of both eyes was performed: BCVA was determined for the patient, intraocular pressure was measured according to the air puff method (non-contact air tonometry) and fundus examination. The diagnosis was confirmed using the non-invasive imaging method of OCT, DRI OCT Triton, Swept Source OCT device. OCT images and non-contrast angiography were also done in the region of the macula lutea of the retina. For measuring the SHRM weigh and high manual segmentation was performed.

Results: SHRM at the entry of our study is a present finding in 47 respondents (58%). The mean value of visual acuity in the patients in our study ranged from 3.95 at week 0 to 0.9 at week 52 from the start of treatment. We had foveal localization at the beginning in 13 eyes, parafoveal in 12 eyes and perifoveal in 2, while in 20 eyes it was absent as an initial parameter. At the 6th month, the location of SHRM was foveal in 29 eyes, parafoveal in 8 eyes and perifoveal in 4 eyes, in 47 the finding was absent. In month 12, SHRM was foveal in 28 eyes, parafoveal in 18 and perifoveal in 4 eyes, absent in 28. In 6th month the mean BCVA was 6.90, which was up to 2.95 lines higher than the base line. In 12 months, 63/78 (80%) respondents did not have SHRM, and 16 (20%) of the respondents had. The mean final best corrected visual acuity in month 12 was 0.9. In 12 months in 61, (78%) of the respondents there was no SHRM border, in 4 (5%) there was, and it was unclear in 12 (16%) of the respondents.

Conclusion: SHRM presence and reflectivity at base line, which correlated with the BCVA as one of the newest retinal biomarkers in wet AMD forms, carries important

information about the choroidal neovascularization (CNV) activity. SHRM reflectivity may be very useful for monitoring disease activity as well as important criteria for patient retreatment.

Keywords: *anti-vascular endothelial growth factor therapy; biomarker; macular degeneration; subretinal hyperreflective material.*

Introduction

Age related macular degeneration (AMD) is the leading cause for severe and irreversible visual loss in patients aged 50 years and older in developed countries (1). Wet AMD changes progress rapidly if left untreated leading towards severe visual impairment and irreversible visual loss at the end (1). In the pathogenesis of the wet form of AMD, we have the creation of new blood vessels from the existing blood vessels that reach the subretinal space through Bruch's membrane and the retinal pigment epithelium. Newly growing blood vessels with discontinuous integrity release fluid, at first a transudate, and then an exudate that accumulates under the retinal pigment epithelium (RPE), and the neurosensory retina and is responsible for the disruption of the RPE and photoreceptor cells, with the ultimate consequence - damage to central vision. Classically, the abnormal blood vessels in wet AMD arise from the choroidal or choriocapillaris circulation. It is also one of the reasons why they have a pronounced fenestration and a large transudative potential, similar to small blood vessels of the choriocapillaris (2). Choroidal neovascularization (CNV) can be visualized with fluorescein angiography (FA). Although it is an imaging method that visualizes leakage from the vessels, the advent of spectral-domain optical coherence tomography (SD-OCT) allows more accurate visualization of all retinal layers (3). The two imaging methods provide different but complementary information for all retinal vascular disorders.

The hyper-reflective material (HRM) is retinal diagnostic monitoring biomarker for CNV in nAMD changes. The association of HRM with omission in active CNVs especially with a subretinal CNV network has been established (4). Additional studies show the presence of the so-called undefined HRM, ("gray" hyper-reflective material or subretinal hyper-reflective exudation) in untreated forms of nAMD (5). SHRM on OCT is defined as presence of hyperreflective deposition material located in the subretinal space and has been correlated with classic CNV on FA (6). Although SHRM typically correspondents to fibrovascular tissue as in CNV type 2, it can also be included fibrin, blood, lipid, fluid or scar as components that can change in time under anti-VEGF treatment (7). The presence of subretinal hyper-reflective material (SHRM) is of increasing relevance as new biomarker (8). Recent studies have indicated that the presence of this so called subretinal tissue may have stronger implications on visual acuity than the other retinal parameters, and thus may provide additional prognostic disease information (9). However, the

anatomical response to anti-VEGF treatment and the functional outcomes can vary markedly among patients with n-AMD (10).

