“LUCIAN BLAGA” UNIVERSITY OF SIBIU

FACULTY OF MEDICINE

SUMMARY OF THE PhD THESIS

CONTRIBUTION OF FLUORESCEIN ANGIOGRAPHY IN DIAGNOSIS AND TREATMENT OF DIABETIC RETINOPATHY

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1. INTRODUCTION

Diabetes mellitus is a metabolic disease known since ancient times, characterized by hyperglycaemia with its severe variations, secondary to the decrease of endogenous insulin efficiency. Diabetes has become an epidemic in this century, while increasing the standard of living.

Diabetic retinopathy is the most common retinal vascular disease. It is a micro vascular complication of diabetes and the leading cause of blindness in the working population in most industrialized countries.

The main causes of severe visual loss in diabetes are the complications of proliferative diabetic retinopathy and macular edema, respectively. This has meant that in recent years, the interest in diabetic eye complications increased exponentially.

Fluorescein angiography was successfully used in ophthalmology since the early 1960s. It is a method of recording the dynamic vascular perfusion, minimally invasive, with particular importance in treatment planning of the clinically significant macular edema in all cases where visual damage is not explained by fundus picture (ischemic maculopathy) and in the differentiation of retinal neovascularization of intraretinal microvascular abnormalities. Also, early retinal changes in diabetes are visible on angiofluorography because they consist of increased vascular permeability.

Ocular optical coherence tomography is an innovation in ophthalmology, the objective standard for identifying macular edema, a noninvasive investigation, fast and non -contact which tends to replace the conventional subjective methods. In recent years, optical coherence tomography has evolved from a research tool to a procedure commonly available in ophthalmic surgery for the diagnosis and monitoring of patients with macular disease and also for obtaining the measurements used in clinical trials.

In this paper, I aimed at evaluating the importance of fluorescein angiography in early diagnosis and correct treatment of diabetic retinopathy, as well as at contributing to a better understanding of the etiopathogenesis of the disease, offering a medical advice as accurately and useful for the diabetic patients.

2. REASONS FOR CHOOSING THIS TOPIC

The management of diabetic retinopathy is a common theme addressed in ophthalmology, and the decision regarding the maximal treatment is subjective and difficult to standardize.

As an ophthalmologist, I encounter in my practice, many cases of patients with diabetic retinopathy who are interested in the evolution of the disease and the possibilities of investigation and treatment, and fluorescein angiography, although very valuable, is apparently losing its place still irreplaceable by any other investigation.

Recent studies have attempted to demonstrate that fluorescein angiography is now more optional rather than mandatory. Approximately 50% of patients in the Diabetic Retinopathy Clinical Research Network study (DRCR Network) and up to 80 % of the patients in the developed countries are not treated with AFG, probably there is even a higher percentage for the less developed countries. [77,111]

Diabetic retinopathy behaviour, the continuous and unpredictable evolution, with the possibility of aggravation and the final loss of visual function, high frequency of severe forms of the disease, the risk of duplication the painful and debilitating conditions, the impossibility to control this disease with the entire current therapeutic arsenal and the chance to prevent complications through a proper diagnosis and treatment, are all reasons for choosing this study subject.

The need for this subject also derives from the following requirements of the management of diabetic retinopathy:

• periodic ophthalmologic examinations, with the establishment of the investigations indicated for a correct diagnosis and treatment, focusing on proper diet, and observance of the individual therapeutic management.

• medical treatment established in a multidisciplinary manner, depending on associated diseases.

• laser treatment, in this case fluorescein angiography is mandatory, being the only investigation able to identify retinal hypoxic areas and which can properly guide the laser treatment.

• intravitreal injections in selected cases.

3. PURPOSE OF THE PAPER

In this study, I aimed at highlighting the problems that can occur in early diagnosis, accurate staging of diabetic retinopathy and fluorescein angiography importance in monitoring diabetic patients.

In this paper, I also tried to evaluate the sensitivity and specificity of retinal fluorescein angiography in patients with diabetic retinopathy and thus, the contribution of this method in the diagnosis and optimal therapy for these patients, to the extent in which this method could be considered another routine diagnostic test for the patients with diabetic retinopathy.

In elaborating the paper, I aimed at finding fluorescein angiography changes present in infraclinical diabetic retinopathy, as well as the angiofluorography changes existing in the advanced forms of the disease.

The purpose of this paper is to establish an algorithm for diagnosis and treatment as close to ideal, ensuring optimum results, with the reduced incidence of immediate or late complications that arise in the development of diabetic retinopathy.

This special part of the work indicates the importance of recognizing diabetic retinopathy in an early stage in order to establish the appropriate therapy and to prevent the risk of blindness.

The objective of this paper is to observe the presence of all fluorescein angiography changes in diabetic retinopathy in different evolutionary stages. Fluorescein angiography has the advantage that it is a minimally invasive method, repeated with a high reproducibility.

The information obtained is particularly useful in completing the clinical picture in the patients with diabetic retinopathy in general and especially for diabetic maculopathy.

I also wished to point out that “the patient should be one of the most important parts of the treatment itself” [29]. I take into consideration both the advantages and the disadvantages of each method of investigation and treatment and like patients, I prefer the non-invasive methods, and when I consider it necessary and do not have a clear result, in agreement with the patient, I perform the angiofluorography method.

4. MATERIAL

This paper is a retrospective study, begun in 2009, conducted on a sample of 248 patients hospitalized in the Department of Ophthalmology within the Clinical Emergency County Hospital of Sibiu, in the period from 2009 to 2012, with a diagnosis of diabetic retinopathy at different stages of development or diabetes mellitus diagnosed 10 years ago.

Of the 248 cases, 64 cases were also investigated through angiofluorography. I took into consideration the following characteristics:

- patients' age;
- gender;
- area of origin;
- the time since the diagnosis of diabetes;
- the type of diabetic retinopathy, ETDRS classification (Early Treatment Diabetic Retinopathy Study) before and after angiofluorography;
- type of diabetic maculopathy before and after angiofluorography;
- macular volume and central macular thickness measured by OCT
- visible changes on angiofluorography;
- complications and associated deficits.

I used the Carl Zeiss retinophotograph (figure no. 14) of the "Dr. Stănilă" Medical Centre of Sibiu and for every patient, I used 5 ml of 10% fluorescein from the original sterile container. For pupil dilation, I used drops of 1% tropicamide (Mydriacyl) and 2.5 % to 10 % phenylephrine (Neo - Syn - efrina). As a general protocol, I originally used a drop of each solution in each eye. If after 10 minutes, I did not get proper pupil dilation, I added another set of drops. A third row of drops was needed especially in the patients with very dark eyes, in patients with glaucoma medication or following cataract surgery.

All investigations were made in the presence of a physician specialized in intensive, care, equipped with emergency oxygen source, stethoscope, sphygmomanometer, intubation kit, intravenous needles, fluids, intravenous medication, epinephrine and an antihistamine.

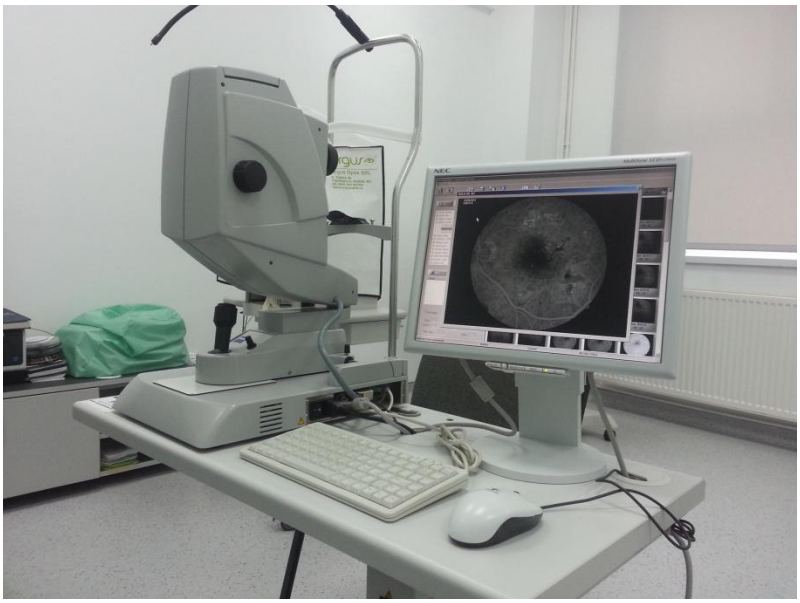


Figure no. 1. Carl Zeiss Retinal photograph
(Dr. Stănilă Medical Centre -Sibiu)

For the quantitative assessment of macular edema, the 64 patients were OCT investigated. To achieve tomography scans, I have used the Carl Zeiss Cirrus HD-OCT (Figure no. 2) of the Dr. Stănilă Medical Centre in Sibiu.

Figure no. 2. Cirrus HD-OCT Carl Zeiss Tomograph
(Medical Centre Dr. Stănilă-SB)

5. METHOD

Patients' data in the study were taken from the records and daily admissions register of the Department of Ophthalmology within the Clinical Emergency County Hospital Sibiu and from the consultations record of the Dr. Stănilă Medical Centre in Sibiu.

I established the inclusion and exclusion criteria in the study, in order to obtain a homogeneous sample of patients (Table 1):

Inclusion criteria	**Exclusion criteria**
The diagnosis of diabetic retinopathy	Severe chronic renal failure
Patients diagnosed with diabetes 10 years ago	Significant changes in ocular media transparency (cataract, corneal leukoma, vitreous hemorrhage)
Follow-up in time	Pregnancy
Patients' agreement to cooperate in the study	History of allergy

Table no. 1 Study inclusion and exclusion criteria

Initial ophthalmologic examination of the people with diabetes included:

- history (type of diabetes, duration of diabetes, glycemic control, current medications, a history of systemic and ocular pathology);
- corrected visual acuity testing, visual disturbances occur early if the lesions are produced in the macula region and the sudden loss of vision may be due to large retinal hemorrhages interesting the macula, vitreous bleeding or retinal detachment;
- biomicroscopy examination of the anterior pole before mydriasis to identify iris neovasculature;
- ophthalmoscopy, including stereoscopic examination of the posterior pole;
- intraocular pressure measurement;

Regarding the complex methods of investigation, I used fluorescein angiography (FA) and optical coherence tomography (OCT) in a group of 64 patients who were informed and explained about the importance and necessity of these investigations, obtaining their written consent (patient data sheet model - table no. 2).

PATIENT INFORMATION SHEET

RETINAL FLUORESCEIN ANGIOGRAPHY

Dear patient,

Special retinal examination has been recommended to you, using a contrast agent, an investigation called angiofluorography.

In view of this examination, pupil dilation is required prior, by using some drops. Contrast substance, called fluorescein is injected intravenously and will spread in the blood and in few seconds, it will reach the eye.

Choroidal and retinal circulation will be photographed with a special camera and the images will be recorded in the computer.

Angiography is a method of eye examination and not a therapeutic manoeuvre.

In most cases, both eye drops and the injected contrast agent are well tolerated by patients. Occasionally, the following adverse effects may occur: dizziness, vomiting and rarely acute glaucoma or circulatory collapse.

After angiography, a temporary skin colouring in yellow is found, as well as darkening of the urine, effects that disappear within 24-48 hours.

If you have any questions about this examination, please consult your ophthalmologist before performing fluorescein angiography!

Date: Signature of the physician performing the investigation

PATIENT'S NAME:

ADDRESS:

Please answer the following questions:

- Do you take medications?

  If yes, what and in what dosage?
- Do you suffer from any of the following diseases?
  - Movement disorders, hypertension?
  - Heart Disease
  - Liver Disease
  - Renal disease
  - Diabetes
  - Diseases of blood
- Are you allergic?
- For women: Are you pregnant or breastfeeding?

I have carefully read the explanations and the questionnaire above and I understand them. I asked the ophthalmologist about the concerns that I had about this investigation.

Following the explanations received, I decided to perform / not perform the above examination.

Date, hour: Patient's signature:

EYE EXAMINATION

Visual acuity	OD	OS
Refraction	OD	OS
Magnification	OD	OS
Tonometry	OD	OS
Retinophotography	OD	OS
Optical coherence tomography	OD	OS

Table no. 2. PATIENT DATA MODEL

5.1. Fluorescein angiography technique

As with any medical procedure, fluorescein angiography (FA) outcomes are improved when patients are explained the technique, so that they understand what will happen during the test. These explanations have the role to banish any fears and preconceptions, being an integral part of the informed consent necessary before the examination.

We prepared the patient for at least 30 minutes before the instillation of the mydriatic procedure, so that the pupils are dilated sufficiently to allow good quality pictures.

Fluorescein injection (5 ml of 10%) was achieved successfully through a central line catheter of 21 to 25 gauge needle attached to a 5 ml solution containing fluorescein.

More photographic protocols have been proposed for use in the study of diabetic retinopathy and macular edema. [25,86]

In the presence of diabetic retinopathy, it is recommended a protocol of 7 visual fields. It allows good coverage of the posterior pole and the upper and lower arches. Figure no. 3 shows the location of these fields and we can appreciate that using a 30-40 degree camera, the coverage area could extend to 90 degrees diagonally from side to side.

