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APPLICATION OF OCT-ANGIOGRAPHY IN PATIENTS WITH AGE-RELATED MACULAR DEGENERATION AND CHOROIDAL NEOVASCULARIZATION

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The dissertation work has been examined, discussed and directed for public defense to an extended departmental council at the Department of Ophthalmology, ENT, LCH with HS at Medical University - Pleven.

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The public defense of the dissertation work will take place on September 17, 2024 at 13:00. in the "Parvum" hall - Second Clinical Base.

The materials on public defence are available on the website of the Medical University - Pleven, as well as in the secretariat of the same.

Abbreviations used

AMD - Age-related macular degeneration

Anti- VEGF – anti- Vascular Endothelial Growth Factor

OCT - Optical Coherence Tomography

OCT-A - Optical coherence tomography - angiography

RPE – Retinal Pigment Epithelium

FA - Fluorescein angiography

FTD - Photodynamic therapy

GA – Geographic atrophy

HNV – Choroidal neovascularization

FAZ – foveolar avascular zone

MMPz – Matrix metalloproteinases

The numbering of the figures in the abstract does not correspond to that in the dissertation.

CONTENTS

IN INTRODUCTION

Age-related macular degeneration is a disease that is gaining more and more social importance due to the aging pyramid of the population, especially on the Old Continent, and also in our country. In its late stages, the disease is highly debilitating, disrupting patients' quality of life and their ability to fully care for themselves.

The neovascular form of AMD affects many layers of the outer and inner retina, the RPE and the choroid. Multimodal imaging techniques (FA, indocyanine angiography, OCT, OCT-angiography) are used in the diagnosis and follow-up of patients. Due to the possibilities of segmentation and microcirculation analysis at different levels, OCT-A has become an indispensable part of the diagnosis and follow-up of patients with AMD. OCT-A improves the ability to identify neovessels and complements structural OCT, FA and indocyanine angiography. The non-invasiveness, short examination time and high informativeness of OCT-angiography in relation to AMD make it possible to identify the individual morphological types of neovascular membranes to serve as prognostic markers for the development and course of the disease. The discovered regularities for remodeling of the vascular wall could serve as a basis for developing new therapeutic schemes and individualizing the approach to the "naive" type of NV membrane at the time of diagnosis of neovascular AMD.

PURPOSE AND OBJECTIVES

The aim of the present work is to study the possibilities of OCT-A in the diagnosis and monitoring of the treatment effect of patients with AMD and choroidal neovascularization.

Task 1: Defining the type of neovascular membrane, according to OCT-A images and proposed by *Coscas et al* classification

Task 2: Application of anti- VEGF intravitreal therapy in patients with AMD and CNV, determined by the type of OCT-A. Assessment of neovascular membrane type change.

Task 3: Defining the OCT-A markers of progression and activity of the neovascular membrane

Task 4 : Effect of ongoing anti- VEGF therapy on membrane activity and OCT-A markers of progression and activity

Task 5 : Assessment of the impact of vascular remodeling after anti-VEGF therapy on visual acuity.

Task 6 : Compilation of a therapeutic protocol

MATERIALS AND METHODS

The medical facilities that served as a base for collecting materials are:

- SHAOD "Zrenie" - Sofia
- Society d'experience liberal des ophthalmologists Barathon - Montluçon , France
- Clinic for eye diseases UHAT "Dr. Georgi Stranski" - Pleven

As the main group for the study of the set goals and objectives, all cases of newly diagnosed neovascular AMD were identified.

The study was retrospective and included 119 patients, 61 of which were cases from France.

Table 1: Demographics of the Followed Cohort:

Years	Men	Women	Total
Middle-aged	76	76	76
SD	9.65	9.46	9.61
Median	77	77	77
Scope	53	66	119

The collection of information covers the period November 2018 - December 2020. The study was carried out in three centers in two European countries, three cities. The algorithm for making a diagnosis, evaluating patients and determining therapeutic behavior is fully consistent with the adopted model of work in France.

The patients included in the cohort were newly diagnosed cases of AMD. All underwent a full ophthalmological examination at their first visit, including:

- Detailed history - family history, risk factors, systemic diseases;
- Determination of near and far visual acuity, tonometry, biomicroscopy, indirect ophthalmoscopy, fluorescein angiography (the so-called " gold standard "), structural OCT and OCT-angiography.

The object of main interest of the present scientific work is the vascular remodeling after anti- VEGF therapy, evaluated by OCT-angiography. In the study, we used 3 of the devices available on the market, from leading companies in the field of optical coherence tomography:

- Heidelberg Engineering SPECTRALIS OCT-A
- Zeiss AngioPlex OCT/Cirrus HD-OCT 5000
- Nidek RS-3000 Advance 2

After performing all the above-described examinations and confirming the diagnosis, a detailed examination of the OCT-angiographic images in their three different depths is carried out. The type of neovascular membrane was determined as described above and presented by *Coscas et al* classification.

The cohort of 119 patients was divided into several groups based on the morphological appearance of the neovascular membrane from the OCT-A image:

Patients with:

1. CNV-membrane type "Coral" - with an eccentric feeding vessel, massive trunks with thin capillary ramifications

2. Medusa-type CV-membrane - has a massive feeding vessel with centrifugal vascular trunks with thin capillaries
3. "Dead tree" type CV-membrane - has a massive main trunk and ramifications varying in size and caliber
4. CV-membrane type "Lace" - highly anastomosing vascular network, without a main vessel
5. CV-membrane type "Filaments" - composed of many intertwining, thread-like vessels

6. Indeterminate CNV-membrane type - whose OCT-A view does not correspond to any of the above types

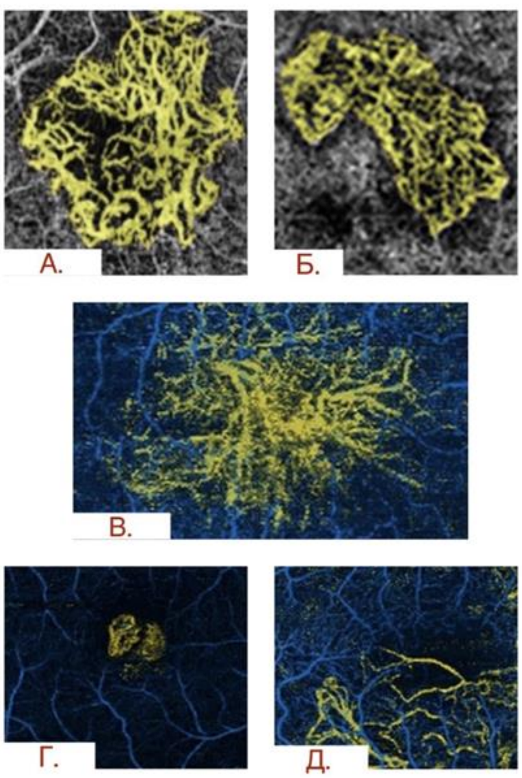


Fig. 3 : *OCT-angiography, own archive, Eye Clinic " Vision" ; Nidek OCT-A RS-3000 Advance. A.) CNV type "Coral"; B.) CNV type "Medusa"; C.) CNV type "Dead tree" ; D.) CNV type " Lace"; E.) CNV type "Filaments"*

After a detailed evaluation, the patient is offered treatment with an intravitreal application of an anti-VEGF preparation . All patients included in the present study were treated with Eylea (Bayer).

VEGF injection procedure is performed in an operating room under sterile conditions. Control, including determination of visual acuity, tonometry, biomicroscopy, ophthalmoscopy, structural OCT and OCT-angiography, is performed on the 25th day after the injection. According to the therapeutic protocol adopted for work in the present study, the next injection should be received at an interval of one month from the first, and the patient is given three "loading" doses with an interval of one month from each other (" loading" doses).

Statistical analysis:

With the aim of easier and better tracking, the collected data are summarized and formatted in a table in Microsoft Excel. Each individual patient is entered in a new row, and the indicators of morphological appearance of the naïve CNV-membrane, morphological appearance of the CNV membrane after three intravitreal applications of anti- VEGF , assessment of progression marks and visual acuity before and after therapy, are entered in columns. Statistical analysis was performed using the SPSS software package, 13.0 (SPSS Inc., Chicago IL). All values with $P<0.05$ were accepted as statistically significant. The chosen critical level of significance is $\alpha=0.05$. The corresponding null hypothesis is rejected when the P-value is less than α. Microsoft Excel and Microsoft Word programs *were used to maintain* tables and graphs.

RESULTS

Demographic Outcomes – Pre-treatment

In the present study, 119 patients with newly diagnosed, hitherto untreated neovascular form of AMD were prospectively followed. The study was conducted in two European countries (Bulgaria and France), in three cities with a population of over 10,000 people for a period of one year .

Sixty-four (64) of the patients were French (European race), and fifty-five (55) were Bulgarian (Caucasian race) . All patients included in the study live in urban centers. Fifty-three (53) of the patients were male (44.5%), and sixty-six (66) were female (55.5%).

The mean age of the patients was 75. 45 years, with the youngest patient at 45 years and the oldest at 97 years.

The mean age of the men was 75.75 years, with the youngest patient at 59 years and the oldest at 94 years.

In women, the mean age was 75.80 years, with the youngest patient at 45 years and the oldest at 97 years.

On Tabl. 3 shows the age-sex structure of the studied cohort.

Table 3 – Age-sex structure of the studied cohort

Group	gender	Number/% of patients	Age				
			Mean	SD	Median	Min	Max
MDSV	men	53/44.5%	75.75	8.65	76	59	94
	women	66/55.5%	75.80	10.33	77	45	97

	in general	119/100%	75.45	8.24	77	45	97

In patients from France, the mean age was 76.56 years with the youngest patient at 62 years and the oldest at 91 years.

The average age for French males was 75.24 years and they made up 45.31% of the study cohort.

The average age for French females was 77.66 years, and they made up 54.69% of the French cohort.

The results are presented in Table 4.

Table 4: Age-sex structure of the studied French cohort

Group	gender	Number/% of patients	Age				
			Mean	SD	Median	Min	Max
MDSV	men	29/45.31%	75.24	8.62	76	62	91
	women	35/54.69%	77.66	9.87	76	54	97
	in general	64/100%	76.56	8.21	76	54	97

In patients from Bulgaria, the mean age was 74.87 years with the youngest patient at 45 years and the oldest at 94 years.

The average age for male Bulgarians is 76.37 years and they make up 38.18% of the studied cohort.

The average age for female Bulgarians is 73.70 years, and they make up 61.82% of the French cohort.

The results are presented in Table 5.

Table 5: Age-sex structure of the studied cohort of Bulgarians

Group	gender	Number/% of patients	Age				
			Mean	SD	Median	Min	Max
MDSV	men	21/38.18%	76.37	8.62	76	59	94
	women	34/61.82%	73.70	9.87	66	45	86
	in general	55/100%	74.87	8.21	70	45	94

According to the OCT-A morphological characteristics of naive neovascular membranes, patients were divided into the following groups: "sea fan", "medusa", "dead tree", "lace", "filaments" and "indeterminate".

