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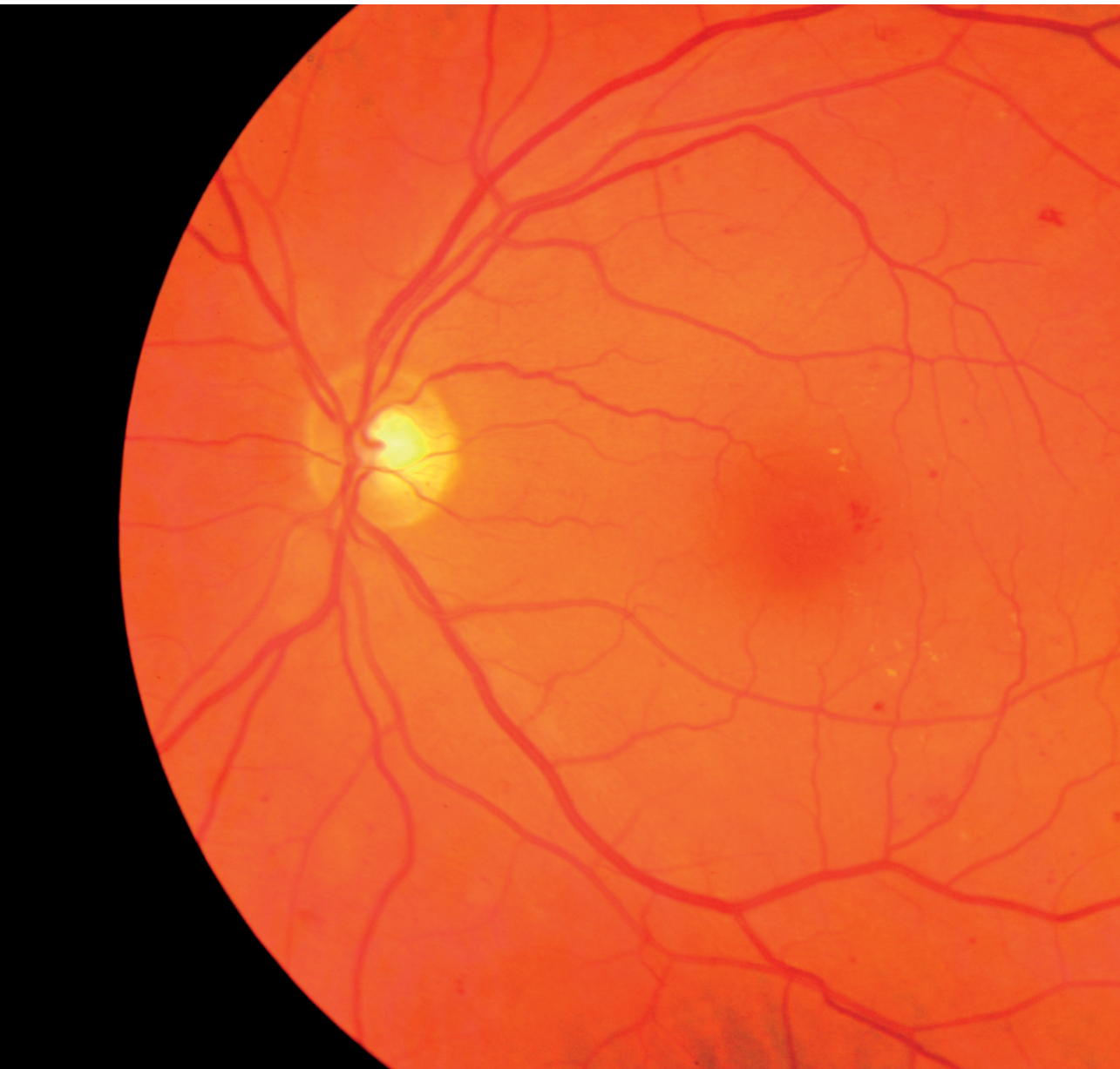
Diabetic Retinopathy

New Perspectives for Personalized Management

JOSÉ CUNHA-VAZ



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Biografia / Autor

José Cunha-Vaz, M.D., Ph.D. is Emeritus Professor of Ophthalmology of the University of Coimbra, Portugal, and President of the Association for Innovation and Biomedical Research on Light and Image (AIBILI), a not-for-profit Clinical Research Organization. He is now Chief Editor of Ophthalmologica, Coordinator of the Diabetic Retinopathy and Retinal Vascular Diseases Section of the European Vision Institute Clinical Research Network (EVICR.net) and Secretary-General of the European Academy of Ophthalmology.

Dr. Cunha-Vaz moved in 1979 to the United States to become a full-time Professor of Ophthalmology and Director of the Retina Service at the University of Illinois at Chicago until his final return in 1986 to Portugal. He was for many years Professor of Ophthalmology and Head of Department of Ophthalmology of the University of Coimbra (1972-2008) and President of Institute of Biomedical Research on Light and Image (IBILI) (1994-2007).

Dr. Cunha-Vaz's work has included laboratory and clinical research in retina and intraocular fluids with special emphasis on blood-retinal barriers and diabetic retinopathy. He was the first to describe the Blood-Retinal Barrier and initiated clinical vitreous fluorometry and multimodal macular mapping. He has also identified different phenotypes and novel biomarkers of diabetic retinopathy. He was PI for research grants funded by NIH (USA), FCT (Portugal) and European Union Research Programmes. He participated as PI in a large number of studies, especially involving therapies for retinal diseases (ETDRS, RESOLVE, EOP1013, etc).

Dr. Cunha-Vaz has authored and co-authored over 470 peer-reviewed papers and books. He was elected member of Academia Ophthalmologica Internationalis in 1994. He is a founding member and General Secretary of the European Academy of Ophthalmology. He has received the Helmholtz Gold Medal of the European Society of Ophthalmology, the Gian Battista Bietti Gold Medal of the Italian Society of Ophthalmology, the Paul Henkind Award of the Macula Society, the Alcon Research Institute Award, the Eva Kohner Award from the European Society of Diabetes, the 2012 BIAL Award for Clinical Medicine and the 2014 Weisenfeld ARVO Award. He has been elected an honorary member of Portuguese Society of Ophthalmology, Spanish Society of Ophthalmology and International Retina Society - Club Jules Gonin. Dr. Cunha-Vaz has received the Orders of Merit and Henry the Navigator from Portugal.

Introduction

This is an integrated perspective of diabetic retinopathy based on my most recent personal contributions which are a continuation of research performed for a period of over fifty years and were initiated in London in 1963.My contributions to the pathophysiology, diagnosis and management of diabetic retinopathy started during my stay at the Institute of Ophthalmology and Moorfields Eye Hospital, London (1963-1966), continued in Coimbra (1971-1978), and then for a period of approximately eight years during my stay in Chicago, in the Illinois Eye and Ear Infirmary and Lions of Illinois Eye Research Institute (1978-1981 and 1984-1986). Finally, when I returned to Coimbra, it was possible to assemble laboratory and clinical research projects by associating the Research Centre of Ophthalmology of IBILI (Institute of Biomedical Research on Light and Image) and AIBILI (Association for Innovation and Biomedical Research on Light and Image) with the Department of Ophthalmology of the University Hospital of Coimbra. It is essentially the work performed in Coimbra in the last ten years that is reported here.

It all started in London where I was challenged by Norman Ashton to focus on diabetic retinopathy and pathophysiology of retinal vascular disease. I accepted this challenge and since then diabetic retinopathy, its understanding and treatment have been my main scientific interest.

When still in London, I was able to demonstrate that there were specific characteristics in the retinal vessels that could explain the initial alteration of diabetic retinopathy. We were looking for signs of specificity in the retina that could explain why diabetes was affecting specifically the retina. We were able to demonstrate that the retinal vessels were different from other vessels of the body and only comparable to the brain vessels. They offered a barrier to small molecules that was only similar to the blood-brain barrier.

The concept of blood-brain barrier was well established but the ophthalmological literature still, at the time, considered the retinal vessels to have similar permeability to most vessels in the body. The relevance of the blood-retinal barrier became even more apparent when we were able to identify in the retinal vessels “tight-junctions” between the endothelial cells that explained well the restrictive permeability of the retinal vessels and their barrier properties, behaving like an epithelium because of the tight-junctions of the “zonula occludens” type (Shakib and Cunha-Vaz, 1966). This finding

was of major relevance because it explained the blood-retinal barrier and indicated that an alteration of this barrier was likely to be at the basis of the initial changes occurring in diabetic retinopathy. It is also worth noting that the presence of tight-junctions in the blood-retinal barrier demonstrated for the first time by our group was later confirmed to occur also at the blood-brain barrier level (Reese and Karnovski, 1967).

After we were able to identify the morphological basis of the blood-retinal barrier it was of clear interest to understand its function, i.e., to characterize its permeability.

We developed new methodologies to study the permeability of the blood-retinal barrier and we were able to measure the permeability of the retinal vessels to fluorescein and demonstrate the presence of an active transport for organic anions like fluorescein in the retinal vessels (Cunha-Vaz and Maurice, 1967 and 1969). These studies were performed in the rabbit retinal vessels which are in the vitreous, free from the surrounding glia and using a slit-lamp fluorometer. The permeability coefficient for fluorescein across the retinal vessels was $0.14x10^{-5}$ cm. sec^{-1}, similar to the value obtained for sucrose, which is a molecule of similar size, by Crone (1965) in the blood-brain barrier, $0.16x10^{-5}$ $cm.sec^{-1}$, and to the value obtained by Davson and Danielli for the permeability of an isolated cell, $0.14x10^{-5}$ $cm.sec^{-1}$ (Davson and Danielli, 1952).