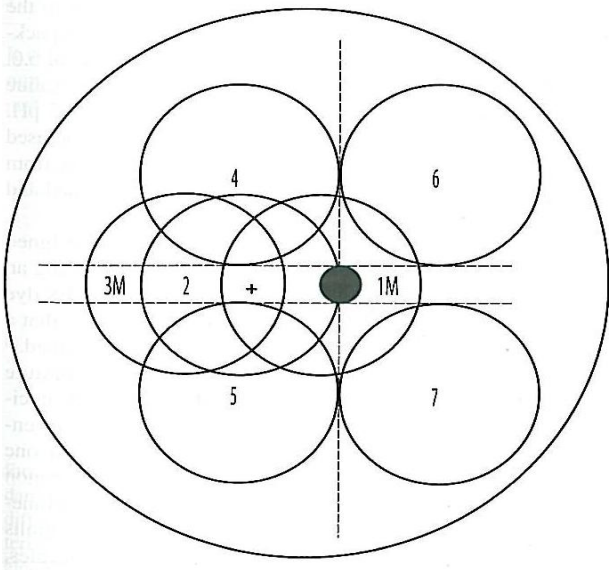


Figure no. 3. Modified protocol with seven standard fields for the analysis of diabetic retinopathy (J. Fernnando Arevalo - Retinal Angiography and Optical Coherence Tomography)

This is a modified protocol with 7 visual fields used in 1980 and it is derived from the Early Treatment Diabetic Retinopathy Study (ETDRS). [28]

After dye injection, I quickly obtained (one second) a series of digital photos. This allowed a proper study of early filling and angiography transient frames.

Eyeball with priority interest has been identified and positioned at camera at the beginning of angiography. After about 45 seconds of photos obtained from the first sight, I switched to the other eye in order to record intermediate frame images.

The final framework images were then recorded some minutes later from each eyeball, in order to capture the late times of fluorescein movement. [87]

The first retinal changes that can be detected on fluorescein angiography are: localized or generalized vasodilatation, segmental occlusion, microaneurysms and permeability changes. [26,43,64]

5.2. Optical coherence tomography

OCT is a noninvasive method of choice, which detects, monitors and evaluates quantitatively macular edema, retinal thickness being correlated with increased reflectivity changes in the retinal layers and the emergence of the nonreflective cystic fluid or low reflectivity in the external retinal layers (due to the attenuation of the optic signal entering and reflecting at this level). [2,7,22]

Regarding the purchase protocol, I used the 512x128 macular cube. Within the analysis of the cube, an automated algorithm is used specifying the internal limiting membrane and the retinal pigment epithelium, layers used as the basis for the measurement of macular thickness and macular volume.

5.3. Statistical analysis

For statistical data processing, I used the SPSS software, version 19.

Methodology: The patients were studied as a global group, taking into account the parameters listed above, but also as separate batches, divided by the type of diabetic retinopathy.

For data analysis, I used frequency tables that include two columns: one for the distinct values of the analysed variable and the other for the frequencies of these values. For the

presentation of the frequencies, I also used contingency tables, the variables having multiple values; in tables, I presented the frequencies of the occurrence of the ordered pairs of values. I used these tables to study the relationship between two or more variables.

Checking the condition of normality of the studied variables was done using the "One Sample Kolmogorov -Smirnov Test" with the Null Hypothesis "variable distribution is normal" and the Alternative Hypothesis "variable distribution differs from the normal one " level of significance values greater than 0.05 indicating a normal distribution of the variable, and values less than 0.05 indicating that the variable values do not follow the normal distribution.

The comparison of the averages of two groups, in the case of the independent samples, in which values distribution was normal was made by using the "Independent -Samples T Test" with the Null Hypothesis "there are no significant differences between averages" and the Alternative Hypothesis: "there is a significant difference between the average" level of significance values greater than 0.05 indicating a significant difference between averages and values less than 0.05 indicating that the averages of the two groups did not significantly differ . When comparing the average of multiple groups, I used the "ANOVA" test, the interpretation of the significance level being similar. If the distribution of values was not normal, I used the nonparametric tests based on ranks: "Mann -Whitney" with the same level of significance of 0.05.

The relationship between the quantitative variables was performed by Pearson correlation coefficient analysis. In case of a combination of a risk factor and the occurrence of a disease, the RR (relative risk) was calculated, and if the RR is 1, the risk is the same in both groups, if the RR is less than 1, the risk in the non-exposed is higher and if the RR is higher than 1, the risk in the exposed is higher.

Data representation was performed using pie charts, bars charts, charts with bars grouped for association tables, lines, histograms, boxplot for highlighting the indicators of central tendency and variables distribution.

Descriptive statistics (mean value and standard deviation) was used to show the basic features.

Statistical analysis, data processing, the graphs and tables were made using the Microsoft Office computer programs.

6. RESULTS AND DISCUSSIONS

Between January 2009 and December 2012, in the Department of Ophthalmology within the Clinical Emergency County Hospital Sibiu, 4924 patients were hospitalized with diverse ocular pathology.

Of these, 221 cases (138 women and 83 men) were diagnosed with diabetic retinopathy patients, representing 4.49% of admissions (120 patients with NPDR and 101 patients with PDR) and 27 cases were patients diagnosed with diabetes mellitus 10 years ago without diabetic retinopathy changes, representing 0.54% of admissions (figure no. 4).

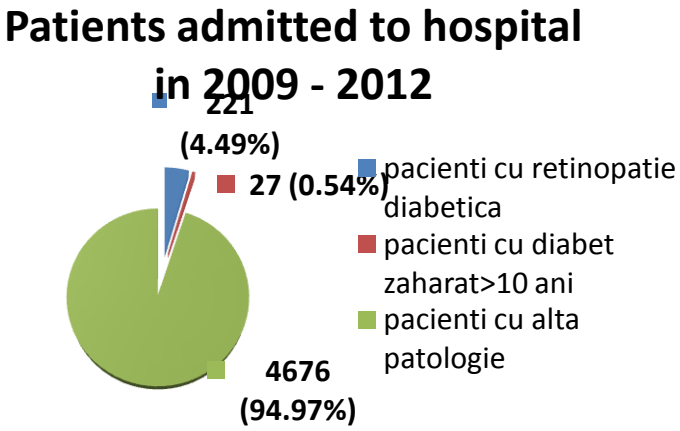


Figure no. 4. Patients admitted in the Ophthalmology Department during 2009 – 2012

Of the 248 patients with DR and type 2 diabetes diagnosed 10 years ago, I performed the angiofluorography in a number of 64 patients (Figure no. 5): after slit lamp fundus examination, 61 patients were diagnosed with diabetic retinopathy, and in 3 patients diagnosed with type 2 diabetes over 10 years ago, I did not find ophthalmoscopic changes characteristic of diabetic retinopathy.

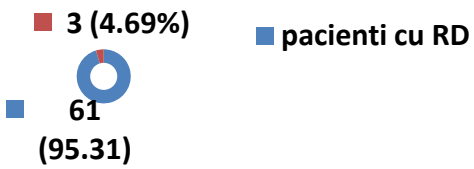


Figure no. 5. Patients examined by FA

Of the 64 patients admitted to the Department of Ophthalmology within the Clinical Emergency County Hospital of Sibiu from 2009 to 2012, FA investigated, 36 cases (56.25%) were women and 28 cases (43.75%) were males (Figure no. 6).

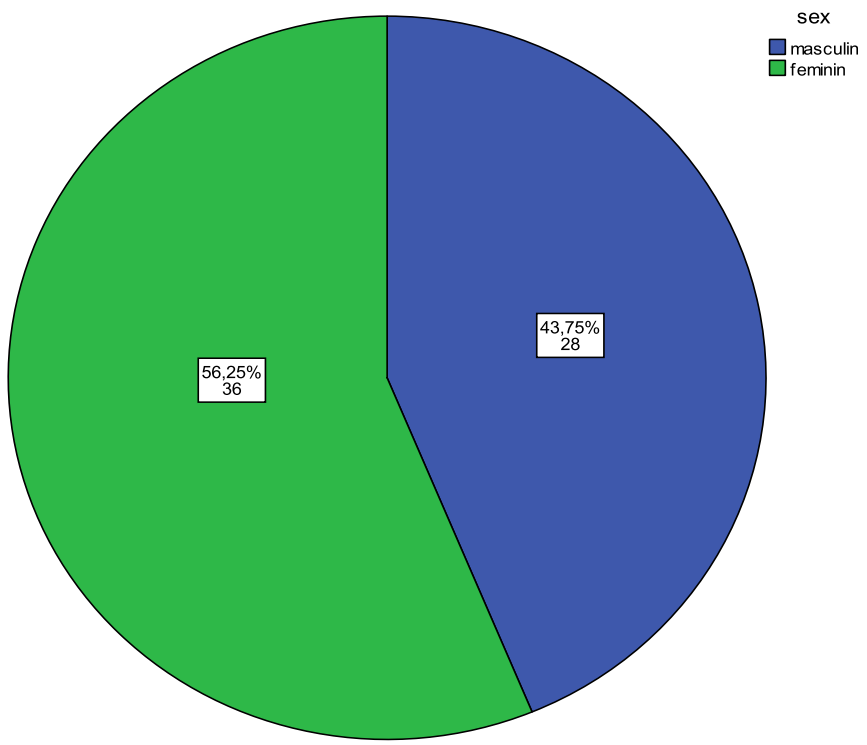


Figure no. 6. Gender distribution of the number of cases investigated by FA with DR and diabetes >10 years

A slightly higher incidence is observed among women (up 12.5%). This is due to the fact that the female population registers a higher number (51.40% women, 48.59% men in the general population) and there is a higher average lifespan in women - 76.06 years, compared to 68.96 years for males.

Of the 221 patients with diabetic retinopathy hospitalized in the Department of Ophthalmology within the Clinical Emergency County Hospital Sibiu from 2009 to 2012, 126 patients were from urban areas and 95 patients were from rural areas. With reference to FA investigated cases, 42 patients were from urban areas, representing 65.6% of the total of 64 patients and 22 patients were from rural areas, representing 34.4% of the total (figure no. 7).

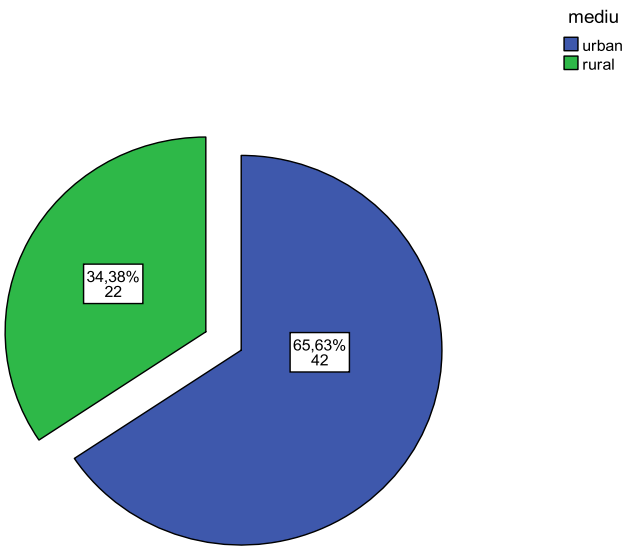


Figure no. 7. Patients' distribution according to area of origin and to the number of FA investigated cases with DR and diabetes >10 years

Also, the ratio of the general population of Sibiu, depending on the area of origin is of 67.44% to 32.55% for the urban environment.

The average age of the patients investigated by angiofluorography was of 59.73 years old (SD = 8,156) and the results of the distribution by age groups were the following:

- Under 40 years - 1 patient (1.56%)
- Between 40 years old and 49 years old - 4 patients (6.25%)
- Between 50 years old and 59 years old - 29 patients (45.31%)
- Between 60 and 69 years old - 21 patients (32.81%)
- Between 70 years old and 79 years old - 8 patients (12.5%)
- Over 80 years old - 1 patient (1.56%) (Figure no. 8).