The largest number of patients was in the "sea fan" type group (40 pcs), followed by "lace" (25 pcs), "filaments" (23 pcs) and "medusa" (19 pcs). The distribution is presented graphically in Fig. #4

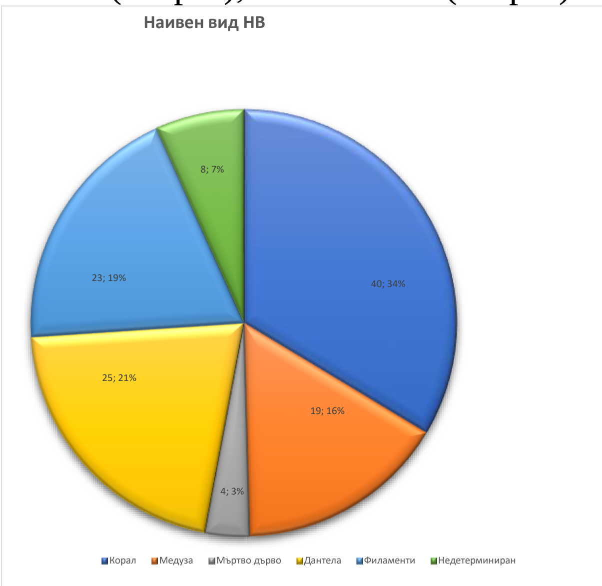


Fig. 4 *__Distribution by number of the naïve neovascular membrane form, according to OCT-A__*

The distribution of individual types of naïve CNV was also analyzed among patients of different sexes.

__No statistically significant relationship was found between the gender of the patients and the shape of the "naïve" neovascular membrane.__

Among men, the number of patients with naïve CNV type "sea fan" (17 pcs) is the largest, followed by CNV type "lace" (15 pcs) and "filaments" (12 pcs).

In women, the most frequent types of "coral" (23 pcs), "medusa" (14 pcs) and "filaments" (11 pcs) CNVs stand out.

"Sea fan" CNV occurs almost equally among both sexes, with a slight predominance among women - 57.50% versus 42.50% among men.

"Medusa" CNV occurs significantly more often among female patients - 73.68% of all "medusa" CNV patients are female, while only 26.32% are male.

The percentage distribution by gender is similar for the "dead tree" CNV: 25% men and 75.0% women.

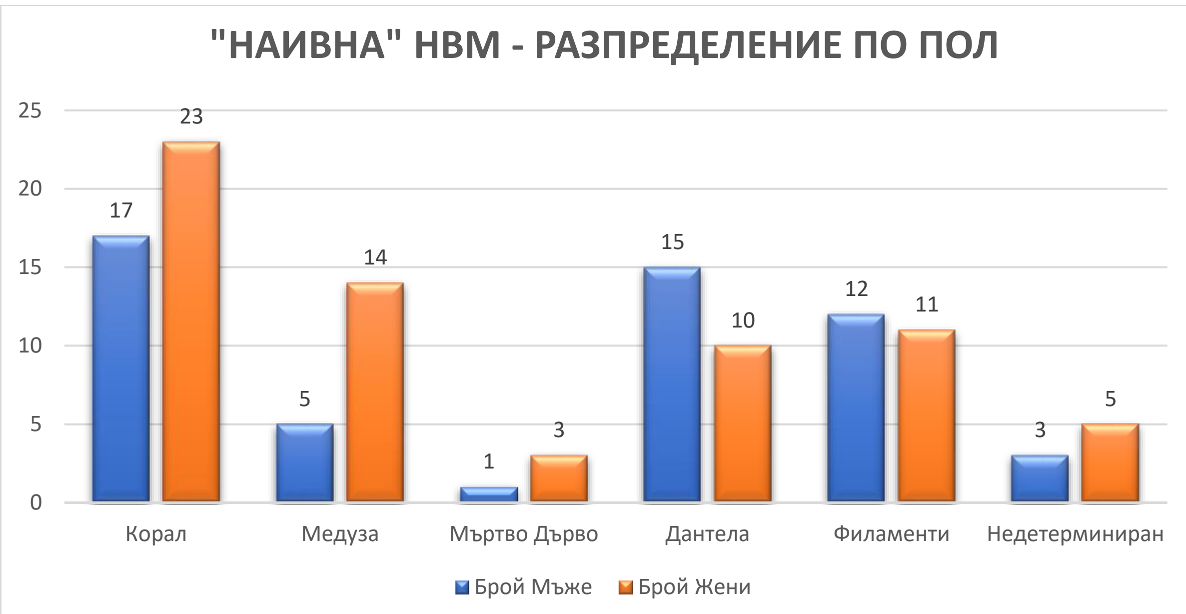
"Lace" type CNV is more common in men - 60% compared to female patients (only 40% of them have "lace" CNV).

CNV type "filaments" are more common among men - in 52 . 17%, compared to women – 47 . 83% of the study cohort.

The "indeterminate" type of CNV in 62.50% of cases occurs among female patients.

In Fig. 5 shows the distribution of patients with different morphological variants of naïve CNV by gender.

Fig. 5 – Distribution of CNV before therapy by sex



On the table 6 presents the data on the distribution of CNV by gender and average age for the individual types of membranes before therapy

Table 6: Distribution of morphological variants of CNV before therapy by gender and age

NV naïve	Number of Men	Number of Women	Total No	Male % of group	Women % of group	Middle-aged
Coral	17	23	40	42.50%	57.50%	76.35
Medusa	5	14	19	26.32%	73.68%	77.58
Dead Tree	1	3	4	25.00%	75.00%	74
Lace	15	10	25	60.00%	40.00%	71.56
Filaments	12	11	23	52.17%	47.83%	76.48
Indeterminate	3	5	8	37.50%	62.50%	80.75

The results of the present study indicate that the youngest patients were with "lace" type CNV - mean age 71.56 years, followed by patients with "dead wood" CNV, and the oldest were "indeterminate type" HVM: 80.75 years.

According to age, patients are further divided into four subgroups:

1. First age group: 45 – 65 years.
2. Second age group: 66 – 75 years.
3. Third age group: 76 – 85 years.
4. Fourth age group: Patients over 86 years of age

In the First age group (45 - 65 years), the largest number are patients with naive "lace" CNV (7 pcs), followed by "sea fan" CNV type (6 pcs), "medusa" type (2 pcs), "filaments" (2 nos.), "dead tree" (1 nos.) and patients from the "indeterminate" CNV group did not occur.

In the second age group (66 - 75 years), patients with "sea fan" type CNV prevail (10), followed by "lace" and "filament" CNV - each with 8 patients, "medusa" CNV

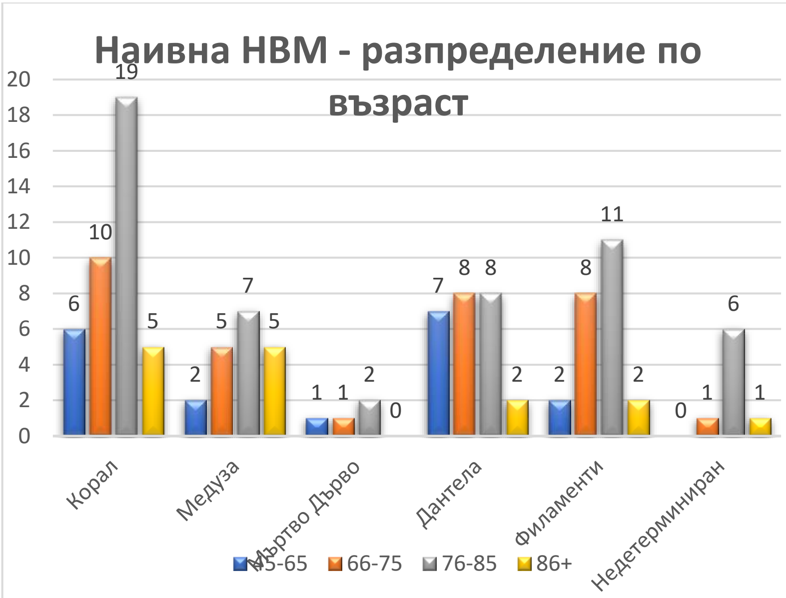
(5 pcs.), and one patient is included in each of the "dead tree" and "indeterminate" types of CNV.

In the third age group (76-85 years) there are the most patients with "sea fan" type CNV (19 pcs), the second place is the "filament" type CNV (11 pcs), followed by the "lace" type CNV " (8 pcs.), "medusa" type CNV (7 pcs.), "indeterminate" type CNV (6 pcs.) and "dead tree" type CNV (2 pcs.).

Among the oldest patients of the Fourth Group (over 86 years old), the most common morphological variants of CNV are "coral" and "medusa" - each of them with five patients, then for the corresponding age group, CNV type " lace" (2 pcs.), "indeterminate" variant - one patient. In the Fourth age group, there are no "filament" and "dead wood" type CNV patients.

Fig. 6 presents the distribution of the different morphological types of CNV ("sea fan", "medusa", "dead tree", "filaments", "lace" and "indeterminate") in four different age groups 45 - 65 years, 66 - 75 years. , 75 – 85 years, over 86 years of age.

Fig



. 6 Distribution of the type of naive CNV in the different age groups

The correlation analysis performed did NOT reveal a statistically significant relationship between the age of the patients and the type of untreated (naïve) neovascular membrane.

After treatment with three intravitreal applications of an anti- VEGF preparation (Eylea, Bayer) with an interval of a month between them, the distribution of the individual types of treated CNV among patients of different sexes was analyzed . Subjected to a correlation analysis was the factor "gender" and the morphological variant of the treated CNV:

No statistically significant relationship was found between the gender of the patients and the morphological form of the neovascular membrane after therapy.

Among men, the largest number of patients with treated CNV type "filaments" (14 pcs), "medusa" (13 pcs), followed by CNV types "dead tree" and "lace" - each of them with 10 patients, CNV type "sea fan" (6 pcs.).

Among women, the most frequent types of "dead tree" (24 pcs), "medusa" (22 pcs), "lace" (13 pcs), "filaments" (4 pcs), "sea fan" types stand out. " (3 pcs.)

Patients with CNV morphological variant "indeterminate" are not found in either men or women.

Post-therapy sea fan-type CNV occurs predominantly among men (66.76% men, 33.3% women), while among naïve forms of CNV “sea fan” occurs almost equally among both sexes, with a slight predominance among women (see above) .

"Medusa" type CNV after therapy, as well as among naive forms, occurs more often among female patients (although with a changed percentage expression, compared to the distribution in naive CNVs) - 62.86% of all patients with "medusa" CNV are female, while 37.14% are male.

The percentage distribution by sex and the "dead tree" CNV type after therapy remained almost unchanged compared to naive forms: 29.41% men and 70.59% women.

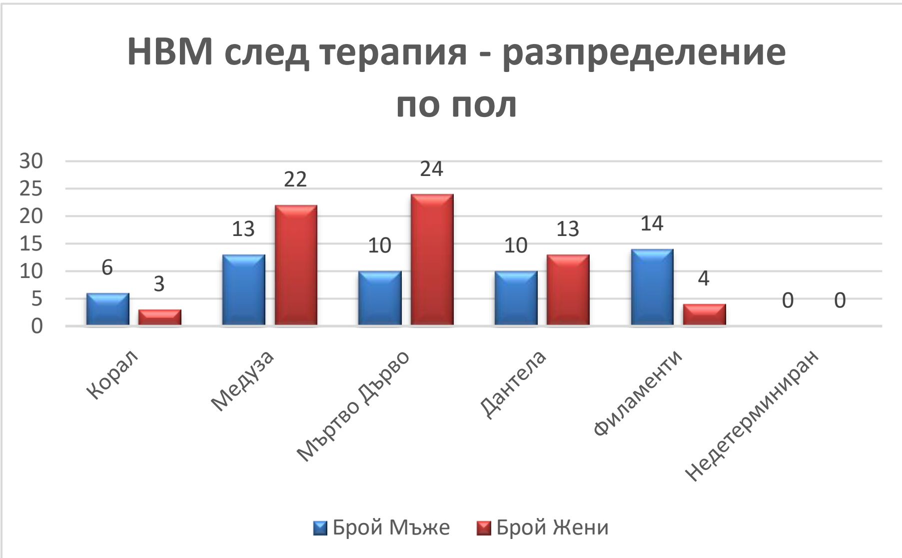
"Lace" type CNV before therapy is more common in men - 60%, while after therapy the percentage distribution changes with a slight predominance of the female sex: 43.48% men versus 56.52% women.

