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CHOROIDAL THICKNESS AND MORPHOLOGY ANALYSED BY OPTICAL COHERENCE TOMOGRAPHY AS A METHOD TO APPROACH DIABETIC OCULAR DISEASE PROGNOSIS AND PROGRESSION

DOCTORANDO:

Lilianne Gonçalves Duarte

Director and Co-Director

Maria Dolores Pinazo-Duran, MD, PhD

José Salgado-Borges, MD, PhD

Roberto Gallego-Pinazo, MD, PhD

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"CHOROIDAL THICKNESS AND MORPHOLOGY ANALYSED BY OPTICAL COHERENCE TOMOGRAPHY AS A METHOD TO APPROACH DIABETIC OCULAR DISEASE PROGNOSIS AND PROGRESSION"

Doctoral thesis presented to obtain the PhD degree by Lilianne Gonçalves Duarte,

medical doctor, with the passport number: 566209506

Signature: Lilianne Gonçalves Duarte

Valencia, July the 31st

Directors:

Prof. María Dolores Pinazo Durán

Prof. José Salgado-Borges

Prof. Roberto Gallego Pinazo

Dña. María Dolores Pinazo Durán

Profesora Asociada de Oftalmología. Departamento de Cirugía de la Facultat de Medicina y Odontología de la Universitat de València,

D. José Manuel Salgado Borges

Doctor en Oftalmología por a Facultat de Medicina de la Universitat de Porto, Portugal. MD, PhD, FEBO.

D. Roberto Gallego Pinazo

Doctor en Medicina y Cirugía por la Universitat de València, Especialista en Oftalmología. FEBO. Subespecialista en mácula.

INFORMAN:

Que la Sra. Doña Lilianne Gonçalves Duarte ha realizado el presente trabajo de Tesis Doctoral bajo nuestra supervisión, y habiendo revisado la versión final la encontramos apropiada para ser presentada y defendida ante tribunal para optar al grado de Doctor de la Universidad de Valencia en su modalidad de Tesis Internacional.

En Valencia a 31 de Julio de 2018

M D Pinazo Duran ***J M Salgado Borges*** ***R Gallego Pinazo***

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GLOSSARY OF ABBREVIATIONS AND ACRONYMS

ACCORD	Action to control cardiovascular risk in type 2 Diabetes
AGE	Advanced glycation end products
BP	Blood pressure
CCD	Charge-coupled device
CFRT	Central foveal retinal thickness
CHEDV	Centro Hospitalar Entre o Douro e o Vouga
ChS	Choriocapillaris/Satler
CL	Choroidal layers
CME	Cystoid macular edema
CRF	Case Report Form
CSME	Clinically significant macular edema
CT	Choroidal Thickness
DME	Diabetic macular edema
DO	Drop out
DR	Diabetic Retinopathy
DRIL	Disorganization of retinal inner layers
EDI	Enhanced depth imaging
ETDRS	Early Treatment Diabetic Retinopathy Study
FD	Fourier domain
IRMA	Intraretinal microvascular abnormalities
LFU	Lost of follow up
ME	Macular edema
MIT	Massachusetts Institute of Technology
NDR	No diabetic retinopathy
NPDR	Non-proliferative diabetic retinopathy
NSD	Neurosensorial Detachment
NVD	New vessels on the disc
NVE	New vessels elsewhere
OCT	Optical Coherence Tomography

OD	Oculus dextrus - right eye
OP	Ocular pressure
OS	Oculus sinister - left eye
PDR	Proliferative diabetic retinopathy
PKC	Protein Kinase C
RPE	Retinal Pigment Epithelium
RT	Retinal Thickness
SCI	Sclero-choroidal interface
SD	Standard deviation
SD-OCT	Spectral Domain Optical Coherence Tomography
SFCT	Sub foveal choroidal thickness
SLD	Super Luminescent diode
SMD	Sub macular detachment
SS-OCT	Swept Source Optical Coherence Tomography
TD-OCT	Time Domain Optical Coherence Tomography
UKPDS	United Kingdom Prospective Diabetes Study
VA	Visual Acuity
VEGF	Vascular endothelial growth factor

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SUMMARY

TITLE

CHOROIDAL THICKNESS AND MORPHOLOGY ANALYSED BY OPTICAL COHERENCE TOMOGRAPHY AS A METHOD TO APPROACH DIABETIC OCULAR DISEASE PROGNOSIS AND PROGRESSION

INTRODUCTION

Diabetic choroidopathy (DC) in-vivo clinical evidence has been difficult to demonstrate clinically due to the lack of means to visualize the choroid. Advances of Optical Coherence Tomography (OCT) technology allowed in-vivo visualization of the choroid. Controversial results in Diabetes for choroidal thickness (CT) and lack in the identification of morphologic changes makes DC identified with OCT still to be understood.

PURPOSE

To evaluate whether CT measurement alone is a reliable parameter for characterize DC, to characterize choroidal morphological findings using the OCT and correlation with CT and the diabetic retinopathy (DR).

METHODS

A cohort, prospective, longitudinal and observational study, where diabetic and healthy controls were followed in consecutive visits. Inclusion criteria were: healthy and Diabetic patients without or with any stage of DR, aged 18 or more, signed voluntarily informed consent. Exclusion criteria were: previous treatment of any kind

for DR; previous or ongoing ocular disease or history of ocular surgery; relevant media opacities; refractive errors greater than ± 6 Diopters; systemic chronic steroid or immunosuppressive medication; any serious and uncontrolled systemic condition. Previous studies were performed to identify choroidal morphological findings on OCT to be used as classification method. Quantitative assessment of retinal and subfoveal choroidal thickness (SFCT), qualitative assessment of choroidal morphological findings, and correlation with the retinal diabetic disease state. Evaluation of progression and predictive relevancy.

RESULTS

One hundred and ninety-six diabetic eyes and seventy-eight healthy eyes were included. From choroidal morphologic analysis we found: on healthy 78% were normal, and abnormalities found were always on the great vessels layers with preserved choriocapillaris/Sattler (ChS) layers. Most of diabetic eyes with no abnormalities had no and initial stages of DR. Focal choroidal vascular changes were correlated with the subjacent retinal disease. Atrophy of the ChS layer or the great vessels layer was well corelated with more advanced stages of DR and maculopathy. Progression of choroidal morphologic changes was well evidenced and correlated with progression of retinal disease. From statistical analysis: SFCT showed huge variability with multifactorial dependence. Statistical significance of correlation with SFCT was found only for age for both groups and choroidal layers abnormalities for diabetics and systolic blood pressure for healthy.

CONCLUSION

SFCT showed to be dependent of multifactorial variables with a wide range of values showing not to be a reliable parameter alone for DC evaluation. With OCT images we can identify and classify as normal or abnormal choroidal morphologic findings. Abnormal choroidal findings are well correlated with suprajacent retinal diabetic disease and can be a marker of evolution or progression of the disease of treatment effect.

RESUMEN

TÍTULO

ESPESOR COROIDEO Y MORFOLOGÍA ANALIZADO POR TOMOGRAFÍA DE COHERENCIA ÓPTICA COMO MÉTODO PARA ABORDAR LA PROGNOSIS Y LA PROGRESIÓN DE LA ENFERMEDAD OCULAR DIABÉTICA

INTRODUCCIÓN

La evidencia clínica in vivo de coroidopatía diabética (DC) ha sido difícil de demostrar clínicamente debido a la falta de medios para visualizar la coroides. Los avances de la tecnología de la tomografía de coherencia óptica (OCT) permitieron la visualización in vivo de la coroides. Los resultados controvertidos en la Diabetes para el grosor coroideo (CT) y la falta de identificación de los cambios morfológicos hacen que la DC identificada con OCT aún no se conozca.

PROPÓSITO

Evaluar si la medición por CT sola es un parámetro confiable para caracterizar la DC, para caracterizar los hallazgos morfológicos coroidales usando la OCT y la correlación con la CT y la retinopatía diabética (DR).

MÉTODOS

Estudio cohorte, prospectivo, longitudinal y observacional, donde se siguieron los controles diabéticos y sanos en visitas consecutivas. Los criterios de inclusión

fueron: pacientes sanos y diabéticos sin o con cualquier estadio de RD, de 18 años o más, firmados voluntariamente con consentimiento informado. Los criterios de exclusión fueron: tratamiento previo de cualquier tipo para DR; enfermedad ocular previa o en curso o antecedentes de cirugía ocular; opacidades de medios relevantes; errores de refracción mayores que ± 6 dioptrías; medicamentos séricos crónicos o inmunosupresores sistémicos; cualquier condición sistémica grave e incontrolada. Se realizaron estudios previos para identificar los hallazgos morfológicos de la coroides en OCT que se utilizarán como método de clasificación. Evaluación cuantitativa del grosor de la coroides retiniana y subfoveal (SFCT), evaluación cualitativa de los hallazgos morfológicos coroidales y correlación con el estado de la enfermedad diabética retiniana. Evaluación de progresión y relevancia predictiva.

RESULTADOS

Se incluyeron 96 ojos diabéticos y setenta y ocho ojos sanos. A partir del análisis morfológico de la coroides, encontramos que el 78% de los sanos eran normales y las anormalidades encontradas siempre en las capas de grandes vasos con capas preservadas de coriocapilar / Sattler (ChS). La mayoría de los ojos diabéticos sin anomalías no tenían y las etapas iniciales de DR. Los cambios vasculares coroidales focales se correlacionaron con la enfermedad retiniana subyacente. La atrofia de la capa de ChS o la capa de grandes vasos estaba bien correlacionada con etapas más avanzadas de DR y maculopatía. La progresión de los cambios morfológicos coroidales se evidenció y se correlacionó con la progresión de la enfermedad retiniana. A partir del análisis estadístico: SFCT mostró una gran variabilidad con la

dependencia multifactorial. La significación estadística de la correlación con SFCT se encontró solo para la edad para ambos grupos y las anomalías de las capas coroideas para los diabéticos y la presión arterial sistólica para la salud.

CONCLUSIÓN

SFCT mostró ser dependiente de variables multifactoriales con una amplia gama de valores que muestran que no es un parámetro confiable solo para la evaluación de DC. Con las imágenes de OCT podemos identificar y clasificar como hallazgos morfológicos coroidales normales o anormales. Los hallazgos anormales de la coroides están bien correlacionados con la enfermedad diabética retiniana suprayacente y pueden ser un marcador de evolución o progresión de la enfermedad del efecto del tratamiento.

CHAPTER 1 - INTRODUCTION

1 - INTRODUCTION

Diabetes Mellitus has been increasing in prevalence over the last decades. According to the last report by the International Diabetes Federation, there were 425 million diabetic patients in the adult population worldwide in 2017. This number projected to the year of 2045 will arise to 629 million[1]. Due to this epidemic prevalence of Diabetes, the World Health Organization emphasized on the World Health Day of 2016 the need to increase awareness on Diabetes and its morbidity and burden, and to stablish specific and effective actions to tackle Diabetes.[2]

Diabetes is a metabolic disease associated to hyperglycemia. Severe acute or prolonged hyperglycemia leads to the macrovascular and microvascular diabetic disease affecting several organs and tissues. Microvascular complications are responsible for the diabetic nephropathy, neuropathy and retinopathy, while macrovascular complications affect mostly the heart and peripheral vascular systems and can be associated with stroke[3]. It is important to understand the pathophysiology of vascular damage due to hyperglycemia and the changes in vessels so that we can look for signs of risk to progression and ways to prevent and/or treat the vascular complications, such as in Diabetic retinopathy.

According to a large systematic analysis, Diabetic retinopathy was responsible for 1,9% of moderate to severe visual impairment globally and 2,6% of blindness in 2010[4]. Another meta-analysis demonstrated a worrying increase from 1990 to 2010 of 27% in blindness and 64% of moderate to severe visual impairment due to Diabetic retinopathy[5]. Higher risk and prevalence were found in older populations and high-income regions, such as Western Europe, North America and Australasia[5].

Diabetic retinopathy was first described by Eduard Jäger, in 1856, as a finding with the new ophthalmology instrument developed just few years earlier, the opthalmoscope.[6–8] Acceptance of a specific pattern of diabetic lesions in the retina only started on the 40's with Ballantyne's description[9] and later with Ashton and Cogan.[7,9–11] In the 60's with the publication of the new technique fluorescein angiography and its progressive use in ophthalmology, brought about new insights into the pathophysiology of diabetic retinopathy and allowed new advances in diagnosis and treatment guidelines.[12,13]

For about 3 decades, the only ophthalmic imaging systems available for examination and diagnosis were fundus photography and angiography. In the early 90's David Huang presents a paper on the Optical Coherence Tomography.[14] It was a breakthrough in Ophthalmology and in the diagnosis, treatment and follow up for ocular diseases, with special focus on retinal diseases. Since the initial development, commercialization of the first device for clinical purpose till now, the Optical Coherence Tomography (OCT) systems have been improving in acquisition systems, hardware and software for better resolution, increased speed and increased information.[15,16] The OCT has allowed, with a noninvasive ophthalmological exam, to have qualitative and quantitative information of the retina, with anatomical and in-depth information. It has also been very important in the workup of Diabetic retinopathy, in the diagnosis and classification of macular edema, in guiding treatment indications, efficacy and follow up, decreasing the need of the invasive ophthalmological exams, such as fluorescein angiography.

The first OCTs, due to a limitation of the system, were unable to give information of deeper structures such the choroid. In 2008 Spaide et al. described a novel imaging technique using the OCT Spectralis system that allowed to improve choroidal visualization, the Enhanced Depth Imaging (EDI).[17,18] Since then with the evolution of the OCT systems with increased acquisition speed, increased resolution and the availability of the Swept-Source OCT, choroidal visualization has been improved. New data from retinal and choroidal diseases have emerged with this additional feature of the OCTs.

Since the first publication by Spaide describing the EDI, several papers have been published regarding choroidal thickness and its correlation with retinal disease. Several look for a correlation of choroidal thickness changes with the diabetic retinopathy trying to find signs of a diabetic choroidopathy. The results between the papers vary and sometimes are controversial.

In this Section a review of current data was presented as the rationale for this study.

1.1. VASCULAR COMPLICATIONS OF DIABETES

Diabetes and hyperglycemia cause disease in several tissues by affecting the vascular system. Great vessels and smaller/microscopic vessels affected by hyperglycemia injury lead to two separated diabetic vascular disease: the macrovascular and the microvascular diabetic complications.

It is important to understand how vascular complications occur, since the pathophysiology of diabetic retinopathy undergoes from them. The choroid is a

mainly vascular structure, so it is expectable to have a diabetic choroidal vascular disease leading to a diabetic choroidopathy.

Macrovascular diabetic disease affects primarily greater vessels with atherosclerotic process and hypercoagulation states increasing the risk of thrombosis, vessel occlusion and stroke. The exact mechanism how Diabetes increases the risk of atheroma formation is not well understood. Several studies had shown the association of Diabetes with an increased risk of cardiovascular disorders.[3,19–24] Type 2 Diabetes is a complex metabolic clinical condition that comes with increased blood glycose, hypertension, overweight, dyslipidemia, all of these contributing to a higher risk of cardiovascular disorder. So the association of Diabetes with cardiovascular disorder seems to be a part of a multifactorial condition. Nevertheless, intensive control of blood pressure and lipid levels in Diabetic patients have shown a decrease in cardiovascular events.[22,24–26] In type 1 Diabetic patients, usually thinner, younger with less propensity to high blood pressure or dyslipidemia, hyperglycemia was correlated to higher heart rate, a risk factor for cardiovascular disease.[3,21,26] Macrovascular diabetic disease is associated to increased death risk.

Microvascular diabetic disease tends to cause intrinsic damage on the anatomy and function of the affected organs. It refers to the disease affecting vessels with an internal diameter of 100 or less microns. The most important manifestations of diabetic microvascular disease are the Diabetic retinopathy, nephropathy and neuropathy, being the retinopathy the most common complication. Microvascular disease in Diabetes occurs due to a particular vessel cellular response and damage caused by increased and sustained hyperglycemia.[27–29] Although several studies

demonstrate that hyperglycemia is a major risk factor for microvascular damage in Diabetes, other causes underly such concomitant pathologies (e.g. hypertension and dyslipidemia) and genetic predisposition[30–32].

Microvascular disease includes endothelial dysfunction, pericytes or podocytes death, increased vascular permeability and angiogenesis, basement membrane thickening, cell growth and apoptosis, vascular occlusion and dilatation, extracellular matrix expansion and dysfunctional enzymatic activity. From the increased metabolic products of the glycolytic pathway in hyperglycemic state, modulated by genetic predisposition and local tissue response, several mechanisms are involved into the microvascular lesion: the activation of specific enzymes, such as the protein kinase C (PKC), phospholipase, adenosine triphosphatase and others, leads to altered cell signaling or molecule transduction; the activation of the polyol pathway, the formation of advanced glycation end products (AGE) and the production of superoxide and reactive oxygen species, leads to oxidative and endoplasmic reticulum stress, and the increase in inflamatory cytokines. All of these changes cause abnormal turnover and vascular and cell dysfunction leading to the diabetic retinopathy, neuropathy and nephropathy.[27,33,34]

1.2. THE DIABETIC RETINOPATHY – A VASCULAR DISEASE

Since the earliest description of diabetic retinopathy with ophthalmoscopy we have been able to identify vascular involvement of the diabetic disease by observing microaneurysms (small capillaries dilatations), intra-retinal hemorrhages, signs of exudation, vessel abnormalities and new-vessels. Fluorescein Angiography allows

us to observe the anatomical and dynamic (flow and perfusion) changes in the vascular retina occurring in the diabetic disease. Optical coherence tomography demonstrates the presence of anatomical and thickness changes as the result of permeability alterations with fluid accumulation, cysts, and chronic sequalae as atrophy and loss of tissue and fibrosis. The classification of diabetic retinopathy has been evolving since the first, the Arlie House classification, to more simplified classifications such as the International Clinical Disease Severity Scale for Diabetic Retinopathy. The severity of the diabetic retinopathy is classified according to the extent of the vascular changes encountered.[35–38]

Klein et al. described as earliest changes observed in diabetic retinopathy the widening of retinal venules, even before the appearance of microaneurysms and blot hemorrages.[39] In a most recent review it is summarized how retinal microvasculature measured and quantified can indicate the risk of progression of diabetic retinal disease in pre-clinical stages.[40] At the early clinical stages of diabetic retinopathy where findings are essentially microaneurysms, the turnover (or rate of appearance and disappearance) and count over time of the microaneurysms are biomarkers of disease progression.[41–46] Also the turnover of microaneurysms and presence of foveal avascular zone abnormalities were described as characteristics allowing to determine patterns or phenotypes of risk of progression to a more advanced disease.[47–49] As the diabetic retinopathy progresses to more advanced stages, signs of alteration of the blood-retinal barrier permeability appear with more extensive retinal hemorrhages, hard exudates (representing lipidic deposition in the extravascular retinal space as a consequence of the lipoprotein leakage from the vessels), leakage and edema. More extensive vascular lesion can be observed with

anatomical changes such as tortuosity, beading, loops, and infarction of the nerve fiber layer appearing as whitish or cotton wool spots. In severe or proliferative stages non-perfusion/ ischemia leads to neovascularization in the superficial retina with high risk of bleeding to the vitreous. Although some authors claim retinal neurodegenerative diabetic changes, it is not clear if they precede microvascular disease or are a consequence.[50–54] It remains that the most important changes occurring in the retina and responsible for the morbidity and visual function disability are related to the vascular disease.

Hyperglycemia is the trigger in Diabetes for the development of diabetic retinopathy. As previously described it leads by several biochemical, cellular, physiological and inflamatory mechanisms to vascular changes as the pericyte loss, endothelial cells damage, basement membrane thickening, capillary occlusion, blood-retinal barrier disfunction and increased permeability, increased circulating growth factors and oxidative products. However other concomitant conditions can act as incrementing risk factors for the microvascular disease, such as hypertension, dyslipidemia and genetics. The UKPDS showed benefit and decrease in the risk of progression of diabetic retinopathy in lowering blood pressure in uncontrolled hypertension.[22] But the ACCORD did not find any benefit in lowering blood pressure in mild hypertensive or normotensive patients in order to prevent diabetic retinopathy progression.[55] Some studies suggest that the control of lipid serum levels might have a small effect on diabetic retinopathy progression, but the physiologic mechanism is not clear.

1.3. THE CHOROID - A COMPLEX VASCULAR STRUCTURE. THE DIABETIC CHOROIDOPATHY

1.3.1. ANATOMY AND FUNCTION[56–68]

The choroid is a part of the uveal tract of the eye. It is an extensive vascular network involved by connective tissue that goes from the ora serrata up to the optic nerve, and lies between the sclera and the retina. From histology data the choroid varies in thickness from 220µ posteriorly to 100µ anteriorly.

The choroid is supplied by ciliary arteries derived from the first branch of the internal carotid artery that pierce the sclera more anteriorly by the long posterior ciliary arteries and peripapillary by the short posterior ciliary arteries, until the choriocapillaris. The venous drainage is made primarily by the vortex vein system that ultimately drain to the superior and inferior ophthalmic veins.

The enervation of the choroid has parasympathetic and sympathetic nerves, but only the latter has an autoregulatory influence in the choroidal blood flow.

The choroid has a very specific anatomic architecture. Histologically it is divided in four layers: the Bruch's membrane, the choriocapillaris, the stroma and the suprachoroid.

The Bruch's membrane separates the retinal pigmented epithelium (RPE) from the choriocapillaris. It is essentially a collagenous and elastic fiber tissue.

The choriocapillaris, described for the first time in the 18th century by Hovius, corresponds to the choroidal layer with the smallest vessels, with a 10 to 30 µm thickness. It is composed of modified capillaries with an unusual larger diameter (up

to 40µm) comparing with capillaries elsewhere in the body, and with the particularity of fenestrated walls. We can find gap junctions between endothelial cells but there are far fewer pericytes as those we find over retinal vascular endothelial cells. The support for the vascular system is mostly provided by the involving connective tissue. The whole choriocapillaris has an elaborated architecture in various functional independent lobules. Each lobule is supplied in its center by a precapillary arteriole to the flat and one-layer capillary network that drains into postcapillary venules. These characteristics have influence on the high speed of blood flow leakage from the capillaries. The choriocapillaris blood flow was found to have up to more 77% higher seed than retinal capillaries.

The stroma is composed of larger vessels than those found on the choriocapillaris, and the vessel diameter increases from the choriocapillaris adjacent side to the more posterior or scleral one. Involving the vessels there is connective tissue, melanocytes, nerve fibers, fibroblasts and non-vascular smooth muscle, macrophages, lymphocytes, plasma and mast cells. It is divided into two vessel layers: the medium diameter vessel layer or Sattler's layer, and the large vessel layer or the Haller's layer. The vessels of the stroma are not fenestrated or organized in lobules, they are rather intertwined and, especially in the Haller's layer, the arteries are similar to those elsewhere with contractile ability due to the presence of internal elastic lamina and smooth muscle. They don't leak as the choriocapillaris. The vessel system is supported by the involving tissue and cells, and it was suggested that the non-vascular smooth muscle and fibroblasts plays a role in the choroidal thickness changes occurring in physiologic states as on retinal defocus.

The suprachoroid separates the Haller's layer from the lamina fusca (pigmented layer of the sclera). It is an avascular layer rich in melanocytes, collagen and elastic fibers, fibroblasts and nerve fibers and cells.

It is important to know the anatomy of the choroid in order to better interpret OCT imaging.

The physiological role of the choroid is highly related to its blood flow. It is responsible for the major supply of oxygen and nutrients to the outer retina with particular influence on the metabolic processes of the photoreceptors and light absorption due to its rich melanocytes content; it has a retinal thermoregulation role; it is important in the immunologic response of the eye since it works as a provider of mast and inflamatory cells to the eye and a barrier for certain kind of molecules; and it is responsible for about 35% of the humor aqueous drainage with a role in the ocular pressure control. Since it is a highly vascular structure with elevated blood flow speed, it is also susceptible to systemic changes. Beside those functional roles, it has been suggested that the changes in choroidal thickness can be correlated with refractive states and even with the eye growth process.

The high and speedy blood flow of the choroid and particularly of the choriocapillaris is necessary to outdo the barriers of the Bruch's membrane and retinal pigment epithelium to provide the increased amount of oxygen required for the extremely metabolically active photoreceptors. The large caliber capillaries of the choroid and the fact that they are fenestrated allow permeability to large molecules that do not pass through the retinal capillaries, such as glucose, proteins and other nutrients.

This is important not only for the contribution of nutrients and molecules for the outer retina, but also for the removal of the waste produced from metabolic processes of the outer retina.

Choroidal and retinal disorders are intimately related due to the high dependence of the normal and efficient outer retina function to choroidal blood flow supply. Systemic diseases affecting vascular system or spread by blood can affect both the choroid and the retina. A malfunction of the choroid will have a strong effect on the outer retina and photoreceptor metabolism. Until now it is not clear if the choroid might have an autoregulation mechanism, but the choroid may react in response to retinal pathology, as a response to an increased or decreased need for nutrition or metabolic input of retinal cells, or to the excessive retinal metabolic products to be expelled to blood circulation.

1.3.2. THE DIABETIC CHOROIDOPATHY

Diabetic retinal disease is much better known than diabetic choroidal disease, a great part because of easier visibility by fundoscopic exam and imaging such as fluorescein angiography and first OCTs.

Searching PubMed, the first paper using the term “Diabetic choroidopathy” was published on 1982 by Sarraco et al., where they described vascular anomalies, microaneurysms, and obliterated zones similar to the retinal changes of diabetic patients.[69] The exact pathophysiology of a Diabetic choroidopathy is still unknown, but several histologic and imaging studies indicate changes occurring in the choroid in diabetic patients.

Hidayat and Fine[70] in 1985 described histologic findings in eyes of subjects who had suffered for long standing Diabetes of capillary dropout and basement membrane thickening. In 1988, Fryczkowski et al [71] using scanning electron microscopy also found a drop-out of the choriocapillaris and they described additional findings such as increased tortuosity, dilation, narrowing, hypercellularity, vascular loop, microaneurysm formation, and sinus-like structure formation between choroidal lobules. Another important histologic study demonstrating evidence of changes in the choroid of Diabetic patients was performed by Cao et al[72] in 1998: they described two types of choriocapillaris degeneration, focal and diffuse, and the evidence of neovascularization and increased basal laminar deposits. Focal choriocapillaris degeneration was found to be more prominent in the posterior pole than in the equatorial or peripheral choroid, not associated to neovascularization and 4-fold greater in diabetics than in nondiabetics. As choriocapillaris degeneration increased from focal to diffuse, neovascularization was identified beneath the RPE cells, and most were in the peripheral choroid. Diffuse choriocapillaris degeneration was associated with increased thickness of Basal Laminar Deposits, suggesting that deposition of this material at Bruch's membrane was related to the atrophy of the choriocapillaris. They also postulate that visual acuity loss can occur due to diabetic choroidopathy before having retinopathy. In 2013, Lutty et all[73] described same findings described previously by Cao et al, but they introduced two types of choroidal neovascularization: the intrachoroidal regarding capillary network near the lamina fusca, and the extrachoroidal located primarily between the retinal pigment epithelium and the Bruch's membrane and within the latter. Neovascularization was found more peripherally.