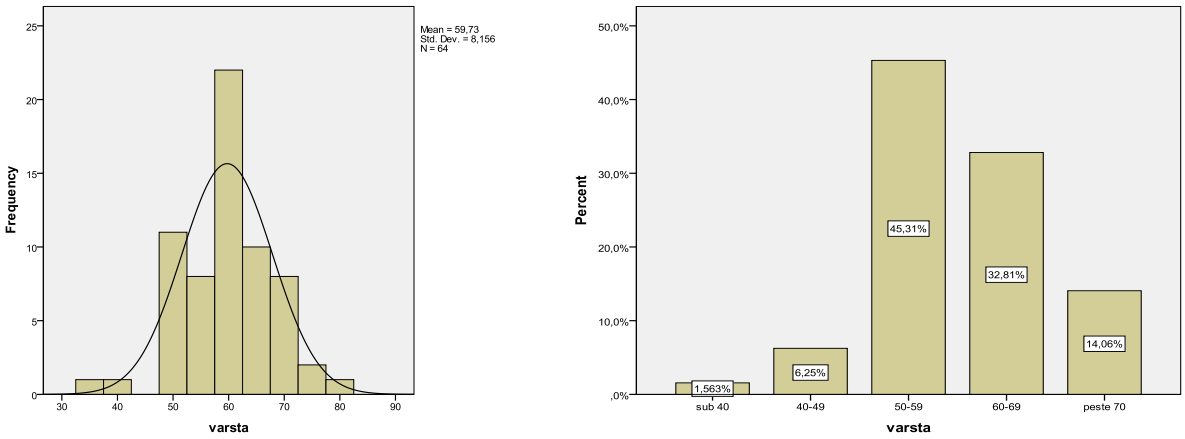


Figure no. 8. Distribution by age groups of the number of cases with DR and diabetes > 10 years

There can be observed a high incidence of diabetic retinopathy in the range of 50-59 years old (45.31% of patients).

I analyzed the central macular thickness and macular volume and I found that there was a significant positive correlation between macular thickness and macular volume (r = 0.830, p = 0.000, CI 99%), without any correlation between the two ones and age (r = 0.226, p = 0.072> 0.05, r = 0.106, p = 0.405> 0.05) (Figure no. 9).

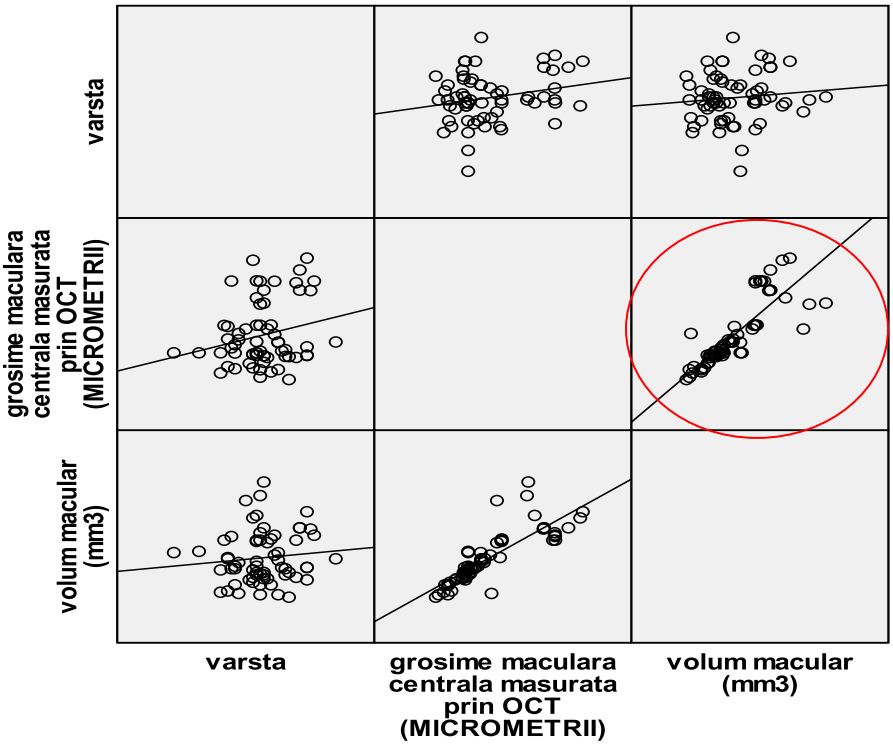


Figure no. 9. Correlation between central macular thickness, macular volume and patient age

I comparatively analyzed eye fundus appearance in the 64 patients before and after fluorescein angiography (figure no. 10 and figure no. 11) and I identified two patients with early signs of diabetic retinopathy which were not detected at the slit lamp fundus examination: 61 cases of diabetic retinopathy by slit lamp examination of the fundus, compared with 63 cases of diabetic retinopathy after FA examination (table 5).

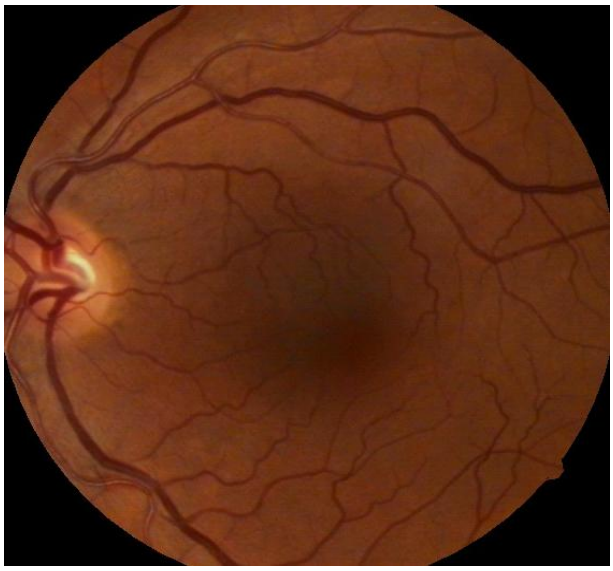


Figure no. 10. Left eye retinal photography (L.D. Dr. Stănilă Medical Centre Archive -SB

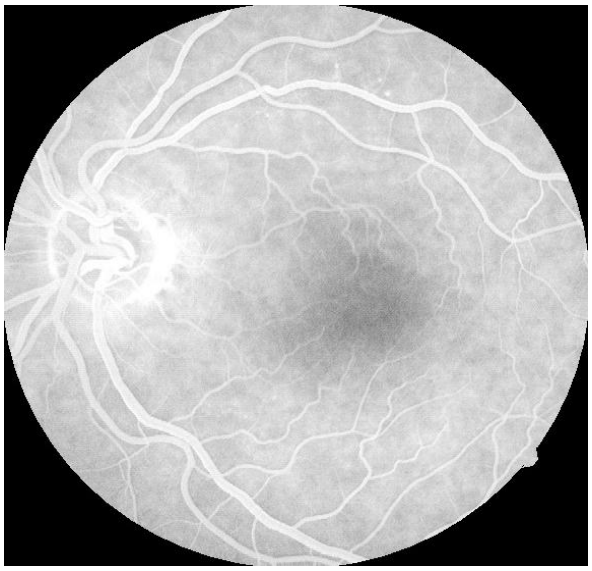


Figure no. 11. FA aspect, left eye middle phase (L.D. Dr. Stănilă Medical Centre Archive -SB)

		Nr.	%
Dg. 1: diagnostic initial (dupa examinarea biomicroscopica a FO)	RD	61	95,3%
	DZ dg. In urma cu peste 10 ani fara semne oftalmoscopice de RD	3	4,7%
Dg. 2 (dupa efectuarea AFG)	RD	63	98,4%
	DZ dg. In urma cu peste 10 ani fara semne de RD	1	1,6%

Table no. 5 Comparative analysis of cases of diabetic retinopathy before and after FA

After slit lamp examination of the eye fundus, I had 68.8% (44 patients) with NPDR, 26.6% (17 patients) with PDR and in 4.7% (3 patients), I found no signs of diabetic retinopathy. Most patients (43.7% -28 patients) were aged between 50 and 59 years old, and of these 79.3% (23 cases) were NPDR and 17.2% (5 cases) were PDR.

After the FA examination, I had 57.8% (37 patients) with NPDR and 40.6% (26 patients) with PDR. Most patients (45.3% -29 cases) were aged between 50 and 59 years old, and of these 69% (20 cases) were NPDR and 31% (9 cases) were the PDR.

I made a comparative analysis of the types of diabetic retinopathy, after slit lamp examination of the eye fundus, FA respectively, by age and I found that there are not significant changes between them (figure no. 12).

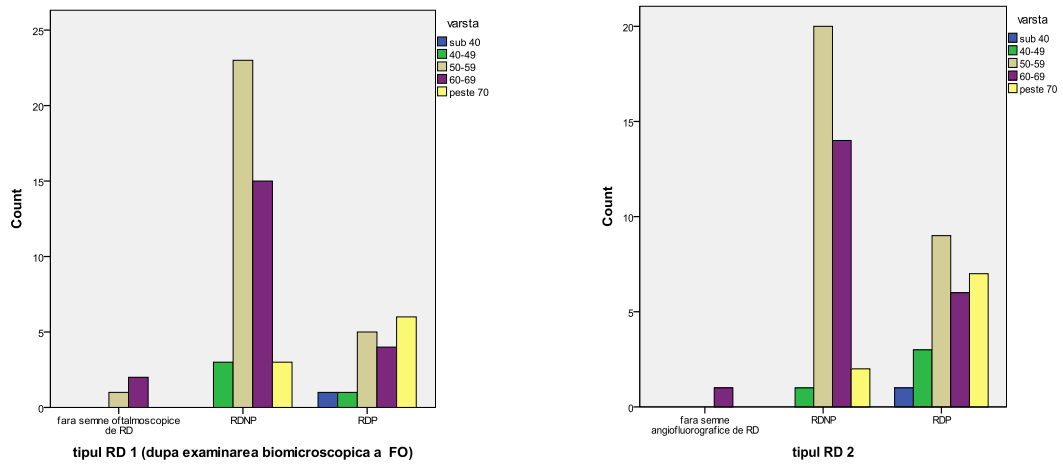


Figure no. 12. Comparative analysis of the types of DR after slit lamp examination of the fundus, FA respectively, by age

I did a statistical analysis between the type of diabetic retinopathy after fundus biomicroscopy examination and fluorescein angiography and I noticed that 22.7% (10 cases) are NPDR, according to the type of diabetic retinopathy after fundus biomicroscopy examination and according to the type of diabetic retinopathy by fluorescein angiography examination, they are PDR (p = 0.000) (Figure no. 13 Figure no. 14, Figure no. 15).

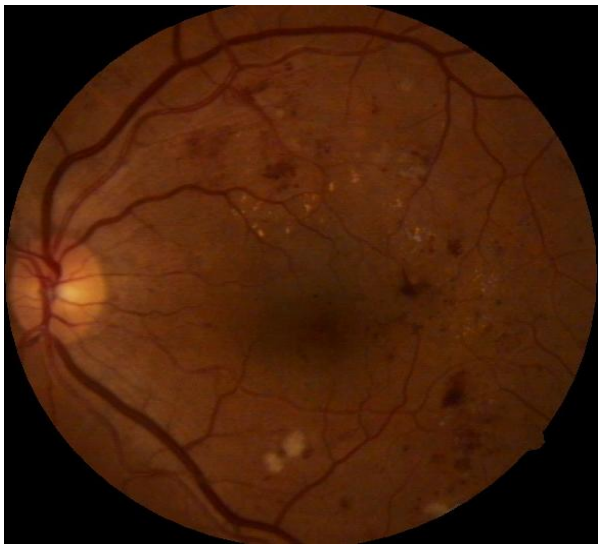


Figure no. 12. Left eye retinal photography (B.C. Dr. Stănilă Medical Centre - Archive -SB

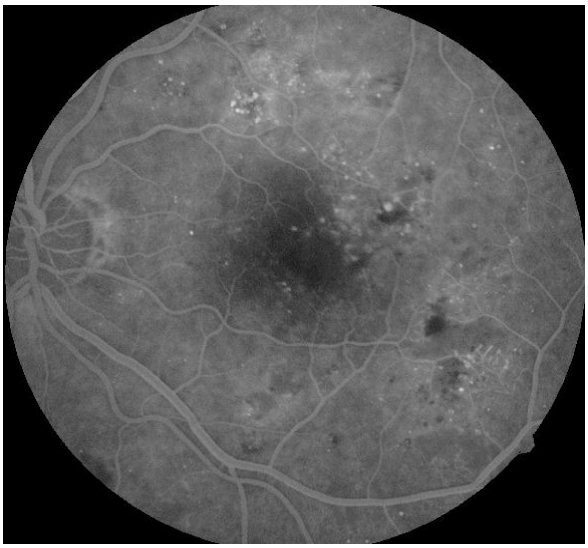


Figure no. 13. FA aspect, left eye middle phase (B.C. Dr. Stănilă-Medical Centre - Archive SB)

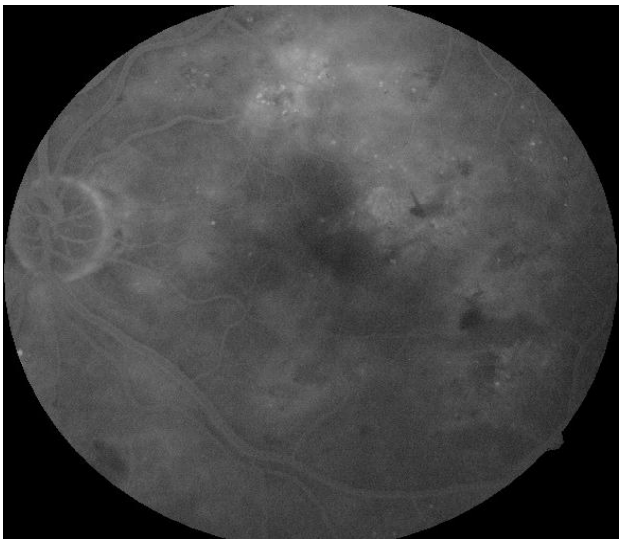


Figure no. 14. FA aspect, left eye late phase
(B.C. M Dr. Stănilă Medical Centre - Archive -SB)