CNV type "filaments" after therapy are more common among men - in 77.78%, compared to women - 22.22% of the studied cohort.

The "indeterminate" type of CNV in 62.50% of cases occurs among female patients.

In Fig. 7 graphically illustrates the above-described distribution of patients with different morphological variants of CNV by gender after intravitreal anti-VEGF therapy.

Fig. 7 – Distribution of the types of CNV after therapy p



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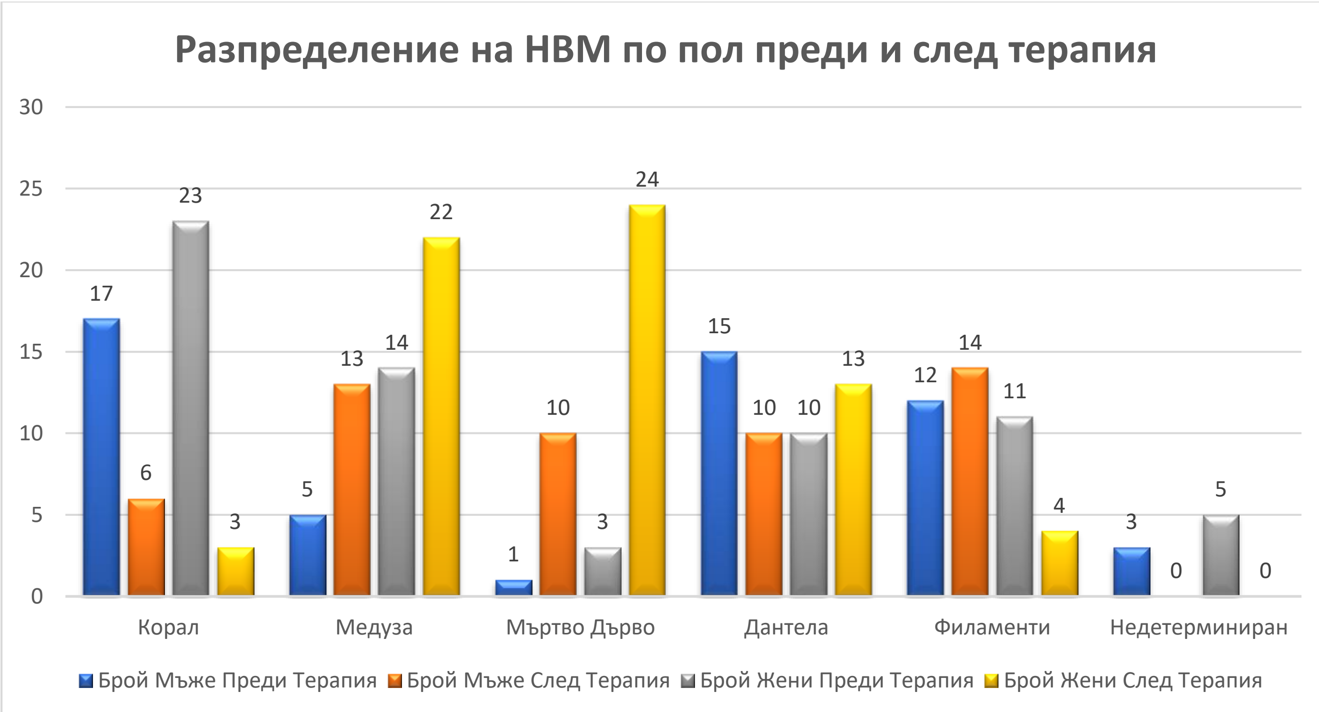
In Fig. 8 graphically presents the distribution by gender before and after therapy of the different morphological variants of CNV



On the table 7 presents the data on the distribution of CNV by gender and average age for individual types of CNV membranes after therapy with three "loading" doses of anti-VEGF.

NV postth	Number Men	Number Women	General Number	Men % of the group	Women % of the group	Average Age
Coral	6	3	9	66.67%	33.33%	75.89
Medusa	13	22	35	37.14%	62.86%	75.37
Dead Tree	10	24	34	29.41%	70.59%	78.38
Lace	10	13	23	43.48%	56.52%	71.65
Filaments	14	4	18	77.78%	22.22%	76.89
Indeterminate	0	0	0	0.00%	0.00%	0

Table 7: Distribution of morphological variants of CNV ***after*** *therapy by gender and age*

The results of the present study show that after therapy, patients with "lace" type CNV are the youngest - mean age 71.65 years, followed by patients with "medusa" CNV - mean age 75.37 years and "sea fan" CNV type - 75.89 , and the oldest are with the "dead tree" type of CNV: 78.38 years.

In order to more clearly present the age distribution of CNV after therapy, the patients were further divided into the four age subgroups used in the presentation of the results for naïve CNV (see above).

In the first age group (45-65 years), the largest number are patients with a morphological variant of CNV after therapy: "lace" (5 pcs), "medusa" (5 pcs), the second place is shared by CNV type "dead tree' (3 pcs.) and 'filaments' (3 pcs.). "Sea fan" CNV in this age group after therapy, there are two patients (2 pcs.)

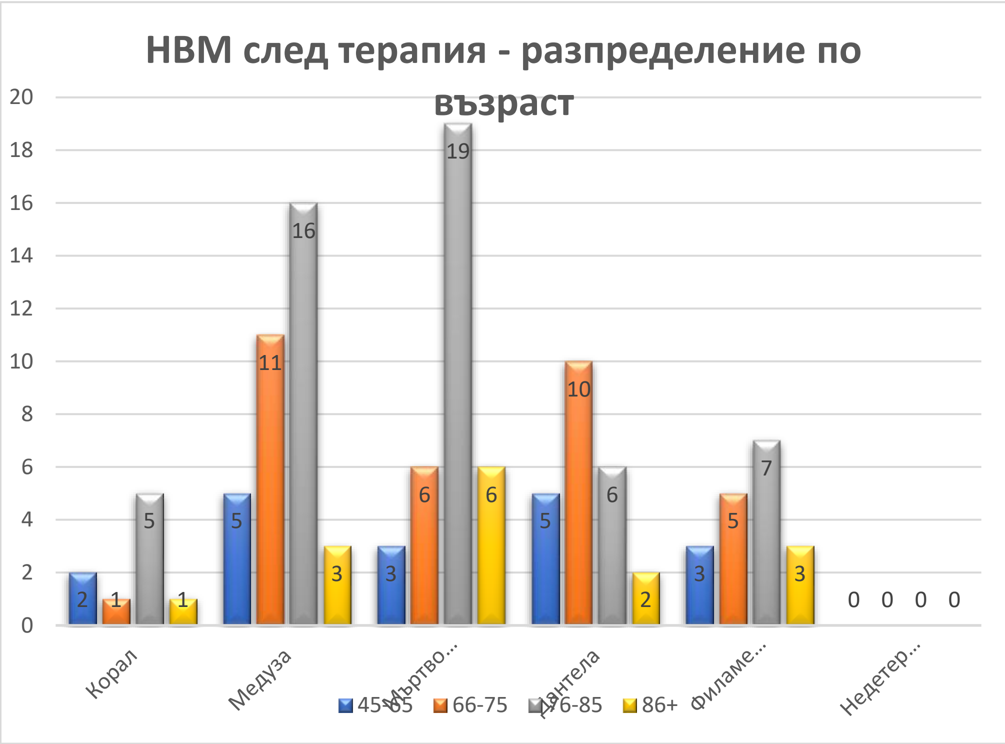
In the second age group (66-75 years), patients with "medusa" type CNV prevail (11), followed by "lace" type CNV (10) and "dead tree" (6). There are 6 CNV type "filaments", and type "sea fan" - only one, after therapy with three "loading" doses of anti-VEGF.

In the third age group (76 – 85 years), after the therapy, there are the most patients with "dead tree" type CNV (19 pcs), "medusa" type CNV is in second place (16 pcs), followed by CNV type "filaments" (7 pcs), CNV type "lace" (6 pcs), and CNV type "sea fan" (5 pcs).

Among the oldest patients of the fourth group (over 86 years old), the most common morphological variant of CNV after therapy is "dead tree" - 6 pieces, the second place is shared by CNV of the "medusa" and "filaments" type - 3 each count. In this age group, there are only 2 cases and 1 case of "lace" type CNVs after therapy. In the Fourth Age Group **before therapy,** patients of CNV type "filaments" and "dead tree" are not found.

Fig. 9 presents the distribution of the different morphological types of CNV **after therapy** ("sea fan", "medusa", "dead tree", "filaments", "lace" and "indeterminate") in four different age groups 45 - 65 years, 66 - 75 , 75 – 85 years, over 86 years of age.

Fig. 9 Distribution of the type of naïve CNV in the different age groups



after therapy

The summarized results from the tables and graphs presented above were subjected to a correlation analysis in order to establish a possible relationship between the patient's age and the morphological variant of CNV after therapy with three "loading" doses of anti-VEGF as described in the "Materials and methods' therapeutic protocol.

The correlation analysis performed did NOT show a statistically significant relationship between the age of the patients and the morphological appearance of the neovascular membrane after therapy.

Correlation analysis between naïve CNV and its morphological appearance after therapy

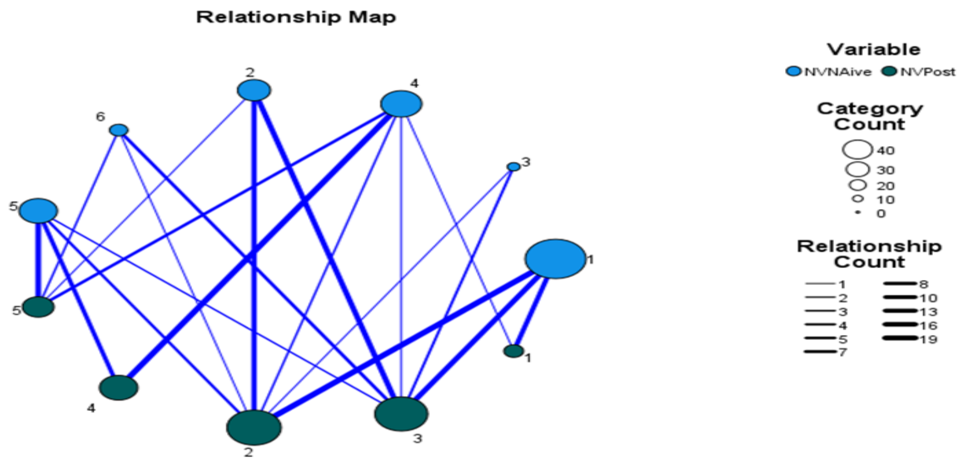
Modern anti-VEGF therapy aims to reduce the activity of the treated membrane and establish control over the progression of the disease. A widely established therapy protocol requires a loading dose of three regular monthly intravitreal applications. This is believed to be sufficient to transition the acute phase to chronic and establish control over the membrane.

A relationship was sought between the initial appearance/naïve neovascular membrane/ and its change after three applications of anti-VEGF intravitreally every other month.