We were also able to demonstrate, together with David Maurice, that there was a much faster movement of fluorescein in the opposite direction, i.e., from the vitreous to the blood circulation through the retinal vessels and that this movement could be inhibited by competitive inhibitors like iodopyracet, probenecid, trypan blue and by enzymatic inhibitors like dinitrophenol and sodium fluoride. This movement showed all the characteristics of an active transport for organic anions, similar to the ones previously described in the liver and kidneys. This transport moved fluorescein against an uphill gradient fulfilling all the characteristics of an active transport.

These studies were followed by further clarification of the blood-retinal barrier morphology by identifying its “inner” and “outer” components, the “inner blood-retinal barrier located in the endothelial cells joined by tight-junctions and the “outer” blood-retinal barrier in the retinal pigment epithelium joined also by tight-junctions (Cunha-Vaz, 1976). Two cell

layers of the retina showing epithelium-like characteristics and protecting the retina and its highly developed functional activity from the constantly variable influences of the body circulation.

An important part of our research work in London involved the characterization of the initial lesions of diabetic retinopathy. This work was performed in unique conditions because of the availability of post-mortem study material from diabetic eyes. We were able to study following specific guidelines and in a semi quantitative manner 101 retinas from diabetic patients showing various degrees of retinopathy. Many of these retinas had been injected post mortem with Indian Ink to improve visualization of the retinal vasculature and others had been digested with trypsin in order to obtain preparations of the retinal vessels that could be examined for their cell content and cellular abnormalities.

An important conclusion of this work was the observation that the initial pathological lesions of diabetic retinopathy, when seen isolated, were nonspecific for diabetes and represented a relatively uniform retinal vascular response to disease. There are, initially, minimal cellular alterations in the retinal vessels, which are focal and localized preferentially to the posterior pole of the retina, these alterations being more visible in the venous side of the retinal circulation together with the first microaneurysms. The endothelial cells show signs of swelling or cell proliferation, whereas the pericytes present signs of early degeneration. The cell proliferation predominates in the venous side, whereas the degenerative cellular changes are located preferentially in the arterial side. Associated with these degenerative cell changes there is progressive vascular closure. Associated with endothelial proliferation there is an increasing number of microaneurysms (Cunha-Vaz, 1967 and 1972).

The retinal endothelial cells and pericytes appear to be involved from the earlier stages of the disease even before there are any ophthalmoscopic alterations. Which of these cells is affected first? Are these cells of the retinal vessels affected because there are even earlier changes in the retinal neurons and glia?

Our studies were not conclusive and this has remained a controversial issue. After one year in Lisbon and two in Angola I returned to the University of Coimbra in 1971. There were at the time no conditions to continue our

research work, but the same questions remained to be answered and I wanted very much to help solving them. In this time interval there were a few publications but no major contributions to the field. By then Carolina Mota and Jose Rui Faria de Abreu joined me and a small research group was assembled in the Department of Ophthalmology of the University of Coimbra.

The Centre of Ophthalmology of the University of Coimbra was initiating its first steps.

Fluorescein angiography had just been introduced into ophthalmological practice and was followed with great interest by everyone. This method which consists in the photographic recording of the retinal vascular tree after intravenous injection of fluorescein and using appropriate filters, showed that one of the most frequent alterations occurring in retinal vascular disease was an alteration of the blood-retinal barrier. The concept of blood-retinal barrier was essential for the interpretation of fluorescein angiography. Our experimental observations were confirmed in daily clinical practice and the role of the blood-retinal barrier in retinal disease became widely accepted.

Fluorescein angiography identified localized alterations of the blood-retinal barrier, showed their frequency, how they appeared in the earlier stages of retinal vascular disease like diabetic retinopathy, but these alterations were qualitative and it was difficult to compare and follow their progression or improvement.

We were then able to develop and initiate, in Coimbra, a new method, vitreous fluorophotometry, to quantify the alterations of the blood-retinal barrier and applied it to study diabetic retinopathy (Cunha-Vaz et al, 1975a). This publication in the British Journal of Ophthalmology was listed in 23rd place in the top 100 most cited classics in Ophthalmology and it is the most cited from European authors (Ohba et al, 2007).

We saw in the literature a description of a relatively simple instrumentation based on a slit lamp developed to measure fluorescein concentration in the anterior chamber. I managed to modify it with the limited means at our disposition in Coimbra and to perform for the first time, clinically, measurements of fluorescein in the boundary vitreous-retina and to register concentration gradients of fluorescein in the vitreous, next to the retina, following an intravenous administration of fluorescein. The first results

were extremely promissing, because it was possible to show an alteration of the blood-retinal barrier in the early stages of diabetic retinopathy and in some cases even before there were any fundus alterations detectable either by fundus examination or fluorescein angiography. Furthermore, vitreous fluorophotometry was able to quantify this alteration and to allow its follow-up with the evolution of the retinopathy (Cunha-Vaz et al, 1978a and 1979).

It is becoming more and more apparent, from the data available from a variety of large longitudinal studies and from clinical experience, that the evolution and progression of diabetic retinopathy vary, not only according the type of diabetes involved, but showing differences among different patients even when belonging to the same type of diabetes and furthermore, that the retinopathy does not necessarily progresses in every patient to the development of sight-threatening complications, center-involved macular edema or proliferative diabetic retinopathy.

Diabetic retinopathy has been characterized on clinical examination using ophthalmoscopy as disease of the microvasculature being identified by the presence of microaneurysms, microhemorrhages and exudative changes (hard exudates). However, the recent identification of different phenotypes of progression by our research group suggests that diabetic retinopathy is indeed a spectrum of retinal responses to the hyperglycemic insult of diabetes resulting from different initial alterations in the retinal neurovascular unit.

Our more recent studies suggest that in diabetic retinal disease we are dealing with a complex systemic disease that is affecting in each patient predominantly different components of the retinal neurovascular unit. It is as if there are different subtypes of retinal disease caused by diabetes, with different clinical patterns of disease progression depending on the cell tissue predominately affected by the systemic disease.

In the eyes of different patients different disease mechanisms appear to predominate, showing different risks of progression to sight-threatening complications.

A new concept of diabetic retinopathy emerges from our most recent studies here reported. Diabetes targets the entire retinal neurovascular unit. In different patients, possibly by genetic predisposition, different components of the neurovascular unit are more affected, leading to different patterns of disease progression and different levels of risk for the development of sight-

threatening complications. It is possible to envision now an individualized approach to the management of diabetic retinopathy. A prospective health care plan to prevent and manage diabetic retinpathy may now be considered and planned. This exercise will certainly open novel perspectives for more efficient health care.

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Chapter 1
Impact

1.1. The diabetes epidemic

The number of persons with diabetes worldwide is predicted to grow to 429 million by 2030, due to the rising frequency of obesity, increasing life span, and improved detection of the disease (King et al, 1998).

In India, an estimated 32 million persons had diabetes in 2000 and roughly 79 million will be affected by 2030 (Rema et al, 2005). If the prevalence of complications remains unchanged, approximately 0.7 million Indians will have proliferative diabetic retinopathy and 1.8 million will have clinically significant macular edema. Improved delivery of healthcare reduced the incidence of vision impairment in whites in developed countries (e.g., Denmark, Sweden and United States) but it remains uncertain whether the life style changes that are associated with urbanization in India and other undeveloped countries will result in uncontrolled glycemia, blood pressure and lipid locals and a higher frequency of severe diabetic retinopathy in persons with type 2 diabetes.

The Diabetes Control and Complications Trial (DCCT) showed that intensive metabolic control reduces the incidence and progression of diabetic retinopathy. However, despite advances in diabetes care, complications continue to develop for various reasons. More proliferative diabetic retinopathy and other complications develop after 30 years in up to 20% of persons with diabetes who have been treated with intensive metabolic control (Antonetti et al, 2012) and ideal metabolic control is difficult to achieve because of the increased risk of hypoglycemia and the nonphysiologic route of insulin administration. It must be kept in mind that only 17% of persons in DCCT who were followed in the Epidemiology of Diabetes Interventions and Complications Study had glycated hemoglobin levels less than 7% at their last visit.

In less developed countries, the resources needed to implement good diabetes control are generally unavailable. Therefore, greater emphasis must be placed on preventing complications, which will require both a better understanding of the mechanisms by which diabetes affects the retina and improved means of detecting retinopathy.

The global prevalence of diabetes among adults aged $\leq$ 0 years was estimated to be 171 million (2.8% of the world population) in 2000 and is

expected to rise to 366 million (4.4% of the estimated world population) by the year of 2030 due to: population growth, aging, urbanization, and increasing of obesity and physical inactivity (Wild et al, 2004).

The incidence of diabetic retinopathy increases with the duration of diabetes, and after 20 years, nearly all patients with type 1 diabetes and > 60% of those with type 2 diabetes will develop diabetic retinopathy (Fong et al, 2004).

In the 14-year follow-up of the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), the incidence of diabetic retinopathy was 95.9% in patients with diabetes type 1 and was slightly higher in males than in females (97.7% vs. 94.0%) (Klein et al, 1998).

In persons diagnosed with diabetes before age 30 years, the prevalence of proliferative diabetic retinopathy is around 25% after 15 years and 55% after 20years. In those diagnosed after 30 years age, the proliferative diabetic retinopathy prevalence is 20% after 20 years (Bhavsar, 2002).