I made a comparison between the subtype of diabetic retinopathy after slit lamp fundus examination and after fluorescein angiography examination (figure no. 16):

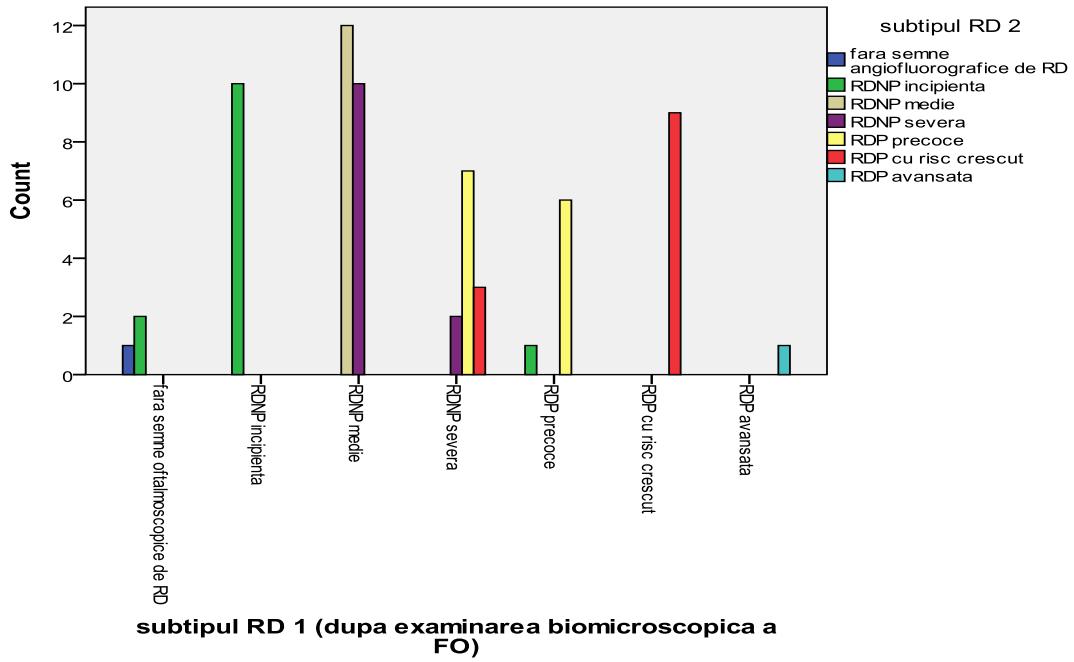


Figure no. 16. Association between the subtype of DR after biomicroscopic examination of the fundus and after the FA examination

I noticed that 45.5% of patients (10 cases) have moderate NPDR, according to the type of diabetic retinopathy after fundus biomicroscopy examination and have severe NPDR according to the type of diabetic retinopathy by fluorescein angiography examination (p = 0.000). The diagnosis of severe NPDR was established using the FA examination which revealed intraretinal micro abnormalities that were not identified on fundus biomicroscopy (Figure no. 17 and Figure no. 18).

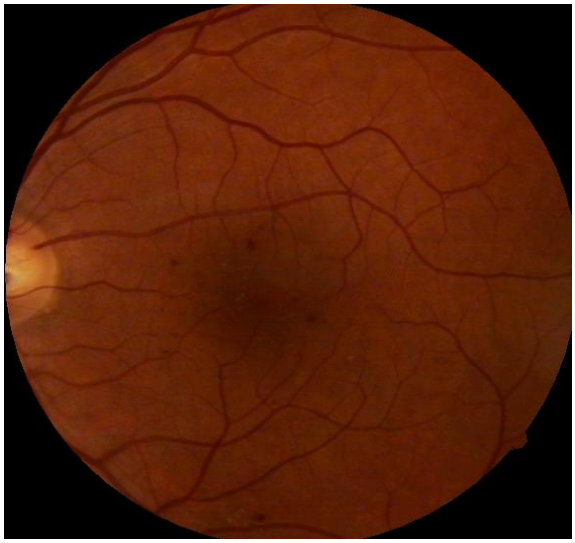


Figure no. 17. Left eye retinal photography (G.R. Dr. Stănilă-Medical Centre - Archive SB)

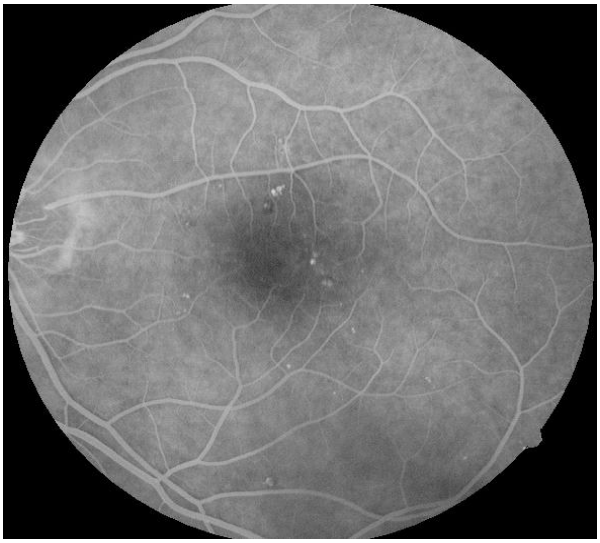


Figure no. 18. FA aspect, left eye medium phase (G.R. Dr. Stănilă Medical Centre - Archive -SB)

In clinically significant macular edema, proliferative diabetic retinopathy, the severe form and the proliferative diabetic retinopathy, immediate laser photocoagulation ("gold standard") is required at the level of the eye fundus in order to prevent complications that could lead to blindness.

Diabetic maculopathy was found on FA and I followed-up the topography and the size of lesions in view of laser treatment:

- Focal maculopathy appears as a circumscribed area of "leakage" with late focal hyperfluorescence (figure no. 19);
- Diffuse maculopathy is seen as an area of generalized "leakage" with early spotted hyperfluorescence of the microaneurysms and late diffuse hyperfluorescence (Figure no. 20);
- Ischemic maculopathy is highlighted as an area of nonperfusion with foveolar broad and irregular hypofluorescence (Figure no. 21);

- Mixed maculopathy - occurs by combining diffuse macular edema appearance ischemia (Figure no. 22);

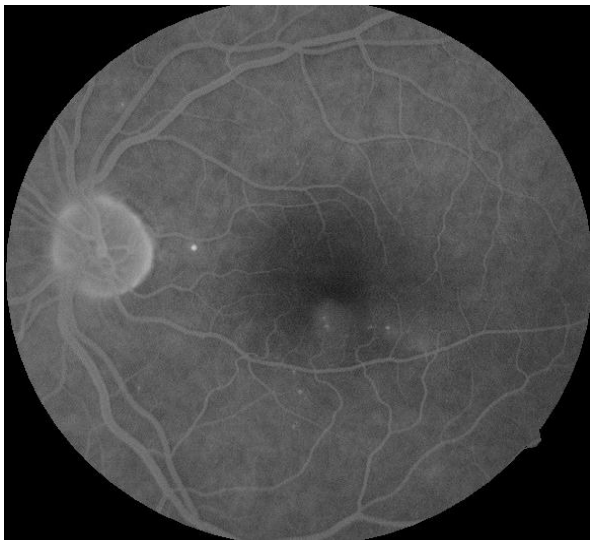


Figure no. 19. FA aspect, left eye medium phase (B.I. Dr. Stănilă-Medical Centre - Archive SB)

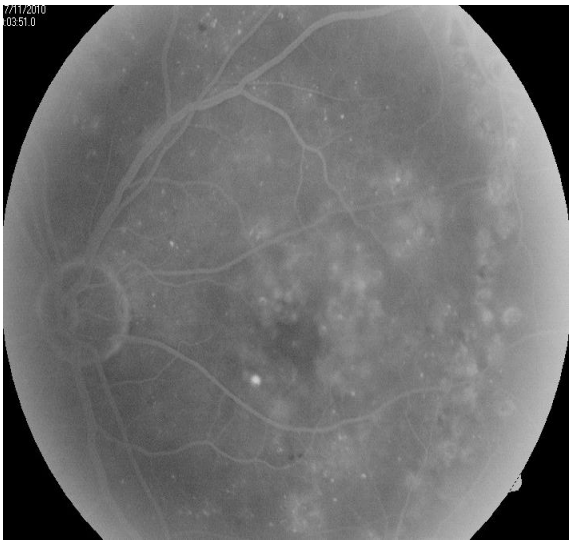


Figure no. 20. FA aspect, left eye medium phase (S.M. Dr. Stănilă Medical Centre - Archive -SB)

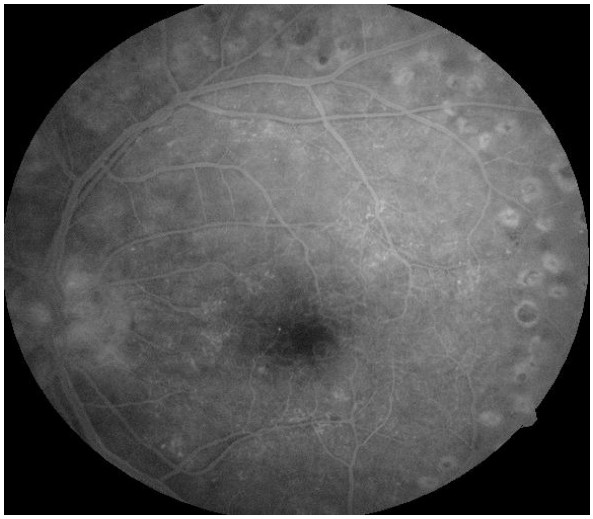


Figure no. 21. FA aspect, left eye medium phase (B.I. Dr. Stănilă Medical Centre - Archive SB)

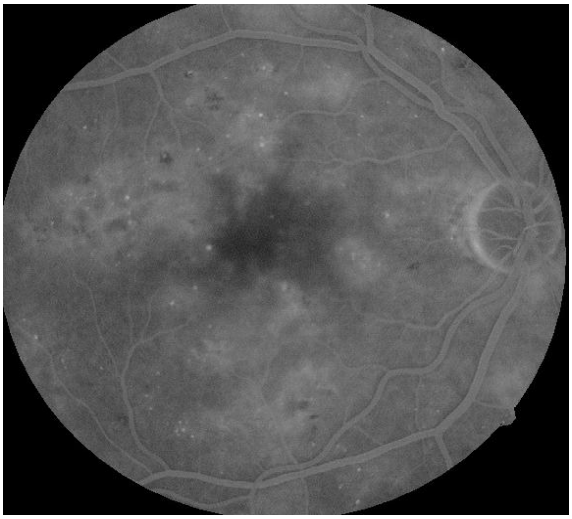


Figure no. 22. FA aspect, right eye medium phase (B.C. Dr. Stănilă Medical Centre - Archive -SB)

The distribution of the types of diabetic maculopathy after fluorescein angiography examination: 48 patients (75%) had diabetic maculopathy by angiofluorography: focal - 10 patients (15.6%), diffuse - 7 patients (10.9%), ischemic - 3 patients (4 , 7%), mixed - 28 patients (43.8%).

I made an association between the presence of diabetic maculopathy after fundus biomicroscopic examination and the presence of diabetic maculopathy after fluorescein angiography examination (Figure no. 23 and Figure no. 24): I identified 11 cases (40.7%) in whom after a slit lamp examination of the fundus, there has not been reported the presence of diabetic maculopathy and after FA examination, I noticed the presence of diabetic maculopathy ($p = 0.000$) (Table no.4).