Correlation analysis on this dependence indicated a **statistically significant relationship** between the morphological form before therapy and that after for the entire cohort of 119 patients: *Spearman's rho: ρ =0.61137, p=0, n=119*

Fig. 10 : Map of the relationships between the naïve type of HB membrane and the post-therapy type

Line thickness is directly proportional to bond strength.



__Legend:__ *NV naïve – "naïve" form of neovascular membrane, before therapy; NV post – shape of the neovascular membrane after three intravitreal anti- VEGF applications with a month interval between them*

1 – Coral; 2 – Medusa; 3 – Dead tree; 4 – Lace; 5 – Filaments; 6 – indeterminate;

For a more detailed and in-depth analysis, naive neovascular membranes were further subdivided depending on the "maturity" of the vessels that make them up. In this way, a connection was sought between the histological organization of the membrane (the components that make up its vascular wall) and the remodeling that occurs after therapy. Subdivision was performed taking into account the OCT-A image and the caliber of the vessels forming the membrane and according to the retinal angiogenesis theory of *De Almodovar et al.*

Thus, two groups were formed: "differentiated" and "undifferentiated" membranes. Among the "differentiated" were "sea fan", "medusa", "dead tree". To the "undifferentiated" - "lace", "filaments", "indeterminate". For the differentiated group (62 pieces), the Spearman's rho test was again applied, which confirmed the statistical correlation between the three morphological types:

Spearman's rho: $\rho=0.32385$, $p=0.01024$, $n=62$.

A chi-square test was also applied - 39.6406, p=< 0.00001 , which also confirmed the statistical relationship

Table 8: Chi-square test results

Results			
	HE is naive form	HE after therapy	***Total lines***
Coral	40 (24.00) [10.67]	8 (24.00) [10.67]	48
Medusa	18 (23.00) [1.09]	28 (23.00) [1.09]	46
Dead tree	4 (15.00) [8.07]	26 (15.00) [8.07]	30
Total columns	62	62	**124** (Total **)**

The study found that "sea fan"-type neovascular membranes most often changed to "medusa" (47%) or "dead tree" (33%) after therapy. Only 20% do not change after therapy. Type "medusa" after therapy in 55.6% turns into "dead tree", and in 44.4% it does not change. In 75% of cases, "dead tree" retains its morphological appearance even after therapy. It is a final, maximally differentiated form (by histological essence) of a neovascular membrane that does not change over time.

Fig. 11 Remodeling CNV type "sea fan", "medusa", "dead tree" after therapy:

For the group of "undifferentiated", immature neovascular membranes, which include "lace" , "filaments", indeterminate", Spearman's rho and chi-square test were again applied :

Spearman's rho: ρ = -0.07683, p=0.57358, n=56 (4-6)

However, they found no correlational dependence between individual forms before and after therapy.

Regardless of the fact that in 64% of cases a naive neovascular membrane of the "lace" type does not change after therapy, the static data for the three types do not allow to define a regularity for the transition from one naive form to another.

In Fig. 9 shows the statistical dependence in the remodeling of the "differentiated" HVMs under the influence of anti- VEGF therapy.

In Fig. 10, based on the obtained statistical results, a map of the dependencies in the group of "differentiated" CNVs is derived.

Fig. 12 – Remodeling process of the " differentiated" CNV under the influence of intravitreal anti- VEGF therapy

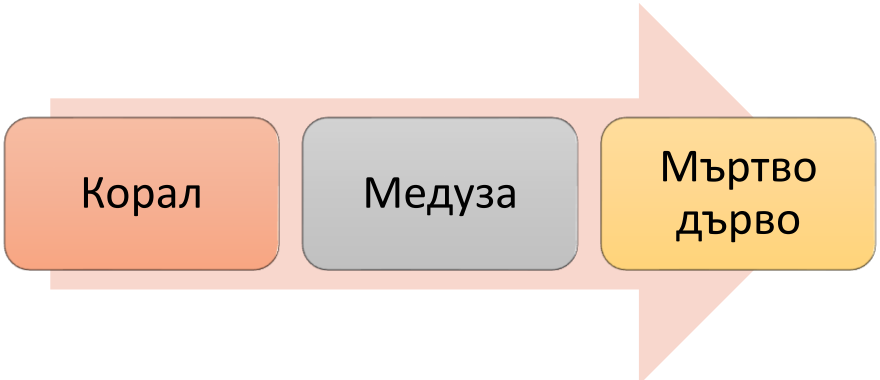
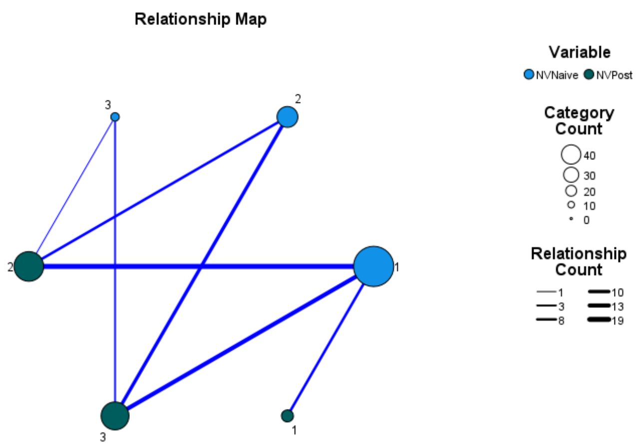


Fig. 1 3 – Map of dependencies/relationships between the naive form of CNV from the differentiated group and its form after anti- VEGF therapy



<u>Legend:</u> *NV naïve – "naïve" form of neovascular membrane, before therapy; NV post – shape of the neovascular membrane after three intravitreal anti- VEGF applications with a month interval between them 1 – sea fan; 2 – medusa; 3 – dead tree;*

Defining OCT-A markers of progression

Identifying signs of progression is key to establishing control over a disease. In the case of OCT-A, it provides repeatable information on high-resolution images. Based on a review and analysis of 52 literature sources from the pubmed database and a search for keywords "OCT-angiography", "macular degeneration", "choroidal neovascularization", the following OCT-A signs of NV-membrane activity were summarized

- perilesional dark halo
- ramifications in the pathological network
- angiographic relationship between the neovascular membrane and the normal choroidal network

Correlation dependences between the signs of progression and the type of CNV before and after therapy

The definition of signs of progression is fundamental for the assessment of the ongoing therapy. The effectiveness of the selected treatment is determined by the control over the signs of disease progression. The changes that occurred in the progression marks during the course of the therapy were analyzed. All OCT-A images of the 119 patients included in the study before the start of therapy and after each of the three doses of intravitreal administration of Eylea were carefully reviewed and the dynamics in the progression marks were tabulated (Tables 5,6, 7 – below in the text):

- presence of **the perilesional dark halo** before and after each one of the doses

- **ramifications** in the pathological network - their reduction or increase

- tracking the presence or not of **an angiographic connection** between the NV-membrane and the normal network

In coiled, untreated CNV, all 119 eyes included in the study had all three signs of activity (dark halo, ramifications, and angiographic connection).

VEGF treatment are presented and carefully analyzed in tabular form .

The tabulated data were subjected to statistical analysis (Spearman's rho, independent samples t-test) and the following results were obtained:

1. No statistically significant relationship was found between the type of naïve NV membrane and the presence of the perilesional dark halo: Spearman's rho: $\rho = 0.1166$, $p = 0.20665$, $n=119$

2. Perilesional dark halo decreases after therapy –

   Spearman's rho: $\rho = -0.68361$, $p = 0.00665$, $n=119$

3. Ramifications in the pathological vascular network decrease after therapy only in the group of differentiated membranes, as the more differentiated the membrane, the fewer the ramifications after therapy :

   Spearman's rho: $\rho = -0.33451$, $p=0.00788$, $n=62$ (1-3)

4. The more differentiated the membrane, the more often there is an angiographic connection after therapy - the results of the correlation analysis are presented in Table 8 and Table. 9. It is evident from them that no statistically significant relationship is established between the undifferentiated membranes after therapy and the presence of an angiographic connection .

Correlation dependences between the type of CNV and visual acuity

The determination of different types of neovascular membranes raises the question of whether they affect visual acuity differently. **The applied statistical analysis of the data from the study did not prove a relationship** (either positive or negative) of any **NAIVE morphological type of membrane, with visual acuity before and after therapy.**

After vascular remodeling occurred, **a statistical relationship was established between the morphological variant AFTER therapy and visual acuity** :

For the group of "differentiated" neovascular membranes ("sea fan", "medusa", "dead tree") the dependence is negative:

Spearman's rho: $\rho = -0.29387$, $p=0.02043$, $n=62$

Which means that the less differentiated the membrane (e.g. "sea fan"), the lower the visual acuity.

For the group of undifferentiated membranes, we again have a statistically significant result, but it has a positive sign:

Spearman's rho: $\rho=0.30498$, $p=0.02228$, $n=56$, i.e. the more differentiated the membrane (eg "filaments"), the higher the visual acuity.

Drawing up a therapeutic protocol

The analysis of the results related to the type of neovascular membrane, its change and the demonstrated signs of progression formed a protocol for conducting individualized and monitored behavior in choroidal neovascularization, in the course of AMD.

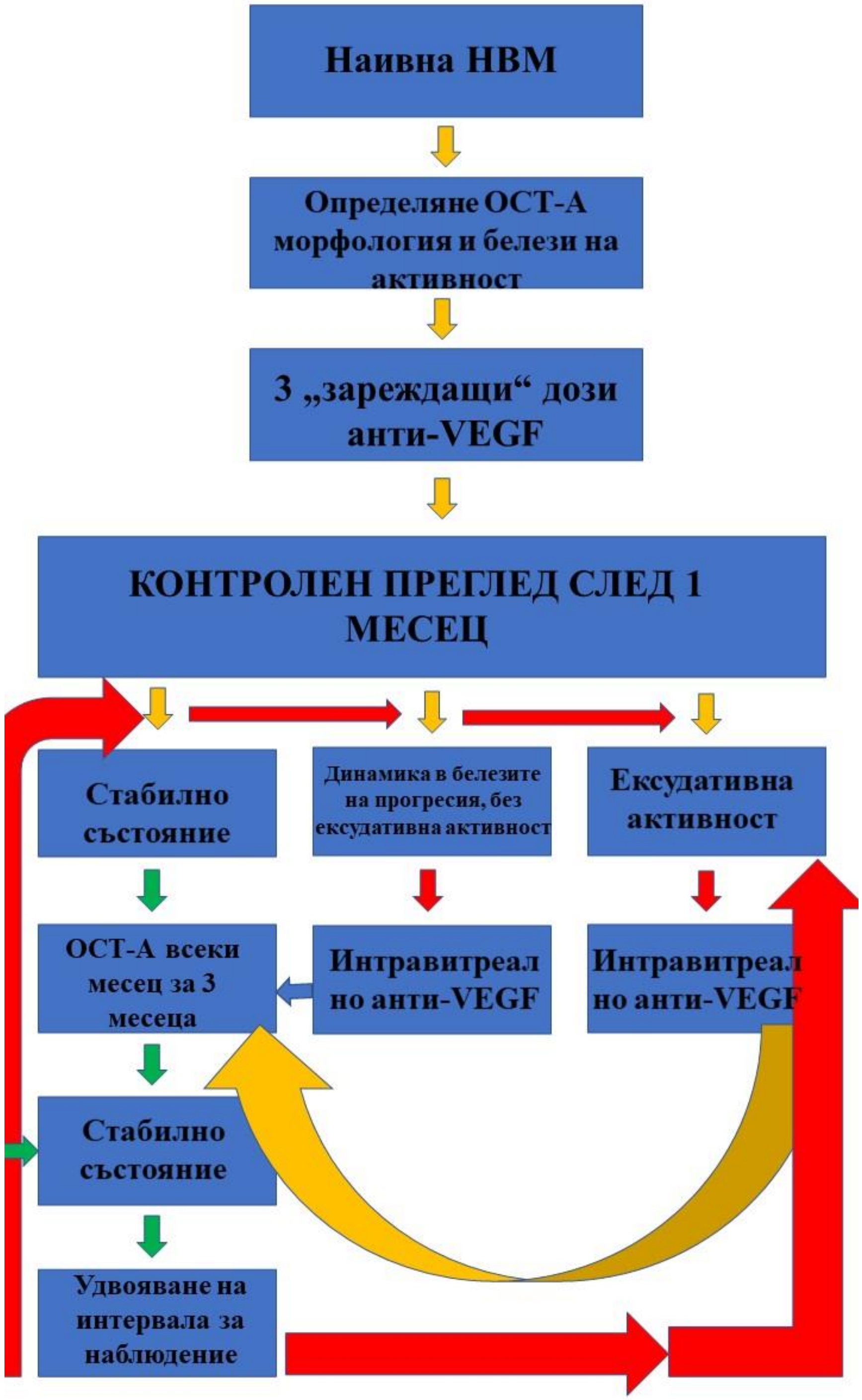
The protocol applied in this study - three "loading" doses (Eylea, Bayer) within a month of each other ("loading" doses) and control of visual acuity and OCT-A findings, proved to be effective in 97.5% of patients. Because of the high percentage of good therapeutic effect, I would suggest it as an initial model of behavior for all patients with newly diagnosed AMD with choroidal neovascularization.