There are a multitude of studies that have examined the prevalence of diabetic retinopathy and diabetic macular edema among patients with diabetes. The prevalence of diabetic retinopathy varies widely among these studies, from as low as 10% and up to 41% (Matsuhashi et al 2001; Varma et al 2004; Massin et al, 2008). However, it is difficult to make comparisons between the studies as there are differences in the dates of data collection, patient ages, duration and type of diabetes.

The prevalence of diabetic macular edema is somewhat less variable, at 3-13%. In the study by (Varma et al, 2004), diabetic macular edema was observed in 10.4% and clinically significant macular edema in 6.2%. A further study, in the United States, has reported clinically significant macular edema rates of 5.9% and 7.5% in patients with diabetes (type 1 or 2) diagnosed at either <30 or30 years, respectively (Hirai et al, 2008). The 14-year incidence of diabetic macular edema in the WESDR was 26.1% (29.7% in males and 22.6% in females), and of clinically significant macular edema 17.0% (21.2% in males and 12.8% in females) (Klein et al, 1998).

Looking at the population as a whole, the United States National Eye Institute has estimated that 4.1 million United States adults (aged 40 years) have diabetic retinopathy (3.4%) and 0.9 million have sight-threatening

diabetic retinopathy (0.8%), using pooled data from 8 population-based studies (Kempen et al, 2004). A national health interview survey of non-institutionalized United States adults 1(8 years) has estimated a lifetime prevalence of diabetic retinopathy of 0.7% (1.3 million) (Ryskulova et al, 2008). Decision Resources, Inc. have estimated there to be 3.2 million cases of diabetic retinopathy in the United States; 2.6 million in Europe and 1.5 million in Japan (Table 1.1). Only around 2/3 of these cases are diagnosed, and <15% treated, depending on the region.

Table 1.1. Diabetic retinopathy patient population, percentage diagnosed and percentage treated in the United States, Europe and Japan, 2006-2016 (Decision Resources 2008) ©

	DR patient population		
	2006	**2011**	**2016**
Major-market total prevalence cases	7,324,696	7,324,696	7,324,696
United States			
Cases	3,190,603	3,813,269	4,429,462
Diagnosed (%)	65.0	70.0	75.0
Drug treated (%)	15.0	40.0	45.0
Europe			
Cases	2,643,552	2,789,058	2,936,861
Diagnosed (%)	60.3	66.9	70.9
Drug treated (%)	10.4	34.2	39.2
Japan			
Cases	1,490,541	1,569,412	1,607,799
Diagnosed (%)	60.0	65.0	70.0
Drug treated (%)	0.0	0.0	35.0

In the 1990s, data from DCCT, United Kingdom Prospective Diabetes Study (UKPDS) and WESDR conclusively demonstrated that intensive glycemic and blood pressure control prevents and slows the progression of diabetic retinopathy complications (DCCT Research Group, 1993; Klein et al, 1998; UK Prospective Diabetes Study Group, 1998a/b). A 10-year follow up of the UKPDS showed that intensive blood glucose control

has a lasting beneficial effect (Holman et al, 2008). This information has had a dramatic impact on practitioners' thinking about control of hyperglycemia and hypertension, and also appears to have reduced the prevalence of diabetic retinopathy. Although the incidence of diabetes is increasing, recent data (Cugati et al, 2006) indicate that incidence rates for any new diabetic retinopathy and for the incidence of vision-threatening stages are lower than those recorded 15-20 years earlier (Mitchell, 1985; Klein et al, 1998), although there were differences in diabetes duration in these studies. In a recent Spanish study (Romero-Aroca et al, 2009), the prevalence of diabetic retinopathy in type 2 diabetic patients decreased from 39.4% in 1993 to 27.5% in 2006; although a decrease was not seen among type 1 diabetic patients (35.5% in 1993 and 36.6% in 2006). In this study, diabetes duration and patient ages were well matched between the older and newer data.

Available forecasts suggest that the number of people with diabetic retinopathy and vision-threatening diabetic retinopathy complications will almost triple during the next 45 years. The number of people with diabetic retinopathy is expected to increase from 5.5 million in 2005 to 16.0 million in 2050, and the number with vision-threatening diabetic retinopathy complications is expected to increase from 1.2 million in 2005 to 3.4 million in 2050. These projections indicate an even larger growth in the number of blacks and Hispanics with diabetic retinopathy and vision-threatening diabetic retinopathy complications, especially among those 65 years or older. It is estimated that 5.9 million whites, 1.0 million blacks, and 2.9 million Hispanics 65 years or older will have diabetic retinopathy in 2050. The projected increases for vision-threatening diabetic retinopathy complications are considerably smaller but also show expected increases to 1.0 million for whites, 0.4 million for blacks, and 0.5 million for Hispanics 65 years or older by 2050.

Projections of eye disease among people with diabetes are based on unchanging prevalences based on data from published studies. Thus, changes in the numbers of diabetic people with eye disease are expected to follow changes in the numbers of people with diabetes. Burden of diabetic macular edema is naturally expected to increase due to predicted rise in diabetes prevalence (Chen et al, 2010).

1.2. The socioeconomics of diabetic retinopathy. Economic and societal burden of diabetic eye disease.

Diabetes affects 5% of the world's population and its prevalence doubles every generation. The International Diabetes Federation estimates that in 2005 approximately 333 million people in the world aged 20 to 79 had diabetes. In the United Kingdom, with a population over 60 million, more than two million people have been diagnosed with diabetes and estimated 750.000 people have undiagnosed diabetes. In this population, more than 250.000 have type 1 disease, while 1.8 million have type 2 diabetes, with the latter group making up about 90% of patients with diabetes (Diabetes UK, 2004).

In Portugal, with a population of 10,144.940, there are 1,059.642 patients with diagnosed diabetes (Pina, 2009; PREVADIAB 2009; Correia et al, 2010) and an estimated 105.000 with some form of retinopathy (Falcão et al, 2008), i.e., approximately 10%. The occurrence of clinically significant diabetic macular edema, one of the vision-threatening complications of diabetic retinopathy has been calculated to be in the order of 3.5% of all diagnosed diabetic patients.

Access to good advice and effective health care is a priority for people who want to reduce risks of diabetic complications, including diabetic retinopathy and the threat to vision. Medical care aims to help to reduce blood sugar levels and blood pressure and to detect and treat complications at an early stage.

Diabetes has a high cost for individuals and the state. For individuals, there are both health and pharmaceutical costs, with the latter comprising the cost of purchasing health care and the loss of earnings. Worldwide it is estimated that the spending on diabetes mellitus and its complications totaled $232 billion in 2007, with more than 50% of this spent in the United States and 25% in Europe.

Current management strategies emphasize screening and risk factor reduction. The International Diabetes Federation Guidelines state that, at a minimum, all people with diabetes should have annual direct fundoscopy or fundus photography and an examination of visual acuity by a trained provider.

Recent data for 2008 state that the economic costs of partial sight and blindness on the United Kingdom total &22 billion, with direct health care costs amounting to £2.14 billion. The research estimated that there were a total of 1.8 million people with partial sight and blindness in the IK adult population in 2008, with 3.5% from diabetic retinopathy (Royal National Institute of Blind People. http/www.mib.org.uk). Early detection and treatment of diabetic retinopathy could potentially avoid or reduce these costs.

In the United States, screening and treatment of eye diseases in patients with diabetes mellitus costs $3190 per quality-adjusted life-year (QALY) saved. This cost is a weighed average (based on prevalence of different subtypes diabetic patients) of the cost-effectiveness of detecting and treating diabetic eye disease in those with insulin dependent diabetes mellitus ($1996 per QALY), those with noninsulin dependent diabetes mellitus who use insulin for glycemic control ($2933 per QALY), and those with noninsulin diabetes mellitus who do not use insulin for glycemic control ($3530 per QALY) (Javitt and Aiello, 1996).

Diabetic retinopathy is a particularly good example of a disease that needs to be addressed on a perspective that includes planning for prospective health care (Snyderman and Williams, 2003).

1.3. Summary

Diabetes is a major health problem which is affecting progressively more people all over the world as a result of the progressive aging of the population and spread of obesity resulting from increased access to sugary foods worldwide.

Diabetic retinopathy is the most frequent and most dreaded complication of diabetes, and, therefore, has tremendous impact on society. This is even more relevant when it is realized that diabetic retinopathy, now the most frequent cause of blindness in working age adults in the western world will progressively involve similarly the entire world as the economic conditions improve in poorer parts of the world.

Diabetic macular edema is the most frequent complication of diabetic retinopathy and the most common cause of vision loss due to diabetes. The

14-year incidence of clinically significant macular edema has been reported as 17%. Identifying the eyes/patients at risk to develop clinically significant macular edema and visual loss and understanding its causes and development is fundamental for its appropriate treatment and, finally, to avoid vision loss due to diabetes.

Diabetic retinopathy is already recognized as a major cause of blindness, but the health and economic trends when considered globally point to its increased relevance in the near future.

Diabetic retinopathy is, therefore, a major health care challenge that needs to be addressed urgently.

Chapter 2
Clinical presentation of diabetic retinopathy

Diabetic retinopathy is composed of a characteristic group of lesions found in the retina of individuals having diabetes, generally for several years. It has serious significance for the affected eye in that the ocular sequellae may progress to vision loss and blindness.

Medical intervention can decrease some of the risk to vision caused by diabetic retinopathy, i.e., the control of glycemia decreases the risk of incidence and progression of retinopathy, but diabetic retinopathy may still in many cases progress to visual loss in well-controlled patients.