Flow studies, whether quantitative or dynamic, also showed evidence of changes in the choroid of diabetic patients suggesting Diabetic Choroidopathy. Dimitrova et al in 2001[74] using Color doppler imaging showed that in early stages of diabetic retinopathy the choroidal circulation is affected with findings suggesting occlusive or hyper viscosity phenomena reducing blood flow. In 2004, Nagaoka et al[75] using laser doppler flowmetry demonstrated that the choroidal blood flow in the foveal region was significantly lower in patients with Diabetes, especially those with macular edema. Pemp et al [76], in a review of literature found some contradictory data but the most consistent was that studies assessing the choroidal blood flow beneath the fovea with the use of laser doppler flowmetry delivered more consistent results, indicating a reduction of choroidal blood flow in patients with nonproliferative and proliferative diabetic retinopathy.

Studies using imaging techniques for choroidal and retinal blood flow also demonstrated changes in diabetic eyes. Shiragami et al[77], found on Indocyanine green angiography choroidal abnormalities that they described as: hypofluorescent spots, small and large hyperfluorescent spots. The severity of diabetic retinopathy was significantly associated with the presence of hypofluorescent spots. Hua et al in 2013[78] also described the same findings and found late choroidal nonperfusion regions more predominant in advances stages of retinopathy (proliferative).They described an “inverted inflow phenomena” referring to a finding where choroidal vessel filling time was longer than retinal vessel filling time.

Both from histologic and angiographic studies we do have evidence that there is a Diabetic choroidopathy. Histologic proof or diagnosis can only be obtained from

enucleated or post-mortem eyes, with only retrospective value. Angiographic exams can be performed *in vivo* with diagnostic and prospective clinical value, but are invasive, and with limited capacity of choroidal changes information due to limited resolution and natural image overshadowing by the pigment of the RPE. Ultrasonography and Doppler flowmetry are another ophthalmological exams that can give limited information when we want to evaluate the choroid.

The technology improvement in Optical Coherence Tomography in the last 10 years has brought about the possibility to evaluate the choroid in a noninvasive exam, giving more detailed and new information correlated to physiologic and pathologic states.

1.4. OPTICAL COHERENCE TOMOGRAPHY – A METHOD TO VIEW THE CHOROID

Almost 30 years ago, ocular and especially posterior pole imaging was revolutionized with a new technology, the Optical Coherence Tomography (OCT) described by Huang et al. from their work at the James Fujimoto laboratory at the Massachusetts Institute of Technology (MIT).[14,15] The technique was an optical imaging modality using the same rationale of ultrasonography of measuring backscattering signal, but using light instead of sound. It allowed in a noninvasive, noncontact, simple and rapid way to image cross-sectional anatomy of the retina. First in vivo images were shown in 1993 and since 1996 when the first commercial device was released, it became widely used and now is an indispensable tool in current clinical practice in Ophthalmology.[15,79] OCT is based on low-coherence

interferometry employing near- infrared light to produce cross-sectional two- and three-dimensional high resolution images.[14,15,80]

The first device was an innovation for retinal clinical practice, but it had some limitations in image quality. Those limitations were related to the scanning speed (100 A-scans/second) and the technology used, the Time Domain (TD). Structures and layers of the retina were identified by software in a false color code to facilitate diagnosis, since the resolution was broad. The quality of images was highly susceptible to movement and other factors. In the subsequent models the TD-OCTs were improved in image quality, resolution and speed of acquisition. It evolved from 100 A-scan/s with 20µ of axial resolution for the 1st OCT to 400 A-scans/s with 10 µ of axial resolution for the last TD-OCT, the SRATUS from Zeiss. With the latest TD-OCT, image resolution of the retina and details improved, but due to highly pigmented retinal pigmented epithelium, signal was unable to penetrate deeper and image the choroid. From the first commercial device, it took about OCT imaging 10 years to make the leap. The technology switched from Time domain to Frequency or Fourier Domain (FD). Basically, the image is obtained from backscattered light as frequency information acquired with a light source, charge-coupled device (CCD) camera, and a spectrometer, the Spectral Domain OCT (SD-OCT) or by sweeping a narrow-bandwidth source through a broad range of frequencies with a photodetector, the Swept source OCT (SS-OCT). These new OCTs use light sources from 880nm for SD to 1060nm for SS. With these improvements in technology, along with improvement and development of software, tracking, averaging, digital image corrections, it is now possible to have devices with axial resolution up to 2µ, acquisition speed of 100 000 A-scan/s. Image resolution and depth penetration of

the signal allow good and detailed visualization of the retina and the choroid, three-dimensional and volumetric information.[15]

The interest and studies of the choroid with OCT were driven with the first description of the Enhanced Depth Imaging by Spaide[17] with the SD-OCT Spectralis from Heidelberg Engineering and later with the SS-OCT. With those technologies the difficulty in viewing the choroid and its scleral limit due to scattering of light by the pigment of the RPE was solved. With both it become possible to visualize the choroid until the scleral limit and sometimes beyond that, with good identification of the different vascular layers and the suprachoroidal space. Choriocapillaris microscopic details still exceeds the resolution capacity of these devices.

Several ocular diseases were studied and important findings were reported in Central Serous Chorioretinopathy, Diabetic Retinopathy, Glaucoma, Tumors, Age Related Macular Disease, High Myopia, Inflamatory diseases and some inherited retinal diseases.[81,82,83]

Since OCT choroidal morphologic or anatomic findings were and still are not well known, most studies tried to correlate the easiest parameter to evaluate the choroidal thickness with pathology.

Until the end of 2014, year of start of our study, the status regarding imaging the choroid with OCT was as described below.

Mrejen and Spaide[84] published in 2013 a very good review regarding Choroidal OCT imaging. They found that in most studies the choroidal thickness was measured with

the anterior limit at the outer edge of the RPE and the posterior limit of the choroid was considered as the inner border of the hyperreflective surface posterior to the large choroidal vessels, named as scleral/choroidal interface. Several studies evaluated and found good intersystem and interobserver reproducibility and repeatability of choroidal thickness measurements.[85–93] Studies in normal or healthy populations were made in order to determine normal thickness values and the effect of various parameters (Table 1).

Author	Year Publication	n eyes	mean age (years)	±SD	range (years)	mean SFCT (µ)	±SD	range (µ)	Correlation age	Country	OCT
Margolis	2009	54	50,4		19-85	287	76		-15,6/10Y	usa	SD
Manjunath	2010	34	51,1		22-78	272	81			usa	SD
Ikuno	2010	79	39,4	16	23-88	354	111	191-573	-14/10Y	Japan	SS
Rahman	2011	100	38		30-49	332		142-563		GB	SD
Hirata	2011	31	64,6	17,3	21-87	202,6	83,5		-30/10Y	Japan	SS
Ding	2011	420	49,73	17,89	20-85	261,93	88,42			China	SD
Ouyang	2011	55	32,85	11,45	20-68	297,8	82,2		-19,5/10Y	usa	SD
Chen OD	2012	100	38	5	30-49	334	94	172-568		australia	SD
Chen OS	2012	100	38	5	30-49	333	90	133-555		australia	SD
Shin	2012	57	45,28	14,8	23-80	270,82	51,4	161-383	-13,1/10Y	Korea	SD
Wei	2013	3233	64,3	9,6	50-93	253,8	107,4	8-854	-33/10Y	China	SD
Read	2013	194	8,2	1,9	4-12	330	65	189-538		australia	SD
Sanchez-Cano	2014	95	23.8	3.2	19-32	345,67	81,8	152-519		spain	SD
Pappuru	2014	221	42,8	13,6	21-80	280,1	46,5		-11,8/10Y	india	SD

Table 1 – Most cited studies of Choroidal thickness in healthy population[18,86,101–104,88,94–100].
Legend: n – number; SD – standard deviation; SFCT – subfoveal choroidal thickness; µ - micron; OCT – optical coherence technology; SD OCT – spectral domain; SS OCT – swept source. Blank spaces mean lack of data on publication.

Table 1 shows that there is a great variability between the published studies regarding the sample size, mean and range of subjects age. But the greater variability is in the subfoveal choroidal thickness (SFCT) measurement, with variable mean values with great standard deviations (SD) and large range of thickness measurements. Not all studies presented all data, missing in some the information regarding SD values or the range of measurements. The studies were performed in different ethnicity populations, but no evidence at the time indicates that it can be a bias factor. Almost all studies reported strong correlation with age, with a decrease in thickness with ageing, some indicating the rate of thickness loss per year.[18,94–99] But Ding et al in their study stratified the subjects by age and found no correlation of choroidal thickness with age in the group less than 60 years old. Negative correlation was found only with the older population.[98] On the other hand, Read et al. in their study in a pediatric population found that choroidal thickness is thinner in the youngest and get thicker until the teen age.[100] In the largest population study, the Beijing Study, they found the greater variability and range of choroidal thickness measurements, and found significant correlation with a multitude of parameters such myopia, axial length, high blood pressure, deep anterior chamber depth and others.[101] The great variability of choroidal thickness was noted by Kahn et al in a comment to the study of Rahman (more than 80% of choroidal thickness variation not explained by age) postulating that other causes may be behind this variability than age alone.[102,86] Choroidal thickness was evaluated in some studies with several measurements in different locations or as macular volume or maps measurements.[89,95,96,103–110,111] The common finding in those studies is that in healthy subjects the thicker point of the choroid is found centrally and the thicker quadrant is

usually the superior and the thinnest the nasal. Correlation of choroidal thickness measurements was found with other factors such as diurnal variation, blood pressure, water intake, coffee, thicker choroids.[83,92,112–117] Some authors tried to look to vascular choroidal morphology.[118–124] Most of these studies are descriptions of software development with no application or reproducibility for use with current available devices used in clinics. Nevertheless Zhang et al. concludes in their work that it is possible to do segmentation of most of the choroidal vasculature with standard SD-OCT.[119] The only study that describes and characterizes normal findings on OCT images of the choroidal vasculature was the one published by Branchini et al.[125] After evaluating images of a wide-age range of healthy subjects (23 to 89 years) they conclude that normal/healthy vascular morphology on OCT images of the choroid includes the following characteristics: 1- choroid is thicker in subfoveal zone; 2- the posterior boundary or choroid-scleral border has a convex or "bowl" shape and is clearly identifiable; 3- there is no focal thinning ; 4- the continuum in size of choroidal vessels axially with "even", i.e., that larger vessels lie closer to the choroid-sclera junction and smaller vessels closer to the Bruch's membrane; 5- and the large vessels are uniformly spaced. Suprachoroidal layer was described only by Rahman[86] and Yiu[122] as the hyporreflective layer or space visualized in some patients, posteriorly to the hyperreflective limit that lays after the large choroidal vessels. It is more frequent in hyperopic eyes and may be a cause of misjudgment of the correct limit for choroidal thickness measurement.

1.5. LITERATURE REVIEW ON DIABETIC CHOROIDOPATHY ANALYSED WITH OCT

The literature search was done using the PubMed, Web of Science databases engines and Google search using selective and advanced search filters with the words: diabetic/Diabetes/choroid/choroidopathy/OCT/optical coherence tomography, to identify all pertinent and peer-reviewed publications until 2014.

A few cross-sectional studies were published looking for choroidal changes in diabetic patients. The majority of authors found a decrease in choroidal thickness in diabetic patients but with different patterns. Esmaeelpour et al. found in type 1 and type 2 diabetic patients that the CT was thinner in diabetic patients when compared with controls, particularly centrally, but with no correlation with retinal thickness. No focal CT thinning was found below retinal lesions[126,127] Regatieri et al. and Unsal et al. found significant CT thinning when compared with controls only in severe cases of diabetic retinopathy (DR) as proliferative diabetic retinopathy (PDR) and macular edema (ME). Mean values of CT for control and early stages of DR were similar.[81,128] Querques et al. also found decreased CT in diabetic patients, even with no evidence of DR. They describe focal thinning of the choroid correlated with suprajacent retinal thickening. In contrast Vujosevic et al. and Lee et al, describe CT thinning but only in patients with evidence of DR, and no correlation was found with clinically significant macular edema (CSME).[129,130] Two groups looked specially for the correlation with diabetic macular edema (DME). Hua et al. compared findings in subjects with DME and submacular detachment (SMD) or DME without SMD. CT was thinner in those patients without SMD.[78] Gerendas et al. showed a significant

decrease in CT in eyes with DME and in the counter-lateral eye with no DME when compared with controls, but no correlation was found between CT and retinal thickness (RT).[131] When looking to younger populations, Sayin et al. found no difference in CT between diabetic and control patients.[132] Duration of Diabetes was found to have a week correlation with CT by Yülek et al.[133] and microalbuminuria was found correlated with CT thinning in pre-retinopathy diabetic patients.[134] CT appeared to increase after intensive control of several systemic and metabolic factors.[135] There was two studies within all those published until 2014, with a large sample of subjects analyzed reporting, controversially, an increase in CT. Kim el al found an increase of CT with increasing severity of the DR. Only subjects with treated PDR had a decrease in CT. They found that CT increased progressively from cystoid DME, diffuse DME to DME with SMD.[136] In the other study, Xu et al. found, after adjusting for other factors, a significant positive correlation between CT and the diagnosis of Diabetes and glycosylated hemoglobin serum values, but none with the presence or severity of retinopathy. Significative correlation was found with a variety of parameters, ocular and systemic, suggesting that CT measurements are multifactorial dependent.[137]

Findings had some discrepancies between studies, probably due to different designs and analysis made. The sample of subjects also was very variable between studies and in several just a few parameters were taken in account (table 2).

		Age			TOTAL			NDR			MNPDR			SNPDR			PDR			DME			DME+SMD		
Author	n	(mean years)	±SD	Range (years)	SFCT (mean µ)	±SD	Range (µ)	SFCT (mean µ)	±SD	Range (µ)	SFCT (mean µ)	±SD	Range (µ)	SFCT (mean µ)	±SD	Range (µ)	SFCT (mean µ)	±SD	Range (µ)	SFCT (mean µ)	±SD	Range (µ)	SFCT (mean µ)	±SD	Range (µ)
Esmaeelpour et al	63			41-82				214	55	114-365	208	49	122-313	205	54	122-321				211	76	117-365			
Hua et al	18																			276		142-351	364		250-400
Vujosevic et al	102	57,5	13,3	31-83				280,6	68,6		279,4	81,6					230,5	25,8							
Regatieri C et al	49	68									222	21,6					162,7	7		169,5	14,7				
Farias et al	62				228,87	14,44																			
Kim et al	235	62,6	12,4					262,3	68,4		244,6	77		291,1	107,7		363,6	74,9							
Lee et al	203	58	12	32-78				219,1	47,8	98-318	158	56,3	111-326	161,2	38,5	101-398	228,5	38,9	164-284						
Xu et al	246	66	9,2		266	108	45-724																		
Unsal E et al	151	60,5	8,2								235,4	84,5					203,8	46,8		206,8	45,4				
Querques G et al	63	65	9	48-83				238,4	47,9		207	55,9								190,8	48,4				

Table 2 – Most cited studies of Choroidal in Diabetic population.

Legend: n – number; SD – standard deviation; SFCT – subfoveal choroidal thickness; µ - micron; NDR – no diabetic retinopathy; MNPDR – mild/moderate nonproliferative diabetic retinopathy; SNPDR – severe nonproliferative diabetic retinopathy; PDR - proliferative diabetic retinopathy; DME – diabetic macular edema; SMD – submacular detachment; Blank spaces mean lack of data on publication

All authors considered mean thickness values to perform the analysis and correlations except on the paper published by Adhi et al.[138] On their work they looked for choroidal vascular changes occurring in diabetic patients. They used the choroidal morphologic features described by Branchini for healthy subjects.[125] They found that more than 90% of the diabetic patients had irregular choroidoscleral contour (or S shaped) in contrast to none on the control group. Diabetic patients had thinning of central CT and focal CT thinning zones, more frequent in DME. None was found in controls. There was a decrease in large choroidal vessel layer thickness in DR eyes.

CHAPTER 2 - REASON OF THE STUDY AND PURPOSE

2 - REASON OF THE STUDY AND PURPOSE

From histological, flowmetry and angiographic studies, we do know that there is a diabetic choroidopathy. Choriocapillaris drop-out, focal or lobular lesion, occlusion and blood flow impairment, vasodilatation, tortuosity and neovascularization are changes occurring in the choroid in Diabetes. We also know that the choroid has an important role in posterior retinal segment function and choroidal malfunction or pathology can be on the origin and/or consequence of the retinal pathology.[69,70,139–141]

Optical coherence tomography has revolutionized imaging of the eye. With the improvement of the technology it is now possible with the commercially available devices to better view the choroid in the means to study ocular pathology. From the literature several studies were published regarding choroid imaging in healthy and diabetic patients. Nevertheless, discrepancies in results are found.

We found a lack of studies trying to correlate vascular morphology of the choroid with the retinal disease. The choroid is a tissue with a particular anatomy where previous studies had demonstrated a certain independence in function of the lobular units of the choriocapillaris and a possible focal effect in the retina immediately above. On the other hand, the choroid as a highly vascularized tissue me be affected by several other factors, in physiological or pathological systemic conditions.

Choroidal thickness is a parameter that increases or decreases with choroidal vascular changes. But from the studies on healthy populations we can find that there is a great variability of measurements, dependent on individual characteristics, age,

ocular and systemic factors, and others so a cut off or mean normal value stays difficult to establish. Beside the limitations of the published studies pointed above, the most important is may be the lack of correlation of morphological findings with thickness measurements and longitudinal or over time evaluations for progression.

2.1. MAIN STUDY PURPOSE

To evaluate whether choroidal thickness measurement alone is a reliable parameter for characterize diabetic choroidopathy, and to characterize choroidal morphological findings using the OCT and correlate them with choroidal thickness and the diabetic retinopathy to determine if diabetic choroidopathy can be identified, classified and used as a biomarker of ocular diabetic disease and progression.

2.2. SECONDARY, ANCILLARY OR PILOT STUDY PURPOSE

- To explore subjects diabetic and control, for ophthalmological facts, as best corrected visual acuity, intraocular pressure, biomicroscopy of the anterior segment, ocular fundus examination and imaging.

- To define vascular morphological changes identifiable on OCT images as possible markers of choroidopathy

- To investigate demographics, systemic and metabolic status.

- Data collection for statistical analysis for each individual and global

- Biomedical translation of our results

CHAPTER 3 - MATERIAL AND METHODS

3 - MATERIAL AND METHODS

3.1. DESIGN OF THE STUDY AND SETTING

The study was performed at the Ophthalmology Department of the Centro Hospitalar Entre o Douro e o Vouga (CHEDV), Santa Maria da Feira, Portugal, from May 2014 to 2018.

It was a cohort, prospective, longitudinal and observational study, where diabetic and healthy controls subjects attending the Medical Retina and General Ophthalmology consultations were submitted to: complete ophthalmological exam; fundus imaging by photography, OCT and Fluorescein Angiography (the later when indicated); familiar/hereditary, systemic and metabolic review; blood pressure registration; and blood collection when required. The subjects were followed in consecutive visits according to the guidelines for the clinical disease state and directed to correct treatment whenever it was indicated. The follow up and evaluation period for the study was intended to be for 2 years.

3.1.1 PILOT OR ANCILLARY STUDY

PILOT 1 - A small sample patients were analyzed for OCT images to define the morphological findings classification to be used in the whole study.

ANCILLARY STUDY – A small sample of images obtained with the swept-source OCT in collaboration with another Ophthalmology Department (Instituto Gama Pinto, Lisboa, Portugal) to have representative images of superimposed retinal e choroidal maps obtained automatically by the OCT software.

3.2. SUBJECTS

The subjects participating on the study were divided in two groups: Group 1 of Diabetic patients and Group 2 of healthy subjects.

3.2.1. GROUP 1 - INCLUSION CRITERIA

For this group were selected patients attending to the Ophthalmology department visits, with the diagnosis of Diabetes, with none or any stage of diabetic retinopathy, aged 18 or more and who have given and signed voluntarily the consent to participate in this study.

3.2.2. GROUP 2 – INCLUSION CRITERIA

For this group were selected non-diabetic patients attending to the Ophthalmology department visits, aged 18 or more and who have given and signed voluntarily the consent to participate in the study.

3.2.3. EXCLUSION CRITERIA (BOTH GROUPS)

Subjects were excluded from the study when one of the following was present: unwilling or inability to give the consent to participate in the study; previous treatment of any kind for diabetic retinopathy (laser, intravitreal injection of anti-VEGF or steroid, vitrectomy); previous or ongoing chorio-retinal disease; history of intra-ocular inflamatory, infectious or oncologic disease; history of ocular surgery; media opacities affecting good visualization of the posterior segment; refractive errors

greater than ± 6 Diopters; systemic oncologic disease; systemic chronic steroid or immunosuppressive medication; any serious and uncontrolled systemic condition.

3.2.4. DROP OUT or LOST OF FOLLOW UP (DO or LFU)

After subject inclusion in the study, if any of the exclusion criteria occurred, as the need of treatment for DR or ocular surgery, for example, the patient/subject was dropped out from the study. If the patient failed to attend any visit other than baseline during the study period for any reason, he/she was considered as loss of follow up.

3.3. CLINICAL EVALUATION

All clinical and exams data were collected and registered in the source document (the hospital file) and recorded for the study in the Case report forms (CRF).

The subjects were submitted to clinical history collection for ophthalmological, systemic and familiar anamnesis. All were submitted to complete ophthalmological exam including: Best corrected visual acuity using ETDRS equivalent charts; External ocular evaluation; Complete Slit-lamp biomicroscopic exam; Goldman tonometry; Gonioscopy when indicated; dilated fundus exam with auxiliary fundus lenses for posterior pole and periphery observation. Systemic evaluation consisted in registration of current medication, recent (within 3 month) blood analysis results including glycosylated hemoglobin for diabetics, performed by the family or assistant doctor. When not available, these exams were requested. Measurement of blood pressure and recording of time of measurement.

Diabetic retinopathy stage was graded and recorded based on fundoscopy and fundus photography observation according to the International Clinical Disease Severity Scale for Diabetic Retinopathy[38], as following: No apparent diabetic retinopathy (NDR) - no findings found; Mild non-proliferative diabetic retinopathy (Mild NPDR) – only microaneurysms (Ma); Moderate non-proliferative diabetic retinopathy (Moderate NPDR) – more than just Ma and less than severe NPDR; Severe non-proliferative diabetic retinopathy (Severe NPDR) – more than 20 intraretinal hemorrhages in the 4 quadrants and/ or venous beading in 2 or more quadrants and/or prominent IRMA in 1 or more quadrant with no signs of proliferative DR; Proliferative diabetic retinopathy (PDR) – neovascularization of the disc or elsewhere and/or vitreous/preretinal hemorrhage. Macular edema was based on OCT findings.

These evaluations were performed in all subject visits.

3.4. IMAGING ACQUISITION AND ANALYSIS

Imaging exams consisted in Fundus photography, Optical Coherence Tomography and Fluorescein Angiography. All exams were performed by experienced Imaging Technician/Orthoptist.

3.4.1 FUNDUS PHOTOGRAPHY

In all visits the subjects of the study were submitted to dilated posterior pole photography performed with the Non-midriatic Retinograph TRC NW8® from Topcon

Corporation. When Fluorescein Angiography was done, fundus photography was performed using the Angiograph (see below).

3.4.2. FLUORESCEIN ANGIOGRAPHY

Whenever it was clinically indicated, fluorescein angiography was performed according to normal procedures for the exam[142], using the Digital Angiograph FF 450 plus® from Carl Zeiss Meditec. Image analysis and report was done always by an Experienced Retina Specialist.

3.4.3. OPTICAL COHERENCE TOMOGRAPHY (OCT)

3.4.3.1. OCT DEVICES

Most of study OCT image acquisitions were performed using the OCT CIRRUS HD-OCT® 4000 from Carl Zeiss Meditec. It is a spectral domain OCT using a super luminescent diode (SLD) at 840nm, with a scan speed of 27000 A-scans/s, an A-scan depth of 20 µ, an axial resolution of 5 µ and transverse resolution of 15 µ. The device had un upgraded software with EDI and Fast-track.

Pilot studies were performed using the:

- OCT RS-3000 Advance® from NIDEK Corporation, Ltd. It is a spectral-domain OCT using a SLD at 880 nm, with a scan speed of 53 000 A-scans/s, with and enhanced retinal and choroidal acquisition protocols and a tracking software.

- Spectralis OCT2 ® from Heidelberg Engineering. It is a spectral-domain OCT using a SLD at 870 nm, with a scan speed of 85 000 A-scans/s, with eye-tracking software, increased depth resolution and automated retinal layer segmentation.

- DRI OCT-1 Triton plus® from Topcon corporation. It is a swept-source OCT using a light source of 1050 nm, with a scan speed of 100 000A-scans/s. It has the particularity in its software to have automated layer segmentation including the choroid with the ability to provide topographic maps.

3.4.3.2. ACQUISITION PROTOCOLS

OCT image acquisition was made for all study subjects in all visits.

Image acquisition protocols were defined to have 3 dimensional or cube/volume information for retinal thickness maps and macular edema location, and line scans with best resolution for retina and choroid morphologic evaluation and depth penetration for good sclero-choroidal interface identification.

For the CIRRUS OCT, we performed to all patients the protocol acquisition Macular CUBE 512x128 centered in the central fovea to obtain the macular thickness map. Then a HD line scan with EDI and the maximum averaging of 20 was obtained passing through the fovea and in the presence of extra-foveal macular edema through the maximum retinal thickness zone. The best line scan for posterior choroid limit was selected for analysis.

For the RS-3000 OCT we used the protocol acquisition Macular CUBE 512x128 centered in the central fovea to obtain the macular thickness map. Then a Macula cross scan in the choroidal mode with an averaging of 30 was obtained passing through the fovea and in the presence of extra-foveal macular edema a line scan was obtained passing through the maximum retinal thickness zone. The best line scan for posterior choroid limit was selected for analysis.

For the Spectralis OCT we performed also a macular cube and a line scan with an averaging of 20 to 30 following the same guidelines for the other devices.

The DRI OCT-1 Triton image acquisitions were performed in another Ophthalmology department from the Instituto Gama Pinto, Lisboa, Portugal, included in a sub-study. Acquisitions were performed to obtain macular thickness maps, line scans with choroidal definition in the foveal and macular edema zones, and automated choroidal thickness maps.

3.4.3.3. IMAGE ANALYSIS

Analysis was made for the retina and the choroid.

Retinal analysis was to evaluate findings correlated with diabetic retinopathy and to identify and locate macular edema. Morphological findings were changes in the normal anatomy of the retinal layers (hyper/hyporreflectivity, atrophy, cysts, disorganization – DRIL, photoreceptors layer integrity, new-vessels, subretinal fluid and others). Retinal thickness maps were evaluated to identify Macular edema and classify as central (foveal involvement) or extra-foveal (no foveal involvement).

Central foveal retinal thickness (CFRT) value was registered. Any other abnormality found was also registered.

Choroidal analysis was made separately for thickness measurements and morphological evaluation. Only sub-foveal choroidal thickness (SFCT) measurement was recorded. Measurement was made manually, and boundaries were defined as going from the posterior limit of the RPE-Bruch Membrane complex to the outer limit of the great choroidal vessels before the hyperreflective layer (figure 1). The measurement was made always by the same experienced Retina specialist. SFCT measurements were used for the statistical analysis. Images with low resolution or quality turning difficult to identify morphological features of choroidal posterior boundaries were excluded from the study (figure 2).