Follow-up and therapy are the key to good long-term success result. It should be tailored to both the functional and anatomic response to therapy, taking into account the presence or absence of signs of CNV activity and progression.

Ideally, the individualized protocol in the fight against neovascular AMD should include:

1. Three "loading" doses of anti- VEGF within a month of each other ("loading" doses)
2. Control examinations with OCT and OCT-A every month within 6 months, taking into account the signs of progression and activity (perilesional dark halo, ramifications, presence of angiographic connection). This is necessary to establish all possible signs of progression.
    - 2.1. When stability in the condition is established during the first 6 months - the period for control examinations increases
    - 2.2. Upon detection of reactivation of signs of progression (for example, an increase in the dark halo, or the number of ramifications) - even in the absence of intraretinal and subretinal fluid, the patient is re-injected
    - 2.3. When exudation is detected - intravitreal anti- VEGF application

Fig. 14 – Proposal for a therapeutic behavior protocol consistent with the OCT-A



finding

DISCUSSION

1.1. Demographic characteristics

As a proportion of patients were from France (European race), the largest French study on incidence and incidence of MDS was consulted. The LANDSCAPE Study (Creuzot- Garcher CP et al, 2022) is a retrospective study conducted in France, based on the identification of patients with a neovascular form of AMD in the registry of the National Health System of the country. Data from the LANDSCAPE Study (Creuzot- Garcher CP et al, 2022) are also supported by another French study - ALIENOR Study (Delcourt C et al, 2010), which covers a four-year interval and is again retrospective. The average age in the ALIENOR Study (Delcourt C et al, 2010) was 79.7 years, and the female gender predominated – 62.7%. Another large-scale European study was conducted in Finland and covered a period of 15 years (Ida Korva -Gurung et al, 2023). The mean age of patients in the Finnish study was 78 years, with again a female predominance.

The earlier age at diagnosis in the group examined in this study, compared to previous data from the literature, can be explained by facilitating access to medical care (especially in Bulgaria) and improving diagnostic possibilities.

The distribution by gender has a significantly greater predominance of women, compared to the above results of the cited studies.

In conclusion, the results of the present study are close and support the data from the world literature.

2. Defining the morphology of the neovascular membrane - classifications

Macular neovascularization is associated with invasion of vessels and/or materials into the outer retina, subretinal space, or sub-RPE space in various combinations. The FA-based classification for retinal neovascularization describes its anatomical location.

The first OCT-A morphological characterization of NV-membranes was described by colleagues from *Creteil* , France. To date, this is the only one that, although at first glance a subjective classification of NV membranes, has shown high repeatability when observed by different examiners. It is for this reason that it forms the basis of the present study.

In the literature, until now, there are no studies on the predominance of the type of HB membranes by morphological appearance among newly diagnosed patients, as well as on the distribution of individual types by age and sex.

In the present study, among untreated patients with neovascular AMD, the most common morphological variant was “sea fan”, followed by “lace”, “filaments”, “medusa”, “dead tree”.

The determination of the most frequent morphological type is an important initial step in the study, so that dependencies can be sought in the performance of the following tasks - namely, the occurrence of vascular remodeling of a given morphological type as a result of the ongoing anti- VEGF therapy.

This statistical trend is necessary as a starting point and to be able to look for a relationship between the aggressiveness of the disease and a specific naïve type of NV-membrane.

No dependences were found for the age distribution of the different morphological types, nor for a relationship between gender and type of membrane.

The unification of standards for OCT-A description of NV-membranes is of particular importance, and one of the aims of this study is to show the advantages and disadvantages of the only proposed classification of OCT-A images of choroidal neovascularization in the context of AMD.

The classification proposed by Coscas (Coscas F. et al, 2019) is well applicable, recognizable, easily digestible, sufficiently objective and descriptive. In this case, it was not considered in parallel with the FA classification, since the focus of the study is exclusively OCT-A as a new and non-invasive method in the diagnosis of AMD.

3. Correlational dependences between naive CNV and its morphological appearance after therapy

Choroidal neovascularization is typical for neovascular AMD, and according to its anatomical location, two forms are observed (classification according to FA characteristics, which is generally accepted in world clinical practice):

- Occult - between the RPE and Bruch's membrane
- Classic – between the RPE and the neurosensorium

Secretion of VEGF leads to increased levels of profibrotic factors such as tumor growth factor-β (TGF-β) and connective tissue growth factor (CTGF). When the balance between angiogenic (VEGF) and fibrogenic (CTGF) factors reaches a certain ratio, the so-called "angiogenic switch" first described by Folkman and Hanhan in 1991 (Folkman et al, 1991) and further clarified as a concept in 2000 by Hanhan and Weinberg (Hanhan et al, 2000) . Depending on which direction the ratio between proangiogenic and profibrogenic factors is drawn, either enhanced angiogenesis or its replacement by fibrosis is observed. It is the angiogenic shift in favor of fibrosis that underlies the principle of operation of anti- VEGF intravitreal medications.

In 1994, the American Journal of Pathology first reported that the hypoxic retina produced VEGF and suggested its role in eye diseases with neovascularization.

The interest in anti- VEGF increased around 2002, mainly in the field of oncology, from where the innovations regarding the fight against neoangiogenesis started. The first registered product for use in oncology practice is bevacizumab (Avastin) – a humanized antibody against all isoforms of VEGF. Bevacizumab was approved by the FDA for the treatment of breast and colorectal carcinoma in 2004. In 2004, the first anti- VEGF preparation for eye use - pegaptanim (Macugen , Eytech Pharmaceuticals, New York, NY) - was approved . It neutralizes VEGF-165 (probably also VEGF-188 , but unproven) . Phase II and III studies demonstrate that its use reduces visual acuity loss from neovascular AMD (Vinores SA et al, 2006) .

Meanwhile , a small, single-center study demonstrates the positive effect of bevacizumab on visual acuity in AMD and its minimal systemic effects (Shima C. et al, 2008) .

Despite the encouraging " off l a bel" results for bevacizumab, it was initially thought that the drug did not diffuse well enough through the retina to reach effective concentrations in the choroid. It is for this reason that in 2006 the FDA approved for human use Genentech - Renimizumab (Lucentis) - a monoclonal, humanized Fab antibody that neutralizes all active forms of VEGF. The MARINA studies (Rosenfeld PJ. et al, 2006) and ANCHOR (Brown DM. et al, 2009) demonstrated a reduction in visual acuity loss at two-year follow-up with Lucentis therapy in classic and occult NVMs. The SATT study (Maguire MG. et al, 2016) compared the effects of Avastin and Lucentis and found a similar result.

In November 2018, the FDA approved aflibercept (Eylea, Regeneron Pharmaceuticals. Tarrytown, NY), a chimeric fusion protein designed to improve pharmacokinetics, for use in neovascular AMD. Is ylea (Bayer) blocks VEGF-A by binding the tyrosine kinase receptors VEGF-R1, VEGF-R2 as well as the Fc fraction of human placental IgG1. VIEW1 (Kaiser PK. et al, 2017) and VIEW2 (Schmidt-Erfurth, Ursula et al, 2013) studies demonstrated the longer half-life and longer duration of effect of Aflibercept compared to Ranibizumab.

In October 2019, the FDA approved the drug Beovu (brolucizumab- dbll , Novartis) , which binds the three major VEGF isoforms – VEGF-110, VEGF-121, VEGF-165). The HAWK and HARRIER studies (Dugel PU et al, 2019) demonstrated its similar effectiveness in terms of visual acuity in patients with neovascular AMD and a follow-up period of one year. However, numerous publications have also appeared demonstrating vasculitic manifestations after administration of the preparation.

In 2022, the FDA approved for intraocular use the first monoclonal antibody blocking both VEGF and angiopoietin-2, namely faricimab-svoa (Vabysmo , Roch /Genentech). The six-month TRUCKEE study (Khanani AM. et al, 2023) demonstrated improvement and maintenance of visual acuity in patients with neovascular AMD, along with rapid improvement in anatomical parameters. Good tolerance to the preparation and a low incidence of intraocular inflammation have been reported.

Recently, in August 2023 , the FDA approved Eylea HD (Regeneron Pharmaceuticals), an 8 mg boost formulation for which the CANDELA study (Wycoff CC. et al, 2023) suggested a better potential therapeutic effect in the future, in the absence of proven statistically anatomical and functional such and absence of new side effects ocular and systemic manifestations (and respectively absence of higher incidence of side effects with increasing dose).

Although the arsenal of the modern ophthalmologist in the fight against MDS is growing promisingly, the choice of medicinal preparation in the present study was dictated primarily by a number of administrative requirements in the two countries in which it was conducted. Namely: accessibility - reimbursement from national funds and proven good therapeutic effect in world literature. Unlike France, where all preparations approved for use on the territory of the European Union are reimbursed, in Bulgaria at the start of the study the only reimbursed preparation was Eylea (Bayer). All patients included in the study were treated with Eylea.

The next step was the selection of a therapeutic regimen. Various therapeutic schemes have been described in the world literature. Very often, different anti-VEGF preparations according to their pharmacokinetic characteristics predispose to the choice of a certain therapeutic regimen.

The main species described in the literature are:

1. Fixed regimen – one application of anti- VEGF intravitreally every month. The effectiveness of the fixed regimen of receiving therapy has been proven in numerous randomized trials. However, it is associated with a high burden on both ophthalmologists and patients.

Current understanding is that to minimize the adverse effects of a therapy, a balance must be found between the functional and anatomical positives and the adverse side effects (including the atrophy that accompanies anti- VEGF therapy).

2. Pro Re Nata (PRN) - the patient receives three " loading" doses with an interval of one month between them, and the subsequent injections are when new signs of activity are detected during follow-up controls (monthly controls).