Klein and Klein (2003) stated that though maintaining normal blood glucose levels from the onset would be ideal, any improvement in glycemic control at virtually any stage in the course of retinopathy seems to be associated with a decreased risk of progression. This question is still a matter of controversy and there is much information indicating that good metabolic control is not effective when the retinopathy has progressed to a point of no return. In this situation rigorous control may even worsen the retinopathy (Lauritzen et al, 1983; Canny et al, 1985). It has been stated that the abnormalities that characterize diabetic retinopathy occur in predictable progression with minor variations in the order of their appearance. This is clearly not the case and there is much variation in the rate of progression of the retinopathy between different individuals, even when they have been submitted to similar levels of glycemic control (Ribeiro et al, 2003). The different steps of the Early Treatment Diabetic Retinopathy Study (ETDRS) classification do not necessarily follow in an orderly fashion and it is possible to find different evolutions of the retinopathy, such as: regression from moderate to mild nonproliferative or rapid progression from mild to proliferative retinopathy. Nonproliferative retinopathy is the retinal disease resulting from diabetes mellitus. As it progresses it may, latter, develop two major complications, dependent on different mechanisms of disease progression, macular edema and proliferative retinopathy.

We propose that the involvement of retina in diabetes may, therefore, be divided into:

- Preclinical stage
- Clinical retinopathy (background or nonproliferative diabetic retinopathy)

- ❖ Complications of Diabetic Retinopathy
  - ○ Diabetic macular edema
  - ○ Proliferative retinopathy

This categorization is based on a clinical perspective and takes particularly into account two major routines of ophthalmological practice: ophthalmoscopy and evaluation of visual acuity. The preclinical stage is mainly identified by lack of alterations detected by ophthalmoscopy. The last stage, complications, are directly associated with the occurrence of vision loss (Figure 2.1).

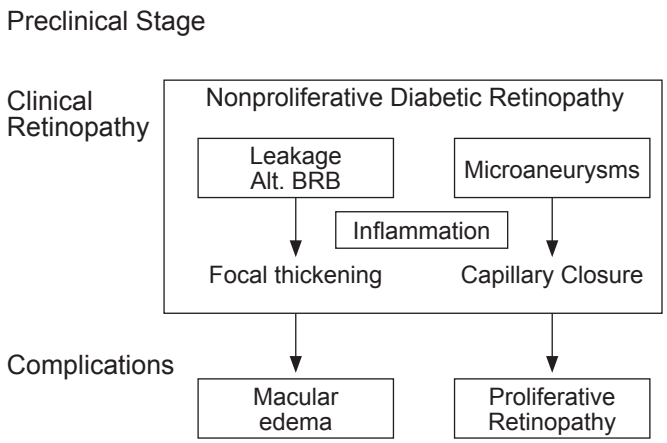

Figure 2.1. Diabetic retinopathy stages.

2.1. Preclinical stage

This stage is characterized by the absence of lesions on ophthalmoscopic examination. However, alterations preceding ophthalmoscopic changes have been demonstrated by a variety of other more sensitive methods of examination and by histological examination.

The initial pathologic findings are characterized by endothelial proliferation in the capillaries and venules and endothelial swelling and degeneration on the smallest arteriolar branchings. Pericyte damage is also widespread and has been

considered to be characteristic of diabetic retinal disease but its distribution is irregular. The apparent predominance of pericytic lesions when compared to the alteration of the endothelial cells may be explained by the different anatomical location of these cells, encased in basement membrane. The damaged pericytes remain in place for longer periods of time compared to the endothelial cells, becoming, therefore their damage more conspicuous (Cunha-Vaz, 1978a).

Fluorescein angiography confirmed the histopathological findings of diabetic retinal disease in a dynamic manner. It was the first technique to document in vivo the abnormal leakage of fluorescein through the walls of the retinal vessels. This leakage of fluorescein was demonstrated by our research group for the first time to be due to breakdown of the blood-retinal barrier (Cunha-Vaz and Maurice, 1967).

We developed vitreous fluorophotometry in order to quantify the alteration of the blood-retinal barrier (Cunha-Vaz et al, 1975b). This sensitive method has confirmed that alteration of the blood-retinal barrier (fluorescein leakage) is one of the earliest changes to occur in the diabetic retina, again suggesting the importance of the retinal endothelial damage.

The alteration of the blood-retinal barrier (fluorescein leakage) was detected by vitreous fluorophotometry, with values higher than 2 SD above the normal in approximately 30% of the eyes that did not show any ophthalmoscopical changes (Cunha-Vaz et al, 1979).

When evaluating neuroglial damage and detecting its occurrence in the preclinical retinopathy stage, it is important to consider changes in retinal function and in particular macular function since diabetes retinal disease affects primarily the central posterior pole of the eye. In this respect it must be kept in mind that assessment of standard visual acuity is not expected to be very rewarding since acuity remains stationary until ~50% of the neuroretinal pathways are affected (Frisen, 1976) and the foveal avascular zone is frequently enlarged in diabetes without any sign of change in visual acuity (Arden et al 1986).

Another problem associated with detection of functional changes in the retina is that diabetic microvascular retinal disease is initially focal in nature, which renders electrophysiological methods that measure the global response of photoreceptors rather unpromising approaches.

However, there are a series of reports indicating that electrophysiological methods are able to detect alterations before the detection of any sign

of vascular damage, at least in about one third of the cases (Shirao and Kawasaki, 1998).

Prospective analysis of focal multifocal ERG have identified functional abnormalities in eyes of diabetic patients in areas without signs of vascular disease (Bearse et al, 2004). Multifocal ERGs may identify, therefore, changes in the neuronal component of the retinal neurovascular unit in the preclinical stages of diabetic retinal disease.

2.2. Clinical diabetic retinopathy

Background or clinical nonproliferative diabetic retinopathy is said to be present when alterations of the fundus are detected by ophthalmoscopy.

On ophthalmoscopic examination, the characteristic features of nonproliferative diabetic retinopathy are: microaneurysms, intrarretinal hemorrhages and hard exudates.

Retinal microaneurysms are usually the first ophthalmoscopic sign of diabetic retinopathy. They are located predominantly within the inner nuclear layer and in the deep retinal capillary network (Ashton, 1958). On ophthalmoscopy, fresh microaneurysms appear as small red dots (Figure 2.2). Microaneurysms may become later yellowish due to increased thickening of basement membrane due to the associated leakage. Finally, they occlude.

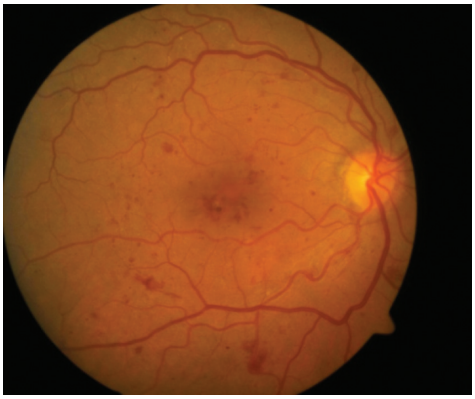

Figure 2.2. Diabetic retinopathy. Fundus photography of the posterior pole showing typical alterations, predominantly microaneurysms and hemorrhages.

Clinical diabetic retinopathy appears to be initially a posterior pole disease, primarily affecting the small retinal vessels. The topographical distribution of its lesions differs markedly from the hematological disorders (e.g. macroglobulinemia and sickle-cell disease) which manifest characteristically peripheral changes, and hypertensive retinopathy, which demonstrates preferential involvement of the arterial side. Diabetic retinopathy, however, can be compared with radiation retinopathy, in which endothelial cell damage predominates.

Fluorescein angiography demonstrates particularly well the microaneurysms as they become hyperfluorescent and leak profusely (Figure 2.3). However, later in the disease process as they become occluded, fluorescein angiography is not able to identify them. Microaneurysm counts on fluorescein angiography are, therefore, only reliable as an indicator of diabetic retinal disease in the initial stages of diabetic retinopathy.

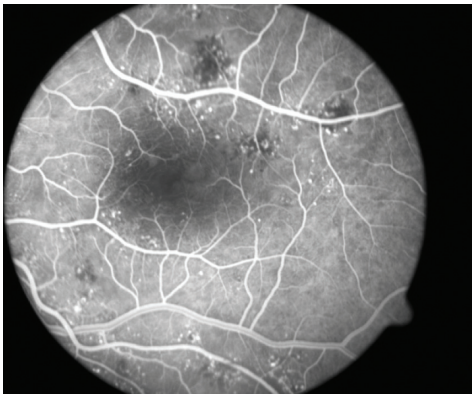

Figure 2.3. Diabetic retinopathy. Fluorescein angiography showing numerous microaneuryms and areas of capillary closure

Intraretinal hemorrhages are another predominant ophthalmoscopical feature of nonproliferative diabetic retinopathy and result from ruptured microaneurysms, capillaries and venules, and are mostly located within the outer plexiform and inner nuclear layers.

In the diabetic eye, retinal intraretinal hemorrhages are characteristically most numerous in the posterior pole. Numerous peripheral hemorrhages should lead one to suspect of another concomitant disease process.

Hard exudates are another ophthalmoscopic feature of background diabetic retinopathy. They are extracellular accumulations of lipoproteins derived from leakage from abnormal vessels. Clinically, these yellowish deposits vary in size from small dots to a confluent arrangement that may even cover most of the posterior pole.