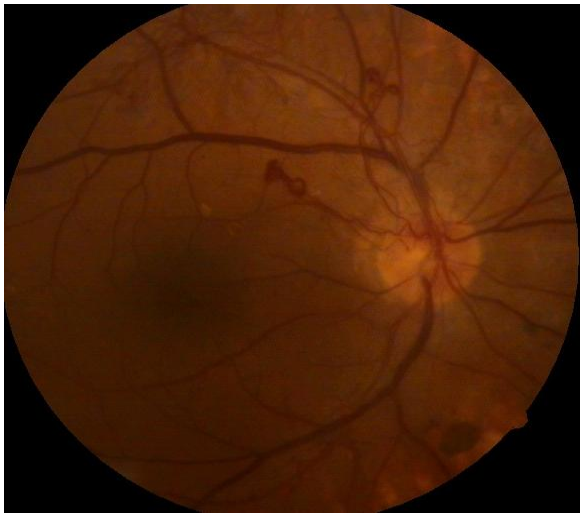


Figure no. 23. Right eye retinal photography (B.I. Dr. Stănilă Medical Centre - Archive -SB)

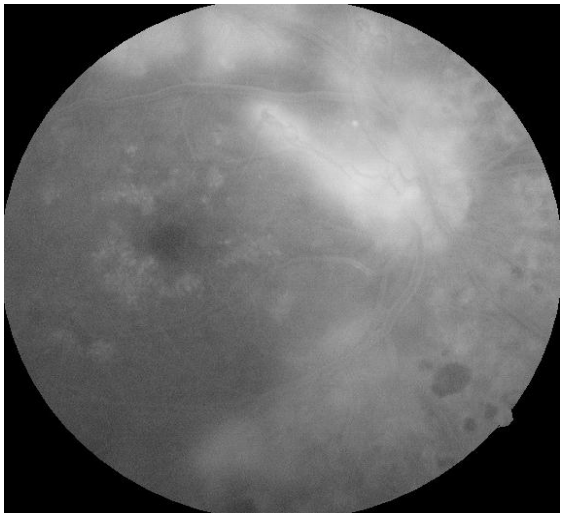


Figure no. 24. FA aspect, right eye medium phase (B.I. Dr. Stănilă-Medical Centre - Archive SB)

			prezenta MD 2		Total
			DA	NU	
prezenta MD 1	DA	Count	37	0	37
		% within prezenta MD 1	100,0%	,0%	100,0%
		% of Total	57,8%	,0%	57,8%
	NU	Count	11	16	27
		% within prezenta MD 1	40,7%	59,3%	100,0%
		% of Total	17,2%	25,0%	42,2%
Total		Count	48	16	64
		% within prezenta MD 1	75,0%	25,0%	100,0%
		% of Total	75,0%	25,0%	100,0%

Table no. 4. The association between the presence of diabetic maculopathy after slit lamp examination of the fundus and after FA examination

I also made an association between the type of diabetic maculopathy after the biomicroscopic examination of the fundus and the type of diabetic maculopathy after fluorescein angiography: it is noticed that there are 11 cases without ophthalmoscopic signs of diabetic maculopathy in whom fluorescein angiography examination shows 1.6% (1 patient) focal

maculopathy, 6.3% (4 patients) diffuse maculopathy, 3.1% (2 patients), ischemic maculopathy, 6.3% (4 patients) mixed maculopathy.

OCT identifies and evaluates quantitatively the macular edema, the retinal thickness being correlated with increased reflectivity changes in the retinal layers.

After optical coherence tomography, 79.7% of patients had signs of diabetic maculopathy.

I made an association between the presence of diabetic maculopathy after slit lamp examination of the eye fundus and after the tomographic examination of the macular area: there are 14 cases (51.9%) in whom the slit lamp fundus examination did not indicate its presence, while the optical coherence tomography shows an increased retinal thickness (p = 0.000) (Table no. 5).

			prezenta MD 3 (dupa efectuarea OCT)		
			DA	NU	Total
prezenta MD 1	DA	Count	37	0	37
		% within prezenta MD 1	100,0%	,0%	100,0%
		% of Total	57,8%	,0%	57,8%
	NU	Count	14	13	27
		% within prezenta MD 1	51,9%	48,1%	100,0%
		% of Total	21,9%	20,3%	42,2%
Total		Count	51	13	64
		% within prezenta MD 1	79,7%	20,3%	100,0%
		% of Total	79,7%	20,3%	100,0%

Table no. 5. The association between the presence of diabetic maculopathy by microscopic examination of the fundus and after the OCT examination

I also made an association between the presence of diabetic maculopathy after FA examination and after the tomographic examination of the macular area: there are 5 cases (31.3%) in whom fluorescein angiography examination did not show the presence of diabetic maculopathy, while the optical coherence tomography showed an increased retinal thickness (p = 0.000) (Table no. 6).

			prezenta MD 3 (dupa efectuarea OCT)		
			DA	NU	Total
prezenta MD 2	DA	Count	46	2	48
		% within prezenta MD 2	95,8%	4,2%	100,0%
		% of Total	71,9%	3,1%	75,0%
	NU	Count	5	11	16
		% within prezenta MD 2	31,3%	68,8%	100,0%
		% of Total	7,8%	17,2%	25,0%
Total		Count	51	13	64
		% within prezenta MD 2	79,7%	20,3%	100,0%
		% of Total	79,7%	20,3%	100,0%

Table no. 6. The association between the type of diabetic maculopathy after FA examination and OCT respectively

In the case of diabetic maculopathy, tomographic examination of macular area was found to be superior to other methods of investigation of the fundus.

I made an association between the presence of diabetic maculopathy after the OCT examination and the macular volume: 94.1% (48 patients) of the cases with diabetic maculopathy had a macular volume of over 10.7 mm3, P = 0.000 (Figure no. 25).

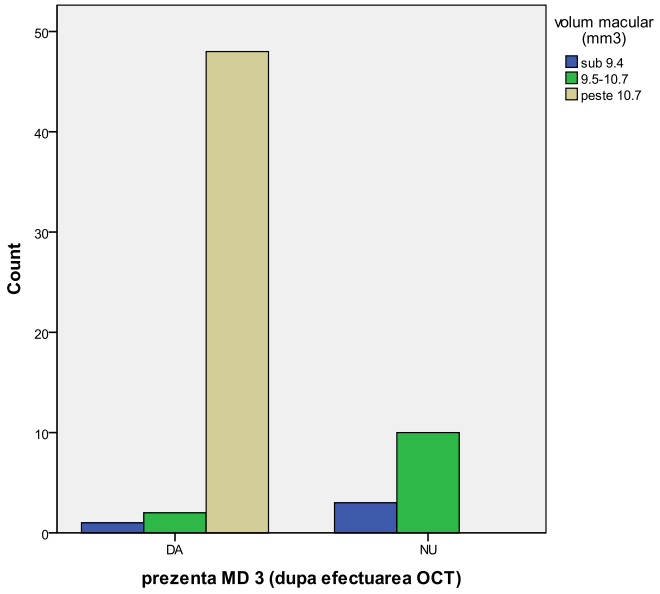


Figure no. 25. The association between the presence of diabetic maculopathy after the OCT examination and the macular volume

I analyzed the presence of changes on angiofluorography (vascular hyperpermeability, microaneurysms, hemorrhages, dilated veins, areas of hypo / non perifoveolar perfusion, hard

exudates, soft exudates, IRMA, neovessels, scars after laser photocoagulation) depending on the type of diabetic retinopathy.

On Fluorescein angiography, vascular hyperpermeability is seen as late hyperfluorescence starting from the retinal vessels, in the patients under study, it occurs in 100% of cases of non-proliferative diabetic retinopathy (37 cases) and proliferative (26 cases).

Microaneurysms are seen under the form of hyperfluorescente points with maximum intensity in the arteriovenous time, disappearing or softening during late venous time; they appear in greater numbers than on ophthalmoscopy and their breaking will result in some diffuse, hiperfluorescences; in the patients in the study, these microaneurysms are found in 100 % (37 patients) of the cases of non-proliferative diabetic retinopathy and in 92.3 % (24 patients), in the cases of proliferative diabetic retinopathy: HR = 1.083 , P = 0.086.

In the literature, there are studies showing that fluorescein angiography has a sensitivity of 82% in detecting microaneurysms [111]

Bleeding occurs hipofluorescent on the FA, and it can be found in 62.2 % (23 patients) in the cases of non-proliferative diabetic retinopathy and in 76.9 % (20 patients) in the cases of proliferative diabetic retinopathy P = 0.215.

Dilated veins are observed in 21.6 % (8 patients) in cases of non-proliferative diabetic retinopathy and in 61.5 % (16 patients) of cases of proliferative diabetic retinopathy: RR (PDR) = 2.038 , P = 0.001 (Figure no. 26).

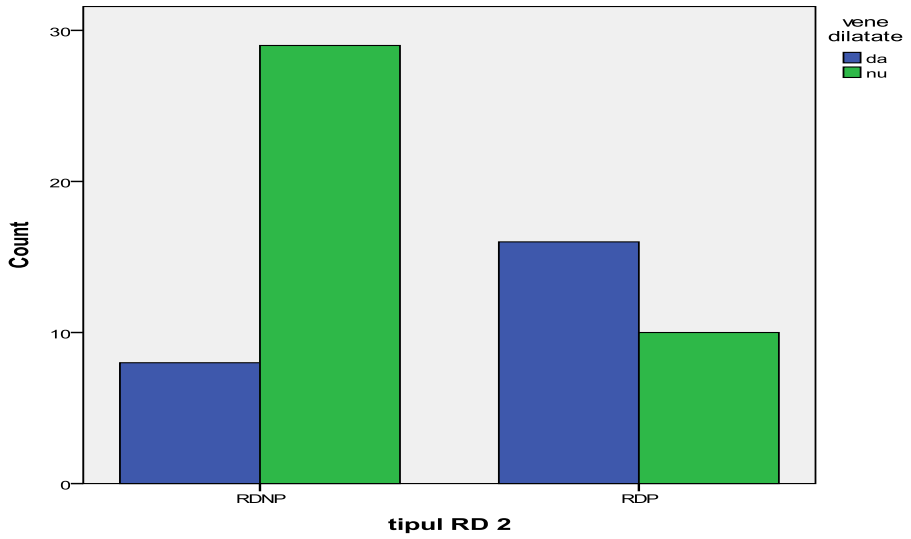


Figure no. 26. The association between the type of diabetic retinopathy and the presence of dilated veins

The areas of hypo / non perfusion is observed in 29.7% (11 patients) of cases of non-proliferative diabetic retinopathy and in 92.3% (24 patients) of cases of proliferative diabetic retinopathy: RR (PDR) = 9.135, P = 0.000 (Figure no. 27).

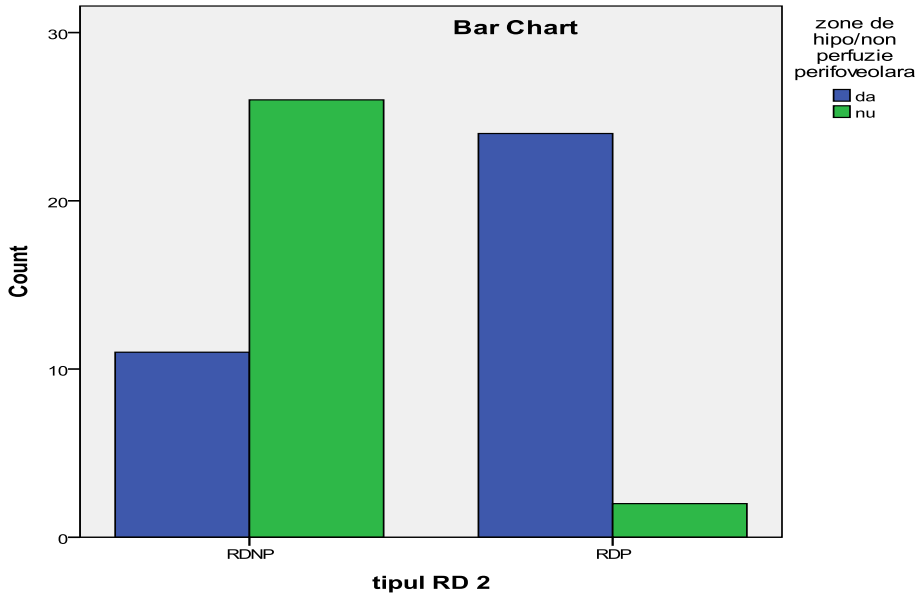


Figure no. 27. The association between the type of diabetic retinopathy and the presence of areas of hypo / non perifoveolar perfusion

Hard exudates are observed in 40.5% (15 patients) of the cases of non-proliferative diabetic retinopathy and in 53.8% (14 patients) in the cases of proliferative diabetic retinopathy P = 0.294.

Soft exudates are observed in 27% (10 patients) of cases of non-proliferative diabetic retinopathy and in 88.5% (23 patients) of cases of proliferative diabetic retinopathy: RR (PDR) = 6.326, P = 0.000 (Figure no. 28).

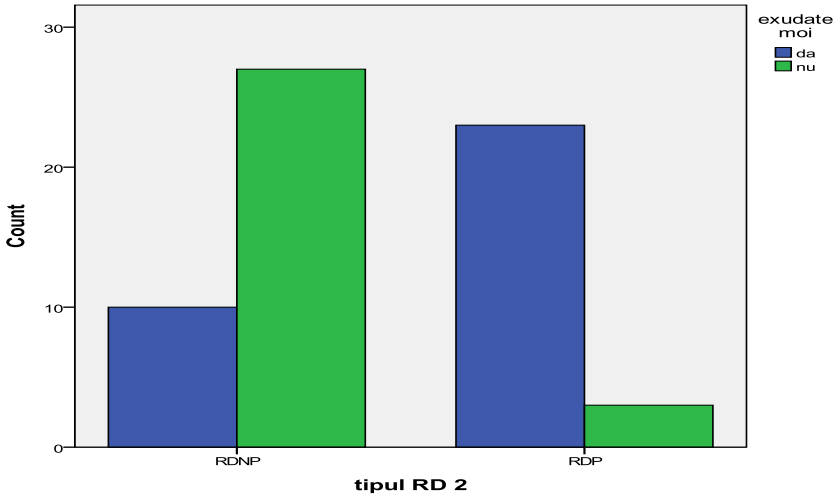


Figure no. 28. The association between diabetic retinopathy type and the presence of soft exudates

Intraretinal microabnormalities are observed in 29.7% (11 patients) of cases of non-proliferative diabetic retinopathy and in 65.4% (17 patients) of cases of proliferative diabetic retinopathy: RR (PDR) = 2.030, P = 0.003 (Figure no. 29).