3. Observe-and-plan (O&P) – is similar in concept to PRN. The patient receives three "loading" doses with an interval of one month between them. Monthly "surveillance" checks follow to determine disease activity. Once the reactivation interval is established, a therapeutic regimen based on it is created with a pre-planned series of intravitreal injections.

PRN and O&P reduce the number of injections, but the antivasoproliferative effect of these regimens may be compromised. In addition, PRN patients need monthly follow-ups, resulting in overworked ophthalmologists.

4. Treat and Extend the regimen is based on the principle of achieving a maximally large interval between individual intravitreal injections, while maintaining the achieved anatomical and functional results of the treatment.

Regarding the present study, the therapeutic regimen was consistent with the VIEW1 and VIEW2 studies, which demonstrated an optimal anatomic and functional effect when aflibercept was administered every month, after an initial three "loading" doses.

After the initial assessment of the patient, determination of the naive type of neovascular membrane, according to OCT-A and determination of the therapeutic protocol, it was time to evaluate the impact of the applied therapy on the coiled HVM.

The present study found correlations between the type of naïve CNV and its remodeling after three intravitreal anti- VEGF applications. As presented in the Results Chapter - the membranes were further subdivided into "differentiated" and "undifferentiated" groups for greater precision in analyzing the results. A number of studies subdivide membranes into "mature/mature" (differentiated) and "immature" (undifferentiated) depending on the caliber of the vessels making up the neovascular complex on OCT-A imaging (Balaskas K. et al, 2018, Coscas F et al, 20 1 8, Vidinova H. and colleagues, 2020), which is also in accordance with the theory of histological maturity of the membranes of De Almodovar et al. (De Almodovar CR. et al, 2007) (more differentiated and histologically mature membranes have larger caliber vessels).

Between the membranes that I referred to the differentiated group ("sea fan", "medusa", "dead tree"), **a correlation and dependence in vascular remodeling after therapy was demonstrated** . It was found that membranes with a smaller caliber of the vessels, after therapy, have the tendency to remodel into a membrane with a larger caliber of the vessels that make it up.

Potente et al (Potente M. et al, 2011) found that the more differentiated vessels an CNV is made of, the more refractory it becomes to anti- VEGF therapy and the more stable it remains over time. That is why, in 75% of cases, the "dead tree" CMV retains its morphological appearance and can be considered as the final, maximally differentiated and mature form of CNV. Potente and collective explain this phenomenon by the fact that neovessels in the early phases lack pericytes and smooth muscle cells. Anti- VEGF therapy in one way "selects" and promotes vessel maturation by destroying immatures with an incompetent vessel wall. However, over time, "screening" vessels whose walls contain pericytes and smooth muscle elements, mature CNVs become VEGF- independent.

In the membranes belonging to the "undifferentiated" group ("lace", "filaments", "indeterminate"), no correlation was found between the naive type of CNV and the type after therapy. On the contrary - in 64% of cases, they do not change their morphological appearance and are extremely refractory to anti- VEGF therapy. In

2017, Takeuchi et al (Takeuchi H. et al, 2017) proved that even in naive CNVs there are vessels that are not affected by anti- VEGF preparations.

To justify the further subdivision of the membranes and to explain their different behavior in intravitreal therapy, it is necessary to refer to the work of Tomita et al (Tomita M. et al, 2008) and propose a different perspective on vascular maturity, apart from the size of the caliber of the builders dishes. The roots of their research are based on the hypothesis that brown bone marrow cells are pluripotent progenitors and can differentiate into different cell types, and that endothelial progenitor cells originate from them (Jiang Y. et al, 2002) . In 2002 it was proven and that endothelial progenitor cells can differentiate into endothelial cells lining blood vessel walls in the event of ischemia following myocardial infarction. In 2003, Grant et al (Grant MB et al, 2003) theorized that retinal neovascularization was due to hematopoietic stem cells.

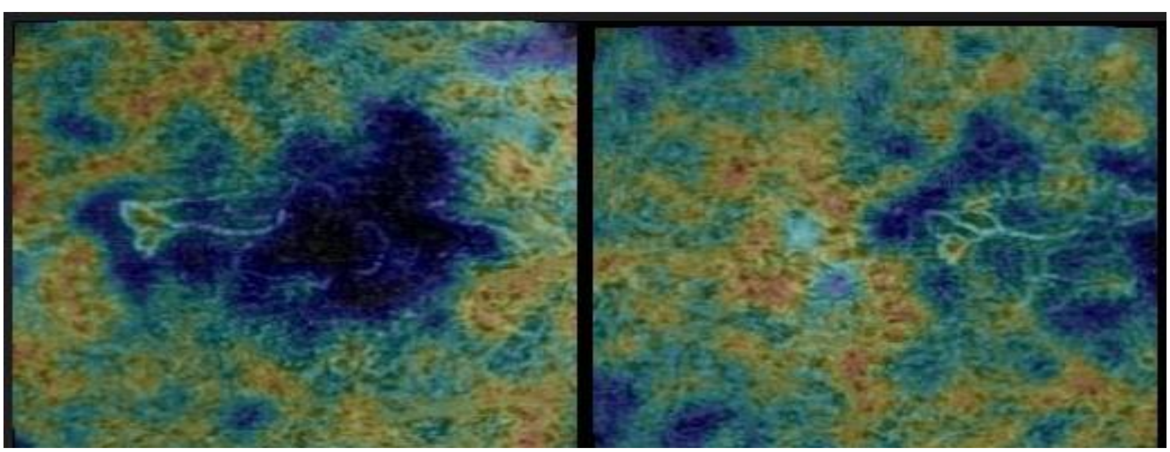
Based on the results of these studies, Tomita et al tested the hypothesis that a fraction of choroidal neovessels originate from the endothelial progenitor cells of the brown bone marrow. In a number of studies in animal models, it has been shown that CNVs originate from existing choroidal vessels (Fabian-Jessing BK. et al, 2023). After performing bone marrow transplantation and inducing Bruch's membrane damage with a krypton laser in chimeric mice, the Japanese team of pathologists and ophthalmologists, led by Tomita , was the first to demonstrate that some of the neovessels in choroidal neovascularization originate from migrated progenitor cells of the brown bone marrow (endothelial progenitor cells). Recently, a study by Wen Deng et al 2023 found higher values of adhesion molecules (Wen Deng et al, 2023), which are chemoattractants for mononuclear progenitor cells, in the intraocular fluid of patients with neovascular AMD compared to healthy controls . Although the purpose of the study of Weg Deng et al. to be another, it indirectly supports the thesis of the Japanese collective Tomita et al. The data presented support the presumed histological difference in the arrangement and maturity of the membranes.

Based on these, I could safely hypothesize that differentiated CNVs originate from existing choroidal vessels, while undifferentiated ones are formed by migrated progenitor cells from the brown bone marrow, under the influence of chemoattractants and VEGF . This would also explain the lack of effect of the anti-VEGF therapy on the undifferentiated membranes, and as suggested by Tomita et al , it would be reasonable in the future to study in detail the origin and histological structure of CNV, so that alternative drugs for treatment could be proposed for development (besides the well-known anti -VEGF).

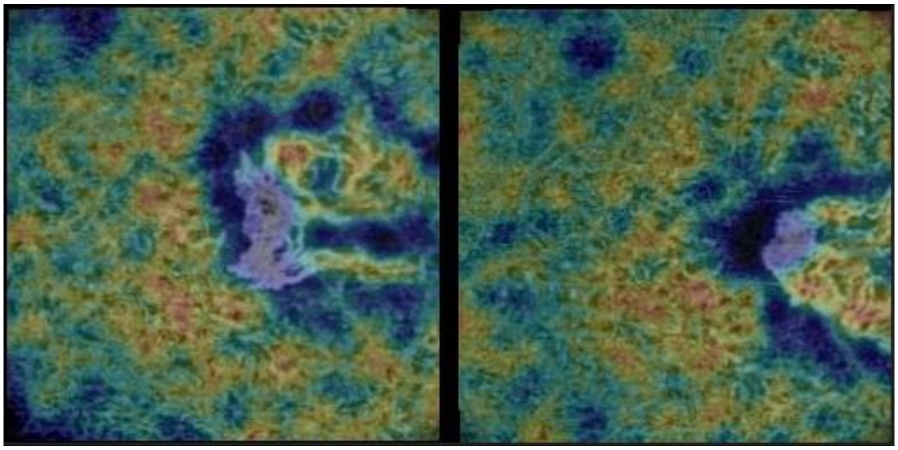
Fig. 1 5 – Vascular remodeling of HVM during anti- VEGF therapy,

traced through OCT-A

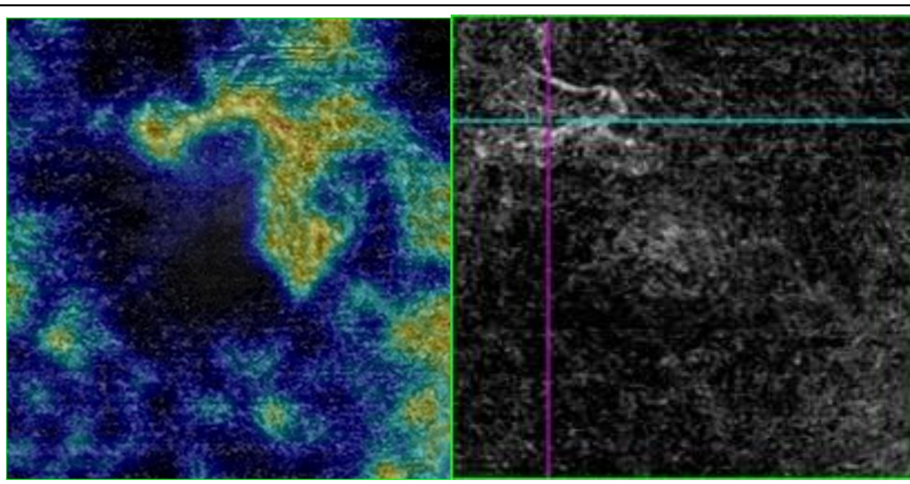
15.1 A CNV “filaments“ BEFORE	15.1 B CNV „filaments“ AFTER anti-VEGF



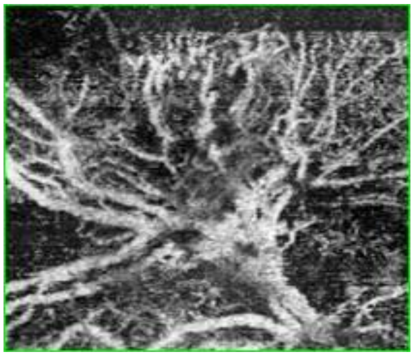
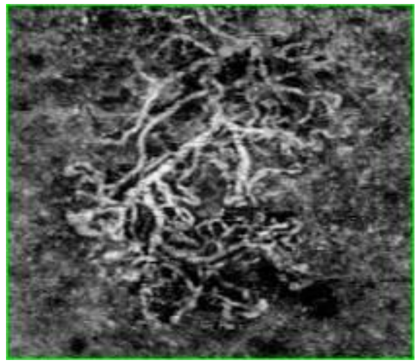
15.2 A CNV “lace“ BEFORE	15.2 B CNV “lace“ AFTER anti-VEGF



15.3 A CNV “medusa“ BEFORE	15.3 B CNV “medusa“ AFTER anti-VEGF



15.4 A CNV “dead tree“ BEFORE	15.4 B CNV “dead tree“ AFTER anti-VEGF

4. Discussion of defined markers of progression

Since OCT-A is a relatively new and developing methodology, a significant inconvenience of working with it is the lack of created classifications that are generally accepted all over the world. CNVs can also be well recognized with conventional imaging techniques. OCT-A provides better visualization of membrane microstructural details at four different scan depths that are often difficult to assess with standard FA or indocyanine angiography due to cumulative imaging and dye leakage from the compromised vessel wall.