The introduction of anti-VEGF treatment in patients with retinal neovascularization little over a decade ago was the first revolutionary leap in treating patients with neovascular AMD and thus preserving their vision acuity (11). The same has reduced the incidence of legal blindness by more than 50% (12). It has been found that SHRM lesion size and location correlates with VA, and SHRM decreases in size with anti-VEGF therapy. The morphological features of SHRM have been studied previously, and it is important to characterize SHRM morphologic features which will enable the treating physician to tailor treatment to provide adequate disease control, minimize recurrence and neurosensory damage, and limit the number of invasive and costly anti-VEGF injections (13).

Our purpose was to assess the response of SHRM on anti-VEGF therapy by monitoring its volume in correlation to visual acuity.

Material and Methods

The conducted study is a prospective cohort. All patients were treated and monitored over a period of one year. The study included 80 eyes that had established, diagnosed with wet AMD. All patients were previously untreated, newly diagnosed with nAMD, with present, developed CNV and reduced visual acuity, best-corrected visual acuity >20/200 according to Snellen optotype. When the patients were included in the study, at the beginning, a complete basic ophthalmological examination of both eyes was performed: the best corrected visual acuity (BCVA) was determined for the patient, intraocular pressure was measured according to the air puff method (non-contact air tonometry) and fundus examination. Imaging and monitoring of fundus changes was performed exclusively on the DRI OCT Triton, Swept Source Optical Coherence Tomography (OCT) device. OCT images and non-contrast angiography were done in both eyes in the macula lutea zone. Subretinal hyperreflective material was visualized and it was measured at the beginning of the treatment and during the treatment on the control period time during the study on week 12, 28 and 52. This was compared to patients' best corrected visual acuity. Patients were treated with the drug aflibercept, which is an inhibitor of vascular endothelial and placental growth factor. The drug was administrated by the so-called regime of treat and extend (T&E regime). All patients received three initial doses of 2mg aflibercept and the 4^{th} dose on the 16^{th} week. Also at this visit, OCTA was performed and BCVA determined and based on the finding (disease activity or inactivity), a further dosing regimen tailored to the needs of each patient was established with further follow-ups. If the activity of the disease was determined, it was continued with the 5^{th} application of the preparation at an interval of 8 weeks. If inactivity of the disease was determined, the application interval was extended for another 2 or 4 more weeks (14). The drug was administrated into the eye vitreous cavity after topical anesthesia in clean and sterile conditions. For statistical processing of the data obtained during the research, a database was created in the statistical program SPSS 21.0. Categorical variables were presented with absolute and relative numbers, and

descriptive statistics (mean, standard deviation) were used to describe quantitative variables. The student t-test was used to compare the analyzed variables between the studied and control groups. Paired sample test, Fisher exact test was used for comparison in 0, 12, 28 and 52 weeks. The x2 test and the McNeamar test were used to examine categorical variables. Values of $p<0.05$ were taken as statistically significant.

Results and Discussion

SHRM at the entry of our study was a present finding in 47 respondents (58%). The mean value of visual acuity in the patients in our study ranged from 3.95 at week 0 to 0.9 at week 52nd from the start of treatment. We had foveal localization at the beginning in 13 eyes, parafoveal in 12 eyes and perifoveal in 2, while in 20 it was absent as an initial parameter. At the 6th month, the location of SHRM was foveal in 29 eyes, parafoveal in 8 eyes and perifoveal in 4 eyes, in 47 the finding was absent. In month 12, SHRM was foveal in 28 eyes, parafoveal in 18 and perifoveal in 4 eyes, absent in 28. When comparing localization with visual acuity, we obtained an increase in visual acuity at the 6th month of treatment compared to 0 week. On this section we had a drop in SHRM height below 175µm and width below 1500µm which was in favor of the obtained higher visual acuity values. In month 6, the mean BCVA was 6.90, that is up to 2.95 lines higher than the base line. At the end of the study, as a result of the progressive decrease in height and width, we also obtained a positive correlation with an increase in mean visual acuity. There was also an increase in the number of eyes where we obtained complete resorption of the deposit material under the retina in 6 and 12 months. In month 12, mean visual acuity was 0.9.