Capillary closure is still another feature of the vascular damage in diabetic patients (Ashton, 1958). It has become recognized that areas of retinal capillary closure, or capillary nonperfusion, as demonstrated by histology and fluorescein angiography, are a frequent feature of diabetic retinopathy. The areas of capillary nonperfusion enlarge as the disease progresses.

Retinal edema is defined as thickening of the macula and is due to accumulation of fluid in the central macular area resulting from fluid leakage due to the alteration of the blood-retinal barrier. Subclinical macular edema is detected frequently in diabetic eyes using optical coherence tomography (OCT).

Severe diabetic retinopathy is an advanced stage of background retinopathy and indicates potential progression to proliferative retinopathy. The presence of soft exudates, venous beading and loops, intraretinal microvascular abnormalities and widespread areas of capillary nonperfusion suggests progression of the retinopathy to a more severe stage (Benson et al, 1988).

Soft exudates or cotton-wool spots are nerve fibre layer infarctions. They are due to obstruction of terminal retinal arterioles. On ophthalmoscopic examination they appear as superficial, whitish, fluffy-appearing patches. A large number of soft exudates (greater than five) often indicates a rapidly progressing retinopathy with high risk of development of neovascularization within 12 to 24 months (Kohner and Dollery, 1975).

Intraretinal microvascular abnormalities are dilated and often telangiectatic capillaries that result from their role as shunts between arterioles and venules in areas of increasing capillary closure. They leak fluorescein although not as profusely as does preretinal neovascularization.

Severe venous dilatation with beading is an important indicator of impending development of proliferative retinopathy because it indicates severe and diffuse hypoxia and a poor visual prognosis. These changes indicate venous thickening that contributes to progressive circulatory stasis and blood sluggishness in the retina.

Widespread and increasing capillary closure is a fundamental feature of the severe retinopathy stage. In severe diabetic retinopathy it is no more the isolated area of capillary nonperfusion that predominates, but large areas of the retina become completely closed to the blood circulation. Retinal non perfusion may be suspected on ophthalmoscopic examination but can only be well demonstrated by fluorescein angiography. This increase in non perfused areas is better seen in the foveal avascular zone which becomes enlarged due to progressive closure of the perifoveal capillaries.

2.3. Complications of diabetic retinopathy

2.3.1. Proliferative diabetic retinopathy

The exact cause of new vessel formation is not known. It is however always secondary to the presence of large areas of capillary nonperfusion, usually associated with nonperfusion of arterioles and venules. It is, therefore, not specific to diabetic retinopathy, as it occurs also in a number of other retinal vascular diseases characterized by marked ischemia, such as sickle cell disease and retinal vein occlusion (Valone et al, 1981; Cunha-Vaz, 1986).

New vessels arise from the optic disk or from the retina (Figures 2.4 and 2.5). Their origin is usually a venule, but they may occasionally arise from arterioles. Retinal new vessels lie initially in the plane of the retina but soon pierce the internal limiting membrane and become preretinal, forming adhesions with the overlying vitreous. While the vitreous is attached to the retina the new vessels are symptomless. However the presence of the new vessels leads to retraction of the vitreous. It is this pulling effect that leads to the progressive complications associated with retinal neovascularization, such as vitreous hemorrhage and progressive visual distortion.

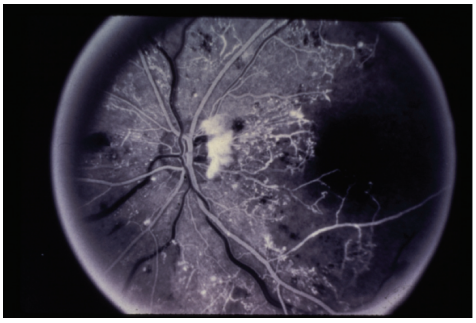

Figure 2.4. Proliferative diabetic retinopathy. Fluorescein angiography showing extensive vascular closure and neovascularization at the optic disk.

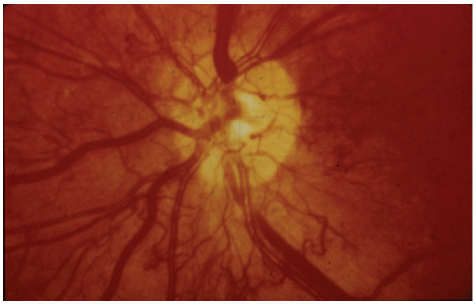

Figure 2.5. Proliferative diabetic retinopathy. Neovascularization in the optic disk.

Characteristically, retinal neovascularization leaks fluorescein profusely demonstrating an abnormal blood-retinal barrier (Figure 2.4). Eyes with new vessels on the disc have always generalized and widespread capillary nonperfusion, again confirming that retinal neovascularization is a direct consequence of generalized ischemia as in other non-diabetic retinal vascular diseases. Proliferative diabetic retinopathy is usually a bilateral disease. Approximately 90% of the persons who present with proliferative diabetic retinopathy have it in both eyes at the time of initial examination (Valone et al, 1981).

Neovascularization elsewhere in the retina, originates from the remaining perfused vessels, almost exclusively venules, next to areas of capillary nonperfusion. Neovascularization elsewhere in diabetes involves

the posterior pole and midperiphery in contrast to other systemic diseases that cause peripheral new vessels elsewhere.

As the vitreous shrinks, possibly due to the abnormal leakage associated with the newvessels, it gradually pulls the neovascular fronds, causing preretinal and intravitreal bleading, a frequent cause of acute vision loss in diabetes. The vitreous alterations associated with fibrovascular tissue contraction and cellular proliferation lead soon or later to localized or generalized detachment. The visual prognosis for eyes with new vessels poor.

Proliferative retinopathy responds well to photocoagulation, but it is essential that it be treated early and adequately, at time when it is symptomless, before tractional complications have developed.

2.3.2. Clinically significant diabetic macular edema

Clinically significant diabetic macular edema is the largest cause of visual acuity reduction in diabetes (Aiello et al, 1998). It may affect central vision from the early stages of retinopathy and is extremely frequent, particularly in older type 2 diabetic patients. Its role in the process of vision loss in diabetic patients and its occurrence in the evolution of the retinopathy is being increasingly recognized.

Diabetic macular edema is frequently, the first alteration occurring in the retina that causes visual loss.

Extracellular edema is directly associated with a situation of open blood-retinal barrier, i.e., results from a breakdown of the inner blood-retinal barrier, one of the earliest alterations occurring in the diabetic retina. The increase in tissue volume is due to an increase in the retinal extracellular space and the breakdown of the blood-retinal barrier is well identified by fluorescein leakage, which can be detected in a clinical environment by fluorescein angiography, vitreous fluorometry or OCT. This type of edema is characterized by its reversibility if addressed in its initial stages. When there is a situation of open blood-retinal barrier, the Starling law governing the movements of fluids applies. With an open blood-retinal barrier, any loss of equilibrium between hydrostatic, oncotic and osmotic pressure gradients across the retinal vessels contribute to further water movements and may result in increased edema formation (Cunha-Vaz and Travassos, 1984).

In this situation, the "force" driving water across the capillary wall is represented by the result of a hydrostatic pressure ΔP and an effective osmotic pressure difference Δπσ. The equation regulating movements across the blood-retinal barrier is, therefore:

$$(\text{driving force}) = Lp\,[\,\Delta P(Pplasma - Ptissue) - \sigma\,\Delta\pi(\pi\,plasma - \pi\Delta Ptissue)]$$

where Lp is the hydraulic conductivity or membrane permeability of the blood-retinal barrier and σ, an osmotic reflection coefficient, Pplasma, the blood pressure, P tissue, the retinal tissue pressure, π plasma, blood omostic pressure and π tissue, the tissue osmotic pressure.

An increase in ΔP, contributing to increased movements of fluids into the retinal tissue and retinal extracellular edema, may due to an increase in Pplasma or a decrease in Ptissue or both. An increase in Pplasma due to increased systemic blood pressure does contribute to retinal edema formation only after loss of autoregulation of retinal blood flow and breakdown of the blood-retinal barrier to as mentioned before.

A decrease in Ptissue is also an important component that has not been given sufficient attention. Any alteration in the cohesion of the retinal tissue due to pathologies, such as localized cell loss, cyst formation and vitreous traction with pulling on the inner limiting membrane of the retina will lead to a decrease in Ptissue thus facilitating fluid accumulation in the retina and an increase in retinal thickness, i.e., retinal edema.

Similarly, a decrease in $\Delta\pi_{+}$ contributes to retinal edema due to protein accumulation in the retina associated with the breakdown of the blood-retinal barrier. Extravasation of proteins and lipoproteins, such as in hard exudates, increase the osmotic pressure in the retinal tissue and draw more water into the retinal extracellular space contributing to edema formation and maintenance. This is the main factor associated with oncotic-driven fluid movements in the retina, as reduction in plasma osmolarity high enough to contribute to edema formation is an extremely rare event.

When there is a breakdown of the blood-retinal barrier the progression of the edema depends directly on the gradient induced by differences between blood pressure and tissue pressure and the oncotic gradient induced by protein accumulation in the retina.

The clinical evaluation of macular edema has been characterized by its difficulty and subjectivity. Direct and indirect ophthalmoscopy may show only an alteration of the focal reflexes. Stereoscopic fundus photography and slit-lamp microscopy have played an important role demonstrating changes in retinal volume in the macular area but they are dependent on the observer experience and the results do not offer a reproducible measurement of the volume change. In a study by Gonzalez et al, 1995, the results from stereofundus photography and slit-lamp examination by experienced observers were compared and an agreement of only 45% was found, supporting the unreliability of the clinical methods to objectively document macular edema.