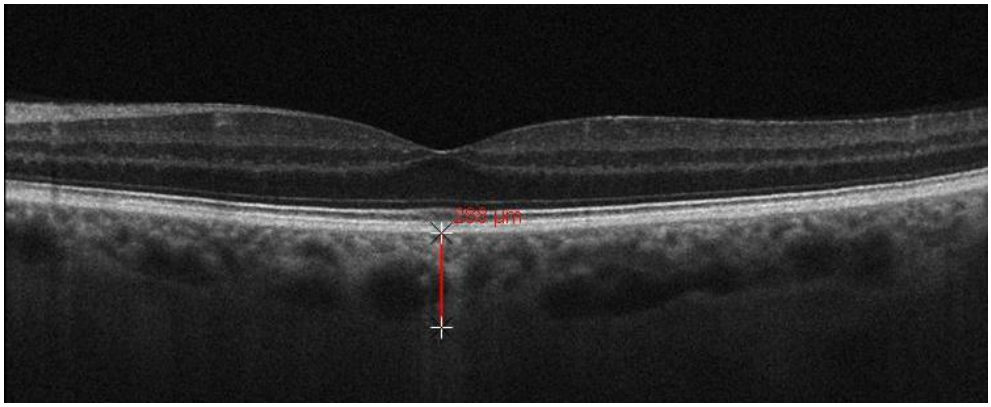


Figure 1 – Example of boundaries for the measurement of SFCT.

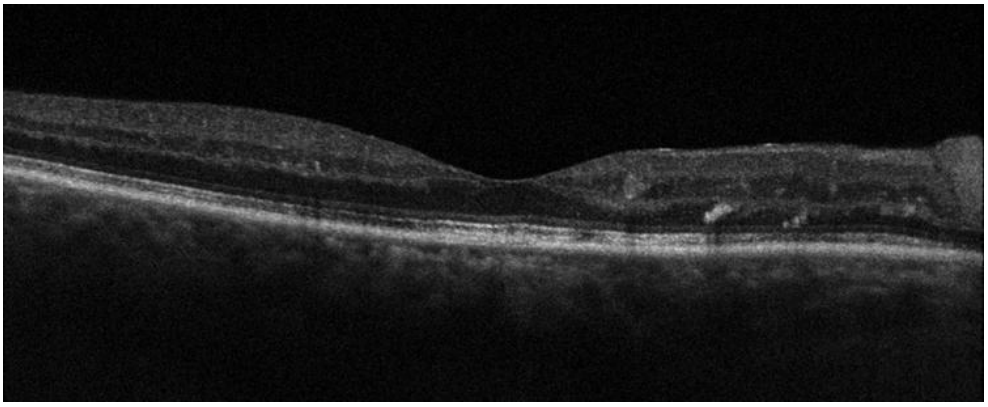


Figure 2 – Example of an image with inability to visualize the sclero-choroidal limit.

All OCT scans were made using the device tracking software and follow up images were obtained at the same location marked as baseline reference.

Choroidal morphological analysis was made based on the findings described by Branchini et al[143] for normal choroid: 1- choroid is thicker in subfoveal zone; 2- the posterior boundary or choroid-scleral border has a convex or “bowl” shape and is clearly identifiable; 3- there is no focal thinning ; 4- the continuum in size of choroidal vessels axially with “even”, i.e., that larger vessels lie closer to the choroid-sclera junction and smaller vessels closer to the Bruch’s membrane; 5- and the large vessels are uniformly spaced (Figure 3). Additionally, we performed a pilot study to characterize abnormal findings in diabetics and establish a classification to use in this study. Morphological findings were analyzed and correlated with suprajacent retina to evaluate any existing anatomical correlation.

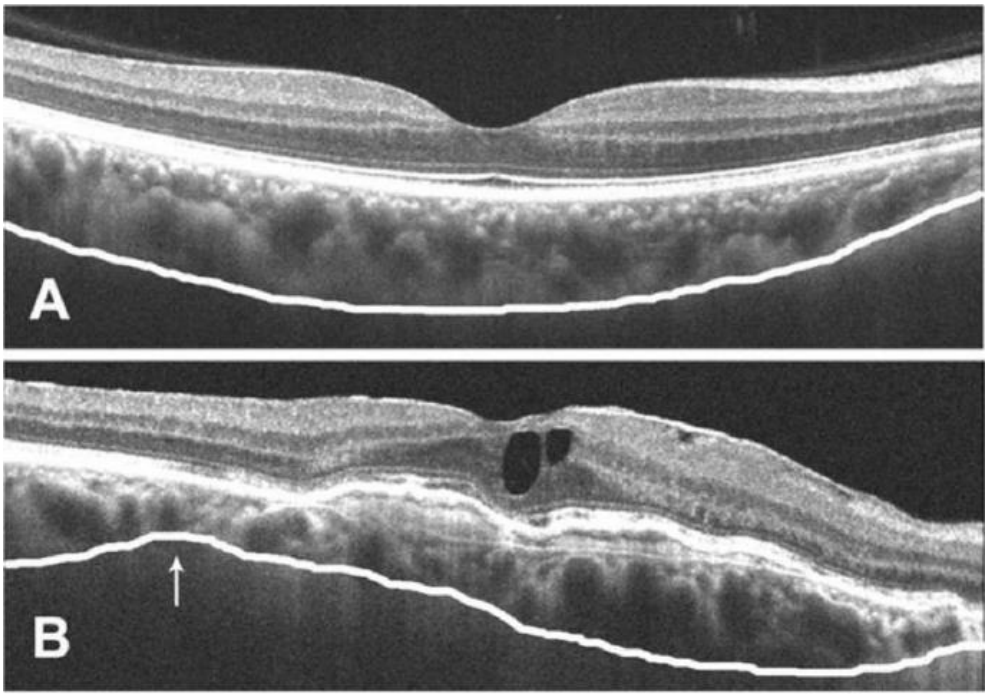


Figure 3 – Choroidal morphological analysis from Branchini et al, in "Analysis of Choroidal Morphology and Vasculature in Healthy Eyes Using Spectral-Domain Optical Coherence Tomography". Ophthalmology, 2013[143]. Reproduced with permission. A – SD-OCT image of a healthy subject with the bowl shape of the sclero-choroidal limit (white line), even distribution of large choroidal vessels in the nasal-temporal plane, even continuum of choroidal vessels size with larger outest in the choroid and smaller in the inner choroid. B – SD-OCT image of a diseased eye with a irregular "S" shaped sclero-choroidal border (white line), a focal thinning of the choroid (white arrow), dislocation of the sub-foveal thickest choroid point and proximity of large choroidal vessels of the EPR/Bruch's membrane posterior border.

Choroidal automated thickness maps (from SS-OCT images) were compared and correlated to superimposed retinal thickness maps and morphologic retina and choroid line scans.

3.5. STATISTICAL ANALYSIS

Statistical analysis was performed by an external statistician, accessing only the coded database and using Sata / SE statistical software version 12.0 (StataCorp LLC).

The normality of the variables was first tested to select the appropriate statistical tests. The normality was tested in general and by groups (control and diabetic) using the Shapiro-Wilk test. Since most of the distribution of variables were not normally distributed, non-parametric tests were selected

For continuous variables, the comparison between two independent groups (as control and diabetic groups) was performed using with the Mann-Whitney test. The comparison between more independent groups (as DR grade) was performed using the Kruskal-Wallis test. For categorical variables the Fisher's test was used. The differences between two dependent variables (as between visits), as well as between OD and OS, were performed using the Wilcoxon test for continuous variables. For categorical variables the McNemar test (for 2x2 tables, e.g., ME) or Stuart-Maxwell (for tables with more categories, as DR grade) were used. Correlations between variables were obtained with the Spearman correlation coefficient.

To identify predictive variables for one continuous variables, and to study the impact these independent variables on a dependent variable (as SFCT), a multivariate linear regression model was performed.

Statistical significance was considered for *p*-values lower or equal to 0.050.

3.6. ETHICS AND CONFIDENCIALITY COMPLAINCE

The protocol of the study with the internal number CHEDV-OFT-01-14 and patient informed consent form was submitted to the Ethics Committee and Administration Board of the CHEDV and approval was given on May 15th, 2014 (copy on the documents appendix).

The study was conducted following the tenets of the Declaration of Helsinki.

Confidentiality of data was assured by coding patient identification on the database for analysis.

CHAPTER 4 - RESULTS

4 - RESULTS

4.1. PILOT STUDIES

A pilot study was performed to identify markers or morphological findings to differentiate normal from abnormal choroid and create a classification system for the study. First evaluation was based on the classification made by Branchini et al[143] and then modified according to our findings. The results of this pilot study were presented on two congresses:

. At the 15th EURETINA Congress, on September 2015 with the poster **"Looking beyond thickness: choroidal findings with the Nidek OCT RS-3000 Advance"**

Thirty eyes of thirty patients were included, where 12 were from healthy controls and 18 from diabetic patients. On the diabetic group, 7 eyes had no DR, 5 eyes had mild NPDR and 6 eyes had ME and/or PDR.

OCT images were evaluated for choroidal morphological changes choroidal findings were classified as:

- Normal (figure 4) – when the sclero-choroidal border had an "inverted dome shape" (the bowl shape from Branchini) with the thickest choroidal point sub-foveal, and the "mottled" vascular layer of the choroid, corresponding to the choriocapillaris and Sattler's smaller vessels layer, had an imaginary line limit to the great vessels layer that lays parallel to the Bruch's membrane and/or the sclero-choroidal limit.

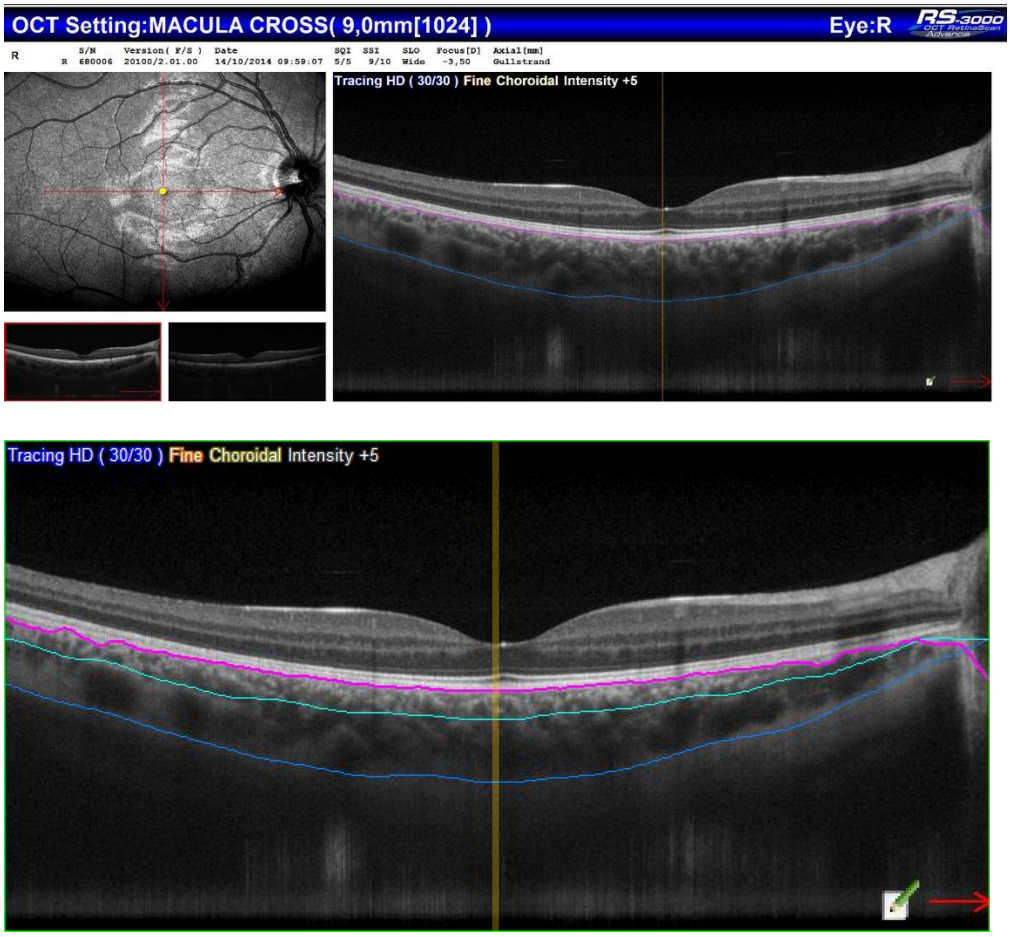


Figure 4– Example of a normal choroid morphology. Upper image showing the normal fundus and the choroid vascular feature. On the bottom the same image with the delineation limits: the Bruch's/RPE posterior border (purple line), the imaginary parallel line separating the mottled choriocapillaris/Sattler's layer and the great vessels layer (turquoise line) and the sclero-choroidal border (blue line).

- Abnormal (figures 5-7) – when there was a loss of the "inverted dome shape" of the sclero-choroidal border and/or the vascular layers had findings such absence or

atrophy of great vessels layer or the choriocapillaris/Sattler's layer, focal or diffuse, leading to a loss of the parallel line, and hyperreflective focal zones instead of the vascular hyporreflective images.

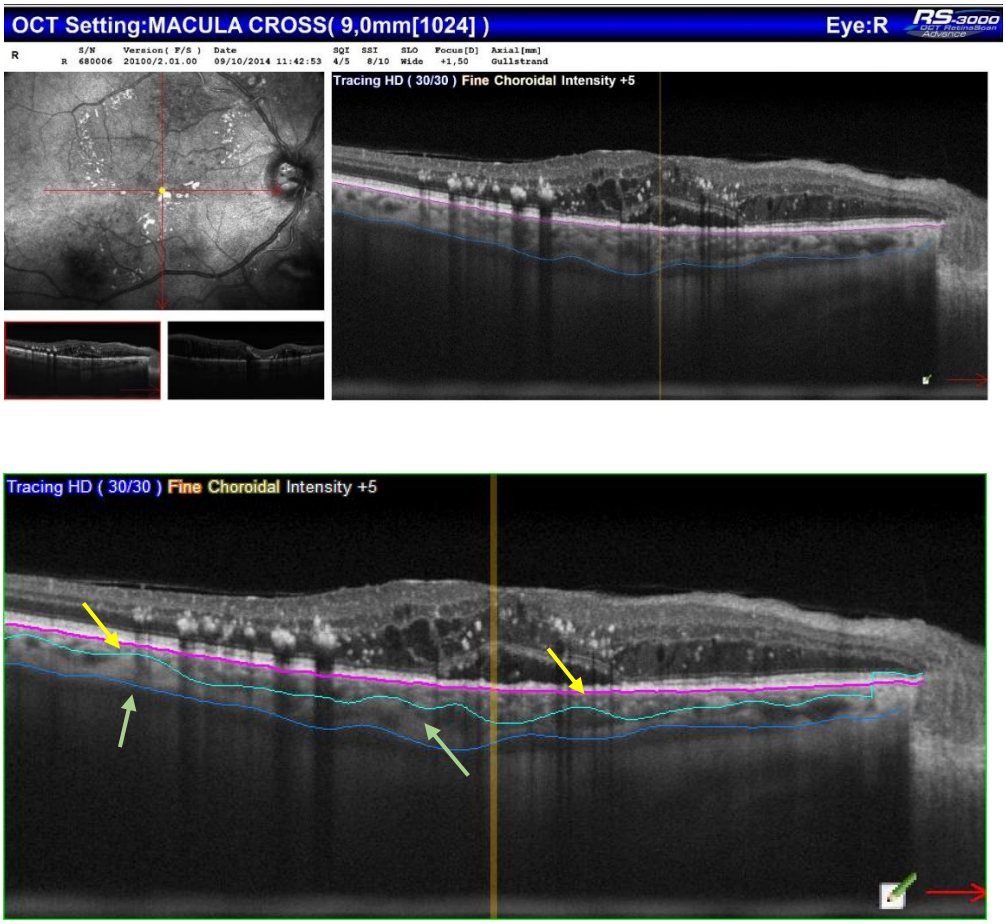


Figure 5 – Example of a Diabetic Macular Edema choroid morphology. Upper image showing the fundus image with CSME and the choroid vascular feature. On the bottom the same image with the delineation limits: the Bruch's/RPE posterior border (purple line), the imaginary line separating the mottled choriocapillaris/Sattler's layer and the great vessels layer (turquoise line) irregular/non- parallel due to focal zones

with atrophy or thinning (yellow arrows), focal lack of great vessels with hyperreflective space (green arrows), and the sclero-choroidal border (blue line) slightly irregular.

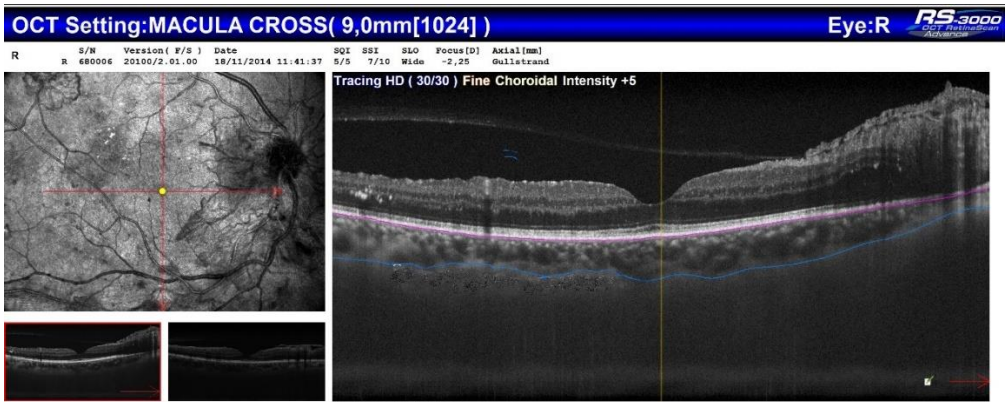


Figure 6 – Example of a PDR choroid morphology. On the fundus image large new vessels of the disc (NVD) and elsewhere (NVE) on the superior temporal arcade are visible. On the OCT line image: the Bruch's/RPE posterior border (purple line), an apparent preserved mottled choriocapillaris/Sattler's layer, the great vessels layer with a focal zone with atrophy and hyperreflective space temporally, and the sclero-choroidal border (blue line) slightly irregular with apparent moderate thinning of choroidal thickness.

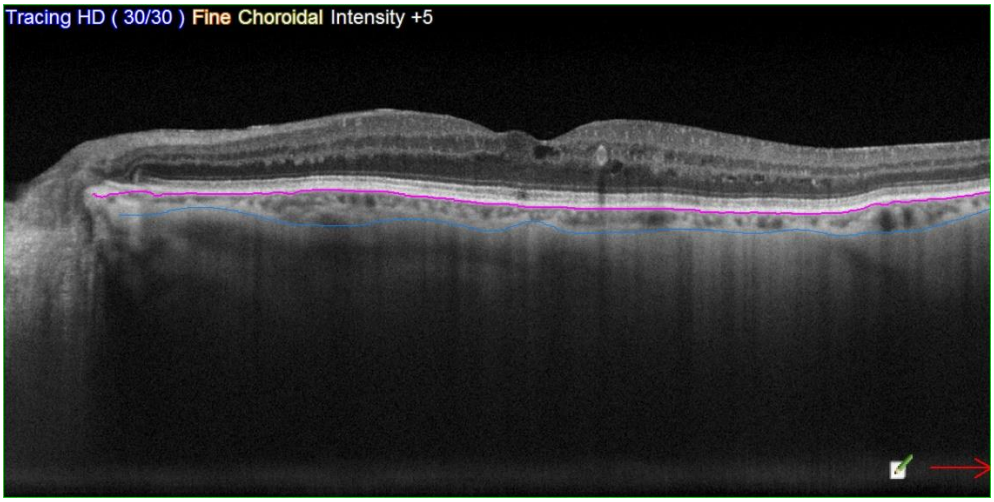


Figure 7– Example of a PDR choroid morphology. This patient had new vessels on the retinal periphery. On the OCT line image: the Bruch's/RPE posterior border (purple line), but a very thin/atrophic choroid with atrophy of all layers and the inability to draw a limit between them. Loss of the "inverted dome shape" of the sclero-choroidal border (blue line).

When choroidal morphological changes were analyzed for the different groups, we found that with the advance of the disease there were a progressive loss of the normal "inverted dome shape" of the sclero-choroidal border and with macular edema focal changes in the vascular layers were noticed suggesting a vascular/blood recruitment of focal retinal suffering zones (figure 8).

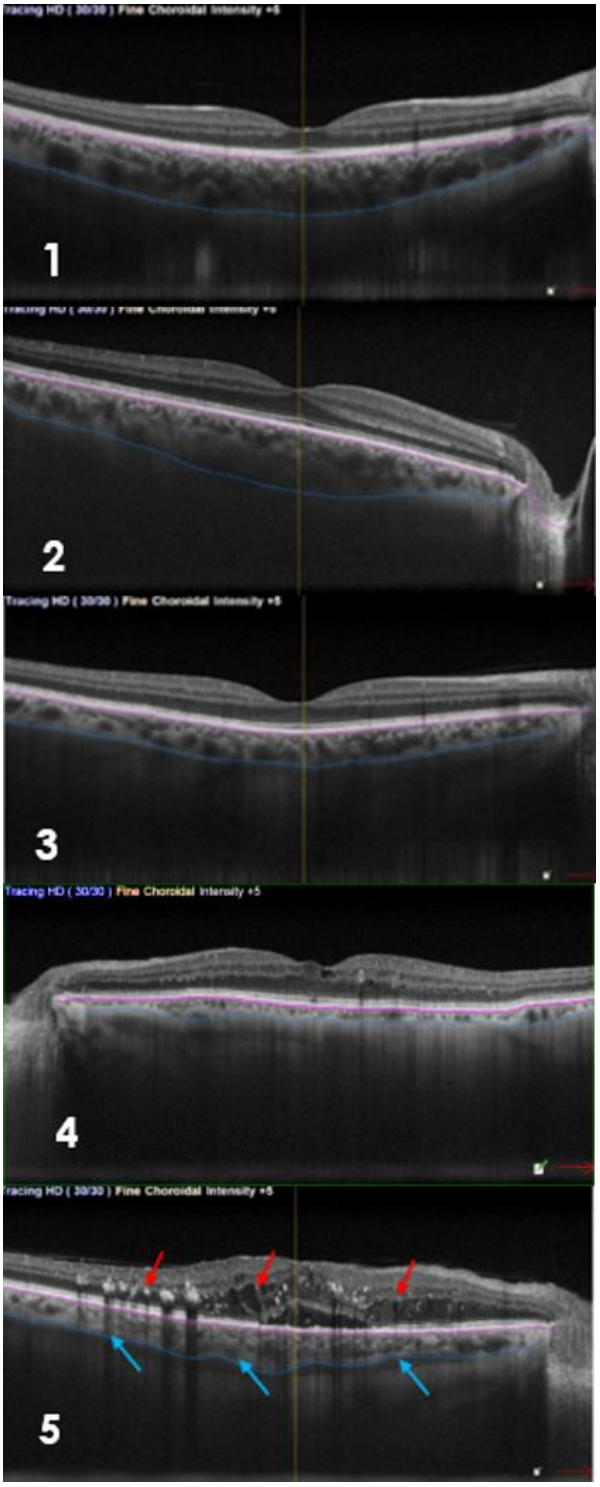

1
2
3
4
5

Figure 8 – Progressive change in choroid morphology in the different groups. 1- Healthy control. 2- Diabetic with no DR. 3- NPDR. 4- PDR. 5- CSME.

. At the EVER Congress on October 2015 with the poster **"Focal Choroidal Changes on Diabetic Macular Edema"** (published abstract on the Acta Ophthalmologica[144]).

Fifty-four eyes were included in this study: 8 with macular edema, 12 with non-proliferative diabetic retinopathy, 22 with no retinopathy and 12 controls. Age ranged from 22 to 80. Choroidal thickness varied from 61µm to 495µm. No correlation was found between SCFT with age, blood pressure or diabetic stage. Choroidal thickness showed high standard deviations values (Table 3).

	AGE	SUB FOVEAL THICKNESS	SISTOLIC BLOOD PRESSURE	DIASTOLIC BLOOD PRESSURE	DURATION DIABETES	HA1c
TOTAL	56,56±13,45	301,65±88,56	138,18±18,57	71,02±8,11		
CONTROL	50,58±12,59	312,08±41,85	132,17±20,14	74,75±19,52		
NDR	53,5±12,59	341,64±87,34	136,95±18,07	77,99±12,53	9,96±6,64	7,18±1,12
NPDR	62,67±9,71	265,42±75,79	137,33±18,44	75,33±8,56	13,42±5,55	7,76±2,05
ME/PDR	64,75±12,01	230,38±107,00	150,63±14,68	74±8,8	14,75±5,42	9,23±1,45

Table 3 – Mean values of SFCT, systemic and metabolic data by group and diabetic retinopathy sub-group.

In this study we found that for advanced stages of retinopathy and macular edema, the changes in the architecture of the choroidal vessels with a tendency of vanishing of the large vessels in favor to the smaller and choriocapillaris and the changes in

the sclero-choroidal border shape, were well related to the subjacent area of retinal active disease and edema.

4.2. PRIMARY STUDY

The patient observation and data collection for the study was from May 2014 to February 2018.

One hundred and eighty-one patients (362 eyes) were selected for this study, where 129 (258 eyes) were diabetic patients (13 with type 1 Diabetes and 116 type 2 Diabetes) and 52 (104 eyes) were controls non-diabetic patients. Eighty-one (88) eyes were excluded to enter the study due to the following: 75 eyes – inability to identify the posterior limit of the choroid on the OCT image; 6 eyes – previous cataract surgery; 2 eyes – epiretinal membrane with traction; 2 eyes – previous focal macular laser; 1 eye – foveal atrophy from other causes; 2 eyes – drusen and foveal vitelliform lesion.

From the 274 eyes included in the study, 31% (85) had a second visit, 8,8% had 3 visits and only 3,9% had 4 visits during the study period. The eyes needing any kind of treatment considered as exclusion criteria were directed to do so and were considered as drop-out of the study. Subjects with eyes with no disease or no need for treatment were counseled to perform a regular visit (6 months or 1 year later). Since it is an observational study, the scheduling of the visits was dependent on the family doctor and/or patient. Therefore, 36 eyes were dropped-out from the study: 4 had to be submitted to cataract surgery, 14 were directed to a loading dose of intravitreal anti-VEGF, 16 were directed to perform macular laser treatment and 2

eyes due to ocular hyperpressure they started medication with Latanoprost drops. 80 patients (160 eyes) did not have any other visit during the study period for unknown reasons so they were considered as loss of follow up (LFU). Due to the small sample of eyes with more than 2 visits, data was not considered for statistical analysis. The interval of time between the baseline visit to the second visit was of mean ± SD: 424 days ± 238 (mean 14 months: minimum 61 days maximum 1012 days) for diabetic patients and mean ± SD: 391 ± 276 (mean 13 months: minimum 105 days maximum 854 days) for healthy controls.

The analysis was made for morphological changes and for statistical results.

4.2.1 MORPHOLOGICAL ANALYSIS

For the morphological analysis all OCT images were observed and classified according to the retinal and choroidal macular anatomical/ morphological features.

Retinal analysis consisted in evaluating and classifying as normal (with no abnormalities) or abnormal with diabetic retinopathy findings. The DR findings could be: evidence of increased thickness (focal or diffuse) in the retinal thickness map, cysts, hyperreflective spots (hemorrhages/exudates), disorganization of the layers, atrophy, fibrosis, new vessels, fibro-vascular proliferation, or others.