1. Evaluation of signs of progression with FA

CNV typically presents as dye “leakage” in classical FA. According to the anatomical localization of the leaking dye, the membranes are classified as "classic", "occult", "mixed" (RAP) (see Chapter "Literature review"). The assessment of signs of progression in CNV with conventional imaging methods is challenging because the evolution of the disease is associated with profound structural changes involving the choriocapillaris, RPE, and outer retina.

Another finding taken to be an FA marker of CNV activity is RPE detachment (RPED), which is a separation of the RPE from the underlying Bruch's membrane. It can be classified as serous or fibrovascular RPED.

Accumulation of serous fluid under or within the retinal neurosensorium is considered a biomarker of disease activity. FA manifests as leakage or accumulation of fluorescein in a petal-like pattern.

Fibrovascular RPED is associated with an area of granular appearance and irregular elevation characterized by persistent luminescence or dye leakage in the late phases of FA.

2. Evaluation of signs of progression with indocyanine green angiography

Indocyanine green has a greater binding affinity to plasma proteins. It shows a lower rate of leakage from the choriocapillaris compared to fluorescein.

This type of angiography is used in order to differentiate the individual subtypes of CNV - both classic and occult, as well as RAP, are well visualized .

Contrary to FA, on indocyanine green angiography, serous RPED exhibits an acto-hypofluorescent finding that persists throughout all phases of angiography.

This imaging technique is especially valuable in the differential diagnosis of polypoidal choroidal vasculopathy: on dynamic angiography with indocyanine green, it is possible to observe the pulsatile filling of the polyp. In addition, the core of the lesion may become hypofluorescent due to washout of the dye, thus forming the ring-like luminescence characteristic of polyps.

In Bulgaria, angiography with indocyanine green is not used, so I will not go into more details about the methodology.

3. Evaluation of signs of progression with structural OCT

Over the past two decades, structural OCT has established itself as a rapid and non-invasive method for evaluating patients with retinal pathology, and since the introduction of anti- VEGF therapy in 2007, the number of follow-up examinations with OCT documenting the patient's condition has grown tremendously.

In the interpretation of OCT signs of AMD, the main descriptive features are "hypo-" or "hyper-reflectivity".

Subretinal and intraretinal fluid are hyporeflective, whereas pigment deposits, lipid exudates, hemorrhages, subretinal fibrosis, and fibrovascular complexes are hyperreflective.

Subretinal and intraretinal fluid are considered markers of activity in both naïve and treated CNV. The subretinal fluid of OCT characterized by an elevation of the neurosensorium and a zone of hyporeflectivity between the RPE and the outer retinal layers. It is usually associated with a type I lesion according to the FA classification. Intraretinal fluid is often associated with a more aggressive type of occult lesions that are of long standing. OCT is characterized by thickening of the retina and formation of cystic spaces.

Hyporeflectivity is not always associated with disease activity. It is extremely important to differentiate subretinal fluid from the so-called "wedge-shaped subretinal hyporeflective lesions", which do not require treatment and are due to local degenerative changes in the retinal layers, mainly in areas of geographic atrophy. Intraretinal fluid due to exudation should be distinguished from intraretinal fluid due to degenerative changes in the neurosensorium. The latter are not accompanied by leakage of dye at FA, but at OCT they are visible in the inner nuclear layer.

Retinal pigment epithelial detachments (RPEDs) have traditionally been classified according to clinical findings and FA, but since the emergence of OCT, new qualitative characteristics have been introduced. By OCT, RPEDs are subdivided based on their reflectivity into "hyporeflective" (considered "hollow, optically empty"), "hyperreflective" ("solid") and "mixed". Studies have shown that hyporeflective RPEDs show a good response to anti- VEGF therapy and are considered a sign of disease activity, while hyperreflective ones demonstrate a poor response to intravitreal application of anti- VEGF.

4. Evaluation of progression markers with OCT-A

OCT-A is a non-invasive alternative to standard FA, which is characterized by high sensitivity and specificity in identifying membranes. The different morphological variants of CNV according to OCT-A image and according to the classification of Coscas et al , so far have no proven relationship with membrane activity.

OCT-A markers of NVM progression are under study - publications regarding them are few and in the process of confirmation.

PubMed database , 52 scientific papers concerning OCT-A markers of CNV progression and activity were isolated. By analyzing the literature sources identified in Pub Med, a comparison table was prepared showing OCT-A differences between active and inactive CNV

Table 16: Comparison between OCT-A characteristics of active and inactive CNVs

	Active NVM	**Inactive NVM**
1.	High vascular density and ramifications	Greater caliber of building vessels
	Multiple anastomoses	Absence of anastomoses

3.	Greater number of anti- VEGF injections	Regression of the membrane under the action of anti- VEGF therapy

For the first time, an attempt to summarize and classify markers of activity and progression was made by Coscas et al. The French team from Creteil identified the following marks:

1. Anastomoses and loops
2. Highly branched vessels/ramifications
3. Presence of a peripheral arcade/angiographic connection to the normal vascular network
4. Form of CNV
5. Presence of perilesional dark halo

Later, the same team of authors found that the presence of the following signs predict the exudative activity of CNV in 97.6% of cases.

1. Ramifications
2. Perilesional dark halo
3. Angiographic connection

The ones suggested by Coscas and collective three markers of activity have been adopted for follow-up in the course of the present study. Al-Sheikh and team were the first to define them as "OCT-A biomarkers' for disease activity and progression (Al-Sheikh M. et al, 2018) .

Sulzbacher et al (Sulzbacher F. et al, 2017) confirm the Coscas imposed signs of activity . Lumbroso et al (Lumbroso B. et al, 2015) found that after therapy and in the absence of exudative activity of the membrane, the blood flow in the feeding vessel increases and this is accompanied by a decrease in ramifications , which confirms the theory of the French collective.

Fossataro et al (Fossataro F. et al, 2022) for the first time compared the imaging of the perilesional dark halo by two methods - OCT-A and indocyanine green angiography and described the advantages of non-contrast angiography in the assessment of scar progression.

5.3 Discussion of the correlational dependences between progression markers and the type of HVM before and after therapy

In recent years, efforts have been made to integrate uniform terms regarding the interpretation of OCT-A and study the specific modalities of the technology, but the identification of biomarkers in AMD is still a challenge.

Biomarkers for disease activity, as became clear in the implementation and discussion of the previous task, are poorly studied. Publications and scientific reports on the impact of anti- VEGF therapy on OCT-A markers of progression and activity are few.

The present study is the first to aim to analyze the relationships between markers of progression and activity in naïve and post-therapy CNV.

5.5.1 Effect of anti- VEGF therapy on the perilesional dark halo

The first to study the change of the perilesional dark halo was Mario Rispoli. Rispoli et al (Rispoli M. et al, 2018) described the changes in the perilesional halo in 11 eyes with MDS after intravitreal therapy, and found that in 95.4% of the cohort, anti-VEGF led to a reduction in the halo. Kunho Bae et al (Bae K. et al, 2019) investigated the impact of anti- VEGF therapy on both the morphological characteristics of HBM and the activity markers defined by Coscas et al , but failed to infer a relationship. Sulzbacher et al and Al-Sheikh et al also described biomarkers in HBM with exudative activity, but found no relationship between the applied therapy and their persistence, reduction, or regression.

The results of the present scientific work support the data published so far in the world literature, namely: no connection was established between the perilesional dark halo and the morphological variant of the naïve CNV according to OCT-A. The found dependencies confirm previous publications: the perilesional dark halo decreases after applied anti- VEGF therapy.

None of the collectives cited above managed to find a correlational dependence between the type of naïve CNV and signs of activity. The results of this paper also did not find such a relationship. We can assume that the scars (perilesional dark halo, angiographic connection with the normal vascular network and ramifications in the pathological one) are only indicators of the exudative activity and aggressiveness of the disease, and not a characteristic of a certain morphological variant CNV.

5.5.2 Influence of anti- VEGF therapy on ramifications in the pathological vascular network

Perhaps the best studied among the signs of activity is precisely the presence of ramifications in the pathological vascular network.

Spaide et al (Spaide RF, 2020) and Huang et al (Huang D et al., 2015) found that the number of ramifications decreased after therapy. Their studies were also confirmed by Kuehlewein et al (Kuehlewein L. et al, 2015) , who described a reduction in the number of ramifications, but without changing the caliber of the feeding vessel (in contrast to all other studies on the subject). In contrast to the results of Kuehlewein et al regarding the lack of change in the alimentary vessel after therapy, is also the study of Lumbroso et al: the Italian authors found that after therapy the blood flow in the alimentary vessel was greater, which is also indirect evidence of an increase of vessel caliber and redistribution of blood in the pathological network.

The results of the present study coincide with the data from the world literature - after three intravitreal applications of anti- VEGF with an interval of a month between them, the number of ramifications in the pathological vascular network decreases. This decrease is directly proportional to an increase in the caliber of the membrane-forming vessels. These data support the thesis expressed by a number of authors that during the course of treatment, the vessels of the membrane under the action of anti- VEGF undergo a process of selective maturation: the drug destroys the undifferentiated, giving the opportunity for the development of the vessels in the membrane that are already have reached a certain stage of maturity - they contain pericytes and smooth muscle cells (Lumbroso B. et al, 2015, Spaide RF, 2020).

5.5.3 Effect of anti- VEGF therapy on angiographic relationship

A number of authors indicate the angiographic relationship between the pathological and the normal vascular network as a sign of CNV activity. In the world literature, however, there is no data on its modification after anti -VEGF therapy. The results of this study convincingly emphasize that even after therapy, the angiographic connection with the normal network is preserved, especially in the differentiated membranes ("sea fan", "medusa", "dead tree"). This phenomenon can be explained hypothetically with the above-mentioned theory of Tomita et al: namely, that a part of the neovessels in CNV originate not from the normal vascular network, but from migrated pluripotent stem cells (endothelial progenitor cells) under the action of a hypoxic stimulus and in case of Bruch's membrane damage.

5.5.4 Relationship between the type of morphological variant of HVM and signs of activity

None of the collectives cited above managed to find a correlational dependence between the type of naïve CNV and signs of activity. The results of this paper also did not find such a relationship. We can assume that the scars (perilesional dark halo, angiographic connection with the normal vascular network and ramifications in the pathological one) are only indicators of the exudative activity and aggressiveness of the disease, and not a characteristic of a certain morphological variant CNV.

5.4 Discussion of the correlational dependences between the type of CNV and visual acuity

Decreased visual acuity is usually the first symptom that leads the patient to the ophthalmologist. However, as it is well known, CNVs start far from the macula and a significant decrease in visual acuity is reached when significant anatomical changes occur. The relationship between visual acuity before and after anti- VEGF therapy is well-studied, and numerous studies support the effect of different anti-VEGF preparations on the market, with the therapeutic response being comparable between them (Kaiser PK. et al, 2017 , Rosenfeld PJ. et al , 2006 , Brown DM et al, 2009) .

Some independent risk factors (diabetes, smoking), age, gender and race have been proven predictors of the functionally measured therapeutic effect after anti- VEGF application (Kamao H. et al, 2019).