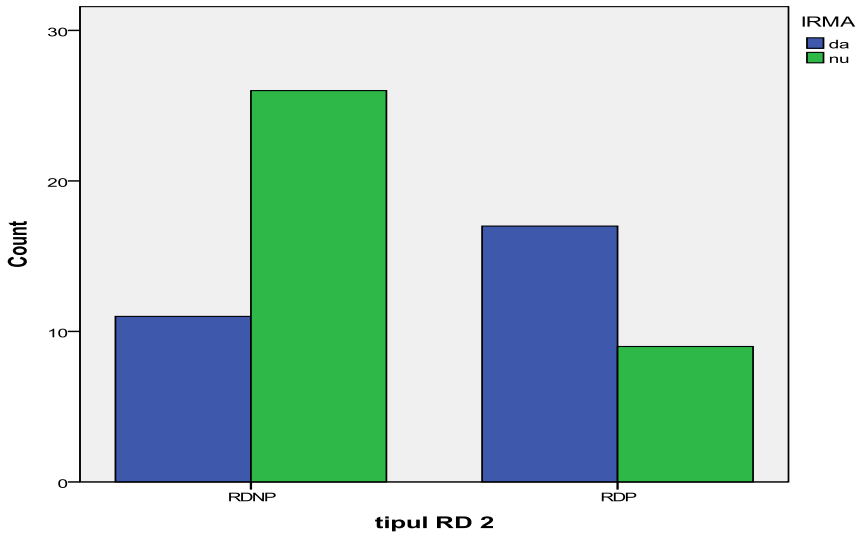


Figure no. 29. The association between diabetic retinopathy type and the presence of intraretinal vascular micro abnormalities (IRMA)

Retinal or disc neovessels, characterized by hyperfluorescente "leakage" areas that grow in size and intensity, are observed in 69.2% (18 patients) of cases of proliferative diabetic retinopathy: P = 0.000 (Figure no. 30).

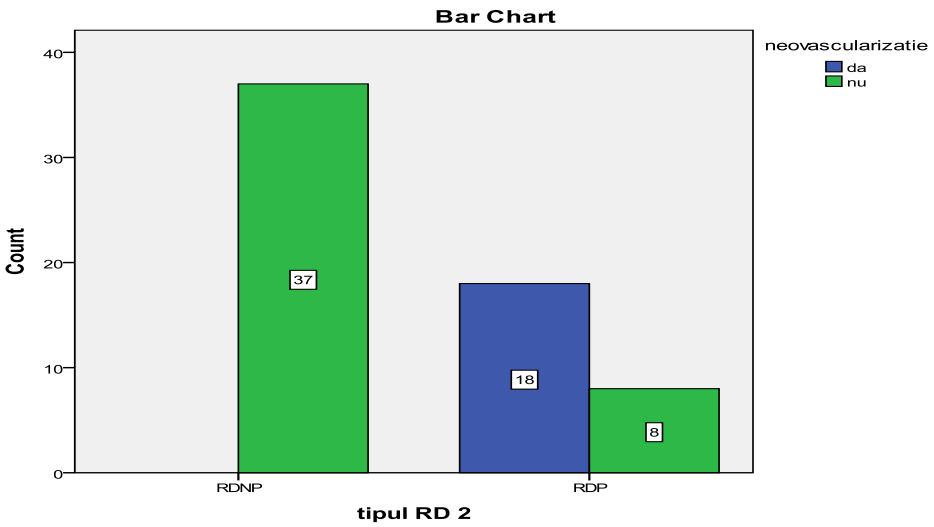


Figure no. 30. The association between diabetic retinopathy type and the presence of neovessels

I analyzed the presence on angiofluorography (vascular hiperpermeability microaneurysms, hemorrhages, dilated veins, areas of hypo / non perifoveolar perfusion, hard exudates, soft exudates, IRMA, neovessels, scars after laser photocoagulation) according to the presence of diabetic maculopathy.

Vascular hyperpermeability was observed in 100% (48 patients) of cases of diabetic maculopathy: RR = 1.067, P = 0.081.

Areas of hypo / non perifoveolar perfusion were observed in 70.8% (34 patients) of cases with diabetic maculopathy: OR = 36.42, RR = 11.33, P = 0.000 (figure no. 31).

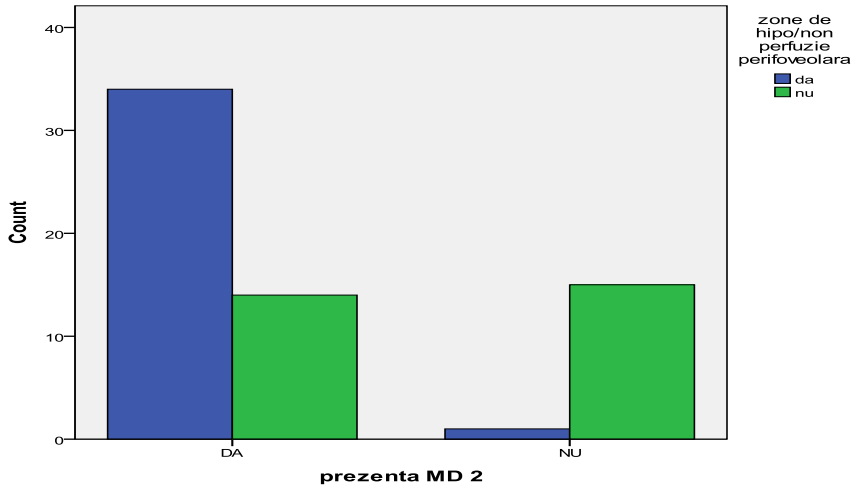


Figure no. 31. The association between the presence of diabetic maculopathy and the presence of areas of hypo / non perifoveolar perfusion

Hard exudates are observed in 60.4% (29 patients) of cases with diabetic maculopathy: RR = 0.396, P = 0.000 (Figure no. 32).

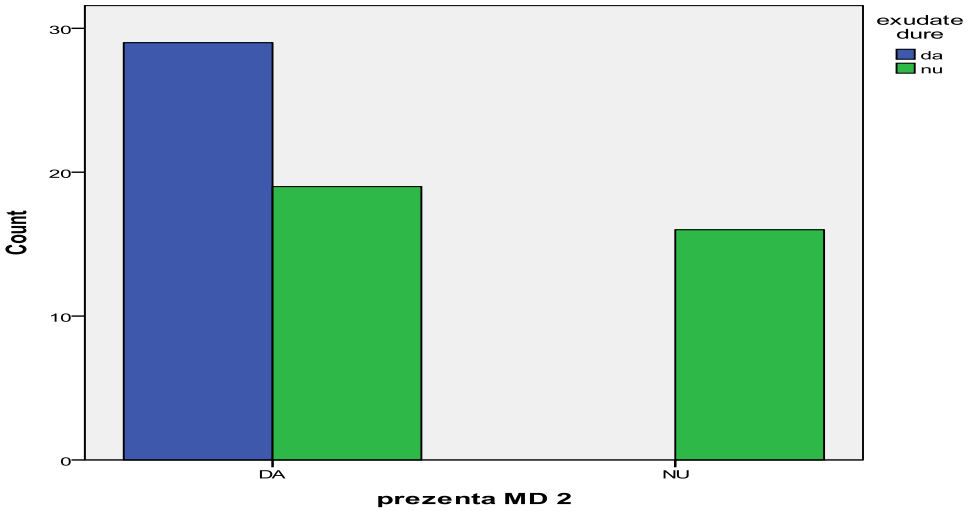


Figure no. 32. The association between the presence of hard exudates and the presence of diabetic maculopathy

Soft exudates are observed in 66.7% (32 patients) of cases with diabetic maculopathy: OR = 30.00, RR = 10.66, P = 0.000 (Figure no. 33).

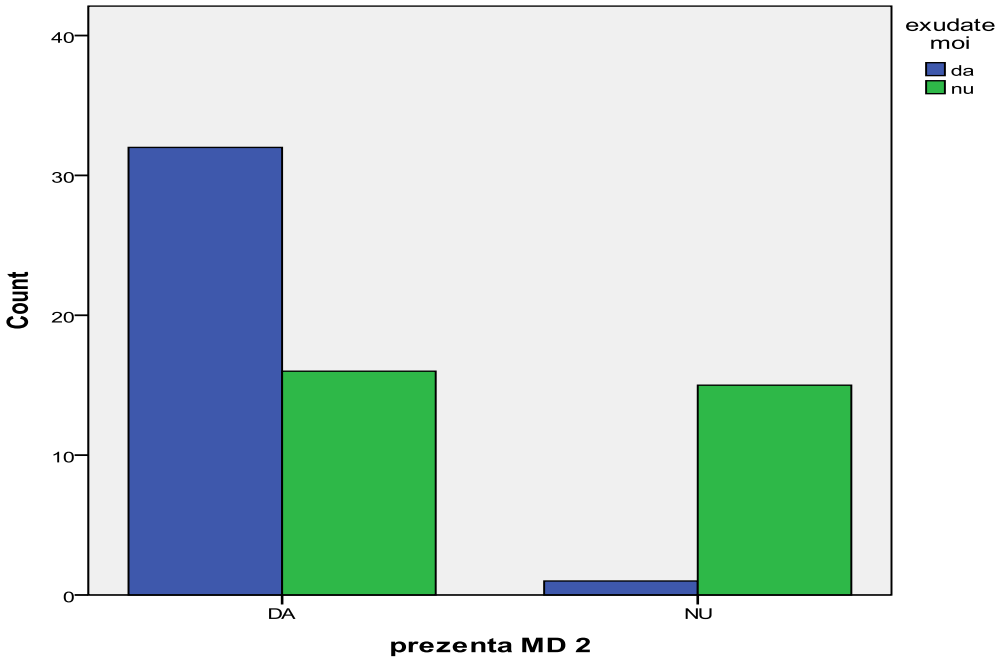


Figure no. 33. The association between the presence of diabetic maculopathy and the presence of soft exudates

Intraretinal microabnormalities are observed in 58.3% (28 patients) of cases with diabetic maculopathy: RR = 0.417, P = 0.000 (Figure no. 34).

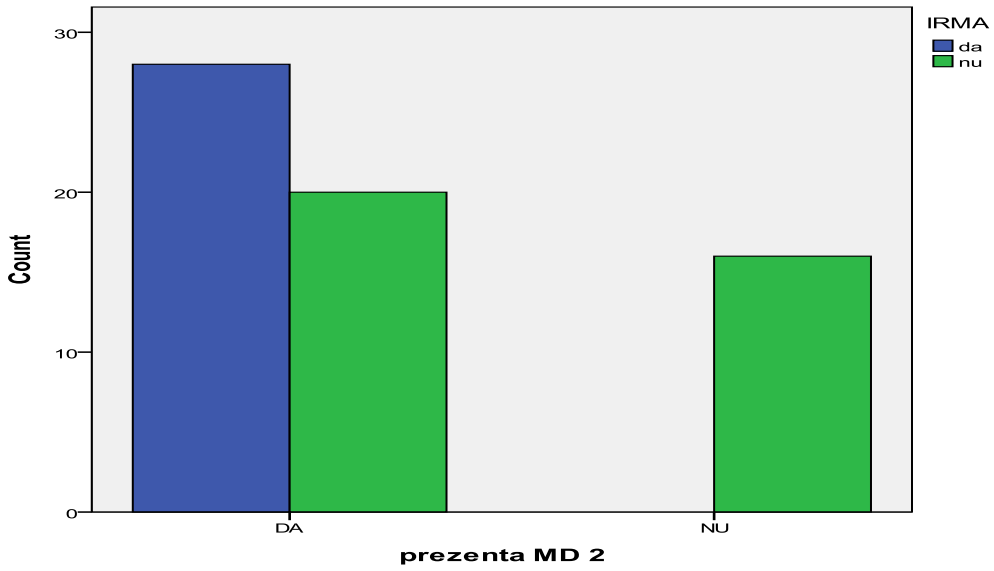


Figure no. 34. The association between the presence of diabetic maculopathy and the presence of IRMA

Retinal and/or disk neovessels are observed in 37.5% (18 patients) of cases with diabetic maculopathy: RR = 0.625, P = 0.000 (Figure no. 35).