Choroidal analysis was based on the findings of the pilot studies and classifying as: normal sclero-choroidal border - with the “inverted dome shape” and the thickest point below the fovea; normal layers - with integrity of the mottled choriocapillaris/Sattler’s (ChS) and parallel to the Bruch’s membrane, homogeneity

of the great vessels distribution; abnormal sclero-choroidal border – with loss of the "inverted dome shape" and irregular delineation; abnormal layers – with loss of the integrity of the mottled choriocapillaris/Sattler's and parallel to the Bruch's membrane, focal atrophy or increase thickness of vascular layers, loss of even distribution, hyperreflective spaces on the vascular zone.

The choroidal findings were correlated with the retina above.

For the 203 diabetic and 78 healthy eyes included at baseline the choroidal morphologic features are summarized by groups in table 4.

Choroidal morphologic features	Healthy eyes n (%)	Diabetic eyes n (%)
N-N SCI – CL	61 (78,2)	106 (54,1)
N-AN SCI - CL	8 (10,3)	34 (17,3)
AN-N SCI - CL	2 (2,6)	8 (4,1)
AN-AN SCI - CL	4 (5,1)	48 (24,5)
AN- Imp SCI - CL	1 (1,3)	-
N- Imp SCI - CL	2 (2,6)	-
Total eyes (n)	78	196

Table 4 – Distribution of choroidal morphologic features by group. Legend: N – normal; AN – abnormal; SCI – sclero-choroid interface; CL – choroidal layers; Imp – impossible to evaluate.

About 78% of the healthy had all choroidal morphological features classified as normal. When analyzed individually the greater cause of some abnormalities was due to vascular layer abnormalities (12 eyes – 15,4%) and what we observed was it was always due to some irregularities in the great vessels layer, being the

choriocapillaris/Sattler's layer always preserved. The irregularity of the SCI was a consequence of localized thinning of the great vessels layer. The 3 cases where it was impossible to classify CL it was due to the extremely thin choroid and it was on the oldest patients (all with 79 years old). Examples are shown in the figures 9 to 11 below.

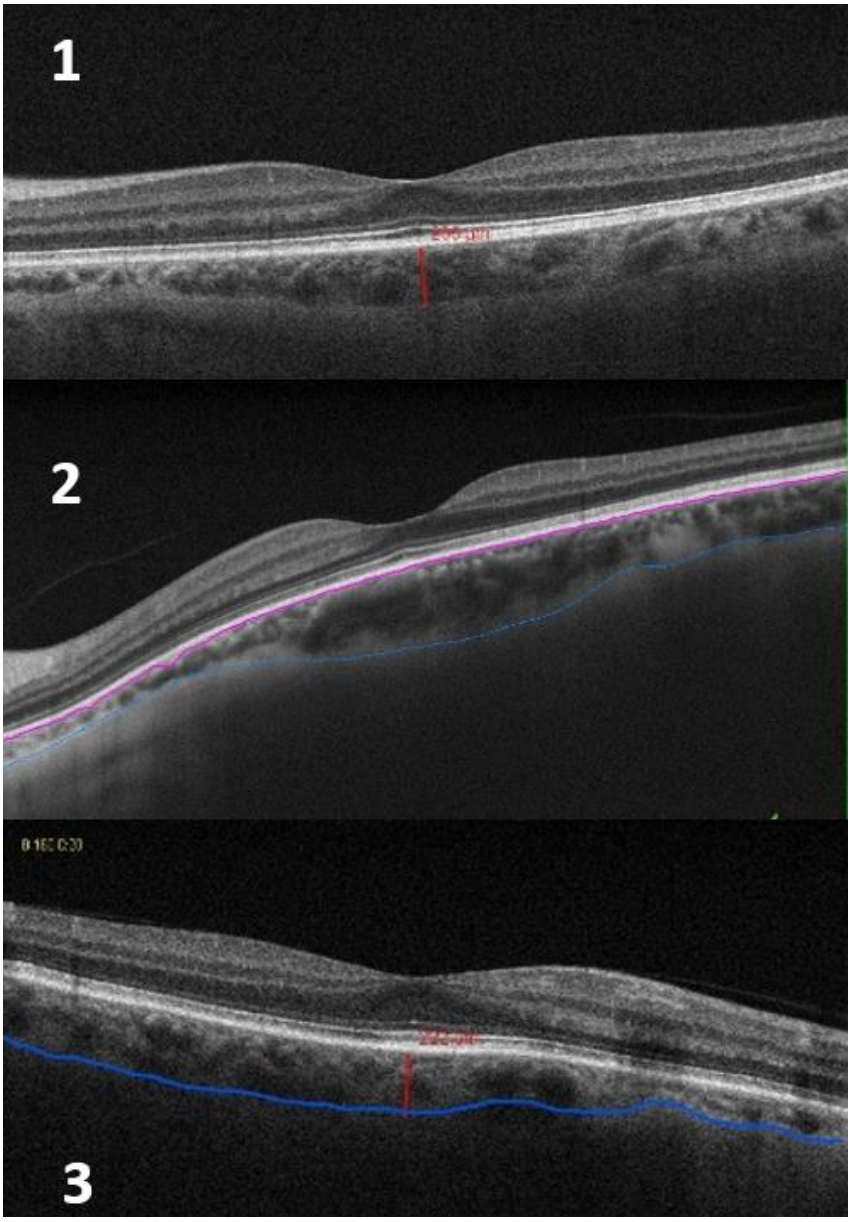


Figure 9 – Three examples of irregularity of the SCI due to localized atrophy of the great vessels layer (most frequently nasal located) or loss of great vessels with hyperreflective zone (temporally on the OCT image 2).

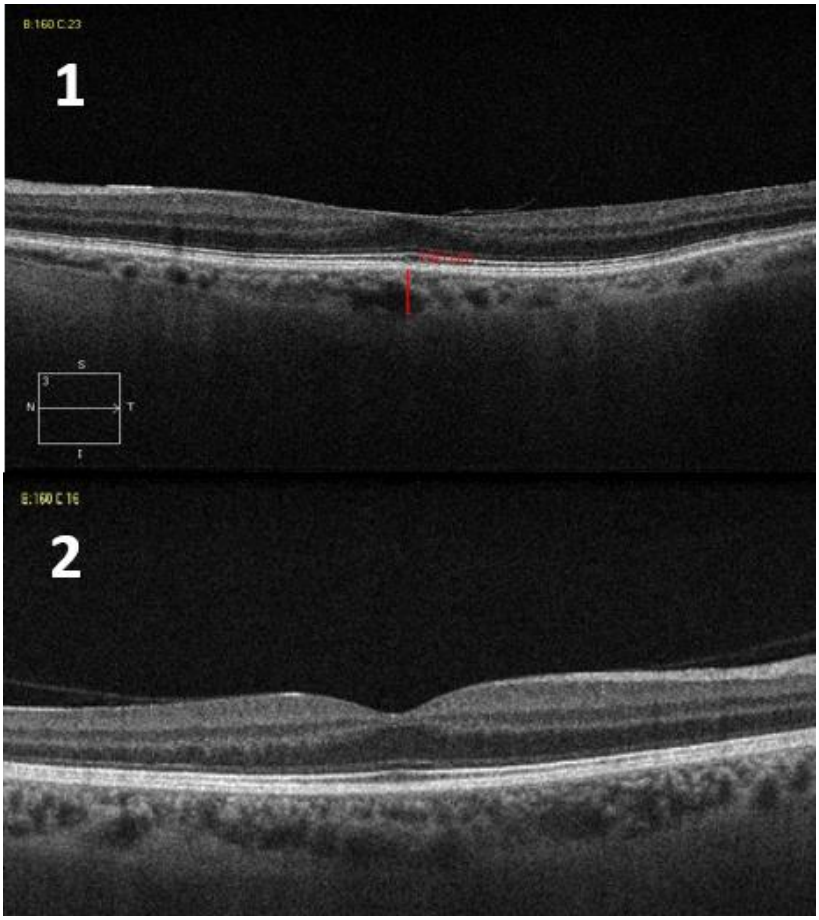


Figure 10–Two examples of normal contour of the SCI with abnormal CL due to localized atrophy of the great vessels layer and/or loss of great vessels with hyperreflective zone, but with preservation of the choriocapillaris/Sattler's layer.

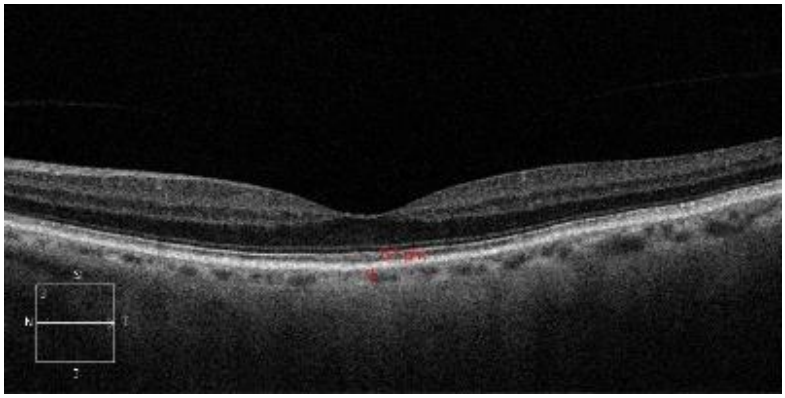


Figure 11 – Example of a very thin choroid where it is impossible to differentiate the layers boundaries.

On the diabetic group, 54,1% of the eyes had no choroidal morphologic abnormalities. Most eyes with choroidal abnormalities had vascular changes (41,8%) compared with those with just sclero-choroidal irregularities (4,1%). The sclero-choroidal border was always correlated or due to changes in the normal distribution of the great vessels (enlargement, atrophy or loss), except in one case where we found an anatomical variation with a triangular shape sub-foveal (figure16).

SCI-CL	NDR	MildNPDR	ModNPDR	Sev/PDR	Maculopathy
N-N	**71,7%**	19,8%	8,5%	0	18,5% (5)
N-AN	50%	29,4%	20,6%	0	29,6% (8)
AN-N	75%	25%	0	0	0
AN-AN	56,3%	16,7%	18,7%	8,3%	**51,9% (14)**

Table 5 – Correlation of choroidal morphologic findings with the retinopathy status.

When correlating choroidal morphological findings with the retinopathy grade and the presence of maculopathy, we found that most of the diabetic eyes with no choroidal abnormalities had no or initial stages of DR (table 5). Five of these had extra-foveal focal macular edema, and only in those focal locations choroidal changes were evident (figure 22). Choroidal vascular abnormalities were more prominent in the presence of macular edema or diabetic retinopathy. More advanced choroidal morphological changes with both SCI and Vascular layers affected was associated to more advanced retinal disease.

From individual OCT image observation, we found that:

- Changes in the great vessel layers are the major responsible for the irregular sclero-choroidal border, usually due to focal atrophy or thinning, loss of vessels with hyperreflective spaces occupying the vessels space or hypertrophy.

- In the absence of evident retinal disease and changes in the choriocapillaris/Sattler's layer, great vessels layer abnormalities are usually by focal loss or atrophy, more frequently nasal to the fovea, or loss of even distribution.

- Changes on the choriocapillaris/Sattler's layer as focal atrophy/loss with approach of the great vessels to the Bruch's membrane, hypertrophy associated with focal thinning or loss of subjacent great vessels, are correlated to changes in the retinal anatomy above. It becomes more evident in the presence of macular edema specially if the photoreceptor layer is affected.

- With maculopathy, we can find focal thinning of the choriocapillaris/Sattler's layer below the affected retina (ex- initial Cystoid macular edema with mild lesion of the retinal layers) with prominent choroidal great vessels, or more frequently associated to the presence of hard exudates, disorganization of retinal layers, lesion/atrophy of photoreceptors, we can find a focal enlargement of the choriocapillaris/Sattler's layer below the affected retina with thinning or loss of the great vessels layer.

- Independent of the retinal stage, if there is a severe choroidal thinning, it becomes difficult to analyze and classify choroidal morphological changes.

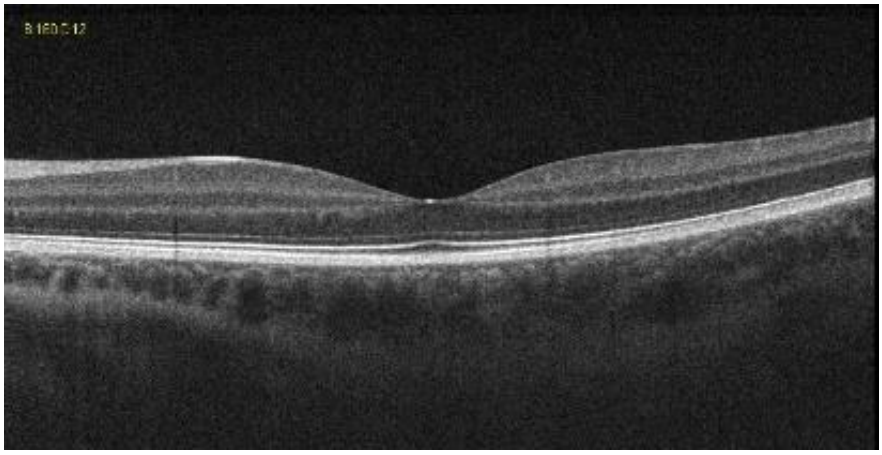


Figure 12– A NDR eye with integrity of the choriocapillaris/Sattler's layer but with a nasal thinning of the great vessels leading to a nasal thinning of choroidal thickness and irregular SCI.

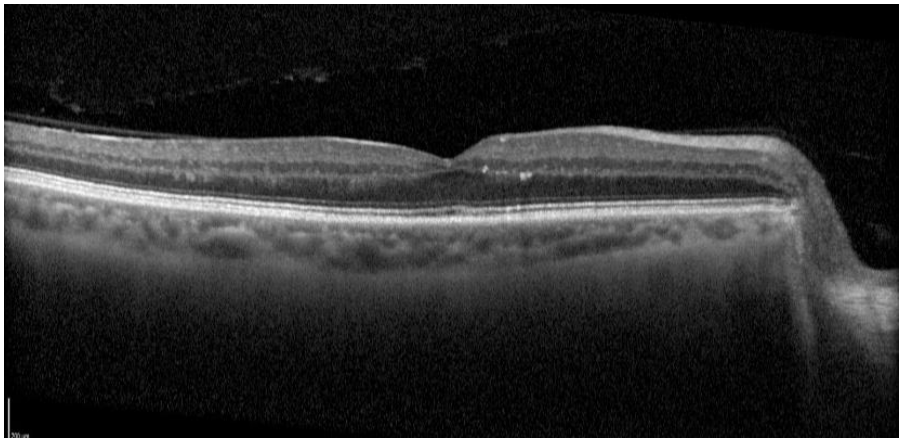


Figure 13– Mild NPDR eye with small retinal lesions foveal and nasal to fovea with subjacent loss of great vessels and hypertrophy of the choriocapillaris/Sattler's layer, and temporally to the fovea some irregularities of the retinal outer plexiform layer with subjacent slight thinning of the choriocapillaris/Sattler's layer with an enlargement of the great vessel.

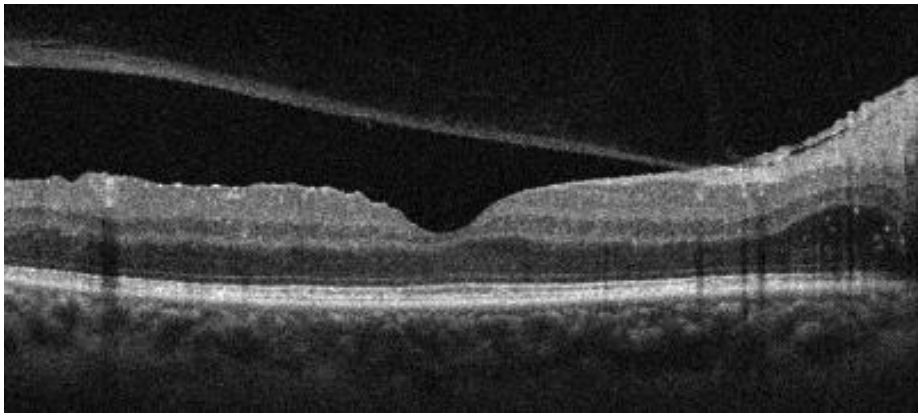


Figure 14 – PDR where the most affected retina is in the internal layers with integrity of the outer retina. Note the regular choroid with no morphologic changes.

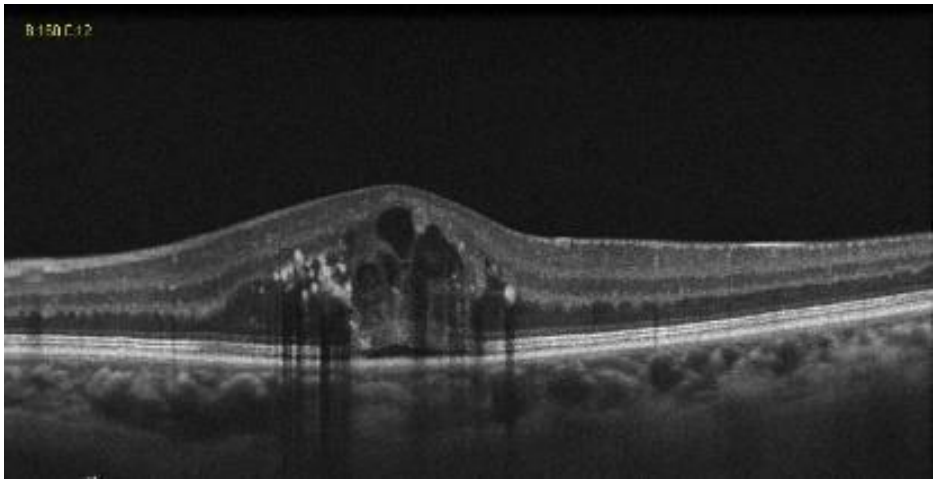


Figure 15 – A Macular edema with slight neurosensorial detachment (NSD) and some chronicity signs. Note the lack of great vessels below the NSD and nasally below the hyperreflective lesions in the retina a slight atrophy of the choriocapillaris and great vessels enlargement.

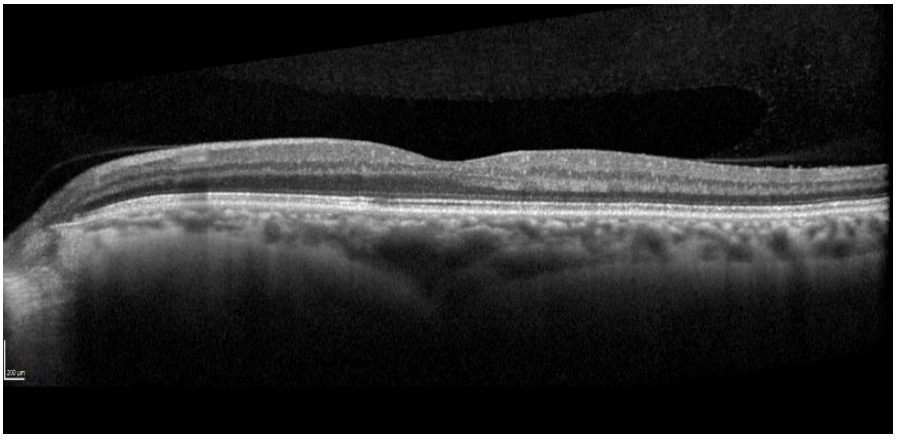


Figure 16 – The case of the anatomical variation with a triangular shape of the SCI.

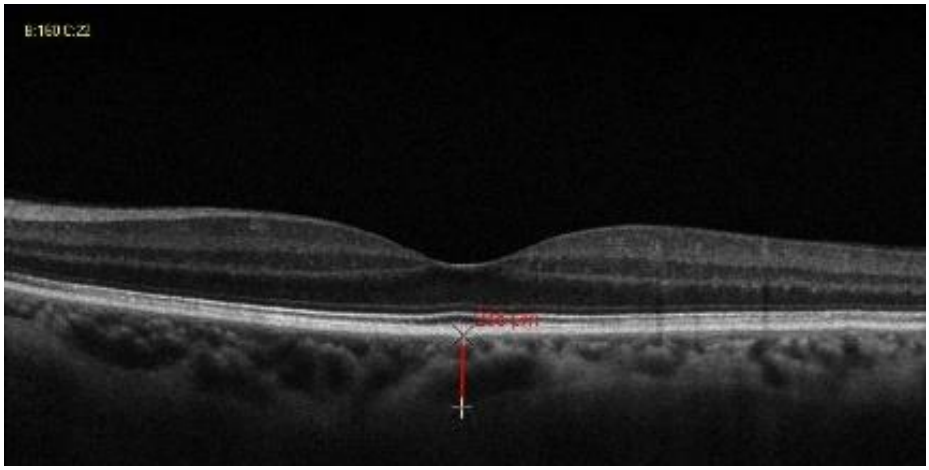


Figure 17 – Mild NPDR with temporal macular Microaneurysms and small hemorrhages on fundoscopy, and the OCT image showed an irregular SCI with loss of the inverted dome shape due to a temporal thinning related to loss/atrophy of temporal great vessels.

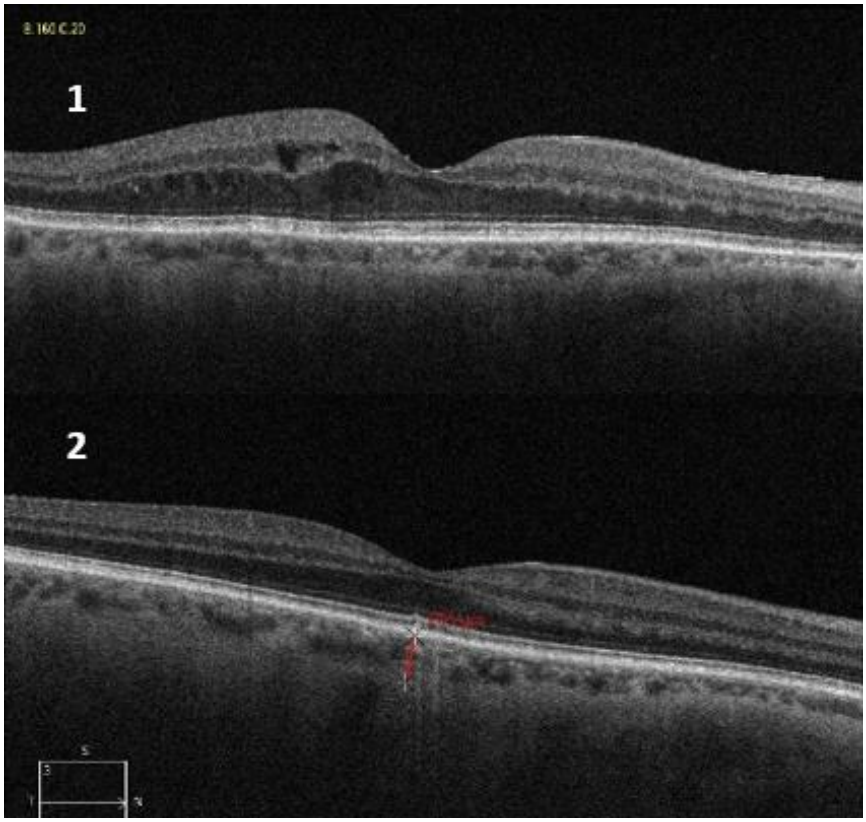


Figure 18 – Two examples of older patients with very thin choroid where morphological classification is difficult.

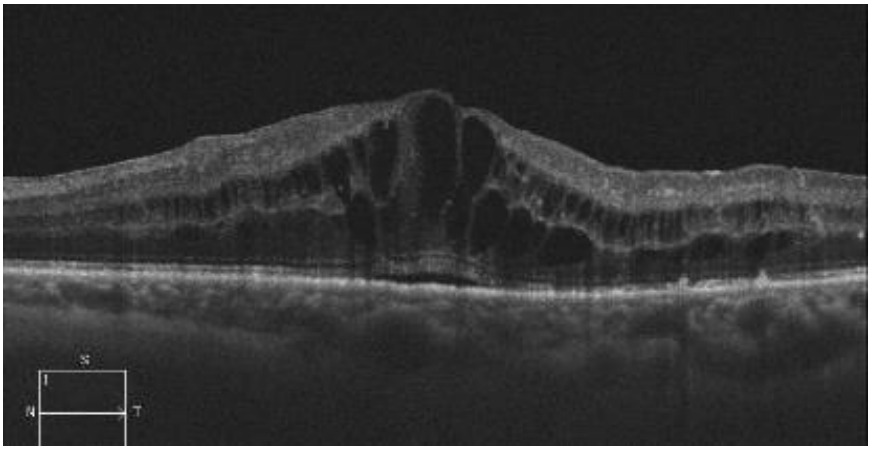


Figure 19 – An exuberant cystoid macular edema with greater cysts centrally, with a small NSD, slight inner retinal layers edema, and temporally to the fovea focal zones of atrophy of the photoreceptors layer. Under the nasal retina where the outer retina (from the outer nuclear layer to the EPR) has no relevant changes, the choroid is thinner with lack of great vessels and hypertrophy of the choriocapillaris/Sattler's layer. Under the NSD there is an enlargement of the great vessels. Under the focal zones temporally with atrophy of the photoreceptors there is a focal atrophy of the choriocapillaris/Sattler's layer immediately below with invasion of a great vessel.

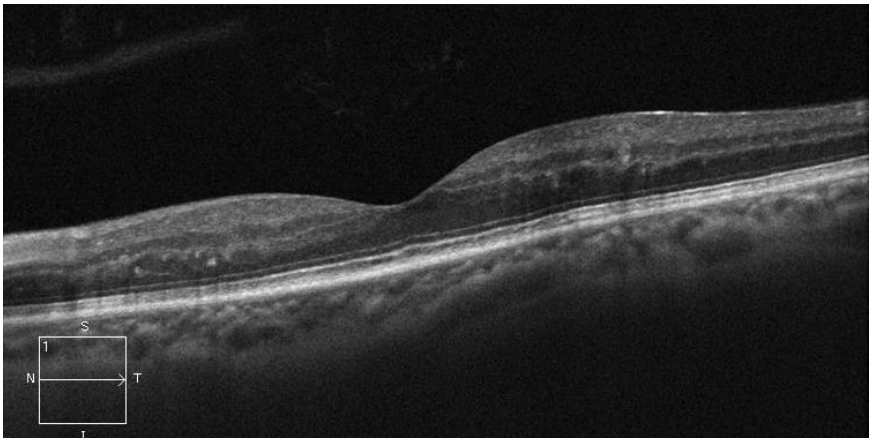


Figure 20 – Focal retinal DR changes with corresponding focal thinning of the choroid right below associated with loss/atrophy of great vessels.

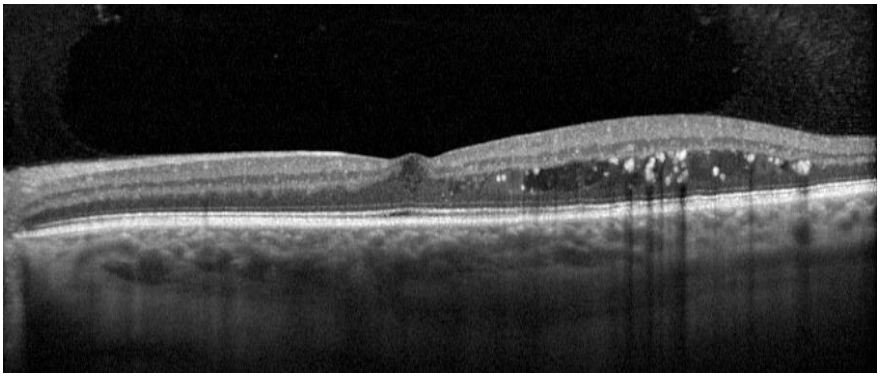


Figure 21– A temporal macular edema with some exudates and cysts. Below the more fluidic cysts (hyporreflective content) no relevant choroidal morphologic features are noted, but below the more chronic/ organized retinal lesions (exudates and cysts with lightly hyperreflective content) there is a lack of great vessels and thickening of the choriocapillaris/Sattler's layer.