The ETDRS, made an effort to establish some guidelines to define "clinically significant macular edema" in order to establish an outcome when designing clinical trials to test the efficacy of treatment for diabetic macular edema (Figure 2.6). They paid special attention to the involvement of the center of the macula taking into the consideration the associated visual loss, with its clinical significance.

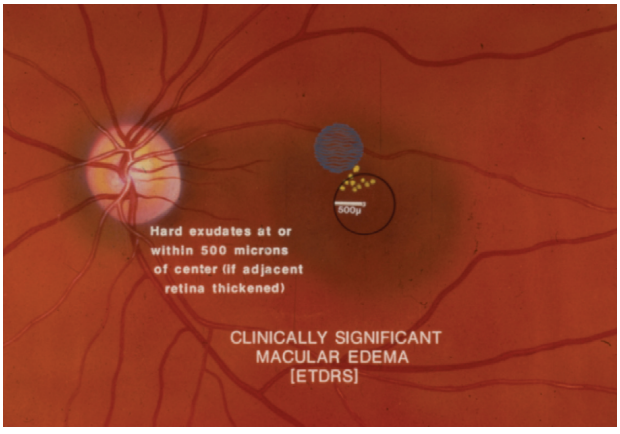

Figure 2.6. ETDRS classification of clinically significant macular edema.

The ETDRS classification of clinically significant macular edema is a follows:

1. Thickening of the retina (as seen either by slitlamp biomicroscopy or by stereofundus photography) at or within 500 microns of the center of the macula.
2. Hard exudates at or within 500 microns of the center of the macula, associated into the thickening of the adjacent retina (but not residual exudates remaining after disappearance of retinal thickening);
3. A zone, or zones, of retinal thickening one disc area or larger size, any part of which is within one disc diameter of the center of the macula.

The problems associated with these guidelines are self-evident, taking into account the subjectivity of the evaluation regarding "abnormal" thickening, the presence of "hard exudates" which are not "residual" and the relative involvement of the central 500 microns circle of the macula.

Recently, two methodologies capable of meaning objectively changes in retinal thickness became available, OCT and the Retinal Thickness Analyzer (Zeimer et al, 1989; Puliafito et al, 1995). The advent and development of OCT changed dramatically our understanding of the incidence, evolution and rates of progression of diabetic macular edema.

We are now able to measure changes in retinal thickness and identify, using non-invasive instrumentation in a clinical setting, macular edema. Diabetic macular edema needs now to be identified regarding its type and distribution, its evolution, its pathophysiology, and degree of involvement of the central macular area, central 500 microns circle of the retina (Figure 2.7).

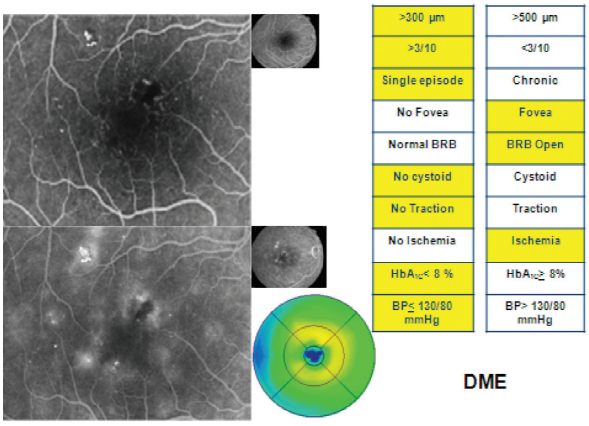

Figure 2.7. An example of characterization of diabetic macular edema. Yellow indicates the alterations identified.

It is, therefore, necessary to consider:

1. The degree of increase in thickness of the retina and its extent (OCT measurement);
2. The best corrected visual acuity;
3. The evolution of the edema and its the response to treatments (OCT information);
4. The degree of involvement of the central fovea (OCT information);
5. The presence or absence of a situation of "open-barrier" (fluorescein angiography or OCT information);
6. The presence or absence of cystoid spaces in the retina demonstrating decreased retinal tissue pressure (OCT information);
7. The presence or absence of vitreous traction (OCT information);
8. The presence of signs of ischemia such as loss of continuity of the capillary net surrounding the foveal avascular zone or the presence of extensive areas of capillary closure (fluorescein angiography information, if necessary);
9. The level of metabolic control;
10. Blood pressure levels.

2.4. Summary

Diabetic retinopathy is identified clinically by its ophthalmoscopic picture. Diabetic retinopathy is considered to be present when a patient with diagnosed diabetes shows microvascular abnormalities in the eye fundus when examined by ophthalmoscopy, fundus photography or slit-lamp examination.

The clinical picture of diabetic retinopathy is, therefore, characterized by the methods most frequently used in daily practice: ophthalmoscopy or slit-lamp examination and visual acuity testing. When alterations on ophthalmoscopy or slit-lamp examination are detected in a diabetic patient, we have clinically visible retinopathy. When some degree of visual acuity loss occurs there is a high degree of likelihood that at least one of the two complications, macular edema or proliferative retinopathy, have developed.

There is also evidence that there is a preclinical retinopathy stage with variable alterations of the neuronal and glial retinal tissue and alterations of

the blood-retinal barrier which are present before changes are detected by ophthalmoscopy. These alterations are detected only in some patients partly due to the still limited sensitivity of the methods used or due to the fact that different patients may have different components of the retinal neurovascular unit affected in the earlier stages of retinal involvement. It is possible that two complementary disease processes, neuropathy and microvascular damage occur as a result of diabetes and have different impact in different patients.

The clinical stage of diabetic retinopathy follows a variable pattern dominated by ophthalmoscopy visible microvascular lesions which occur in the absence of vision loss frequently for many years until the two major retinopathy complications develop: clinically significant macular edema and proliferative retinopathy. The above mentioned late complications of diabetic retinopathy are associated with vision loss and therefore must be well characterized and identified. Treatment is available and timely intervention has been shown to improve the outcomes.

Chapter 3
Early detection of diabetic retinal disease

Early detection of diabetic retinopathy, before the development of fundus lesions that are visible on ophthalmoscopy or slit-lamp examination, and disease progression in the initial stages of the retinopathy, are major research priorities. It is fundamental to be able to follow the initial stages of the retinopathy, when the retinal alterations may still be reversible and may, therefore, be controlled by medical therapy and adequate metabolic control.

The predominant causes of vision loss in diabetic retinopathy are advanced macular edema and proliferative diabetic retinopathy, both complications of the retinopathy. Visual acuity examination is, therefore, not an appropriate method to follow the initial stages of diabetic retinopathy, and must be kept in mind that vision loss due to diabetic retinopathy is a sure indication that the retinopathy has already reached a stage of no return and at present, the only option of the treating doctor is to improve temporarily, stabilize and delay the disease progression.

3.1. Fundus photography

Color fundus photography is the tool most frequently used to document retinal disease and its evolution in diabetic patients. It is used for tracking disease progression and is still accepted as the best screening method for diabetic retinopathy.

Fundus photographs allow to identify microaneurysms in the fundus, the most characteristic initial lesions of diabetic retinopathy. They also show the development of hemorrhages, hard and soft exudates and, finally, show well the major changes occurring in the retinal venules and arterioles as the disease progresses (Klein et al, 1985).

Fundus photographs or fundus digital images must be produced in a consistent manner following well defined protocols, in order to allow comparisons between different examinations performed on different occasions.

Because much of the diabetic clinically significant retinal pathology occurs around the disc and macula, a standard for photographic composition has evolved, with a 30º field that includes the entire macula, the entire optic disc, and the major vascular arcades recorded in one view (Figure 3.1).

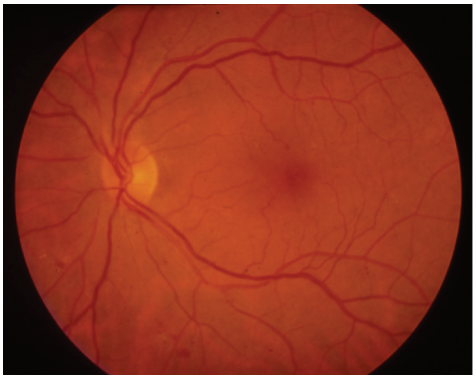

Figure 3.1. Photography of central field 2 central showing entire macula, the entire optic disc, and the major vascular arcades.

Seven standard fields of 30 degrees were proposed for the Arlie House Classification and have been used to classify diabetic retinopathy according to the ETDRS standards.

3.1.1. Grading diabetic retinopathy from color fundus photographs

Fundus photography has been the method of choice to follow diabetic retinopathy, because (1) it is nonivasive, technically easy and well accepted by patients; and (2) its usefulness has been demonstraded in a large-scale randomized clinical trial, the Diabetic Retinopathy Study (DRS), which showed the benefits of photocoagulation in the treatment of proliferative diabetic retinopathy.

Fundus photography was chosen to monitor retinopathy in the Diabetes Control and Complications Trial in the USA and United Kingdom Prospective Diabetes Study Trial. Elaborate fundus photography gradings were developed from the original Airlie House Diabetic Retinopathy Classification to document progression and delineate the natural history of retinal disease, from the earliest visible alterations to more advanced stages, eg, maculopathy and high-risk proliferative diabetic retinopathy.

These gradings however, offer little information on the initial stages of the disease. Microaneurysms appear in the second step of the scale #20. Although they are an important and frequent alteration in the retinal circulation in diabetes,

they are not counted. Furthermore, microaneurysms formation and disappearance is a dynamic process. Microaneurysms need to be counted to assess progression of retinopathy and new microaneurysms should always be added to those previously identified in the same retina (Torrent Solans et al, 2004). It must be realized that the disappearance of a microaneurysm is not a reversible process, but indicates vessel closure and progressive vascular damage.