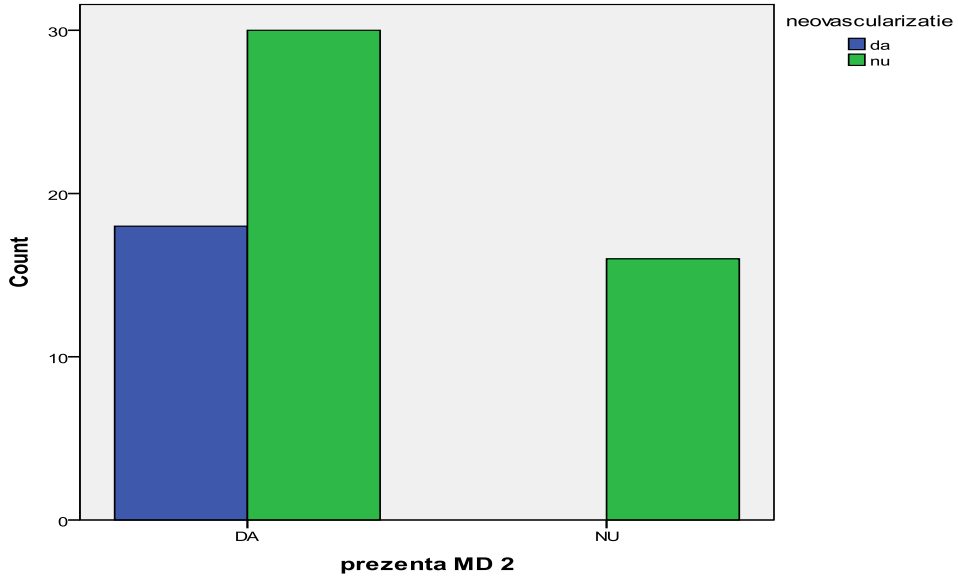


Figure no. 35. The association between the presence of diabetic maculopathy and the presence of neovessels

Regarding the diagnostic method, in the literature, FA has a sensitivity of 87 % for diabetic maculopathy, being superior to retinophotography and ophthalmoscopy (48% - 66%) [111]

The fluorescein angiography changes in diabetic retinopathy represent the mirror of the control of the preexisting systemic disease. In terms of treatment, we should reduce or eliminate hypoxia because this is one of the key factors in diabetic retionpathy. During hypoxia, gene expression of vascular endothelial growth factor increases and the activity of the protein is modulated by other factors including inflammatory cytokines, insulin growth factor, reactive oxygen species and advanced glycation end product [19,98].

Today, laser is the gold standard for the therapy of diabetic retinopathy and for diabetic macular edema, particularly. Laser impacts determine burns that destroy retinal ischemic territories, thus inhibiting the neuro-vascularization process [18,21].

To locate ischemia responsible for neuro-vascularization, fluorescein angiography is compulsory, being the only investigation able to identify these hypoxic retinal areas and which can properly guide the laser treatment [12,109,113] .

The treatment of proliferative diabetic retinopathy by laser panphotocoagulation became the standard treatment for this condition since the years 1972-1975, when the Diabetic Retinopathy Study took place. Then, it was concluded that panphotocoagulation reduces the risk of severe loss of the visual acuity at 2 years by 50%. The complications of this therapy, which included decreased visual acuity with at least one line in 11 % of eyes, visual field loss and reduction of the scotopic vision were considered mild [2111114].

Browning and his colleagues, in a study of 547 eyes, showed that simultaneous panphotocoagulation does not adversely affect the results of focal laser treatment. The association with focal macular photocoagulation is necessary and effective in the patients who present the initial combination of proliferative diabetic retinopathy and clinically significant macular edema [34 104].

Macular laser treatment may be ineffective, especially in cases with proliferative diabetic retinopathy, knowing that panphotocoagulation may exacerbate macular edema [96,97].

In case of a resistance to laser treatment, intravitreal injection of triamcinolone acetonide may be a modern therapeutic options [59,60,61,62].

FA provides a topographic image of the retina that helps establishing treatable lesions, highlights changes in vascular permeability and microaneurysms even from the infraclinical stage of diabetic retinopathy. There are studies showing that 21% - 42% of diabetic patients without signs of diabetic retinopathy on ophthalmoscopy show changes in vascular permeability changes on FA [111].

It is also responsible for identifying retinal ischemia responsible for neurovascularization, being the only investigation able to correctly guide the laser treatment. [5,9,10,12]

We comparatively analyzed the adverse reactions after fluorescein angiography and optical coherence tomography (table no. 7): only 1.6% of the investigated cases (1 patient) reported nausea that persisted for about 15 minutes after angiofluorography.

		No.	%
adverse reactions after the FA	YES	1	1,6%
	NO	63	98,4%
adverse reactions after the OCT	YES	0	,0%
	NO	64	100,0%

Table no. 7. Comparative statistical analysis of adverse reactions after FA respectively OCT

In the literature, the incidence of these minor reactions after angiofluorography was cited as being the highest in 15% and the lowest in 0.6% [28].

In the case of Optical Coherence Tomography, there were no side effects.

There are studies confirming the damage of the retinal capillary glicocalix with phenomena of "leakage" in diabetic retinopathy.

Sulodexide acts on the endothelium, restores the endothelial glicocalix structure with therapeutic facets of the overall protection of vessels. It has an antithrombotic effect, as well as pleiotropic, endothelial and hemoreologic. [35,46,54]

Over a period of six months, I actively participated in a follow-up study of the effectiveness of sulodexide in a dose of 2 to 4 caps / day in 40 patients (of these, 27 patients took part in the study of this PhD thesis). All were monitored by retinal photos: baseline (T0), 3 months (T3) and 6 months (T6).

I divided the 40 patients into 5 groups according to the stage of DR and the therapeutic dose of sulodexide and I obtained the following results:

- Subgroup 1 – mild NPDR - 20 patients - sulodexide 2 caps / day 6 months; in 10 patients, I observed the reduction of the number of microaneurysms (Figure no. 36 and Figure no. 37) and 10 patients were stationary.

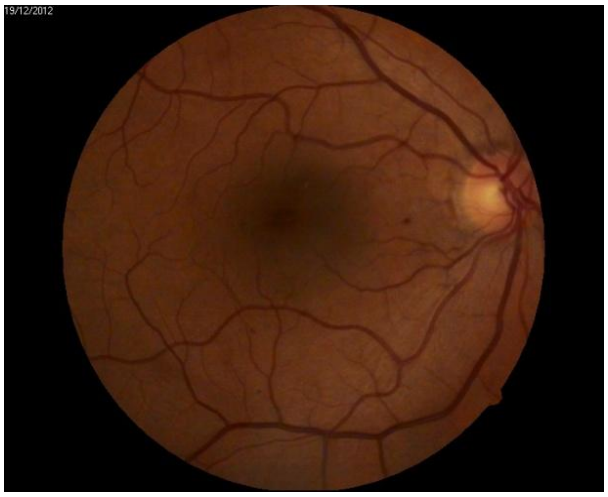


Figure no. 36. Right eye retinal photography (P.F. Dr. Stănilă Medical Centre - Archive -SB)

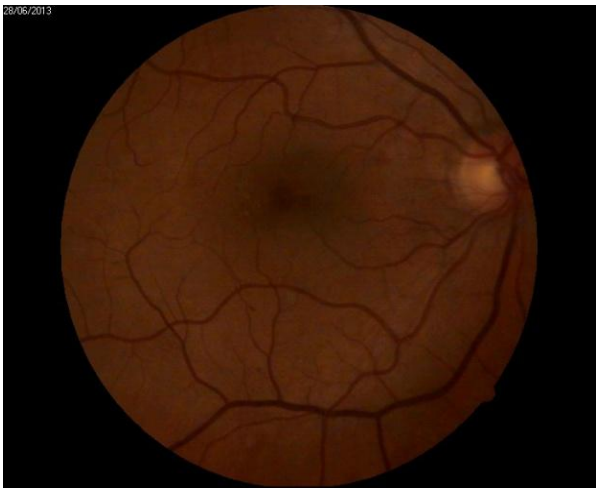


Figure no. 90 Right eye retinal photography 6 months (P.F. Dr. Stănilă Medical Centre - Archive -SB)

- Subgroup 2 - moderate NPDR- 9 patients - sulodexide F 2 caps / day 6 months; in 4 patients I observed the reduction in the size of hemorrhage, microaneurysms, soft exudates, 3 patients at stationary level and 2 patients - progression.
- Subgroup 3 - moderate NPDR - 5 patients - sulodexide inj. 10 days then 2 caps / day 6 months; in patients I observed a reduction in the size of hemorrhage, microaneurysms, hard and soft exudates, 2 patients were at a stationary level and 1 registered a progression.
- Subgroup 4 - moderate NPDR - 5 patients - sulodexide 4 caps / day 6 months; in 4 patients I observed a reduction in the size of hemorrhage, microaneurysms, hard exudates, there was 1 patient at stationary level.
- Subgroup 5 - moderate NPDR - 1 patient - sulodexide inj. 10 days then 4 caps / day 6 months; In 1 patient I observed a reduction in the size of hemorrhage, microaneurysms, hard and soft exudates (Figure no. 38, Figure no. 39, Figure no. 40, Figure no. 41).

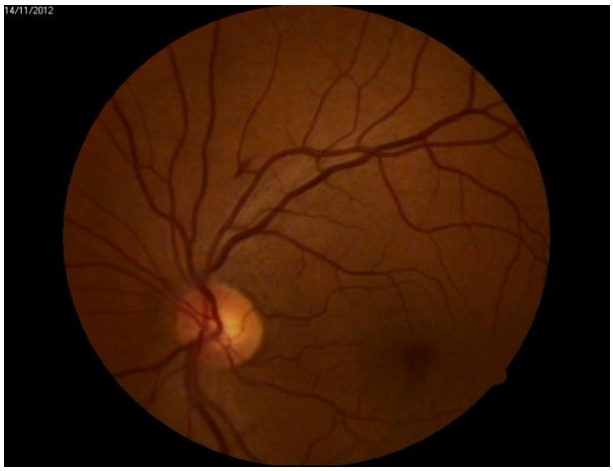


Figure no. 38. Left eye retinal photography (B.I. M Dr. Stănilă Medical Centre - Archive SB)

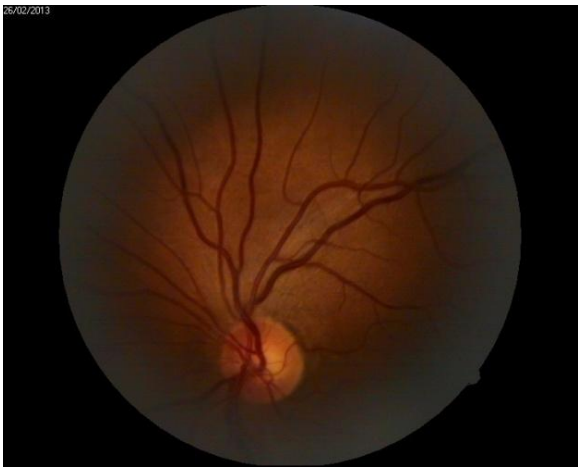


Figure no. 39. Left eye retinal photography, 3 months (B.I. Medical Centre Archive Dr. Stănilă-SB)

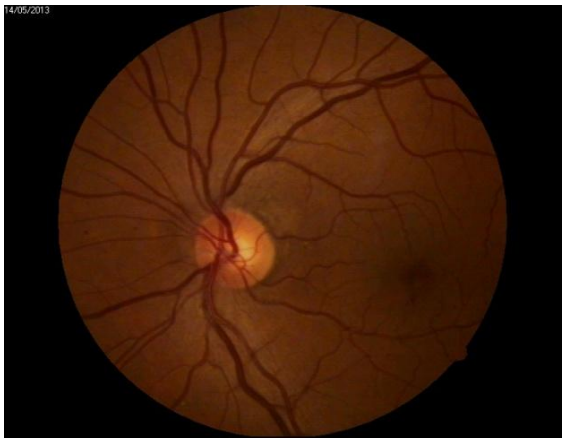


Figure no. 40. Left eye retinal photography, 6 months (B.I. Dr. Stănilă Medical Centre - Archive -SB)