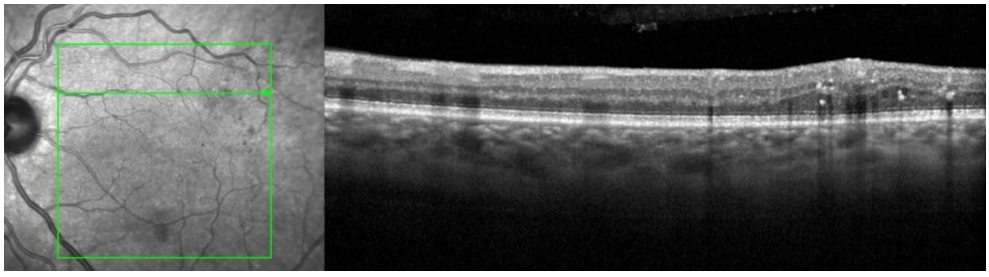


Figure 22 – An example of focal extra-foveal retinal thickening (spared fovea) where in the same scan we can see the choroid below the retinal focal thickening with the

lack of great vessels replaced by hyperreflective space, comparing to the choroid beside (nasal) with some great vessels visible.

We looked for progression changes on diabetic eyes. Eighty-five eyes were analyzed looking for CT increase or decrease from baseline to the second visit. The CT change was correlated with the RT changes and morphological findings.

CT From BL to V2	RT no change	RT increase	RT decrease
Increase	**67,7%**	19,4%	12,9%
Decrease	**50%**	15,4%	36,6%
No change	**81%**	9,5%	9,5%

Table 6 – Correlation of choroidal with retinal thickness

RT From BL to V2	CT no change	CT increase	CT decrease
Increase	21,4%	**43,8%**	34,8%
Decrease	16,9%	21.1%	**62%**
No change	33,3%	41,2%	25,5%

Table 7– Correlation of retinal with choroidal thickness

From table 6 we found that more than half of the eyes having a change in thickness from baseline to visit 2 had no retinal thickness changes. But one third of eyes with choroidal thickness decrease had also a retinal thickness decrease. When inverting the analysis (table 7), we found that more than half of the eyes with decreased Retinal thickness had also a decrease in CT, while distribution was more equitable when a RT increase, or no change was found.

Just a few eyes had moderate thickness changes and even less had huge thickness changes. No pattern stands out from these results.

Following we show some examples of progression of retinal disease and/or choroidal disease.

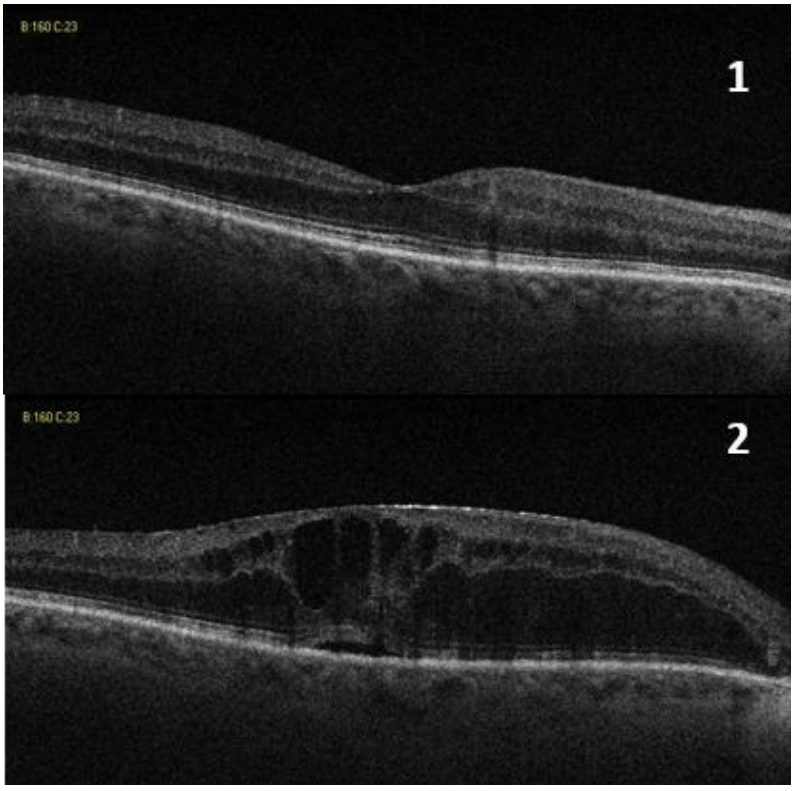


Figure 23 – Image 1 – baseline, image 2 - visit 2 about 1 year later. There was a mild CT increase (<30 µ) and a severe RT increase (+256 µ). Beside the thin choroid that limits the morphologic evaluation we can observe on V2 less vessels with larger caliber, particularly under the central zone of the macular edema.

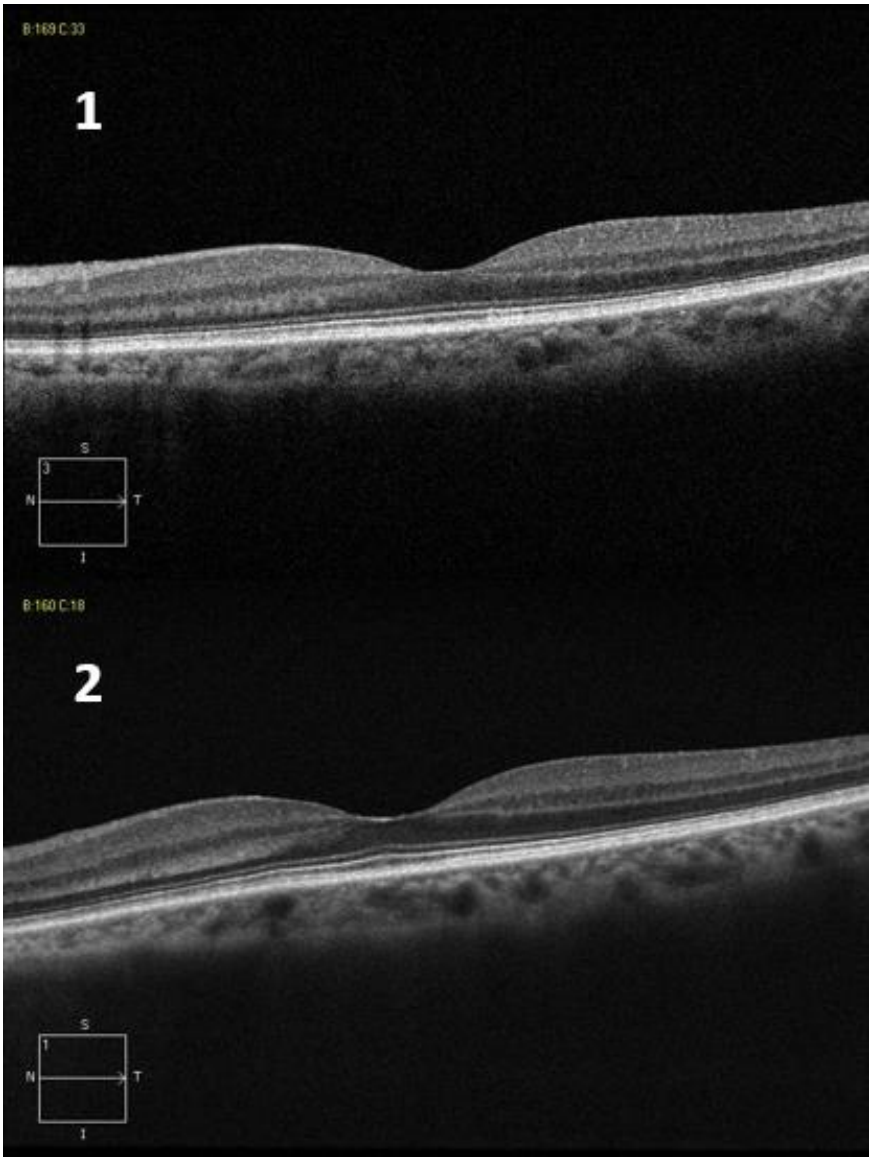


Figure 24 – From baseline (image 1) to V2 (image 2) there was slight increase in CT with no changes in RT. We can observe that there is a slight enlargement in great vessels that can explain the increase in CT.

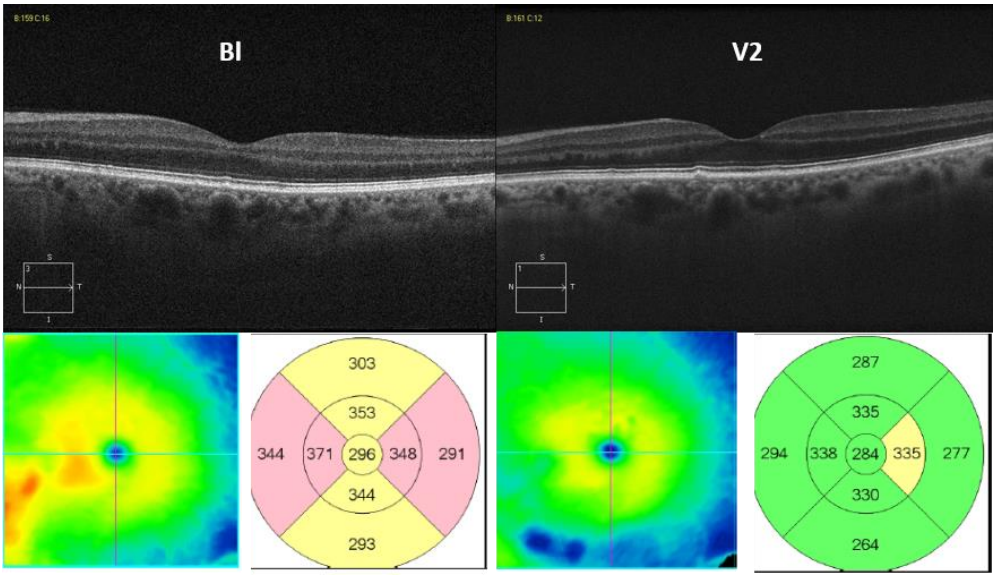


Figure 25 – From baseline (left) to V2 (right) there was a moderate increase in CT (+ 42µ) and focal decrease in RT (Retinal thickness maps below the line scan images). We can observe on BL two focal zones where there is a hypertrophy of the choriocapillaris/Staller's layer (temporal and nasal of the center) that on the nasal one corresponds to some irregularities of retinal layers (Outer plexiform). On V2 there is a recovery with great vessels on the temporal focal zone, but not on the nasal zone where persists the retinal irregularities.

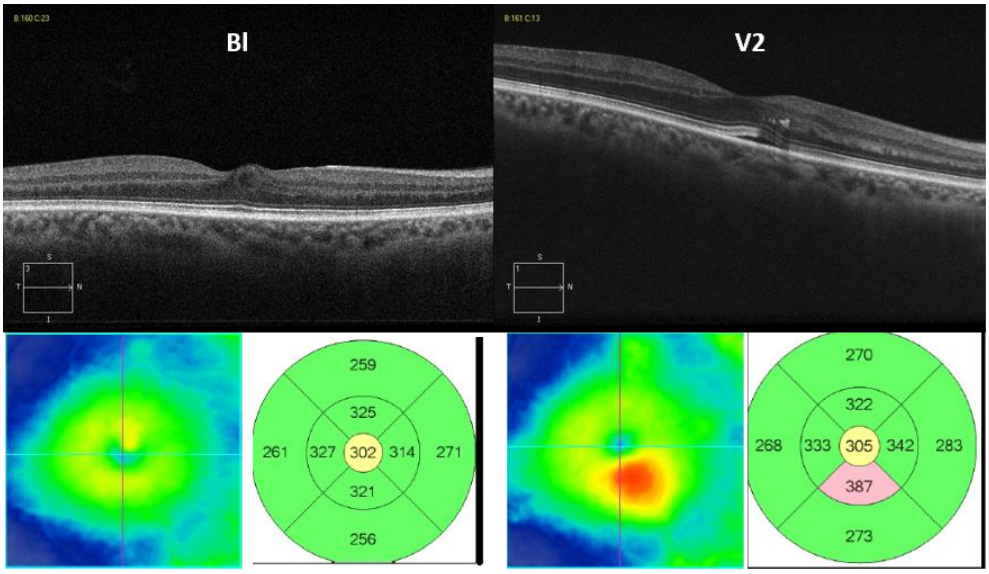


Figure 26– From baseline (left) to V2 (right) there was a moderate focal increase in RT (Retinal thickness maps below the line scan images). Choroidal changes are subtle, but we can observe on BL temporal to the central zone a horizontal great vessel that is replaced on V2 by hypertrophic choriocapillaris/Sattler's layer and hyperreflective space. Under the NSD and exudate zone there is an atrophy of the choriocapillaris/Sattler's layer losing the mottled aspect and being more plain hyperreflective.

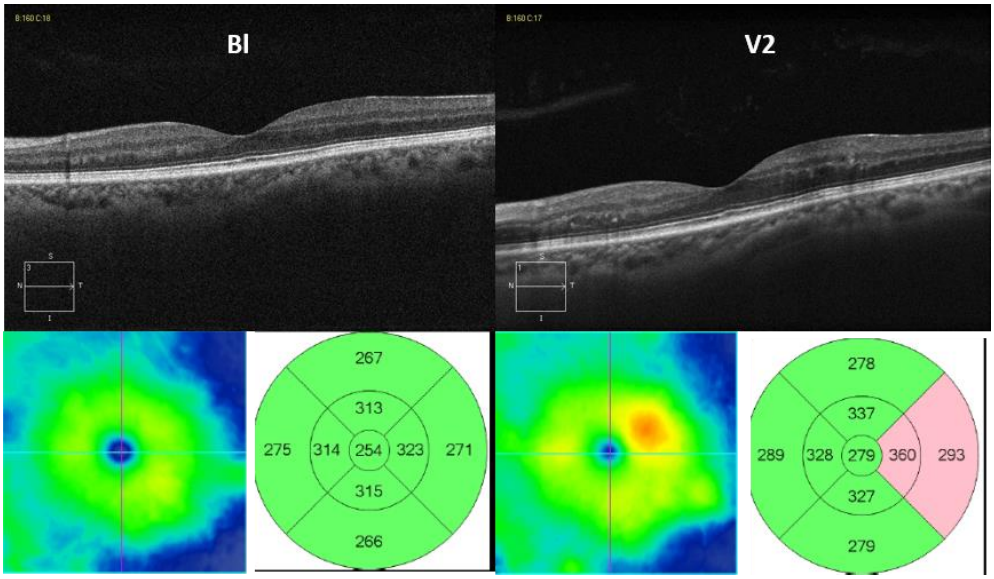


Figure 27 – On baseline image (left) we can observe the loss of the inverted dome shape of SCI, with scarce great vessels with focal loss (nasally and just temporally to the center). One year later, in V2 (right) there was a moderate focal increase in RT (Retinal thickness maps below the line scan images) and outer retinal layers changes nasally. These changes are above the choroidal changes observed on BL and that persists and are more evident. The CT increase from 243µ to 252µ.

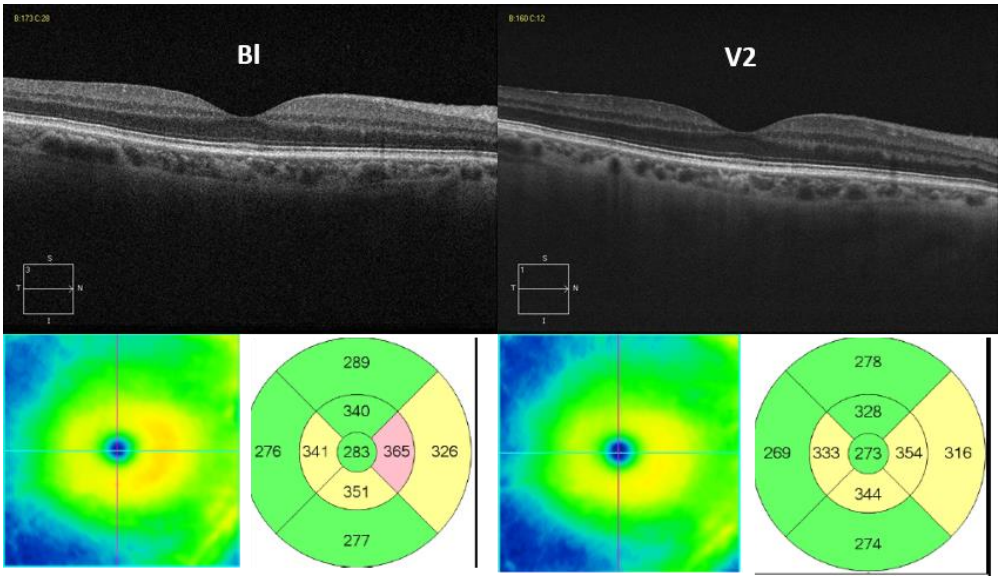


Figure 28 – A case of an eye with non-visible diabetic retinopathy with 2 years of interval between baseline (left) to V2 (right). There was a mild choroidal thinning with focal loss of great vessels temporally, replaced by a hypertrophic ChS layer and a hyperreflective space.

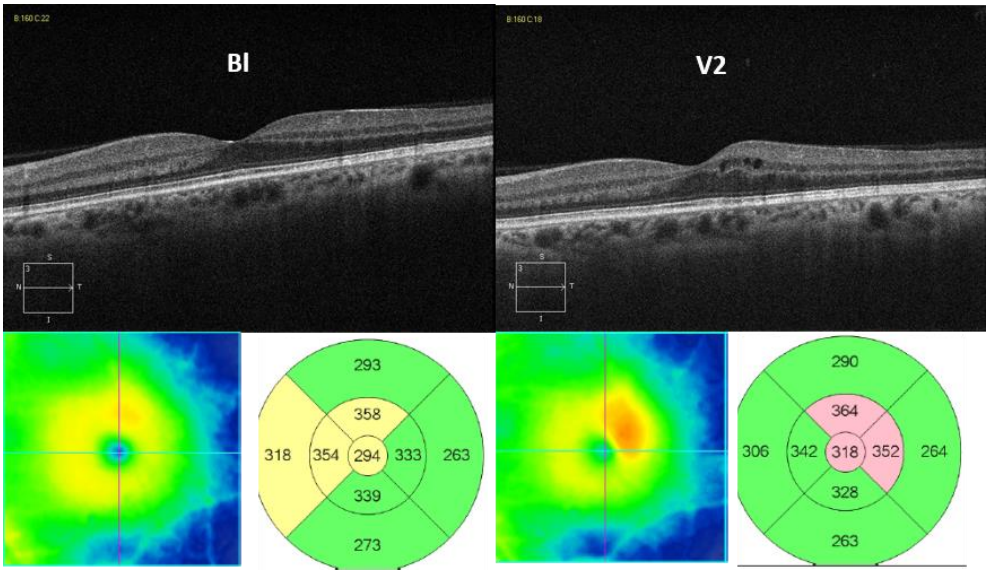


Figure 29 – On baseline image (left), of a mild NPDR eye, we can observe the loss of the inverted dome shape of SCI, irregular vessels layers with focal zones of loss of great vessels, atrophy or hypertrophy of ChS, but with no evident retinal changes. Six months later there was a CME with an increase in CT from 219 to 248µ with a change in choroidal vessels with more larger vessels near the posterior border of Bruch's membrane.

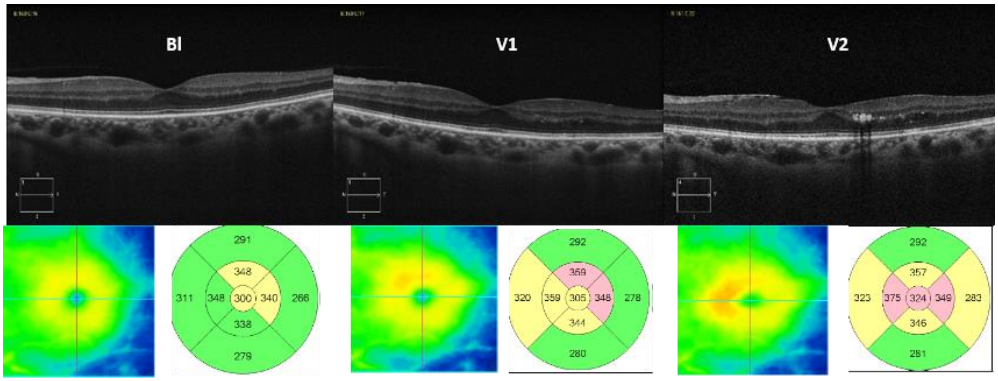


Figure 30 – An evolution with 6 months interval between the visits. DR evolved from NDR to mild NPDR and moderate NPDR (from left to right). The retinal maps below the OCT line images show the RT progression. Observing the images, we can observe on BL focal zones with hypertrophy of the ChS and loss of great vessels, and progression and appearance of retinal diabetic changes occur in the retina immediately above those focal choroidal zones.

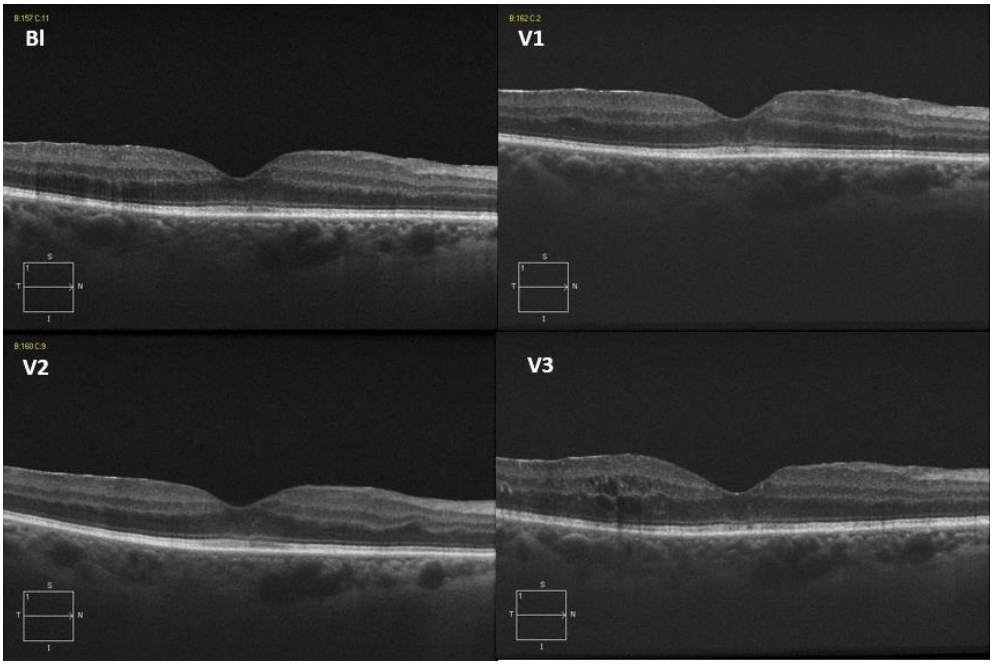


Figure 31 – Evolution during one year of an eye with mild to moderate NPDR evolving to ME. CT was from the first to the last of 344µ ,324µ,326µ and 325µ. The retinal changes are a progression to temporal CME, and some irregularities on nasal retinal layers that regresses on the latest visits. Choroidal vascular changes are a focal increase of the ChS both below nasal and temporal changes zones, but while

nasally it remains with the regression of retinal changes, temporally with the progression to CME the great vessels enlarge and approach to the Bruch's side.

We evaluated some eyes that were dropped out from the study because they were directed to treatment of macular edema. The purpose was to observe what changes occurs in the choroid after resolution or recovery of the ME. Following are the examples.

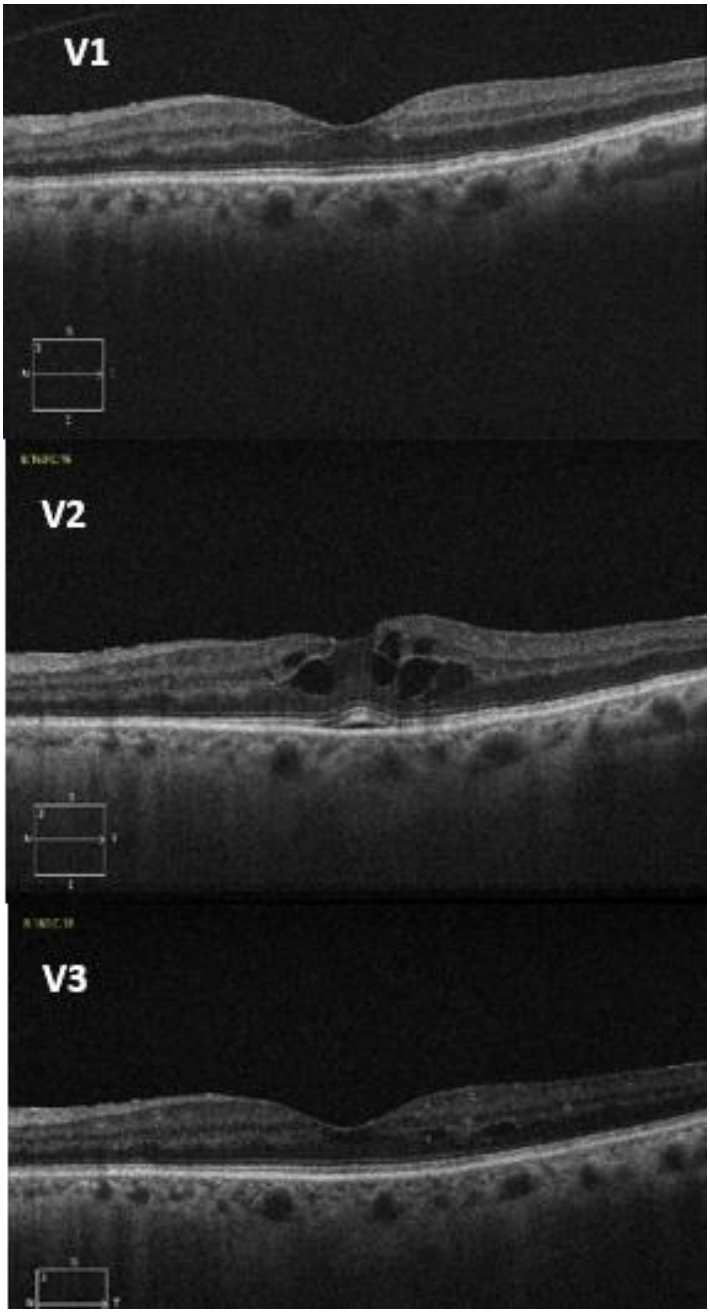


Figure 32 – An eye that evolved to CME with mild NSD that resolved with intravitreal anti-VEGF. On V1 there is a thin choroid with 3 central great vessels pushing up to

the Bruch's the ChS, corresponding to the zone below of where the CME appeared (affected retina). With the progression to V3 the changes that we can observe are a moderate decrease in CT (245µ-240µ-189µ) that we can correlate with the decrease in great vessels caliper.