3.1.2. Monitoring the initial stages of diabetic retinopathy progression. Microaneurysm turnover detected with RetmarkerDR

It is of fundamental importance to monitor the progression of the disease in a specific patient and identify if he/she is a "progressor", i.e., a patient that shows signs of rapid progression. Some eyes/patients need special attention and timely intervention to avoid development of sight-threatening diabetic retinopathy complications, macular edema or proliferative diabetic retinopathy.

The initial alterations that occur in nonproliferative diabetic retinopathy and need to be monitored are: microaneurysms dynamics (their formation and disappearance), and vascular leakage with subsequent edema and hard exudates formation.

Visual function loss occurs characteristically late in diabetic retinopathy , because the eye has a large functional reserve of vision and diabetic retinopathy affects initially the inner layers of the retina away from the photoreceptors. Therefore, structural changes are detected in diabetic retinopathy earlier than functional changes. We have, therefore, to focus on evidence of structural changes if we want to follow progression in the earliest stages of diabetic retinopathy.

One of the best candidates for non-invasive imaging of the eye fundus is clearly fundus digital photography, because retinal cameras are widely available and the data obtained may be supported and analysed by computer-assisted procedures.

To identify progression it is essential to collect sequential series of images and these images must be compared. The need for co-registration of these sequences of images is, therefore, of great relevance. By applying novel image co-registration comparative analysis software it is now possible to perform reliable sequential comparisons of fundus digital photography images (Nunes et al, 2009).

A mandatory step for automatically comparing images is their co-registration, one against the other. This is to say that one of the images needs to be projected to the image space of the other, which acts as a reference image. Following this procedure, both share a common reference being possible to establish a direct pixel-to-pixel correspondence.

In order to achieve the required image co-registration, it is necessary to identify eye fundus natural landmarks, intrinsic fiducial marks, and compute the transformation matrix that, applied to one image, will project it to the image space of the reference image.

Two major steps are incorporated in the above concept. One relates to the identification and classification of the fiducial markers, while the other relates to linking similar fiducial markers between any two images to co-register.

A natural source for fiducial markers is the retinal vascular network, an imprint for each human eye. Vessels characteristics, bifurcations and crossovers, allow establishing possible links between any two images from the same eye. After having found the true links for several fiducial markers, one can compute the respective transformation matrix. These two steps were implemented by a proprietary technique while the vascular network was segmented resorting to contourlets, following an initial approach of using differential geometry (Bernardes et al, 2009).

The RetmarkerDR is a software now available (Critical Health, Portugal) and developed by our research group which is able to automatically detect changes occurring in eye fundus digital images, by comparing successive visits to the reference image, in each eye, based on co-registration and co-localization of the changes (Figures 3.2 and 3.3).

Figure 3.2. This figure illustrates the automatic microaneurysm tracking over time, colour coding each detected microaneurysm as new, old or disappeared (based on proprietary co-registration algorithm).

Figure 3.3. The Retmarker Software automatically calculates microaneurysm formation and disappearance rates. The patient above had a microaneurysm Formation Rate of 5 microaneurysms/year over a 24-month follow-up.

On fundus photography, microaneurysms and small hemorrhages are the initial changes detected in the diabetic retina. They may be counted, and retinal microaneurysm counting has previously been suggested as an appropriate marker of retinopathy progression (Klein et al, 1995a; Csaky et al, 2008).

Retinal microaneurysms are important lesions of diabetic retinopathy and even one or two microaneurysms in an eye should not be regarded as unimportant (Kohner et al, 1999). When examining 1809 patients in the UKPDS cohort that had either no retinopathy or microaneurysms only at study entry they showed that the number of microaneurysms had a high predictive value for worsening retinopathy at 3, 6, 9 and 12 years after entry into the study (Kohner et al, 1999). Similar findings had been presented by Klein et al (1989) who looked at the relationship of retinal microaneurysms to the progression of diabetic retinopathy over a 4-year period. In their study the number of microaneurysms at the baseline examination was positively associated with significant progression of retinopathy. More recently, Sjolie et al (2011) confirmed that microaneurysms counts were predictive of an increased risk of retinopathy progression.

Our studies have shown that it is not the absolute total number of microaneurysms at a certain point in time that may provide the best indication of retinopathy progression, but the rate of microaneurysm turnover in successive visits during a 1 or 2- year period. We have demonstrated that it is possible to use microaneurysm turnover computed from non-invasive color fundus photographs as a biomarker to identify eye/patients at risk of progression for clinically significant macular edema (Nunes et al, 2009). These findings will be presented extensively in chapter 5.

3.2. Fluorescein angiography

Since 1961, when Novotny and Alvis introduced the technique of fluorescein angiography, its routine use has contributed much to our present understanding of diabetic retinal disease.

Sodium fluorescein, which is approximately 80% protein-bound to albumin, is the dye used in fluorescein angiography. Fluorescein, because it is a small molecule that remains unbound in 10-20% of the amount injected, diffuses freely through the choriocapillaries, Bruch's membrane, optic nerve, and sclera. However, its diffusion through the tight junctions of the retinal endothelial cells and of the retinal pigment epithelium which are the inner and outer blood-retinal barriers is minimal. A physiologic inner blood-retinal barrier exists at the level of the retinal vessels due to the "zonula occludens" that unite firmly and tightly neighbouring retinal endothelial cells (Shakib and Cunha-Vaz, 1966). If there is a disruption of the blood-retinal barrier, dye leakage occurs. Similarly, the tight junctions (zonula occludens) between the retinal pigment epithelial cells constitute the outer blood-retinal barrier, which under normal, physiological conditions, does not allow visible leakage of fluorescein from the choroid into the retina.

Understanding the outer and inner retinal vascular barriers is the key to understanding and interpreting a fluorescein angiogram (Cunha-Vaz, 1976).

Another fundamental contribution of fluorescein angiography to our understanding of diabetic retinopathy is the identification of areas of capillary closure or capillary drop-out (Figure 3.4).

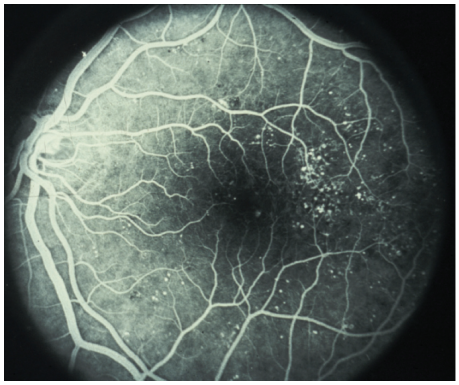

Figure 3.4. Diabetic retinopathy. Fluorescein angiography showing multiple microaneurysms and a few areas of capillary closure.

The normal regular distribution of the capillary network appears interrupted by areas which are not perfused by the dye, identifying well areas outlined by perfused capillaries (Kohner and Henkind, 1970).

Fluorescein angiography quality, however, depends on technique, filters, film, ocular media and patient cooperation. Finally, the information obtained is not quantitative as it depends on all these variables. Fluorescein angiography, because of the need for intravenous injection of fluorescein, is used much less frequently than fundus photography. Although sodium fluorescein is generally safe, and is used in the daily routine of every ophthalmological care centre, severe anaphylactic reactions may occur sporadically (1 in 200.000) – (Yannuzzi et al, 1986).

3.3. Vitreous fluoromety and retinal leakage measurements

The advent of fluorescein angiography confirmed most of what was known about the initial pathological picture of diabetic retinopathy and showed in the initial stages of the disease focal leaks of fluorescein, demonstrating well, in a clinical setting, the existence of focal breakdowns of the blood-retinal barrier.

In 1975, vitreous fluorometry, a clinical quantitative method for the study of the blood-retinal barrier, was introduced by our group (Cunha-Vaz

et al, 1975a), showing that an alteration of the blood-retinal barrier could be detected and measured in diabetic eyes apparently normal fundi. The disturbance of the blood-retinal barrier, as evidenced by vitreous fluorometry, appeared in some patients before microaneurysms or capillary closure could be demonstrated by fluorescein angiography. These results were soon confirmed by Waltman et al (1978a/b).

The development of a quantitative method capable of detecting minimal alterations in the barrier and monitoring their progress has obvious clinical interest.

In vitreous fluorometry, fluorescein, the tracer, is introduced into the blood circulation (first compartment) and from there it crosses the blood-ocular barriers in variable amounts, and penetrate into the intraocular fluids (second compartment). The concentration gradients between the fluorescein present in the blood and the fluorescein that penetrated into the eye namely into the vitreous and aqueous, allowing quantification of the permeability of the boundaries separating the blood from the intraocular fluids, the blood-ocular barriers (Cunha-Vaz, 1985).

3.3.1. Vitreous fluorometry

In the original ocular fluorometer light emitted by a light source is filtered by an excitation that transmits efficiently only wavelengths in the excitation band-width. This light is then focused in the eye by the optics of delivery. Some of the fluorescence emitted in the eye enters the pick up optics which are designed to gather only light originating from the focal plan of the illuminating beam. This intersection of the delivery and pick up optical paths defines a volume inside the eye from which fluorescence is detected by the photodetector. The photodetector outputs a signal that is proportional to the number of photons detected, and this signal, is, in turn, registered by the data processor. The volume in which the fluorophotometric measurement is performed is defined by the intersection of the illuminating beam and the pick up path.