Table 1. Better 12 SHRM0 present

Cross table			
Number			
		SHRM0 presence	
		No	Yes
Better12	No	11	20
	Yes	21	26
Total		32	46

SHRM at the entry of our study was a present finding in 47 respondents (58%).

Table 2. Better12 SHRM0 limitation

Cross table				
Number				
		SHRM0 limitation		
		none	present	unclear
Better12	No	11	17	3
	Yes	22	23	2

Total	33	40	5

There was no SHRM border in 33 (42%) of the patients, 40 (51%) had a border, and 5 (7%) had an unclear border.

Table 3. Better12 SHRM0 high

Cross table				
Number				
		SHRM0high		
		negative	< 175µm	175µm
Better12	No	11	9	11
	Yes	21	13	13
Total		32	22	24

Table 4. Better12 SHRM0 width

Cross table				
Number				
		SHRM0 width		
		negative	< 1500µm	>1500µm
Better12	No	12	11	8
	Yes	21	13	13
Total		33	24	21

Table 5. Better12 SHRM0 reflectivity

Cross table					
Number					
		SHRM0 reflectivity			
		none	Isodense RNFL	Isodense ONL	In between
Better12	No	12	4	13	2
	Yes	20	7	18	2
Total		32	11	31	4

Table 6. Better52 SHRM52 present

Cross table			
Number			
		SHRM52 present	
		No	Yes
Better52	No	10	6
	Yes	53	9
Total		63	15

At 12 months, 63/78 (80%) respondents did not have SHRM, and 16 (20%) of the respondents had.

Table 7. Better52 SHRM52 limitation

Cross table				
Number				
		SHRM52 limitation		
		none	present	unclear
Better52	No	10	0	6
	Yes	51	4	7
Total		61	4	13

In 61, (78%) of the respondents there was no SHRM border, in 4 (5%) there was, and it was unclear in 12 (16%) of the respondents.

Table 8. Better52 SHRM52 high

Cross table				
Number				
		SHRM52 high		
		none	< 175µm	175µm
Better52	No	10	6	0
	Yes	52	9	1
Total		62	15	1

Table 9. Better52 SHRM52 width

Cross table				
Number				
		SHRM52 width		
		none	<1500µm	1500µm
Better52	No	10	6	0
	Yes	52	9	1
Total		62	15	1

Table 10. Better52 SHRM52 reflectivity

Cross table					
Number					
		SHRM52 reflectivity			
		none	Isodense RNFL	Isodense ONL	In between
Better52	No	10	0	0	6
	Yes	46	1	3	12
Total		56	1	3	18

At 12 months from the start of the study, 56/78 or 71% of patients had no greater reflectivity, it was isodense at the RNFL in 1 (1%), isodense at the ONL in 3 (4%) and between the RNFL and ONL in 18 (23%) of the patients.

During the treatment over a period of 2 years in Comparison of Age-Related Macular Degeneration Treatments Trials (CATT), a reduction of subretinal material of up to 45% percent was determined. In another study SHRM was classified as a negative parameter for visual acuity where if the diameter of the expulsion is >1000 μm and involves the foveal center, studies show a difference of 14 letters seen in patients with and without SHRM (15).