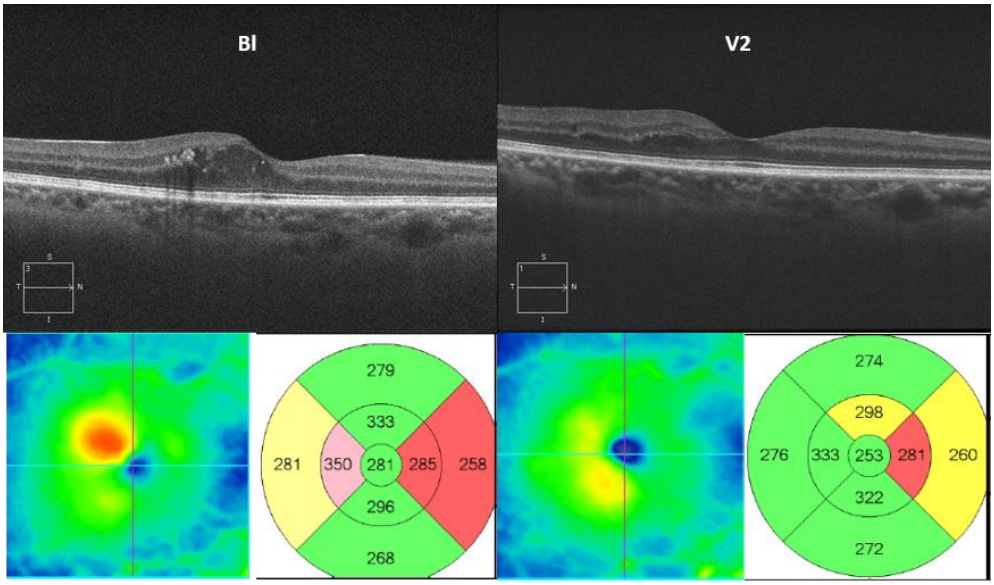


Figure 33 – Baseline with ME and two years after ME treatment. There was an almost complete regression of ME with residual cysts and irregular layers. The initial choroid with loss of great vessels under the CME, recovered some great vessels, but thickness decreased from 289µ to 222µ. Note that there is a slight retinal thinning temporally beside no layers/morphology changes observed, with a corresponding enlargement of a choroidal great vessel pushing ChS to the Bruch's border.

4.2.2. ANCILARY MORPHOLOGICAL STUDY

We evaluated automated retinal and choroidal thickness maps acquired with the

Swept-source DRI OCT-1 Triton to correlate localized retinal changes with superimposed choroidal changes (unpublished data).

The results are in the following figures.

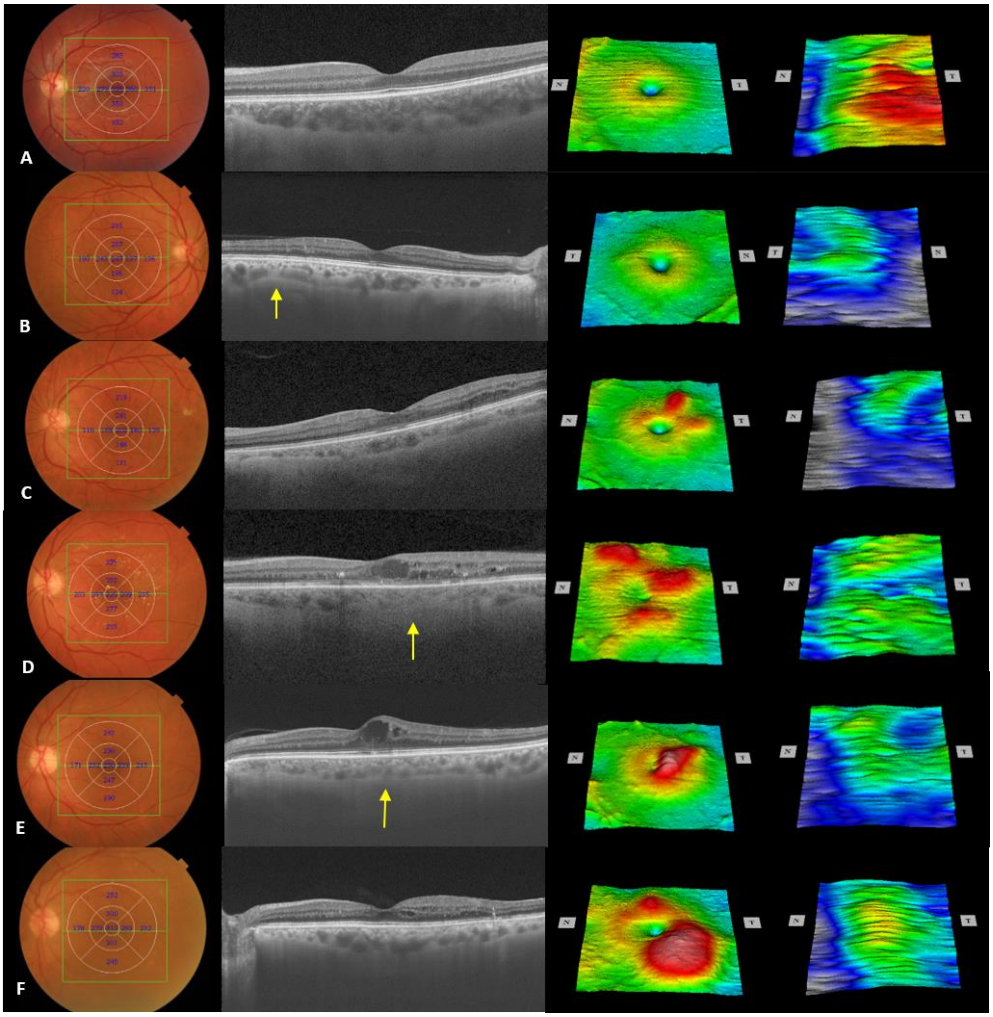


Figure 34 – 1st column – fundus photography. 2nd column – foveal line OCT scan. 3rd column – retinal thickness map. 4th column – choroidal thickness map. A – healthy

subject. B to F – diabetic patients in different stages. Yellow arrows – abnormalities of the choroidal vessels. Detailed description on the text.

Five eyes were analyzed using the SS-OCT retinal and choroidal automated thickness maps. One healthy and five with different types of diabetic retinopathy. Case A – from a healthy 21 years old (y.o.) man. On the line scan we can observe the normal morphology as described previously with the “inverted dome shape” sclero-choroidal border, integrity and parallelism of the choriocapillaris/Sattler’s layer and uniformity of the great vessels layer. The retinal thickness map is normal according to the well described features, with the foveal depression, the slightly thickest circle surrounding the fovea a progressive thinning to the periphery. The choroidal thickness map with thickest zone centrally, superiorly, temporally and inferior to the fovea in a continuous or uniform distribution.

Case B and C – right and left eye of 63 y.o. male diabetic subject with mild non-proliferative diabetic retinopathy. On the right eye (B), with microaneurysm only mostly located temporally and superiorly in the macula, the retinal thickness map is normal, but the choroidal thickness map shows a decreased thickness primarily temporal and inferior comparing to the healthy case. On the line scan there is a focal thinning of the choroid (yellow arrow) corresponding to a zone with less great vessels and minor abnormalities of the retina above. On the left eye (C), microaneurysms are throughout the macula, with a small exudate temporal in the macula. The line scan shows small cysts temporal to the fovea and general decreased thickness of the choroid with atrophy of both group of vascular layers. The retinal map evidence focal increased thickness superior to the fovea. The choroidal thickness map shows

a slightly higher decrease in thickness compared with the right eye, but the location with higher thickness correspond to the retina above with higher thickness.

Case D – a 51 yo diabetic male subject, with moderate diabetic retinopathy presenting microaneurysms, hemorrhages and little macular exudates. The line scan shows some diabetic lesion in the retina with a cystoid macular edema involving the fovea with some loss of integrity of the central photoreceptor layer, and complete vanishing of the choroidal great vessels below this affected photoreceptor zone (yellow arrow). The retinal thickness map shows focal zones with increased thickness, but the choroidal thickness map, despite abnormal compared to the healthy case, does not have a logical thickness distribution correlated with the retinal map. It seems that the thinnest zone corresponds to the retinal zone with more exudates (temporal).

Case E – a 67 yo diabetic woman, with mild NPDR and a CME. The line scan shows a foveal important cyst with apparent almost no lesion of the photoreceptors layer, and integrity of the choriocapillaris/Slater's layer with loss of the inverted dome shape and the subfoveal thickest choroidal point. Retinal map shows the central edema and the choroidal map a general slight thickness decrease.

Case F – a 70 yo diabetic man, with mild to moderate NPDR with ME non-involving the fovea. The foveal line scan shows integrity of the fovea and photoreceptors layers with small cysts and slight disorganization of the layers temporal to the fovea. The choroid has the normal inverted dome shape and the choriocapillaris/Slater's layer is almost normal with only a slight increased thickness on the temporal side

(below the affected retina). The retinal thickness map shows the ME and the choroidal map shows the normal thickest point centrally but with a slight decrease in thickness temporally and inferiorly comparing to the healthy case.

4.2.3. STATISTICAL RESULTS

For analysis both eyes if eligible by the inclusion criteria were included, since the variables to be studied for the principal purpose were specific for each eye and independent of the contralateral eye. Thus, non-ocular variables are presented demographically by patient (age, sex, systolic blood pressure, diastolic blood pressure, HbA1c, duration of Diabetes) while eye variables are presented by eye (best corrected visual acuity, refractive error, ocular pressure, subfoveal choroidal thickness, central foveal retinal thickness, choroidoscleral interface contour, choroidal vascular layers, stage of diabetic retinopathy). To facilitate the statistical analysis, choroidal morphologic features were divided in contour (Sclero-choroidal border delineation) and Layers (vascular layers – great vessels and ChS layer). Both variables could be normal or abnormal.

Visual acuity was recorded on a 20/20 ETDRS chart. For analysis the denominator was registered (example: 20/40 VA was recorded as 40). The refractive error was recorded as spherical equivalent.

4.2.3.1 BASELINE

Group 1 - Diabetics

Baseline demographics for generic, systemic and non-retinal or choroidal variables are show on tables 8 and 9.

Variable	n (eye)	Mean	Std. Dev.	Min	Max
age	196	57.8	12.8	18	81
Gender (F/M)	196	88 (44.9%) / 108 (55.1%)			
VA	196	23.0	9.1	20	100
RE	196	0.1	2.2	-2.5	3.5
SBP	165	140.5	20.0	105	197
DBP	165	76.4	13.6	45	132
OP	174	15.5	3.3	10	27

Table 8 – Demographics. Diabetics (analysis per eye). Gender – F- female, M- male; VA – visual acuity; RE – refractive error; SBP – systolic blood pressure(mmHg); DBP – diastolic blood pressure(mmHg); OP- ocular pressure (mmHg).

Variable	n Subjects	Mean	Std. Dev.	Min	Max
age	100	57.8	12.8	18	81
Gender (F/M) n	100	44 / 56			
SBP	86	141.5	20.2	105	197
DBP	86	76.5	13.5	45	132

Table 9 – Demographics. Diabetics (analysis per subject)

For group 1, diabetic subjects, data regarding SFCT, CFRT, HbA1c and duration of Diabetes is summarized on table 10 and SFCT mean values for each diabetic retinopathy sub-group are in table 11.

Variable	n	Mean (µ)	± SD	Min (µ)	Max (µ)
SFCT	196	273.4	80.9	96.0	495.0
CFRT	196	271.3	45.3	209.0	517.0
HbA1c	183	7.4	1.8	5.1	13.6
Diabetes (years)	196	12.6	8.3	0.5	40.0

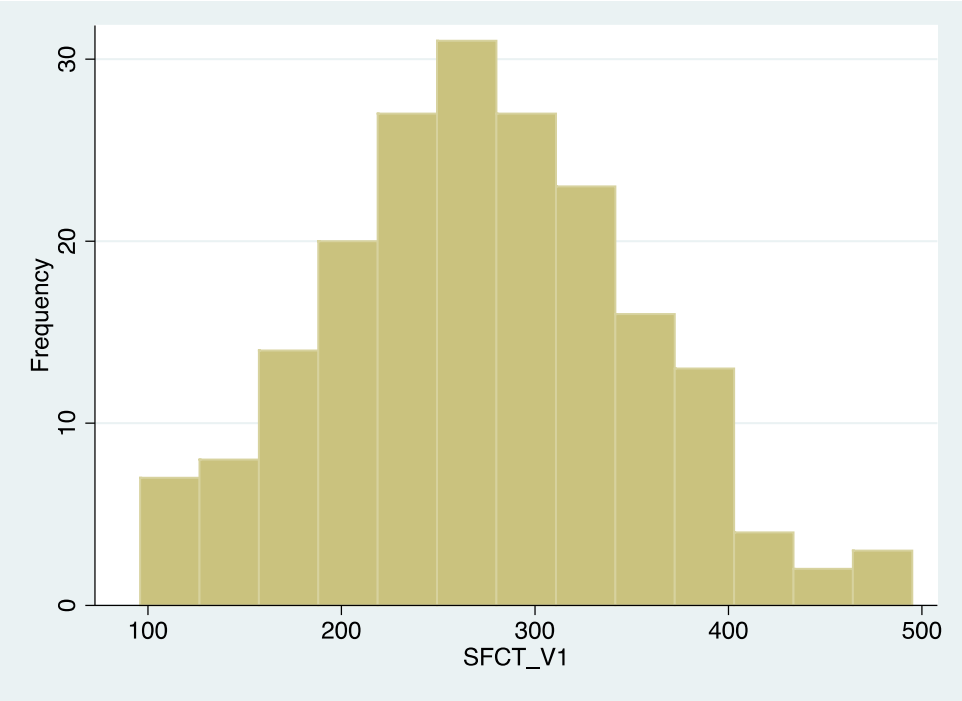
Table 10 -Diabetic - Mean values for SFCT (subfoveal choroidal thickness), CFRT (central foveal choroidal thickness), HbA1c (serum glycosylated hemoglobin) and duration of Diabetes

DR stage	n (eyes)	Mean SFCT (µ)	± SD	Min	Max
NDR	125	264.32	81.08	96	495
MildNPDR	42	262	65.60	113	391
ModNPDR	25	265.64	85.46	100	423
SevNPDR/PDR	4	336,75	55,57	268	404
Maculopathy	27	262.81	85.10	100	461

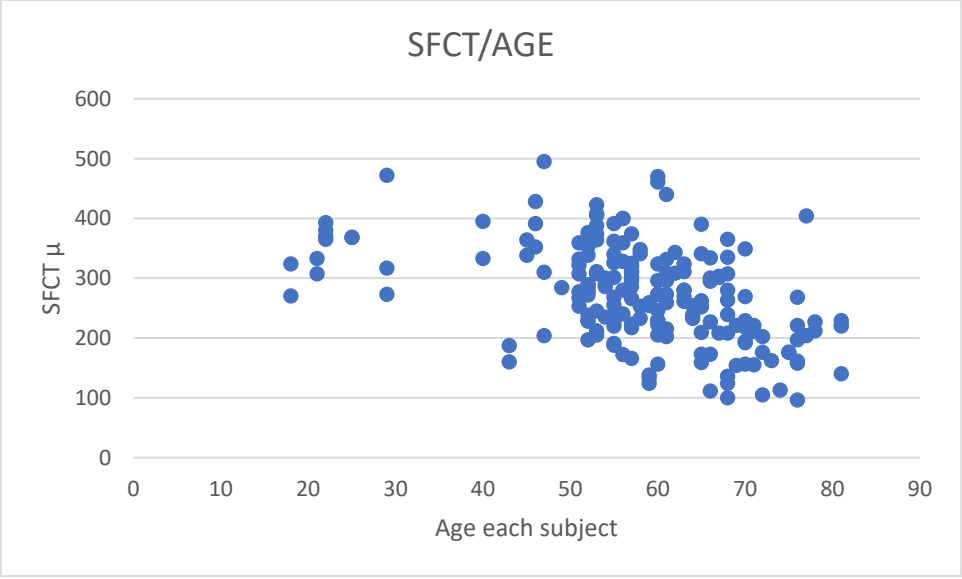
Table 11 -Diabetic - SFCT for each Diabetic retinopathy (DR) stage. NPDR – nonproliferative diabetic retinopathy; Mod – moderate; Sev – severe; PDR – proliferative diabetic retinopathy

On thirteen patients the HbA1c failed to be registered for other reasons (failure to do the analysis with the family doctor). Patients were in global relatively well controlled for Diabetes and blood pressure. Most eyes had no evident diabetic retinopathy or mild NPDR. Only 27 eyes had macular edema (central or focal extra-foveal).

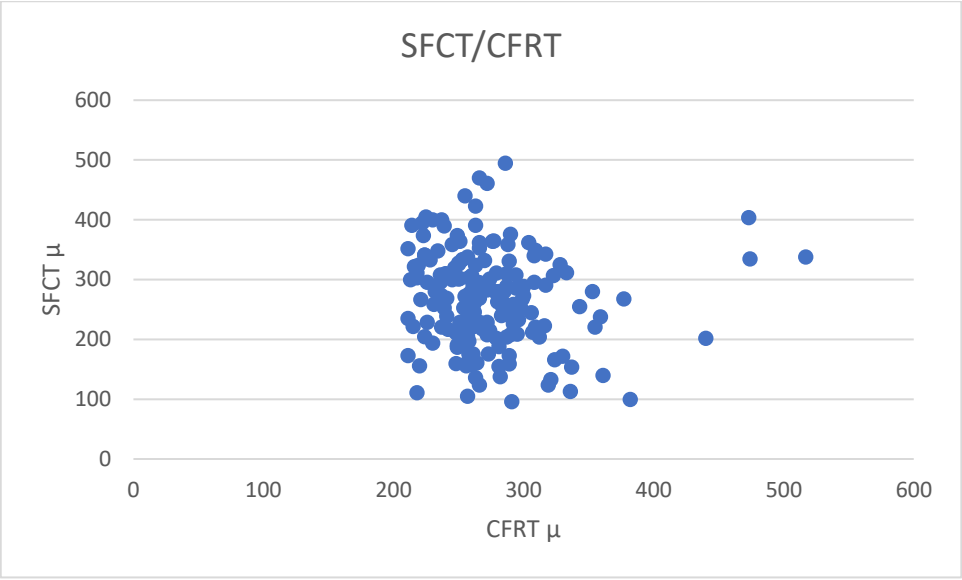
SFCT had a huge range of measurements varying from 96 to 495µ as shown in graphic 1. When analyzing SFCT distribution by age for all eyes we found that there is a great variability and dispersion (graphic 2). When analysis was made regarding the CFRT, we found that, beside the majority of CFRT did not vary much, there was a great SFCT variability (graphic 3).



Graphic 1 -Diabetic – Distribution of SFCT



Graphic 2 -Diabetic – Distribution/dispersion of SFCT for each included eye by age



Graphic 3 -Diabetic – Distribution/dispersion of SFCT for each subject by CFRT

Group 2 – Control

Baseline demographics for generic, systemic and non-retinal or choroidal variables are show on tables 12 and 13.

Variable	n (eyes)	Mean	Std. Dev.	Min	Max
age	78	58.5	14.0	21	85
Gender (F/M)	78	44 (56.4%) / 34 (43.6%)			
VA	78	21.5	4.6	20	40
RE	78	0.7	1.1	-2	3.75
SBP	62	135.3	20.1	102	199
DBP	62	78.4	12.9	52	114
OP	78	14.7	2.9	10	24

Table 12 – Demographics. Controls (analysis per eye) Gender – F- female, M- male; VA – visual acuity; RE – refractive error; SBP – systolic blood pressure; DBP – diastolic blood pressure; OP- ocular pressure

Variable	n (subjects)	Mean	Std. Dev.	Min	Max
age	39	58.1	14.0	21	85
Gender (F/M)	39	24 (61.5%) / 15 (38.5%)			
SBP	31	133.6	18.4	102	191
DBP	31	76.9	12.1	52	111

Table 13 – Demographics. Controls - analysis per subject

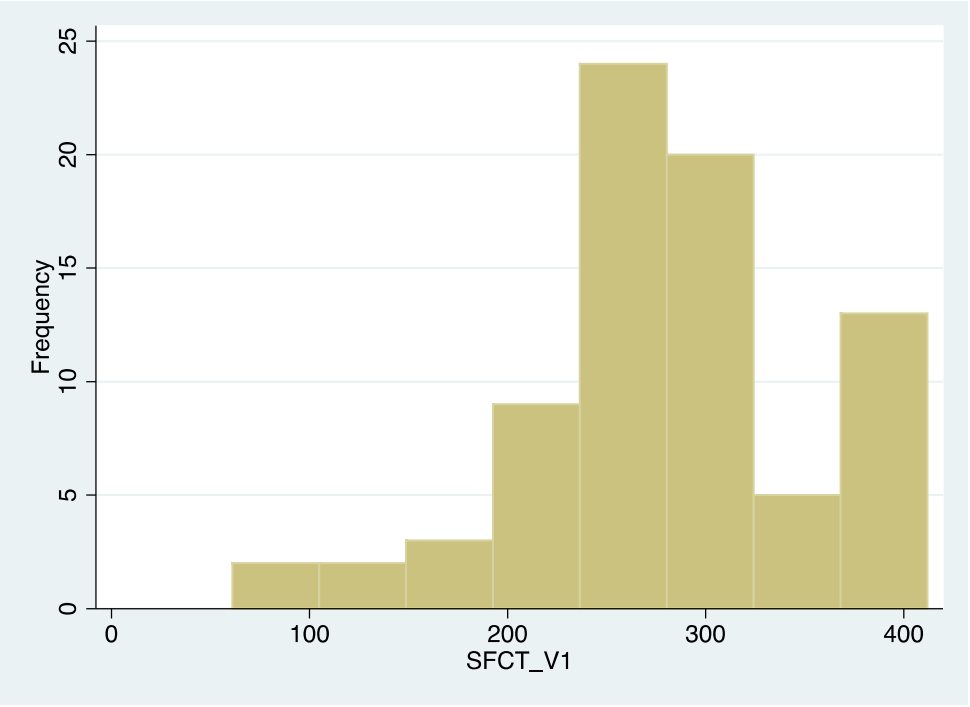
The was a failure to register blood pressure for 8 patients. The patients with a visual acuity lower than 20/20 was due to mild cataract not interfering with imaging acquisition and sufficient quality for analysis.

Data regarding SFCT and CFRT is summarized on table

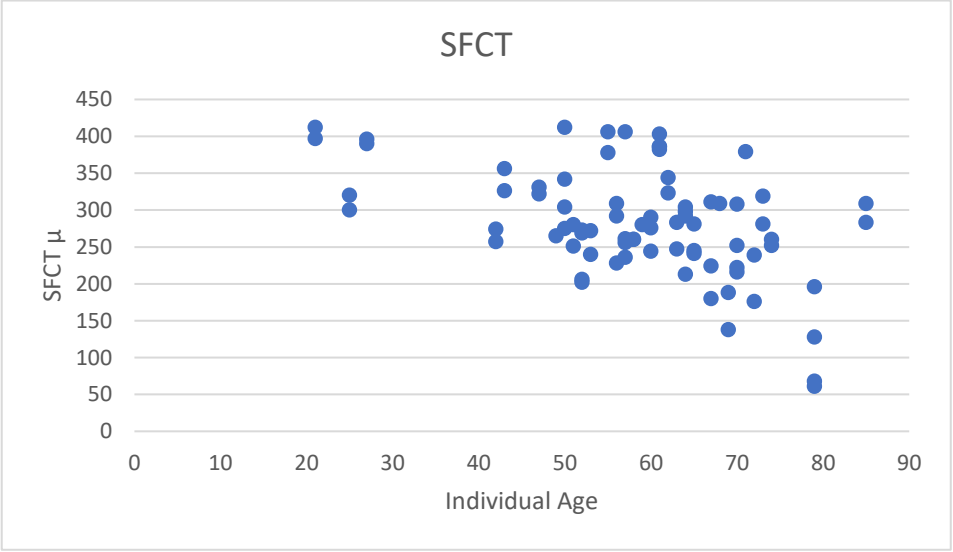
Variable	Obs	Mean	Std. Dev.	Min	Max
SFCT_V1	78	281.8	74.1	61.0	412.0
CFRT_V1	78	258,3	26,7	173	309.0

Table 14 – Controls - Mean values for SFCT (subfoveal choroidal thickness), CFRT (central foveal choroidal thickness)

SFCT had a huge range of measurements varying from 61 to 412µ with a wide dispersion of values by age (graphics 4 and 5). We can observe that youngest ages had the higher SFCT values and that the lowest SFCT values were on the oldest ages. Great variability was found primarily in the intermediate ages (40-70).



Graphic 4 -Controls – Distribution of SFCT



Graphic 5 -Controls – Distribution/dispersion of SFCT for each included eye by age

Baseline Analysis

No statistical significant difference was found between OD and OS in both groups (diabetic and healthy) for ocular variables excluding retinal and choroidal features (tables 15 and 16).

Variable	n (eyes)	Mean	Std. Dev.	Min	Max	p (Wilcoxon)
VA – OD	100	22.3	5.2	20	60	0.431
VA – OS	96	23.9	11.9	20	100	
RE – OD	100	0.30	1.24	-3.75	3.25	0.334
RE – OS	96	-0.13	2.9	-2.5	3.5	
OP – OD	88	15.4	3.2	10	24	0.249
OP – OS	86	15.5	3.5	10	27	

Table 15 -Diabetics-Differences between eyes – there are no statistically significant differences between OD and OS. VA- visual acuity; RE – refractive error; OP – ocular pressure; OD – right eye; OS – left eye

Variable	n (eyes)	Mean	Std. Dev.	Min	Max	p (Wilcoxon)
VA OD	39	21.7	4.6	20	40	0.317
VA – OS	39	21.4	4.8	20	40	
RE – OD	39	0.61	1.08	-2	3.25	0.254
RE – OS	39	0.72	1.18	-1.75	3.75	
OP – OD	39	14.7	2.7	10	21	0.611
OP – OS	39	14.8	3.2	10	24	

Table 16 -Controls-Differences between eyes – there are no statistically significant differences between OD and OS. VA- visual acuity; RE – refractive error; OP – ocular pressure; OD – right eye; OS – left eye

Using the Shapiro-Wilk normality test, no normality was found for the variables in general and by group. Thus, non-parametric test was used to evaluate if there was statistically significant difference between the two groups. Statistically significant difference was found between control and diabetic group for visual acuity (mean ±SD: 21,5±4.6 versus 23,3±9.5: p=0,010), systolic blood pressure (mean ±SD: 135,3±20.1 versus 142,3±19.8: p=0,010) and ocular pressure (mean ±SD: 14,7±2.9 versus 15,6±3.3: p=0,018)- and refractive error (mean ±SD: 0,1± 2,2) - table 17.

Variable	P (control/diabetic)	Test
age	0.612	Mann-Whitney
Gender	0.092	Fisher Chi-Square
VA	**0.0222**	Mann-Whitney
RE	**0.0174**	Mann-Whitney
SBP	**0.0461**	Mann-Whitney
DBP	0.2861	Mann-Whitney
OP	**0.0367**	Mann-Whitney

Table 17 -Statistical results comparing the 2 groups for statistical difference

When comparing SFCT and CFRT between controls and diabetic no statistically significant differences was found ($p > 0.05$) – table 18.

Variable	p	Test
SFCT	0.2978	Mann-Whitney
CFRT	0.1631	Mann-Whitney

Table 18 – Comparison of SFCT and CFRT between the two groups.

For group 1 of diabetic subjects there was no significant statistical difference regarding SFCT between the different stages of DR (Kruskal-Wallis; p=0,3459) and between with or without ME (Mann-Whitney; p= 0,5184) - tables 19 and 20.