The relationship between the OCT-A morphological variant of the CNV and visual acuity is poorly studied in the literature, and the data are contradictory.

In 2020, Douglas et al described higher visual acuity after therapy in sea fan and medusa CNV. In the same year, TB Tew et al described better functional outcomes after anti- VEGF in patients with CNV corresponding to the morphological description of "lace". In 2022, Hsu et al (Hsu CR et al, 2022) investigated the predictors of good visual acuity after anti- VEGF therapy in 34 patients with neovascular AMD, but failed to demonstrate a correlation between OCT-A morphological appearance of the membrane and visual acuity after treatment.

In the present work, for the first time, the membranes are divided into a group of "differentiated" and a group of "undifferentiated", which helps to increase the precision and accuracy of the results, since the membranes of the two groups in the present study have been proven to have different behavior in time and response of ongoing therapy.

As discussed above, a number of regularities, which the present work proves, are closely related precisely to the differentiation of CNVs and their maturity - respectively, with their histological origin and structure.

Membranes that are more mature and differentiated have a more stable vascular wall, their exudation decreases over time under the influence of intravitreal drug applications and visual acuity is higher.

Immature vessels are histologically prone to exudation – which leads to poorer visual acuity, more frequent relapses and a greater number of intravitreal injections, which in turn lead to atrophy in the long term (Hsu CR et al, 2022).

5.5 Discussion of the proposed therapeutic protocol

The currently accepted therapeutic regimens are mainly based on mathematical models that are related to the biochemical properties of the administered anti- VEGF medication and their effectiveness has been confirmed in randomized trials such as MARINA and ANCHOR (for the effect of ranibizumab and bevacizumab) and VIEW 1. 2 (for the effect of aflibercept).

For the first time an attempt to individualize the therapeutic regimen was made by Asten et al (Asten van F, 2018) – evaluating 47 patients who were resistant to ranibizumab treatment. Their team managed to derive prognostic dependencies based on independent risk factors such as: gender, age, initial visual acuity, diabetes mellitus, etc. The described protocol does not gain popularity in scientific practice and remains of scientific-theoretical importance.

Everywhere, in clinical practice, the therapeutic regimes are used: fixed, Pro Re Nata, O&P, TET , which are sufficiently schematized and simplified and ensure that therapeutic decisions are made quickly and easily according to the model previously selected by the relevant specialist.

Each of these modes has both its advantages and disadvantages. The fixed regimen provides stability in the achieved functional and anatomical result, but places a huge burden on both the clinical practice of ophthalmologists (need for monthly injections and controls) and on the patient (emotional and time burden related to commitment and financial costs of treatment). The financial costs associated with the treatment of AMD are enormous and worldwide the aim is to reduce the number of injections.

Pro Re Nata and O & P regimens are based precisely on reducing the number of injections, but judging by the results of the literature data, this is often at the expense of a worse final anatomical and functional result, which returns the patient to a starting position with reduced faith and hopes regarding the ongoing therapy. In addition to poorer functional outcomes, these two regimens are also associated with poorer behavioral and emotional response from the patient.

Perhaps the most widely used currently is the TET regimen, which aims to maximize the interval between intravitreal injections, after an initial three loading doses of anti-VEGF and gradually increase the interval between injections by 2 to 4 weeks, up to 16 weeks.

In this dissertation, for the first time, an objectified scheme for an individualized approach to the treatment of patients with neovascular AMD, based entirely on OCT-A marks, is proposed. The suggested follow-up visits provide maximum control over signs of progression and provide monitoring of the disease. The presented protocol for a personalized approach is a modification of TET - starting with 3 loading doses of an antiangiogenic agent. The decision on further behavior is then based on:

1. VEGF injections are continued with an interval of a month between them, until remission is reached.
2. Absence of exudative activity, but dynamics in signs of activity/progression (perilesional dark halo, ramifications in the pathologic network, and presence

of nagiographic connection) - although exudation is absent, the membrane is perceived as "active" and injection proceeds.

There is published evidence in the literature that the appearance of dynamics in activity/progression marks precedes the appearance of exudation by an average of 3 weeks (Marchese A. et al, 2022).

3. Stable condition - in such a case, control examinations with OST-A are performed monthly during the first three months. In the absence of scurf and signs of membrane activity, the interval for follow-up examination and OCT-A assessment is doubled (eg, every 2 months, then every 4, etc.) until a relapse occurs, at which time therapy is initiated.

The key to success in combating the disease is finding the balance between the number of injections, reducing visits and maintaining the achieved therapeutic effect. Such could hardly be achieved with regimens that are not subject to the individual characteristics and course of the disease in each individual patient.

Since the number of patients with AMD is large and the workload of ophthalmologists significant, the proposed protocol for personalized therapy has all the requisites to help achieve the "golden ruke":

1. It is based on objective parameters
2. Individual characteristics are taken into account during the course of the disease
3. The goal is to increase on the interval between anti- VEGF applications with maximum assurance of preservation of the achieved anatomical and functional result.

5.6 Conclusions

1. The analysis performed on the naïve type of CNV, determined by OCT-A and the patient's age, did not establish a statistically significant relationship.

2. The analysis performed on the naïve type of CNV determined by OCT-A and the gender of the patient did not establish a statistically significant relationship.

3. The study indicated a directly proportional relationship between membrane differentiation, determined by OCT-A, after therapy ("sea fan", "medusa", "dead tree") and the observed visual acuity after therapy.

4. The study indicated a significant relationship between membrane differentiation determined by OCT-A and the number of ramifications.

5. Analysis of the relationship between the perilesional halo and the type of naïve CNV determined on OCT-A did not establish such a statistically significant relationship. The demonstrated relationship is in the reduction of the perilesional halo after therapy.

6. The study of the angiographic relationship between the CNV and the normal blood network highlighted its persistence after therapy. This is a characteristic feature of the differentiated CNV, determined according to OCT-A.

CONCLUSION

In recent years, OCT-A has established itself as an imaging technique necessary for both diagnostics of AMD, as well as for monitoring the therapeutic effect of treatment with anti- VEGF preparations. OCT-A is an extremely fast and non-invasive technique that does not require the injection of contrast material intravenously. The methodology has proven high sensitivity and specificity in the detection of neovascular lesions, even at the earliest signs of retinal pathology with high image quality . OCT-A has certain advantages over conventional, contrast-dependent imaging techniques. In addition to the non-invasiveness and practically no contraindications for performing the study, OCT-A provides detailed information allowing microstructural analysis of the retinal vascular network. Thanks to the segmentation capabilities, the physician can differentiate the level of the lesion in four different scan depths, unlike classical FA, where the images are cumulative and dye leakage often masks certain structural details in the surrounding retina.

The increasingly widespread use of OCT-A in clinical practice led to the need to introduce a new descriptive terminology that would classify and uniform concepts for medical professionals from all over the world, in order to be able to rely on objectivity and reliability in the interpretation of the results from scientific studies and the clinical practice of different units.

The ophthalmological world was faced with the challenge of creating a new descriptive classification of CNV based on OCT-A imaging (the familiar and widely accepted classification of CNV by FA is not optimal for use in OCT-A imaging, as the principles of operation and levels of scanning are different for both methods). The morphological variants of CNV became the basis for many studies, new OCT-A markers for activity and progression of CNV were described. As the results of this study demonstrate, the type of naïve CNV and the signs of activity/progression can be taken as biomarkers and be an objective basis for building a successful individualized approach in the treatment of neovascular AMD.

CONTRIBUTIONS

The present dissertation has contributions in the following several directions:

Contributions of a scientific and theoretical nature

- For the first time, the vascular remodeling of naïve CNVs in patients with AMD under the action of anti- VEGF therapy was analyzed
- The proven dependencies and differences in vascular remodeling for the "differentiated" and "undifferentiated" membrane groups have been hypothesized for the different origin of the neovessels in AMD (probably one part originating from the normal vascular network and the other from migrated endothelial-progenitor cells). This hypothesis is the basis for future studies.
- For the first time, a correlation between the type of CNV according to OCT-A and visual acuity after anti- VEGF therapy was demonstrated, i.e. the type of CNV can be considered a biomarker predicting the course of the disease
- For the first time, the effect of anti- VEGF therapy on the signs of activity/progression of AMD was studied - also both the membrane type and dynamics in OCT-A the finding of the signs of activity/progression can be considered a prognostic marker for the course of the disease
- This is the first study to find a correlation between the activity/progression marker "angiographic band" and a specific type of CNV
- For the first time, a correlation between the "number of ramifications in the pathological network" as a marker of progression and a certain morphological type of CNV was found

Contributions of a scientific and applied nature

- A protocol for therapeutic behavior in neovascular AMD has been developed that allows for the customization of therapy for each individual patient based entirely on OCT-A findings (naïve CNV, vascular remodeling, activity/progression marker data)

Contributions of a confirmatory nature

- Morphological OCT-A variants of CNV ("sea fan", "medusa", "dead tree", "filaments", "lace") described in previous studies were confirmed

- VEGF therapy on the number of ramifications in the pathological network described in previous studies was confirmed

- The present study did not find a correlation between the perilesional dark halo and the type of CNV, but confirmed that it decreases in size after anti- VEGF therapy .

PUBLICATIONS AND SCIENTIFIC ANNOUNCEMENTS RELATED TO THE DISSERTATION

I.) Publications

1. **R. Kirkova** , S. Murgova , V. Kirkov , T. Dimitrov, G. Balchev , I. Tanev. Optic coherence tomography-angiography – a new technique in the diagnosis and follow-up of patients with age-related macular degeneration – an overview. Journal of Biomedical and Clinical Research, 2024, 17(1): 1-8; ISSN: 1313-6917; Web of Science (CABI)
2. **Kirkova R.** , Murgova S., Tanev I. Makulna age - related degeneration - new diagnostic possibilities . GP medic, 2022, 4(2): 50-52; ISSN: 2603-4719
3. Dimitrov Tsv ., **R. Kirkova** . Ours experience with COVID-19. Bulgarian ophthalmic review , 2022, 1(66): 29-33; ISSN: 1311-0624

II.) Scientific communications

1. 2023 FLORETINA/ICOOR - participation with oral presentation " EFFECTS OF CATECHINS AND EPICATECHINS ON VISUAL FUNCTION AND RETINA PERFUSION" R. KIRKOVA, I. TANEV
2. 2023 SICCSO, Catania, Italy participation with oral presentation "Surgically-induced, pharmacologically mediated endothelial corneal proliferation" R. KIRKOVA, I. TANEV
3. 2022 ICOOR, FLORETINA participation with oral presentation "PERFUSION CHANGES IN OPTIC NERVE HEAD IN GLAUCOMA: AN OCT-A STUDY" R. KIRKOVA
4. 2021 - award for a young ophthalmologist with a scientific contribution in the name of Prof. Dr. Stoimen Dubov with the topic "Vascular remodeling after anti-VEGF therapy in patients with neovascular age-related macular degeneration" - Radina Kirkova
5. 2021 ICOOR/Floretina, Roma - participation with oral presentation OCT-A pattern changes in patients with neovascular AMD" – Radina Kirkova, Ivan Tanev
6. 2021 EVER Festival: poster on: VASCULAR REMODELLING AFTER ANTI-

VEGF TREATMENT IN PATIENTS WITH NEOVASCULAR AGE-RELATED MACULAR DEGENERATION - Kirkova R., Murgova S., Tanev I

7. XXV National Congress of the Bulgarian anatomical society with international participation" oral presentation - topic "Anatomical retinal image analysis with OCT" - Bahar, Balchev, Kirkova, Murgova