In our subjects, the reduction or complete resorption of the drusenoid material after the 3 shock, initial doses of the anti-VEGF preparation was shown to be a positive parameter in correlation with the increase in VA, while further in the 6^{th} and 12^{th} month the presence of existing, persistent SHRM did not produce a statistically significant finding in terms of BCVA change. This finding of marked reduction in the undefined component of HRM by month 3 suggests that the subset of HRM that is diffuse and located in the subretinal space, is the result of an inflammatory reaction in early n-AMD. Regarding visual acuity, a statistically significant association was obtained between the thickness of the subretinal hyperreflective material and its reduction. Studies have found that eyes with undefined HRM at month 12 had the poorest vision which suggests a reactivation of the CNV complex and supports the recommendation to consider undefined HRM as a qualitative criterion for retreatment (16).

In another study, the subretinal deposit material is defined as a predictive parameter for the so-called "non-responders". This study has described the presence of subretinal hyperreflective material in patients with nAMD (17). Other features of HRM such as limitation, organization of SHRM seen on the OCT tomogram before the start of treatment, high reflectivity, zone of separation between HRM and outer retina, layered appearance, subretinal hyperreflective foci, thickness and width of HRM have been shown to correlate with poorer visual acuity outcome (18). In our study, we analyzed the limitation, the reflectivity of the deposited hyperreflective material, where at the beginning before the treatment we had an unclear limitation in 31 eyes (47%), while in 47 (60%) it was with a clear limitation. The reflectivity in week 0 was unclear in 31 eyes (39%), while we had increased reflectivity in the remaining 47 (60%). After treatment, we obtained SHRM resorption in 61 patients (78%), in 4 eyes (5%) there was a clear limitation, while in 13 eyes (17%) it remained unclearly limited. In week 52, we have resolution of the initial clearly defined HRM in 56 eyes (71%) while the remaining 22 (29%) have clearly defined hyper-reflective material. SHRM as an initial finding according to the study by CATT and associates is associated with scar development and significant decline in visual acuity (19). Recurrence of scarring or atrophy is associated with persistent findings of drusenoid material during treatment. Bloch found a drop of 10 letters according to the ETDRS. El-Emam provided data on the significance of the location, i.e. the distance from the foveal center and the edge of the scar. SHRM as an initial finding, according to the study by CATT and associates, is associated with scar development and a significant decline in

visual acuity. In our study the presence of SHRM was associated with worse VA, at all sites, regardless of height or width and when compared to a control group without a finding of HRM. In subgroup analysis, foveal SHRM involvement had worse visual acuity at baseline, 3, 6 and 12 months compared to eyes with absence of SHRM in the central 1mm2 of the fovea. There was a significant correlation between VA and SHRM such as height, width and area. The lowest, best-corrected visual acuity was obtained at baseline before starting treatment. SHRM was located in the fovea with an area greater than 0.24mm2 compared to SHRM outside the fovea region (20). VA was shown to be worse when SHRM included the fovea, and the baseline width was more than 1500 um compared to SHRM outside the fovea. When a foveal area involving SHRM greater than 175 ums in height was present, the baseline BCVA obtained was worse compared to SHRM outside the fovea. In our study, the disappearance of SHRM from the beginning to the 3rd month correlated with a better VA, but it did not show a statistically significant improvement at 6 and 12 months after the start of treatment. SHRM has been found to be a statistically more common finding in Type 2 and Type 3 choroidal neovascular lesions (21).

Conclusion

VA was shown to be worse when SHRM included the fovea, and the baseline width was more than 1500 um compared to SHRM localized outside the fovea. Initially vaguely circumscribed SHRM gave a better response to anti-VEGF treatment and visual acuity in 52 weeks of treatment compared to initially clearly circumscribed and increased reflectivity SHRM. In terms of visual acuity, a statistically significant correlation was obtained between the thickness of the subretinal hyperreflective material and its reduction. The unclearly defined SHRM had greater therapy response during the three initial doses of anti-VEGF. The fluctuations in its width and high have shown to be in correlation with disease activity and a biomarker for patient's retreatment. SHRM definitely can be used to better assess choroidal neovascular membrane activity which will promptly and correctly direct the treatment in patients with wet AMD and preserve satisfactory visual acuity necessary to perform daily activities.

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