	Mean	SD	Min	Max
DR = 0	264.32	81.08	96	495
DR = 1	262	65.60	113	391
DR = 2	265.64	85.46	100	423
DR = 3	336.75	55.57	268	404

Table 19 - SFCT mean values by diabetic retinopathy (DR) stage where: 0= No DR; 1= mild non-proliferative DR; 2= moderate non-proliferative DR; 3= severe or proliferative DR.

	Mean	Std. Dev.	Min	Max
ME = 0	266.26	77.27	96	495
ME = 1	262.81	85.10	100	461

Table 20 - SFCT mean values by presence of macular edema (ME) where: 0= no ME; 1= with ME.

To study the impact in Group 1 of the variables CFRT, contour, layers, HbA1c, duration of Diabetes, SBP, DBP, BP medicated, refractive error, VA, DR stage, age, and OP with the variable SFCT, a linear regression model was performed with the variable SFCT as a dependent variable and the remaining variables as independent variables (table 21).

The model obtained is statistically significant ($p < 0.001$) and explains about 40% of the variance of SFCT ($R2 = 0.402$).

SFCT	Coef.	Std. Err.	t	P>t	[95% Conf.	Interval]
CFRT	-0,11	0.17	-0,65	0,519	-0,44	0,22
contour	-13,21	13,83	-0,96	0,341	-40,59	14,17
layers	**-28,76**	**13,32**	**-2,16**	**0,033**	**-55,15**	**-2,37**
HbA1c	-5,32	4,08	-1,3	0,195	-13,41	2,77
Diabetes duration	0,42	0,73	0,57	0,569	-1,03	1,87
SBP	0,01	0,38	0,01	0,989	-0,74	0,76
DBP	0,31	0,54	0,57	0,569	-0,77	1,39
BP med	25,28	15,02	1,68	0,095	-4,46	55,01
RE	-1,02	2,35	-0,43	0,666	-5,67	3,63
VA	0,17	1,29	0,13	0,896	-2,38	2,72
DR stage	19,09	12,15	1,57	0,119	-4.97	43,16
ME	-32,99	27,68	-1,19	0,236	-87,83	21,84
Age	**-3,74**	**0,62**	**-6,0**	**0,000**	**-4.97**	**-2,51**
OP	0,29	1,82	0,16	0,874	-3,32	3,90
_cons	511,26	70,94	7,21	0,000	370,75	651,77

Table 21 - Multivariate analysis with SFCT as the dependent variable. Contour – sclero-choroidal border morphology (normal or abnormal); layers – choroidal layers (normal or abnormal); HbA1c – serum glycosylated hemoglobin; SBP – systolic blood pressure; DBP – diastolic blood pressure; BP med –medicated blood pressure; RE – refractive error; VA – visual acuity; DR stage – diabetic retinopathy stage; ME – presence of macular edema; OP – ocular pressure.

A higher value of SFCT is significantly associated with abnormal layer morphology ($p = 0.033$).

Considering the association found with age, the correlation obtained between SFCT and age (Spearman coefficient) is negative and moderate ($r = -04667$; $p < 0.001$).

For group 2, to study the impact of the variables CFRT, contour, layers, SBP, DBP, BP medicated, RE, VA, age, and OP with the variable SFCT, a linear regression

model was performed with the variable SFCT as dependent variable and the remaining variables as independent variables (table 22).

The model obtained is statistically significant ($p < 0.001$) and explains about 44% of the variance of SFCT ($R2 = 0.439$).

SFCT	Coef.	Std. Err.	t	P>t	[95% Conf.	Interval]
CFRT	0.51	0.4	1.2	0.246	-0.4	1.4
contour	9.85	24.1	0.4	0.685	-38.6	58.3
layers	-38.05	23.8	-1.6	0.116	-85.9	9.8
SBP	**2.24**	**0.8**	**2.8**	**0.007**	**0.6**	**3.8**
DBP	**-3.41**	**1.4**	**-2.5**	**0.017**	**-6.2**	**-0.6**
BPmed	22.12	17.7	1.3	0.218	-13.5	57.7
RE	-1.62	8.4	-0.2	0.847	-18.4	15.2
ME	1.47	1.8	0.8	0.429	-2.2	5.2
Age	**-2.38**	**0.8**	**-3.1**	**0.003**	**-3.9**	**-0.8**
OP	-0.60	2.9	-0.2	0.838	-6.5	5.3
_cons	220.54	161.5	1.4	0.178	-103.8	544.9

Table 22 - Multivariate analysis with SFCT as the dependent variable. Contour – sclero-choroidal border morphology (normal or abnormal); layers – choroidal layers (normal or abnormal); SBP – systolic blood pressure; DBP – diastolic blood pressure; BP med – medicated blood pressure; RE – refractive error; VA – visual acuity; DR stage – diabetic retinopathy stage; ME – presence of macular edema; OP – ocular pressure.

A higher value of SFCT is associated with a higher value of SBP ($p = 0.007$), lower value of DBP ($p = 0.017$) and at a youngest age ($p = 0.003$).

Considering the association found with age, the correlation obtained between SFCT and age (Spearman coefficient) is negative and moderate ($r = -0424$; $p < 0.001$).

4.2.3.2 PROGRESSION

Group 1, Diabetic

85 eyes were analyzed for V2.

The variables used for analysis of progression for the 2 consecutive visits were SFCT, CFRT, HbA1c, SBP, DBP, DR stage, and correlation with age and duration of Diabetes at baseline was looked for.

No significant statistical difference was found between the 2 visits for SFCT, CFRT and blood pressure. Significance was found for glycosylated hemoglobin, but it is not clinically significant (table 23).

Variable	Obs	Mean	Std. Dev.	Min	Max	P (Wilcoxon)
SFCT_V1	196	273.37	80.94	96.00	495.00	
SFCT_V2	85	267.15	78.42	82.00	430.00	0.1012
CFRT_V1	196	270.55	45.94	209.00	517.00	
CFRT_V2	85	272.31	50.24	197.00	568.00	0.4828
HbA1_V1	183	7.43	1.81	5.10	13,60	
HbA1_V2	85	7.35	1.69	4.40	13.00	**0.0332**
SBP_V1	165	140.53	20.02	105.00	197.00	
SBP _V2	85	134.41	17.38	98.00	193.00	0.0512
DBP_V1	165	76.35	13.65	45.00	132.00	
SBP_V2	85	73.21	10.65	54.00	92.00	0.3896

Table 23 – Progression analysis. V1 – baseline; V2 – second visit. HbA1c – serum glycosylated hemoglobin; SBP – systolic blood pressure; DBP – diastolic blood pressure.

There was a slight and non-significant decrease of SFCT from V1 to V2 (Wilcoxon; p=0,1012), with no correlation with the variables analyzed (table 24)

	SFCT_Dif	CFRT_Dif	HbA1_Dif	SistBP~f	DiastBP~f
SFCT_Dif	1.000				
CFRT_Dif	0.224	1.000			
	0.089				
HbA1_Dif	0.034	-0.148	1.000		
	0.800	*0.264*			
SBP_Dif	0.089	0.005	**-0.276**	1.000	
	0.503	*0.968*	***0.035***		
DBP_Dif	0.113	**-0.377**	-0.003	**0.687**	1.000
	0.393	***0.003***	*0.984*	***0.000***	

Table 24 – Correlation analysis between SFCT variation and CFRT and systemic variables.

The variation of SCFT did not showed any correlation with the duration of Diabetes or age (r =-0155; p = 0.158 for the duration of Diabetes, and r =-0.156; p = 0.155 for Age) - table 25.

	SFCT_Dif	Years Diabetes~s	age
SFCT_Dif	1.000		
Years - Diabetes	-0.155	1.000	
	0.158		
age	-0.156	0.1713	1.000
	0.155	*0.1170*	

Table 25 – Correlation of Variation of SCFT with duration of Diabetes and with age

Comparison between the stage of DR from V1 to V2, for each eye was performed for the 85 eyes with a second visit. In V1 we had 61,9% eyes with grade 0 (no DR); 23,8% with Grade 1 (mild NPDR); 10,7% with Grade 2 (moderate NPDR) and 3,6% with grade 3 (severe NPDR or PDR). In V2 we have 57,1% eyes with grade 0; 34,5% with Grade 1; 4,8% with Grade 2 and 3,6% with grade 3. 79,9% of the eyes maintained the degree of DR from V1 to V2: 52,4% eyes with grade 0; 19% with grade 1; 4,8% with Grade 2 and 3,6% with grade 3.

Statistically there were no significant variance (p= 0,0744: Stuart-Maxwell test) for the DR stage, and for Macular edema (p= 0.6547: McNemar test).

The stage of DR was not analyzed for progression since very few patients had changes from baseline to V2. Four patients had a slight improvement in the DR stage (mild to no visible lesions). For Macular edema, 3 eyes with no ME at baseline had ME on V2. Eyes with macular edema at baseline were directed for treatment and dropped out from the study statistical analysis. The eyes with macular edema were analyzed individually for morphological changes (chapter 4.2.1).

Group 2, Control

14 eyes were analyzed for V2.

The variables used for analysis of progression for the 2 consecutive visits were SFCT, CFRT, SBP, DBP and correlation with age at baseline was looked for.

Variable	Obs	Mean	Std. Dev.	Min	Max	P (Wilcoxon)
SFCT_V1	78	281.8	74.1	61.0	412.0	
SFCT_V2	14	286.9	55.9	193.0	399.0	0.729
CFRT_V1	78	258,3	26,7	173	309.0	
CFRT_V2	14	268.9	26.2	194.0	296.0	0.091
SBP_V1	62	135.3	20.1	102.0	199.0	
SBP_V2	12	124.0	13.7	101.0	144.0	0.664
DBP_V1	62	78.4	12.9	52.0	114.0	
DBP_V2	12	70.67	7.22	58.00	81.00	0.875

Table 26 – Progression analysis. V1 – baseline; V2 – second visit. SBP – systolic blood pressure; DBP – diastolic blood pressure.

There was a slight and non-significant increase of SFCT from V1 to V2 (Wilcoxon; p=0,729), with no correlation with the variables analyzed (table 26).

There was a negative and moderate correlation between the variation of SFCT and CFRT however this correlation was not statically significant (r =-0408; p = 0.188). No correlation was found between the variation of SFCT and age (r=0.130; p=0,657). (table 27).

	SFCT_Dif	CFRT_Dif	SistBP~f	DiastBP~f
SFCT_Dif	1.000			
CFRT_Dif	**-0.408**	1.000		
	0.188			
SBP_Dif	-0.286	-0.170	1.000	
	0.367	*0.590*		
DBP_Dif	-0.332	0.220	0.070	1.000
	0.291	*0.490*	*0.820*	

Table 27 – Correlation analysis between SFCT variation and CFRT and systemic variables.

To study the impact of CFRT variables (v2-v1), SBP (v2-v1), (v2-V1), age, and group (i.e., control or diabetic) with the variable SFCT (v2-v1), a linear regression model was performed with the variable SFCT (v2-v1) as a dependent variable and the remaining Variables as independent variables (table 28).

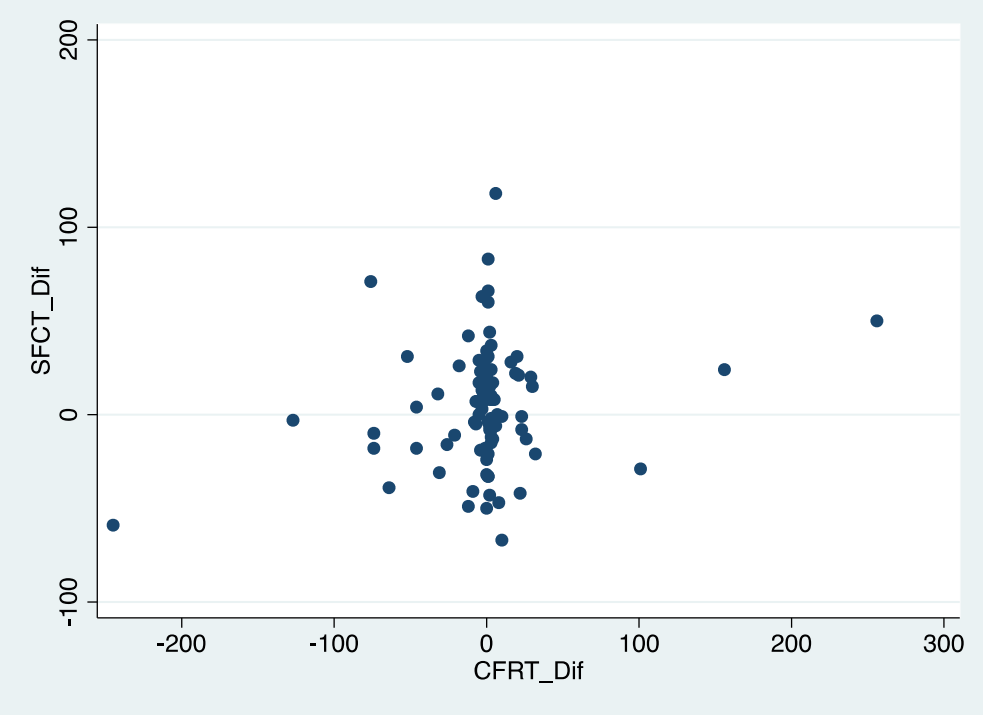
The model obtained was not statistically significant (P = 0.060) and explains about 7.3 % of the variance of SFCT (V2-V1) (R2 = 0.0734).

SFCT_Dif	Coef.	Std. Err.	t	P>t	[95% Conf.	Interval]
CFRT_Dif	0.178	0.065	2.74	**0.008**	0.048	0.307
SBP_Dif	-0.042	0.229	-0.18	0.857	-0.499	0.416
DBP_Dif	0.384	0.392	0.98	0.331	-0.398	1.165
age	-0.411	0.317	-1.30	0.199	-1.044	0.221
Group	10.511	9.117	1.15	0.253	-7.656	28.682
_cons	21.378	18.900	1.13	0.262	-16.290	59.046

Table 28 – Multivariate analysis of progression of variables with SFCT progression.

The only factor associated with the variation of the SFCT is the variation of the CFRT, where a greater variation of SFCT is associated with a greater variation of CFRT

(P=0.008) – graphic 6. But this association is weak (Spearman coefficient: r = 0.0681; p = 0.5097).



Graphic 6 – Correlation of progression of SFCT and CFRT

CHAPTER 5 – DISSCUSSION

5 – DISSCUSSION

The study included an important sample of patients that were followed during about two years. The purpose was to evaluate the correlation of choroidal features and changes with diabetic retinopathy and eventually identify markers of a diabetic choroidopathy in OCT images. Another purpose was to evaluate if choroidal thickness measurement could be considered alone as a reliable parameter for diabetic choroidopathy and if it could be related or justified by morphological findings in the choroidal structure. Very little was known about this at the begin of the study. To avoid eventual bias of choroidal changes caused by any diabetic retinopathy treatment such laser and/or intravitreal injections, or other ocular treatments, since it is still unclear their effects on the choroid, these were considered as exclusion criteria. Published data regarding the eventual effect of treatment on the choroid are unclear e with disparities. While Ohara et al[145] and Zhang et al[146] described a significantly decrease in CT after pan retinal photocoagulation (PRP), Cho et al[147] described an increase in CT after PRP. Kniggiendorf[148] and Lains[149] described a choroidal thinning following treatment with intravitreal Anti-VEGF. Considering previous treatment as exclusion criteria was one of the reasons why most eyes included were in earlier stages or with no diabetic retinopathy. In Portugal we have a National screening program for diabetic retinopathy that allows evaluate, detect and treat almost all patients diagnosed with Diabetes by the family Doctor and presenting diabetic retinopathy, most times in very early stages of diabetic retinopathy. It is not frequent to observe at the sub-speciality Retina consultation of the Ophthalmology department of an end-line treatment Hospital, more advanced cases of diabetic retinopathy with or without macular edema, but naïve of any

treatment. Being an observational study with the intent to evaluate the natural course of progression, the follow-up visits were not planned with a restricted timing as it is done in clinical trials and were dependent on the appointment made by the family doctor and the health system gear. Thus, the follow-up visits interval varied a lot and many patients were lost of follow-up leading to an important decrease on the sample for ensuing visits.

The study included both patient's eyes except in situations where exclusion criteria were found in one eye. The inclusion of the two eyes was part of the principle that the changes to be studied were specific to each eye, independent of the contralateral eye. Thus, the non-ocular variables were presented demographically by patient (age, sex, SBP, DBP, HbA1c, Diabetes in years) while ocular variables were presented by eye (VA, RE, OP, SFCT, CFRT, contour, Layers, DR). The non-ocular variables were selected based on previous published data that refer a possible effect on choroidal thickness. Visual acuity, refractive error and ocular pressure were also recorded to have statistical data of eventual effect on the choroid, but it was not part of the study purpose to correlate choroidal findings with visual function or the other two.

Since one of the purposes of the study was to evaluate choroidal morphologic findings on OCT images, and few or almost none was described previously in literature, we performed two pilot studies to define parameters to evaluate and compare in the main study. Regarding choroidal thickness, to be comparable with other publications, we decided in this study to register the sub foveal choroidal thickness for statistical analysis. But variations in choroidal thickness along the scans were analyzed with the morphologic evaluation of the retina and choroid.

Although it was not described in this study, previously both graders for the OCT images (Lilianne Duarte and Miguel Ruão) had made an inter-grader variability study for choroidal posterior boundaries and choroidal thickness measurements with high agreement (unpublished data). The OCT devices used for this study had not a built-in software doing the automatic segmentation of the choroid boundaries, so it was made manually on the line scans. We could have tried to build a choroidal thickness map manually doing segmentation of each scan of the cube protocol and slide the layers of the retinal thickness map, but it would be time consuming with great risk of slight errors of segmentation. Thus, we performed an ancillary study in collaboration with another Hospital where they have an OCT device that performs choroidal maps by automated segmentation, to get superimposed thickness maps of the retina and choroid.

Only with follow up visits and images it is possible to evaluate if any findings found in a single visit changed with progression of the disease with clinical eventual meaning. So, our study was designed as longitudinal, and morphologic and statistical analysis was made. Although some patients performed more than two visits, the sample was to small to have significance, so we did the analysis comparing two follow up visits. So far as we know, there is no other longitudinal study evaluating choroidal findings in diabetic patients.

As results of our study we found that we can define normal morphologic features of the choroid layers and sclero-choroidal interface. With diabetic disease changes in those morphologic features can be observed, classified, followed up and correlated with the retinal disease. Choroidal morphological changes occur focally related to

the subjacent retina. Automated retinal e choroidal superimposed maps could be an easy way to localize focal zones to be evaluated for morphological changes. Statistical analysis showed a great variability and dispersion of sub-foveal choroidal thickness with an important variation susceptibility to various variables, turning it a low reliable parameter. Nevertheless, some significance was found between choroidal thickness in the correlation with age (decreases with age) for both groups, and in Diabetics also with choroidal morphological changes (corroborating the morphologic study results) and in Controls also with blood pressure.

For both groups, diabetics and controls, ocular but non-retinal or choroidal variables (VA, RE, OP), no statistically significant differences were found between OD and OS. These results give confidence in analyzing all eyes as separate subjects for retinal and choroidal findings.

When compared the 2 groups (control and diabetic) regarding the non-retinal or choroidal variables – and using non-parametric tests due to the non- normality of the variables: Statistically significant differences between the two groups were found for: VA - it makes clinical sense because the presence of pathology in diabetic eyes can interfere with the visual function; SBP - Diabetic patients usually are with more morbidity, sometimes with kidney diabetic disease that can affect blood pressure control; and for RE and OP. The statistical significance finding however is with no clinical relevance (if we look to the mean values they have no clinical significance).

Since there was no significant difference for age, sex, and refractive error, we can consider that the groups were similar for these parameters.

The use of parametric tests is justified by the fact that most variables do not follow normal distribution within each group. Those that were statistically as normal are: age (only for diabetics); gender; Refractive error (only for controls); DBP (only for controls); SFCT (only for diabetics); CFRT (only for controls). To avoid any bias in the statistical analysis, since some variables had normal distribution and others did not, we opted to use non-parametric tests, thus making the analysis more robust.

When comparing both groups, there were no significant differences between SCFT and CFRT at baseline between the 2 groups (diabetic and control), but most of the diabetic (62%) had no retinopathy, being comparable to controls.

But, beside the statistical result, we do have to look to the graphic for individual values to have the perception of the great variability of SFCT measurements by age or CFRT.

Statistical Analysis of diabetics at baseline

We found SFCT mean values of 273,8 µ ± 80,9 (range: 96-495 µ). When analyzing by diabetic retinopathy sub-group we found that values were similar for NDR, mild NPDR and moderate NPDR, but higher in severe NPDR and PDR (respectively: 264,32 µ ± 81,08; 262 µ ±65,6; 265,64 µ ± 85,46; 336,75 µ ± 55,57). With macular edema the value was 262,81 µ ± 85,10. As described previously in the review of literature, mean values of SFCT in diabetic patients vary from studies. A great number of studies showed a decrease in SFCT with the severity of the diabetic retinopathy, while we found higher values in more severe stages, as described by Kim et al in a large sample study[136]. Our results can be biased since the number of

eyes with more advanced disease was too small. But, as the results from all other published studies, what remains is the great variability or dispersion of SFCT measurements, which raise the question if mean value of a single sub foveal measurement is reliable. The choroidal thickness variability can be dependent of systemic, individual or pathologic conditions. Campos et al[150] and Melancia et al[151] made important reviews concerning publications somehow controversial results regarding choroidal thickness in Diabetes. As referred by Campos et al, published studies could not be entirely comparable for choroidal thickness since in their design and variables analyzed they differ from each other. Some studies compared healthy with diabetics without retinopathy[125–129,136,152,153], and others compared with diabetic with retinopathy stage and or macular edema[78,126,128,136,152,154,155]. Increase, decrease or no change of choroidal thickness compared to healthy or related to the retinopathy stage can be found in the literature. Most studies are not longitudinal and are based in one single visit measurement. Few studies looked for correlation of choroidal thickness with other variables.

In our study, when analyzing the impact of various variables on SFCT using the model of linear regression we obtained a statistically significant result (p=0,001) with a R2 of 0,4020 (it explains 40% of the SFCT variance). In another words, the fact that the model is significant means that the selected variables explain and can somehow predict the dependent variable (SFCT), and the value of R2 tells us that the model can explain a lot or a little. More than one third of the variable (SFCT) may be influenced by the remaining variables. When we look at the results of the model, we see that the variables that actually could explain or predict the variable dependent SFCT are the ones that were significant i.e. layers (choroidal morphologic changes)

and age, and the others end up explaining very little. The variable layers could be predictive since their significance level was of p= 0.033. Significance was also found negatively correlated with age. What these statistical results tells us is that, although SFCT can be influenced by various variables, the ones that have a significant impact are an increased thickness correlated to normal choroidal layers and decreased thickness with increasing age. No significant correlation was found with the central retinal thickness, which could be explained by the similar SFCT approximate value for eyes with macular edema comparing to the greater sample of eyes (with NDR or initial stages). Most published studies regarding choroidal thickness in Diabetes does not a multivariate analysis to evaluate the impact of other variables in SFCT measurements. Few looks for correlation with univariate analysis with metabolic state, duration of Diabetes, and age. The only consensual correlated variable is age with progressive decrease of thickness measurements. The choroid is a richly vascular structure with involving fibroblast and muscle meshwork. It is expectable to have contractile or expansion (dilatation) response from these structures to physiologic and pathologic conditions, ocular or systemic. On diabetic patients the metabolic state is altered frequently and associated to other morbidities such high blood pressure. We do know from elsewhere in the body the vascular effect and damage of hyperglycemia and vascular changes with blood pressure disease. Nevertheless, in our study metabolic state and blood pressure, or duration of Diabetes could explain some variability but were not significant for correlation with SFCT. The same result was found for ocular variables except the choroidal ones. Xu et al[153] on their work also did a multivariate analysis and found correlation of SFCT with glycosylated Hemoglobin, age, diastolic blood pressure and the diagnose of

Diabetes, but no correlation with the presence or stage of diabetic retinopathy. These findings also alert to the caution of using choroidal thickness alone as a parameter to determine the diabetic choroidopathy.

Control Baseline Statistical Analysis

We found for the control group mean SFCT values of 281,8 µ ± 74,1 (ranging from 61 to 412µ). Our results are within the mean values of other studies in healthy population (table 1), but as described previously data varies tremendously from paper to paper. Control group also had a great variability in the SFCT values, as on the diabetic group. Comparing with post-mortem data of choroidal thickness (± 220 µ) our mean choroidal thickness is slightly higher, but we should expect a thinning with histologic preparations and dehydration phenomena. Sohn et al in 2014[156] published a histology study demonstrating a normal distribution of choroidal thickness but with a high range of thickness values, corroborating findings in published studies and our study. Furthermore, it is expectable to have a multitude of variables that can interfere with the choroidal thickness measurement, even physiologic conditions.

When analyzing the impact of other variables on SFCT the model obtained was statistically significant ($p<0.001$) explaining about 44% or more of the SFCT variance ($R2 = 0.439$). A positive significant correlation was found only with SBP and a negative correlation with DBP and age. The simple regression model gives us a ratio of SFCT decrease of -38µ/10 years. But using a model of regression analysis taking in account the variables statistically significant (and considering SBP constant), then

the ratio of loss falls to -23µ/10 years. The correlation of choroidal thinning with aging is the only consensual data from all studies, but the ratio for 10 years varies from -11,8µ (Pappuru[99]), -13,1 µ (Shin[97]), -14 µ (Ikuno[94]), -15,6 µ (Margolis[18]), -19,5 µ (Ouyang[96]), -30 µ (Hirata[95]) to -33 µ (Wei[101]). These differences in the ratio might be because just few made corrections for statistical significant variables, and samples were very different in number, age and race (table 1). It is risky to establish a mean ratio of thinning with age since multifactorial factors can affect choroidal thickness. One is blood pressure as in our study we found significance for systolic blood pressure. The choroid as a highly vascular structure can be sensitive to changes in systemic blood pressure. Few studies were published regarding the effect of systemic blood pressure in choroidal thickness, but results are controversial. Akay et al[157] and Ahn et al[158] found a correlation of increased systemic arterial blood pressure with CT (with severe hypertension in the later), but GöK et al[159] concludes on their study that blood hypertension does not affect CT. Samson et al[160] made on their study a multivariate analysis were they found a similar result as we with a correlation only with systolic blood pressure with increase CT. Even if we still don't know the real effect that blood pressure and blood pressure medication could have on CT measurements, it might be a confounding co-factor, especially if severe or uncontrolled. Other studies[161,162] in healthy population demonstrated diurnal choroidal thickness variability, comparing morning to late afternoon measurements on the same individuals and the same day.

Progression statistical analysis

In the progression analysis there was no major or significant variation/progression of all variables, hence it is difficult to find correlations. The multivariate analysis of the impact of the other variables on SFCT was not statistically significant (p=0.060) with a R2= 0.0734 explaining only 7.3% of the variance.

The only factor associated with the variation of the SFCT is the variation of the CFRT: a greater variation of SFCT is associated with a greater variation of CFRT (P = 0.008). Considering the association found with SFCT (Spearman coefficient) this association is however weak (r = 0.0681; p = 0.5097).

No publication was found with longitudinal data (sequential visits) for progression to compare with our results.