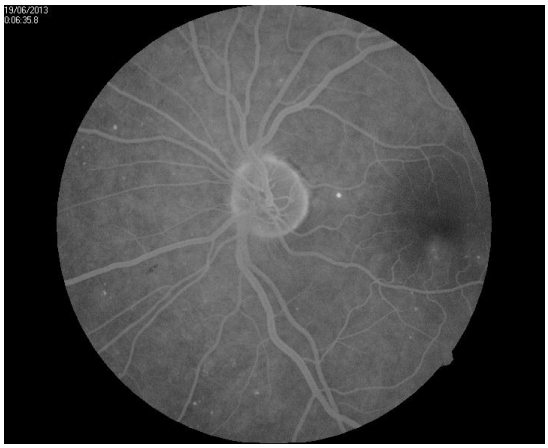
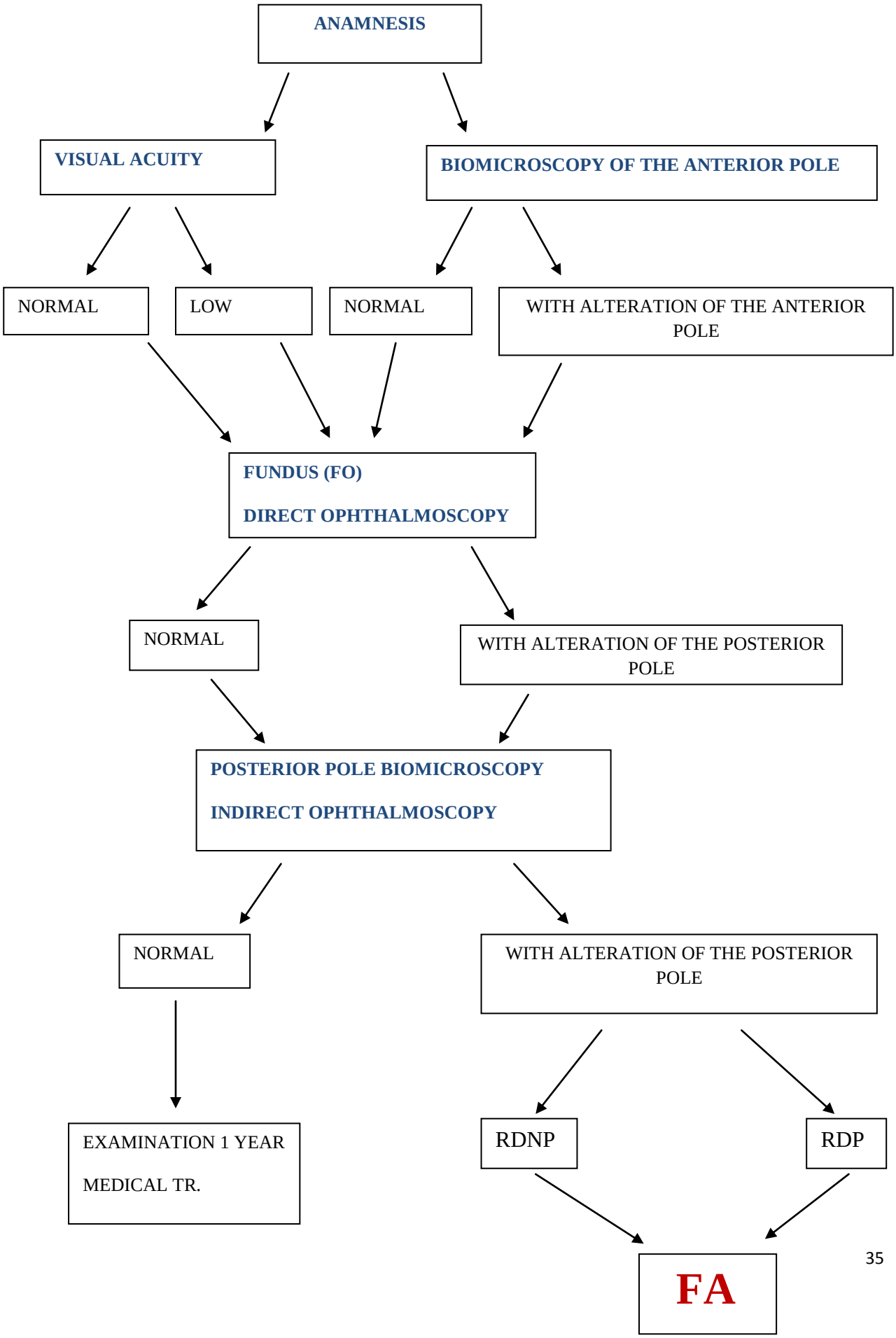
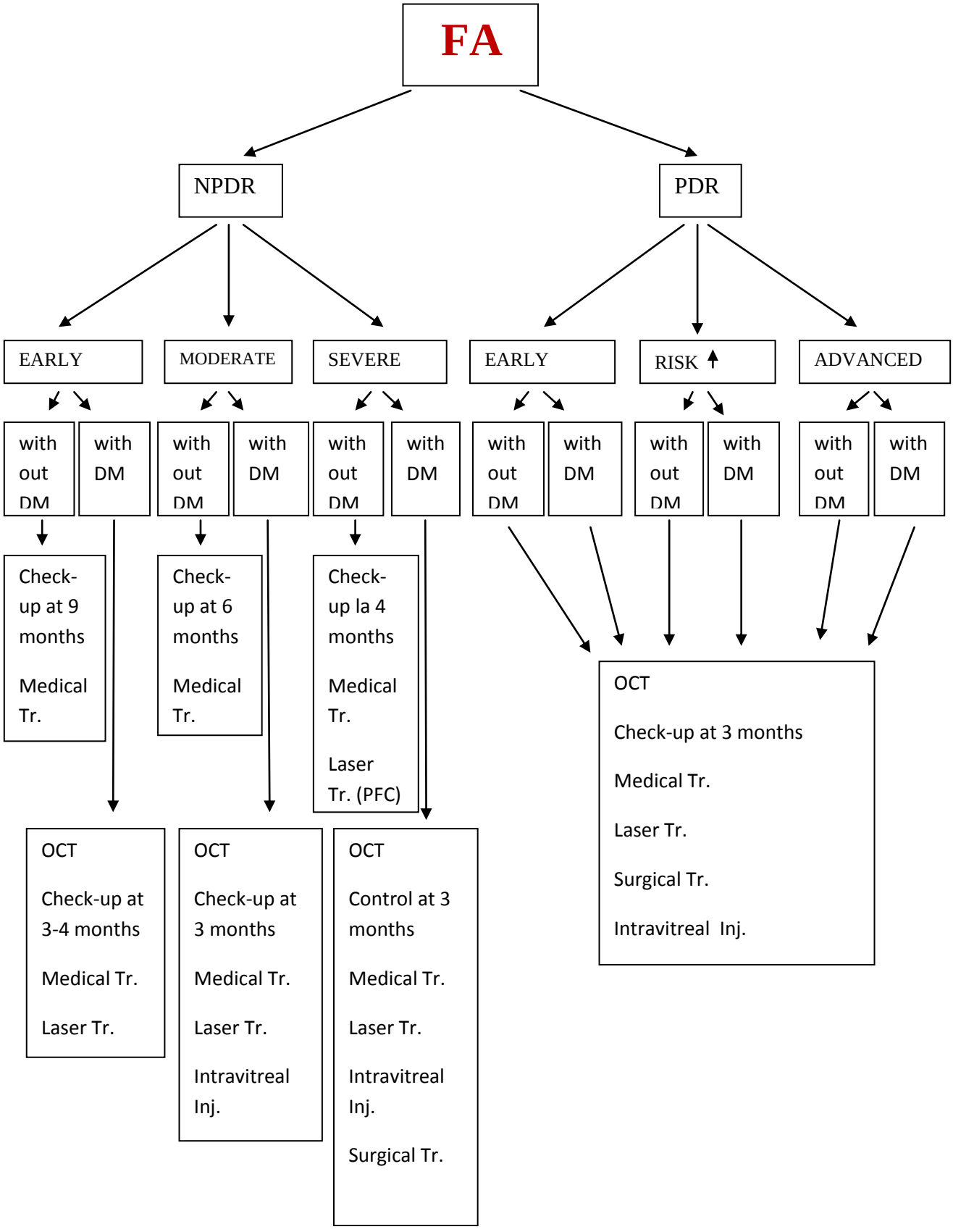


Figure no. 41. FA aspect, left eye (B.I. Dr. Stănilă Medical Centre - Archive -SB)

I believe that fluorescein angiography is useful for all patients with diabetic retinopathy and I suggest an algorithm for the diagnosis and / or treatment of diabetic patients with a view to a certain diagnosis and treatment according to fluorescein angiography changes:


ANAMNESIS
VISUAL ACUITY
BIOMICROSCOPY OF THE ANTERIOR POLE
NORMAL
LOW
NORMAL
WITH ALTERATION OF THE ANTERIOR POLE
FUNDUS (FO)
DIRECT OPHTHALMOSCOPY
NORMAL
WITH ALTERATION OF THE POSTERIOR POLE
POSTERIOR POLE BIOMICROSCOPY
INDIRECT OPHTHALMOSCOPY
NORMAL
WITH ALTERATION OF THE POSTERIOR POLE
EXAMINATION 1 YEAR
MEDICAL TR.
RDNP
RDP
FA


FA
NPDR
PDR
EARLY
MODERATE
SEVERE
EARLY
RISK
ADVANCED
with out DM
with DM
with out DM
with DM
with out DM
with DM
with out DM
with DM
with out DM
with DM
with out DM
with DM
Check-up at 9 months
Medical Tr.
Check-up at 6 months
Medical Tr.
Check-up la 4 months
Medical Tr.
Laser Tr. (PFC)
OCT
Check-up at 3 months
Medical Tr.
Laser Tr.
Surgical Tr.
Intravitreal Inj.
OCT
Check-up at 3-4 months
Medical Tr.
Laser Tr.
OCT
Check-up at 3 months
Medical Tr.
Laser Tr.
Intravitreal Inj.
OCT
Control at 3 months
Medical Tr.
Laser Tr.
Intravitreal Inj.
Surgical Tr.

7. CONCLUSIONS

The study came to the conclusion that diabetic retinopathy remains one of the public health problems in Romania due to the increased risk of blindness, recognized by the Ministry of Health, supported by special programmes, but with a tendency to increase in recent years.

Fluorescein angiography is a method with high specificity and high sensitivity, able to provide important information to complete the clinical picture and the therapeutic orientation in the patients with diabetic retinopathy, because this retinal vascular disease requires an early diagnosis.

Performing fluorescein angiography in the patients with diabetic retinopathy and in those at risk of developing this retinal disease is a minimally invasive method, repeatable and accessible in the clinical practice.

Fluorescein angiography is the only investigation that can identify vascular hyperpermeability and some microaneurysms (92.3% - 100 %, $p < 0.01$) that can be observed in the patients with diabetic retinopathy, even in the early stages.

Fluorescein angiography is the only method by which we can locate retinal hypoxic areas ($p < 0.01$) responsible for neovascularisation and which correctly guide the laser treatment, the different approaches being known depending on the type of macular edema.

Fluorescein angiography has proven its value in identifying intraretinal micro abnormalities ($p < 0.01$) and in their differentiation from retinal neovessels, this aspect being necessary for establishing the proliferative diabetic retinopathy in the proliferative or non-proliferative stage, implicitly for the therapeutic approach.

The areas of hypo / non perifoveolar perfusion are present in mixed diabetic maculopathy ($p <0.01$), fluorescein angiography being mandatory for the diagnosis of this type of maculopathy.

Hard exudates are present in 62.1 % of mixed diabetic maculopathy and soft exudates are present in 78.8% of mixed diabetic maculopathy ($p = 0.000$), fluorescein angiography together with fundus biomicroscopy bringing some additional information in these cases.

There is a strong association of chorioretinal changes in fluorescein angiography with the evolutionary stage of diabetic retinopathy, the patient's age and duration of diabetes.

I believe that fluorescein angiography is useful for all patients with diabetic retinopathy, for the diagnosis and proper treatment of certain changes on fluorescein angiography.

Laser treatment remains the only form of effective long-term treatment of this condition, although recently there have been developed with the advent of optical coherence tomography, microinvasive techniques which remain reserved for the cases resistant to laser treatment.

The preferred therapeutic solution for this disease is prevention, and fluorescein angiography is crucial for the prophylactic treatment of changes in diabetic retinopathy and advanced diabetic complications of the disease.

The continuous improvement of diagnostic and treatment techniques tends to permanently reduce any possible complications that may occur especially in advanced stages of diabetic retinopathy. Knowing and recognizing these possible complications, the accuracy of the medical activities and accurately follow-up the patients will contribute to a positive development of the disease, preserving and / or reoffering the patients' vision capacity.

I suggested that fluorescein angiography should be done regularly within a protocol for diagnosis and / or treatment of the diabetic patients, from which the following items should not be missed: detailed history, visual acuity, biomicroscopy examination of the anterior and posterior pole, intraocular pressure measurement, optical coherence tomography ultrasound in selected cases and periodical check-up, depending on the stage of diabetic retinopathy.

ABBREVIATIONS

DR = diabetic retinopathy

DM = diabetes mellitus

NPDR = non-proliferative diabetic retinopathy

PDR = proliferative diabetic retinopathy

IRMA = intraretinal microanomalies

MD = diabetic maculopathy

FA = angiofluorography

OCT = optical coherence tomography

CSME = clinically significant macular edema

BO = both eyes

RE= right eye

LE = left eye

SD = standard deviation

EF = eye fundus

Dg.1 = initial diagnosis after a slit lamp examination of the eye fundus

Dg.2 = diagnosis after fluorescein angiography

p = level of significance

CI = confidence interval

RD1 = slit lamp examination of the fundus in diabetic retinopathy

RD2 = diabetic retinopathy fluorescein angiography

M = average

MD1 = diabetic maculopathy after slit lamp examination of the eye fundus

MD2 = diabetic maculopathy after fluorescein angiography

MD3 = diabetic maculopathy after optical coherence tomography

RR = relative risk

Tr = treatment

Inj. = injection

T = time

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