Statistically we did not find significant data. Probably because most eyes in diabetic group were at the initial stages or even with no retinopathy, relatively well controlled metabolically, comparable to controls and thus, a mean of two year of follow up might be a to short follow up period for disease progression. Also, with the “lost-of-follow-up” rate and the eyes dropped out for treatment, the sample decreased on the second visit. Data was in general almost the same for baseline and the second visit. Nevertheless, a weak correlation was found between the retinal progression with choroidal thickness change.

Morphological Analysis

From the pilot study we found differences in morphology of the choroid of normal and diabetic eyes allowing to establish a classification system of normal and abnormal that was used for the main study. The pilot study allowed to suggest that:

- In normal, the SCI border tends to be regular, with an inverted dome shape with the thickest point under the fovea, the ChS layer is regular and homogeneous with no focal invasion of great vessels
- In diabetic eyes this regular and homogeneous aspect tend to be disturbed, especially with progression of the retinopathy state, but it is in the presence of Macular edema where we can find a correlation between the choroidal morphological changes and the suffering retina suprajacent. Findings are: loss of the “inverted dome shape” and irregular delineation of the SCI, abnormal layers with loss of the integrity of the mottled ChS and parallel to the Bruch’s membrane, focal atrophy or increased thickness of vascular layers, loss of even distribution, hyperreflective spaces on the vascular zone.

Based on this normal and abnormal classification for SCI and Vascular layers, morphologic analysis was made on the main study.

For the control group of normal we found that:

- The ChS layer was always preserved (mottled, homogeneous with posterior limit parallel to the Bruch´s membrane
- Most eyes had a normal SCI (inverted dome shape with thickest point sub-foveal). When irregular it was always due to great vessels layer abnormalities.
- Thinnest choroids in older subjects difficults evaluation.

For the diabetic group:

- Half of the eyes had no morphologic abnormalities. Probably because most eyes had no DR or were at initial stages of DR.
- When choroidal morphologic changes were found, they were particularly due to vascular changes than SCI changes, and primarily on the ChS layer and in eyes with DR.
- Changes in the great vessel layers were the major responsible for the irregular sclero-choroidal border, usually due to focal atrophy or thinning, loss of vessels with hyperreflective spaces occupying the vessels space or hypertrophy.
- In the absence of evident retinal disease and changes in the choriocapillaris/Sattler's layer, great vessels layer abnormalities are usually by focal loss or atrophy, more frequently nasal to the fovea, or loss of even distribution.
- Changes on the choriocapillaris/Sattler's layer as focal atrophy/loss with approach of the great vessels to the Bruch's membrane, hypertrophy associated with focal thinning or loss of subjacent great vessels, are correlated to changes in the retinal anatomy above. It becomes more evident in the presence of macular edema specially if the photoreceptor layer is affected.

Only two published studies (Branchini[143] and Adhi[138]), were done looking for morphologic findings of the choroid, using cross-sectional OCT images. The first study was made in healthy patients and findings compared with eyes with pathology such Age Macular Disease, and as previously described in this work, it was a reference for the pilot studies. The second was performed in diabetic patients

compared with healthy controls. This study has various similarities with ours. As parameters to evaluate choroidal morphologic changes were defined: the choroidoscleral interface could be regular, convex or bowl shaped (normal) or irregular or "S" shaped; they considered as normal in the thickest point of the choroid was sub-foveal (beneath the fovea or 100µ nasal or temporal); focal thinning of choroidal thickness was considered whenever the thinning was 50% less than the mean value in healthy (previously established) at the same location. But for vascular analysis they established a cut-off value for the diameter of large vessels (> 100µ), identified the large vessel sub-foveal and measure thickness from the anterior to choroidoscleral limits of the vessel to determine great vessels layer thickness by subtraction from the total SFCT, and consequentially the Sattler and choriocapillaris layers. They found irregular contour of the SCI in more than 90% of the eyes with diabetic retinopathy but in none of the healthy. They found a significant thinning in CT in the Sattler and choriocapillaris layers in advanced stages of DR and Macular edema. Our study morphology results are in accordance to these results, but on their study morphology and vascular analysis was made only at the subfoveal location. The method used for vascular analysis using mean values extrapolated from central measurements do not identify focal extra-foveal changes in choroidal morphology. They do not do a direct correlation of the focal choroidal changes with the retina above. It is a retrospective of one visit analysis with no data regarding progression of the findings. A number of patients were previously treated, so we cannot be sure if some findings could be a consequence of the treatment performed.

Other studies[163–165] looking for choroidal morphological findings on OCT used on the other hand, En-face images. On our point of view, those studies had some

limitations, since the choroidal layers were identified by pre-determined thickness slabs based on mean thickness values previously established. If we consider that there is a great variability in thickness of the choroid in healthy, and if in pathology the layers become irregular, we can predict artifacts on the evaluation. Nevertheless, Murakami et al[164] found that diabetic eyes had more frequently than controls areas without definite vascular structures in Sattler's layer, focally narrowed vessels and vascular stump in Haller's layer. But, statistically, they found no association of morphological changes with CT.

Our findings are particularly interesting suggesting a response of the choroid to the retinal disease where it seems that to more acute stages a functional choroid respond to increase the blood flow to nourish the suffering retina, but lately a diabetic choroidopathy occurs with ischemic, atrophic and other changes. Choroidal morphological changes seem to occur in a focal manner and only subjacent to the zone of the retina needing further nutrition and metabolic input.

However, only with consecutive exams in follow-up visits we can validate if the choroidal morphological findings really may represent diabetic choroidopathy and its correlation with the retinal disease. To evaluate that, this study was designed as longitudinal with follow-up visits, looking for progression on choroidal morphologic changes. We found that SFCT vary in subsequent visits despite the CFRT evolution. It was not evident any pattern or correlation of SFCT change (increase or decrease) with the retinal thickness, although a great percentage of eyes with decrease in SFCT had also a CFRT decrease. Even when we stratified for severity of thickness change, no patterns were evident. SFCT should not be considered as a reliable

parameter to evaluate progression or risk of progression, or a parameter to define severity or sequela of the retinal diabetic disease.

From case to case analysis we found that focal changes in the retina tended to have subjacent choroidal changes. This may be explained by the theory of the choroidal function to support the retina, particularly the posterior layers. It seemed to have in some cases of retinal disturbance, the need to improve vascular and blood supply with a reactive or physiologic enlargement or dilatation of the great vessels pushed towards the Bruch's membrane external limit and the retina. When the ocular diabetic disease becomes more advanced, the physiologic response of the choroid fails, and the choroid might start with Diabetes microvascular complications, with ischemic processes leading to focal areas of atrophy of the ChS layer and/or the great vessels layer, which corresponds on OCT images to the focal areas hyperreflective and/or atrophic.

From histology we know that the choriocapillaris is organized in lobules as functional independent units with intervention on the retina immediately above, and only the choriocapillaris leak in the choroid. This may explain the correlation found of focal retinal changes with the subjacent choroid found in our study. Even before the OCT, in histologic studies, Hidayat and Fine[70], Fryczkowsky[71] and Cao[72] described a diabetic vascular choroidopathy with degeneration of the choriocapillaris with capillary drop out, basement membrane thickening, vascular abnormalities such tortuosity, dilatation narrowing, microaneurysms and neovascularization. Cao et al add that focal choriocapillaris degeneration is more initial and becomes diffuse in advanced stages associated to neovascularization.

An interesting work of Zouache et al in 2016 demonstrates that the architecture of the choriocapillaris in independent capillary networks units, the lobules, are correlated with individual unit blood flow rates which can lead to localized implications of the suprajacent retina function. This might be in agreement with the OCT findings of related focal choroidal changes with suprajacent retinopathy.

In an excellent review of diabetic choroidopathy in 2017, Lutty et al[166] postulate that inflammation and inflammatory cells might contribute to loss of choroidal vasculature based on previously demonstrated accumulation of macrophages and PMNs in the retinas of diabetic rats. From histology and histochemistry there are evidence of a Diabetic choroidopathy that somehow leads to morphological changes in the choroid.

Querques et al[152] postulated a mechanism how the choroid could be invloved in the development of diabetic macular edema. A decreased sub foveal choroidal thickness could cause tissue hypoxia with an increase in the level of VEGF, resulting in the breakdown of the blood-retinal barrier, responsible for the development of DR and macular edema[140].

The choroid has an anatomy completely different from the retina. While the retina is composed primarily by cellular layers well-structured and organized, with very little ability or property of changing size, the choroid is a highly and mostly vascular structure involved in connective tissue with high ability and property in changing size and thickness due to contractile and blood flow states. Thus, it is reliable to establish normal thickness values for the retina, since anatomically no extreme variability is

expected to physiologic states. In the other hand, even having choroidal thickness values from histology, on in vivo state it is highly susceptible to physiologic influence, ocular or systemic, with a high variability of measurements, that thickness measurement only makes sense if correlated with morphology and analyzed individually and focally in subsequent visits.

From our findings, focal morphologic changes in the choroid were correlated with focal changes in the retina. We made an ancillary study to evaluate if superimposing automated OCT retina thickness maps with choroid thickness maps would allow to quickly localize focal areas of choroid morphologic changes correlated with the retinal disease. Our results demonstrated that we can superimpose retinal changes with choroidal changes using the maps and easily identify the focal zone to evaluate for morphological choroidal changes in line scans. Normal eye showed the same pattern of choroidal thickness map as described on previous published studies[95,96,103–106,126,129,131], with a continuously decrease in thickness from the superior, to temporal, to inferior to the nasal macula – the STIN pattern. In diabetic eyes different patterns of maps were found, with evidence of changes in choroid even in early stages of DR. These results should be validated with a larger sample study. Unfortunately, till now, only one commercially available OCT (Topcon SS OCT) device has a software for automated choroidal thickness maps. With all other OCTs available, we can build a choroidal thickness map doing choroid segmentation manually for each scan of the macular cube. It is time consuming with great probability of segmentation errors in different scans, becoming not reliable or feasible.

Our study has some limitations or weakness.

The classification used to define choroidal morphologic findings is dependent on quality and resolution of OCT images to allow good identification of SCI border and vascular abnormalities. In most available OCT devices, automatic SCI limit segmentation is not an option and becomes dependent on the training of the grader. When the choroid is very thin it is impossible to evaluate all the parameters described as to be evaluated for classification. Even with our simple classification, in some cases it is not easy to identify the choroidal findings or boundaries. A very clear definition needs to be made. Huynh et al.[167] made a review looking to the posterior boundaries of the choroid in several studies and found a variability and lack of clarity in the definition of the exact choroidal scleral interface, leading to the variability in choroidal thickness measurements. They propose a harmonization classification and suggest for choroidal thickness measurements to consider the posterior boundary as the end of the choroidal vessels. Nevertheless, they advise to measure also the suprachoroidal layer, since it can have clinical relevance in pathology.[167]

Statistical analysis was made only for subfoveal choroidal thickness, but in our own study, we demonstrate that changes in thickness occurs focally, sometimes extra-foveal. Using only the central value for statistical analysis can leave important thickness changes to be taken in account in the statistical data. But this is a limitation of all published papers, and our study was designed so to be comparable with other studies.

To avoid eventual bias of the results due to previous treatment of any kind, the inclusion/exclusion criteria were very strict allowing only eyes naïve of treatment. Although we had a very good sample of eyes, the distribution by diabetic retinopathy severity groups was not equitable, having a predominance of eyes with no or initial stages of DR, and therefore, less probability to have pathologic changes. Very few eyes had advanced stages and a small sample had macular edema, which were the ones that showed on the pilot studies more prominent choroidal changes. On the other hand, one of the main purposes was to evaluate progression but the sample was reduced for follow up visits. A longer period of follow up may be needed to better evaluate progression with the advance of the diabetic disease.

Our study is the first identifying choroidal morphologic changes and correlating them to the retinal disease above. And clearly in the morphologic progression analysis we were able to evidence that there are choroidal morphological changes related to the retinal state that can evolve to recovery or sequela according to the severity of the disease. We think that it became evident that statistical analysis of mean choroidal thickness, especially the central or subfoveal (mostly used is studies) is not a very reliable parameter to evaluate diabetic choroidopathy. But even in the statistical analysis we found correlation with choroidal morphologic changes corroborating the defense that the choroid should be analyzed first for morphologic findings than thickness. Our study was the first introducing as a variable in statistical analysis the presence/absence of morphologic changes in the choroid.

Based on our findings we propose a schematic module of physiologic and pathologic events occurring in the choroid.

NORMAL CHANGES IN CHOROIDAL THICKNESS – PHYSIOLOGIC RESPONSE

Changes in thickness respects the normal anatomy of the choroid, maintaining the inverted dome shape with the thickest point sub-foveal, integrity and homogeneity of the choriocapillaris and Sattler layers. Thickness changes are due essentially to physiologic vasodilatation and vasoconstriction, and it is reversible.

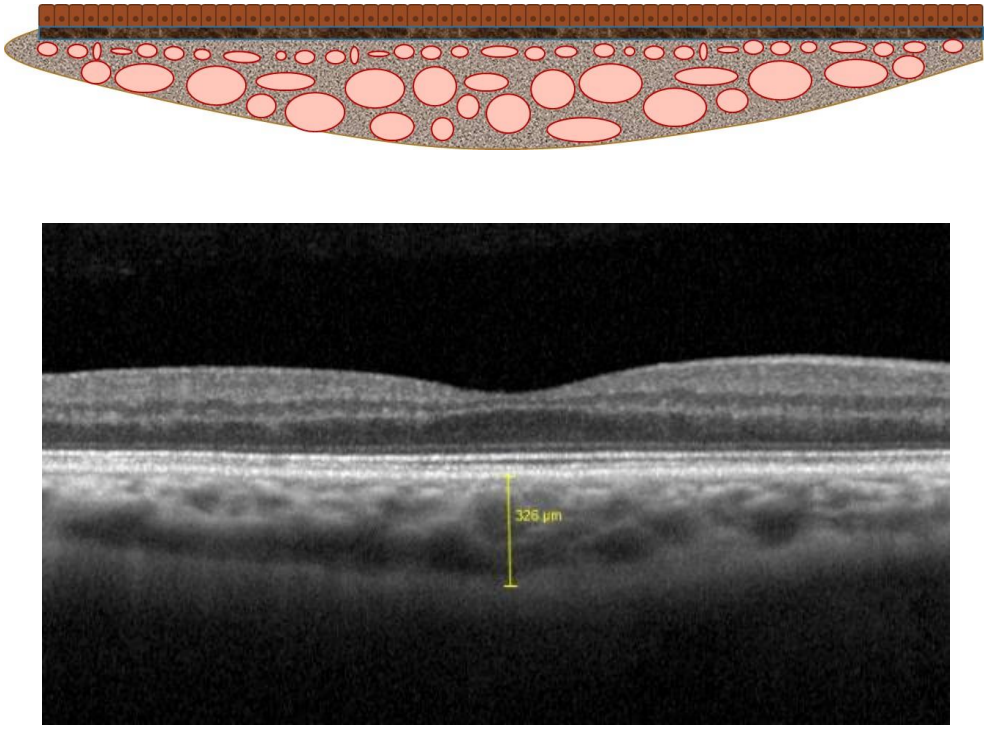


Figure 35 – Schematic and OCT image of normal choroidal morphology.

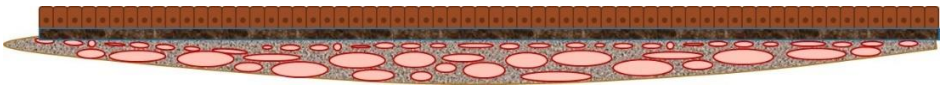


Figure 36 – Schematic image of normal choroidal morphology with vasoconstriction

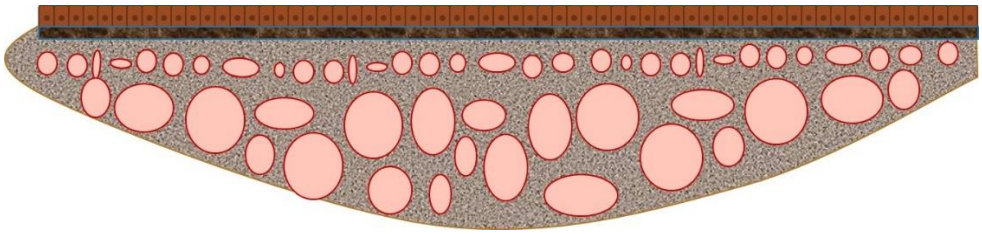


Figure 37 – Schematic image of normal choroidal morphology with vasodilatation

ABNORMAL CHANGES IN CHOROIDAL THICKNESS – PATHOLOGIC RESPONSE

Changes in choroidal morphology with pathologic findings. Focal increase or decrease in thickness causing irregular contour with loss of the inverted dome shape. Focal changes in thickness are correlated with increased vascular caliper and invasion of adjacent vascular layer (great vessel into the ChS layer or vice-versa), atrophic vascular layers and/or disappearance of vessels.

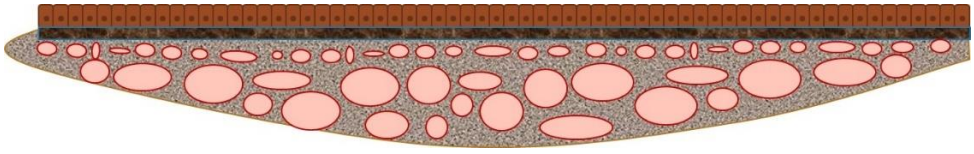


Figure 38 – Schematic image of normal choroidal morphology

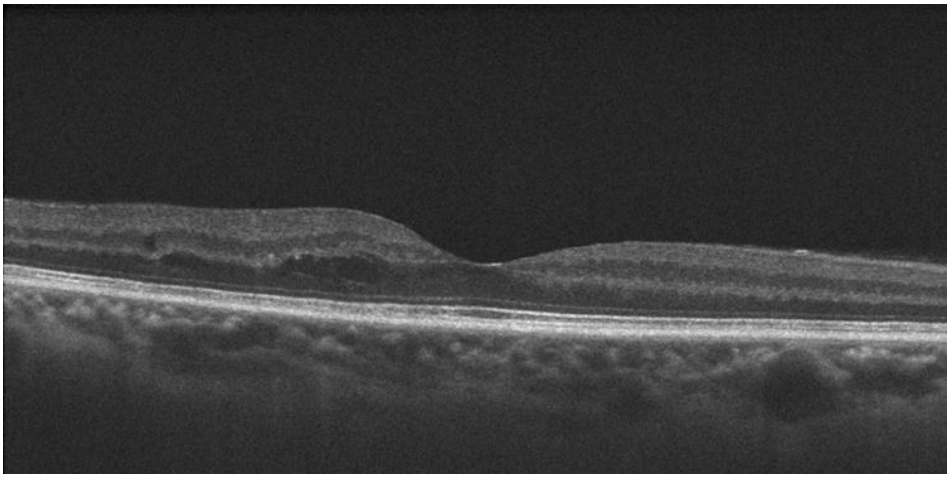


Figure 39 – Schematic and OCT image of an abnormal or pathologic choroidal morphology

Since most morphologic changes in the choroid are vascular, the best way to prove and evidence those changes would be with choroidal vascular architecture maps and flow data.

More recently, with the OCT-Angiography, it is possible to obtain angiographic visualization with higher resolution of the vascular architecture (at capillary level) in a noninvasive manner with no dye.

Some studies looking for vascular index and with OCT-angiography start to be performed and published, and this could be the future complement to better understand the Diabetic choroidopathy[131,168–171].

CHAPTER 6 - CONCLUSION

6 - CONCLUSION

1 – We were able to identify morphologic parameters to be evaluated for normality or abnormality in OCT images of the choroid allowing a simple classification system, achievable with any commercially available OCT device and software.

2 - With diabetic retinal disease we demonstrated the evidence of a Diabetic choroidopathy directly correlated with the retinal disease above, and that changes occur focally, in accordance to data previously described in histology.

3 – Choroidal thickness alone, as a mean value or one-point measurement is not reliable for analysis. Choroidal thickness changes should be analyzed and validated individually, in a map, correlated with choroidal morphologic changes and in subsequent observations.

4 – The software of actual OCT available devices should be improved to allow automated and reliable choroidal segmentation and automated choroidal thickness maps.

5 – A Diabetic choroidopathy can be identified with OCT images, and signs of disease could predict eventual progression or regression with treatment, which could have a prognostic clinical value in the ocular diabetic disease. More consistent data will be available surely in the near future with the advances in OCT technology such the OCT-Angiography.

6- We are the first presenting a longitudinal study in Diabetic choroidopathy using OCT images, where we were able to find that morphological changes in choroid occur related to the progression of the subjacent diabetic retinopathy.

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APENDIX 2 – Patient Informed Consent Form

PROTOCOLO CHEDV-OFT-01-14

FOLHA DE INFORMAÇÃO AO DOENTE

ESTUDO: "ESPESSURA E MORFOLOGIA DA COROIDE ANALISADA COM A TOMOGRAFIA ÓPTICA DE COERÊNCIA COMO MÉTODO DE AVALIAÇÃO PROGNÓSTICA E DE PROGRESSÃO DA DOENÇA DIABÉTICA OCULAR"

Descrição do estudo e Procedimentos do estudo

O estudo proposto é um estudo observacional em que se pretende avaliar as características e alterações que ocorrem na coróide nos doentes diabéticos, comparando com pessoas não diabéticas. A coróide é a parte do olho mais rica em vasos sanguíneos e que está localizada mais exteriormente, daí até agora ter sido de difícil visualização com os métodos de exames complementares de que dispúnhamos. A forma como a Diabetes pode causar doença no olho é através da alteração do normal funcionamento dos vasos sanguíneos. Com a nova técnica de exame complementar, chamada Tomografia Óptica de Coerência, conseguimos de forma não invasiva, avaliar a estrutura das camadas mais profundas do olho.

Neste estudo vamos seguir doentes diabéticos de acordo com os procedimentos habituais e recomendados, fazer uma análise dos exames para avaliar as alterações que ocorrem. Vão ser também observados indivíduos sem o Diagnóstico de Diabetes para se poder comparar as alterações encontradas entre os dois grupos.

Este estudo terá a duração de 2 anos, em que os voluntários serão observados de 6 em 6 meses ou sempre que o quadro clínico o justificar. Sempre que houver necessidade de tratamento serão orientados para o fazer. Em todas as consultas será feito um exame oftalmológico completo que inclui para todos os voluntários a realização da Tomografia Ótica de Coerência. No caso dos voluntários diabéticos, realizarão, de acordo com a prática clínica habitual, a angiografia fluoresceínica, com uma periodicidade anual ou sempre que a evolução da doença o indicar.

Participação voluntária

A sua participação neste estudo observacional deve ser de sua livre vontade.

Se decidir não participar neste estudo, não sofrerá nenhuma consequência negativa no seu futuro tratamento. Isto também se aplica se decidir desistir de participar em qualquer altura. Esta opção está aberta para si a qualquer momento, e não lhe será pedido que forneça explicações por desistir de participar ou continuar o estudo.

Para sua segurança, será submetido a um exame final em qualquer dos casos.

Local de Realização do Estudo

Este estudo será realizado no Serviço de Oftalmologia do Hospital de São Sebastião, em Santa Maria da Feira, Centro Hospitalar Entre o Douro e o Vouga.

Benefícios/Riscos

A sua participação neste estudo pode ter os seguintes benefícios: uma supervisão médica pelo especialista em oftalmologia e tratamento necessário.

Os riscos associados à participação no estudo estão relacionados com o risco da angiografia fluoresceínica. É um método invasivo para avaliação da circulação ocular (na retina), em que se injeta um produto de contraste na veia do antebraço, e que vai circular até ao olho, permitindo, através do registo por fotografias seriadas, avaliar a circulação da retina e as zonas afetadas pela retinopatia diabética e com necessidade de tratamento. É um exame importante e necessário no estudo da retinopatia diabética. Pode apresentar efeitos secundários ligeiros como coloração amarelada da pele e urina nas 24 a 36 horas após a injeção até a eliminação total do produto de contraste (em 100% dos casos), náuseas (em menos de 10% dos casos), vómitos (em menos de 2% dos casos), prurido (comichão) ou reação vagal (desmaio) à injeção (em menos de 1% dos casos). Efeitos secundários graves com reações alérgicas graves são muito raros.

A sua participação neste estudo não deverá causar qualquer inconveniente para além das visitas programadas ao Serviço de Oftalmologia.

Confidencialidade dos registos clínicos

Durante o estudo, os seus dados pessoais serão arquivados. Todos os dados serão tratados anonimamente e serão apenas disponibilizados ao responsável do estudo para avaliação clínica.

Toda a confidencialidade será mantida durante e após o estudo e durante os procedimentos de controlo acima descritos.

Descontinuação involuntária do estudo

O investigador poderá excluí-lo do estudo pelo interesse da sua saúde. Se durante o estudo desenvolver qualquer outra alteração oftalmológica não relacionada com a Diabetes ou doença sistémica grave será orientado para tratamento e sairá do estudo.

Para sua segurança, terá um exame médico final em qualquer dos casos.

Pessoa a contactar

No caso de qualquer problema ou dúvida poderá contactar as pessoas abaixo indicadas:

Drª Lilianne Duarte

Serviço de Oftalmologia

Hospital de São Sebastião/ Centro Hospitalar Entre o Douro e o Vouga

PROTOCOLO CHEDV-OFT-01-14

CONSENTIMENTO ESCRITO

Eu, abaixo assinado, _____, residente em _____, concordo em participar no estudo denominado **"ESPESSURA E MORFOLOGIA DA COROIDE ANALISADA COM A TOMOGRAFIA ÓPTICA DE COERÊNCIA COMO MÉTODO DE AVALIAÇÃO PROGNÓSTICA E DE PROGRESSÃO DA DOENÇA DIABÉTICA OCULAR".**

Foram-me dadas todas as explicações pelo(a) Dr(a). _____, responsável pelo estudo, acerca da natureza, objectivos e duração. Foi-me dada oportunidade de colocar todas as questões relacionadas com todos os aspectos do estudo. Foi-me também dada uma cópia da Folha de Informação ao Doente.

Depois destas considerações, concordo em colaborar neste estudo e com a sua equipa, e informá-la imediatamente se surgir alguma anormalidade. Tomei conhecimento que sou livre para desistir do estudo em qualquer altura, se assim o desejar.

A minha identidade nunca será revelada, e os dados obtidos serão tratados confidencialmente. Concordo que eles podem ser examinados pelos investigadores envolvidos no estudo e pelos representantes das autoridades de saúde. Concordo em não me opor à divulgação dos resultados a que o estudo se propõe.

Data e Assinatura do Investigador

Data e Assinatura do Doente

APENDIX 3 – Hospital Study Approval

Centro Hospitalar
de Entre o Douro e Vouga, E.P.E.

Exma. Senhora
Dra. Lilianne Duarte
Serviço de Oftalmologia
Centro Hospitalar de Entre o Douro e Vouga

CA-286/14-2 **Data:** 2014/05/16
FS/AC

Assunto: "Espessura e morfologia da coroide analisada com a tomografia ótica de coerência como método de avaliação prognóstica e de progressão da doença diabética ocular"

O Conselho de Administração do Centro Hospitalar de Entre o Douro e Vouga, EPE, deliberou em reunião de 15 de maio de 2014, autorizar a realização do trabalho de investigação mencionado em epígrafe.

Com os melhores cumprimentos,

Fernando Silva
Presidente do Conselho de Administração

Sede: Hospital de São Sebastião
Morada: Rua Dr. Cândido de Pinho
4520-211 Santa Maria da Feira

Telefone: 256 379 700
Fax: 256 373 867
NIF: 508 878 462
hss@hospitalfeira.min-saude.pt