Having realized that we are scanning across the eye with a probing volume of finite axial length, it is important to understand that the vitreous fluorometry recording represents the distribution of the fluorescein in the eye (Figures 3.5 and 3.6).

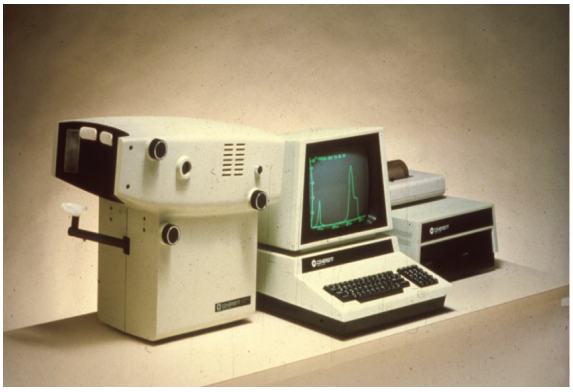

Figure 3.5. Vitreous fluorometer (Flurotron Master).

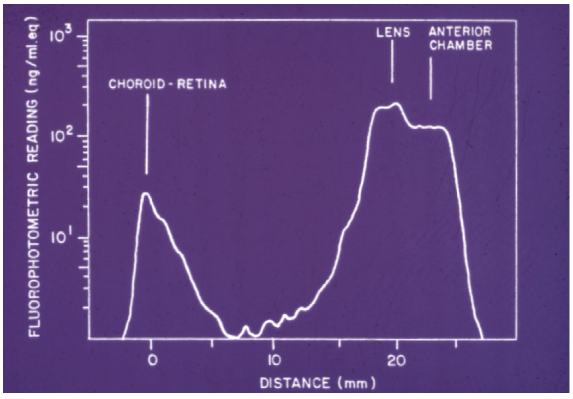

Figure 3.6. Vitreous fluorometry scan.

The inward permeability across the blood-retinal barrier can, therefore, be estimated by calculating the mass of fluorescein that has penetrated posteriorly into the vitreous and the integral over time of the free plasma fluorescein concentration.

3.3.2. Retinal Leakage Analyser

One major limitation of the available commercial instrumentation for vitreous fluorometry (Fluorotron Master Coherent, USA) was associated

with the fact that the permeability of the blood-retinal barrier is measured as an average over the macular area. Accurate mapping of localized changes in the permeability of the blood-retinal barrier would be beneficial for early diagnosis, to explain the natural history of retinal disease, and to predict its effect on visual acuity.

We have since developed a new method of retinal leakage mapping, the Retinal leakage Analyzer, that is capable of measuring localized changes in fluorescein leakage across the blood-retinal barrier while simultaneously imaging the retina. The instrument was based on a prototype Zeiss confocal scanning laser ophthalmoscope that was modified into a confocal scanning laser fluorometer (Lobo et al, 1999). Later on a new version was developed by modifying the commercially available Heidelberg Confocal Scanning Laser Ophthalmoscope (Bernardes et al, 2002).

Retinal and vitreous fluorescence is measured within the first five minutes after the intravenous injection of 14mg/kg of 20% sodium fluorescein and at 30, minutes after the injection. Blood is sampled for plasma fluorescein estimation. The Retinal Leakage Analyzer acquires an entire image of the retina with real three-dimensional information. With use of appropriate software, it is possible to select an arbitrary sized ROI over an image as small as 75mm x 75 μm or the total area, and to build the corresponding fluorescence axial graphic.

Axial graphics of the fluorescence measurements obtained from the vitreous representing a volume of 75μm x 75μm x 2,550μm are converted into retinal leackage maps (RLMaps).

Increased blood-retinal barrier permeability was measured in sites of morphological vascular abnormalities and also in areas where no retinal pathology could be identified.

The method allows localized measurements of blood-retinal barrier permeability discriminating leaking sites of approximately 75μm to 100μm size. This capability offers unique opportunities to examine the focal alteration of the blood-retinal barrier that occurs in the early stages of diabetic retinal disease.

The Retinal Leakage Analyzer, by performing quantitative mapping of retinal fluorescein leakage and simultaneous imaging of the retina, offers a novel approach to examine the role of blood-retinal barrier breakdown in the development and progression of retinal pathology.

3.3.3. Alteration of the blood-retinal barrier in the initial stages of diabetic retinal disease

An alteration of the blood-retinal barrier has been repeatedly reported in experimental diabetes. These studies, initiated by Waltman et al (1978a), showed an alteration of the blood-retinal barrier in rats with streptozotocin-induced diabetes, that was well demonstrated by vitreous fluorometry, soon after induction of chronic hyperglycaemia. Furthermore, this alteration of the blood-retinal barrier was reversed by the administration of insulin and regularization of the glycaemia.

Other studies have confirmed the alteration of the blood-retinal barrier in the rat in streptozotocin-induced diabetes, only one week after the administration of streptozotocin. More recently, our group in Coimbra have demonstrated using confocal microscopy that the breakdown of the blood-retinal barrier occurring in rats one week after onset of streptozotocin-induced diabetes is localized preferentially in the inner blood-retinal barrier (Carmo et al, 1998).

Our clinical studies on the application of vitreous fluorophotometry to diabetes were reported for the first time in 1975 (Cunha-Vaz et al, 1975a). The examination of a series of predominantly adult-onset diabetics with apparently normal fundi revealed the presence of a significant alteration of the blood-retinal barrier. The disturbance of the blood-retinal barrier appeared before microaneurysms or capillary closure could be demonstrated by fundus fluorescein angiography. The fluorescein concentration curves in the vitreous in the diabetic patients followed a typical pattern, the gradient indicating penetration of fluorescein across the blood-retinal barrier.

Our results were confirmed by Waltman et al (1978b), who reported on the vitreous fluorometry examination of a series of juvenile-onset, insulin-dependent diabetics.

Initial studies suggested a direct association between an increase in vitreous fluorometry values and development of the retinal lesions. Higher fluorescein concentrations in the vitreous were observed in eyes showing more pathology. Another aspect of much interest was the observation of a relation between breakdown of the blood-retinal barrier, as shown by vitreous fluorometry, and metabolic control (Cunha-Vaz et al, 1978a/b; Cunha-Vaz et al, 1979).

An European multicentre study involving six different research groups showed that vitreous fluorometry, performed using the Fluorotron Master and following a well-defined protocol is a highly sensitive and reliable method for measuring the permeability of the blood-retinal barrier. An alteration of the blood-retinal barrier appear to be common after development of ophthalmoscopically visible retinopathy and is sometimes present even before the development of clinically visible retinopathy. This breakdown of the blood-retinal barrier increases with duration of the disease and is associated with poor metabolic control (Schaik et al, 1997).

3.4. Optical Coherence Tomography (OCT)

Recently, one methodology capable of measuring objective changes in retinal thickness and give morphological and topographic surface images of the retina became available, OCT, changing dramatically the landscape of diabetic macular edema diagnostic and follow-up.

OCT is a non-invasive and noncontact diagnostic method, well tolerated by patients, that provides important information about the retina. OCT imaging is analogous to B-scan ultrasound imaging, except that it uses infrared light reflections instead of ultrasound. It produces reliable, reproducible and objective cross-sectional images of the retinal structures and the vitreoretinal interface and allows quantitative measurements of retinal thickness.

OCT brought new insights about morphological changes of the retina in diabetic retinopathy and diabetic macular edema. It showed that macular edema may assume different morphologic patterns (Yamamoto et al, 2001; Kim et al, 2006). In addition, a quantitative characterization of macular edema became feasible, as determined by measurements of retinal thickness and volume. OCT has been demonstrated to be more sensitive than slit-lamp biomicroscopy in detecting small changes in retinal thickness (Hee et al, 1995; Yang et al, 2001; Massin et al, 2006; Lang, 2007) and is clearly less subjective. In cases of diabetic macular edema, OCT scans may demonstrate diffuse thickening of the neurosensory retina and loss of the foveal depression; cystic retinal changes, manifest as areas of low intraretinal reflectivity and serous retinal detachment, alone or combined.

Cross-sectional images resemble closely the histological appearance of the retina (Margolis and Kaiser, 2008) (Figures 3.7 and 3.8). The top of the image correspond to the vitreous cavity, which is optically silent, in a normal patient, or may show the posterior hyaloidal face, if there is a posterior vitreous detachment (Cunha-Vaz and Coscas, 2010). Central foveal depression is visible in normal eyes. The anterior boundary of the retina corresponds to the internal limiting membrane, at the vitreoretinal interface, hyperreflective and well-defined, because of the contrast between the nonreflective vitreous and the backscattering of the retina.

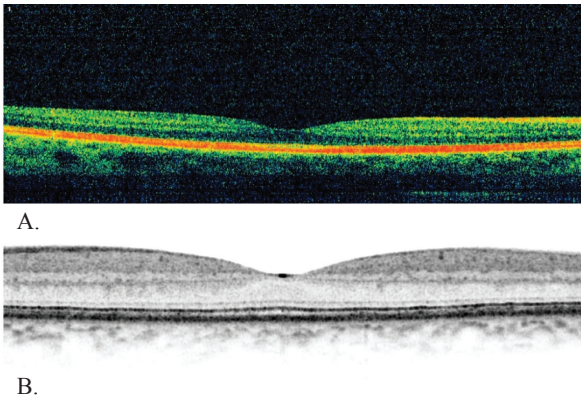

Figure 3.7. SD-OCT- normal cross-sectional macular image; A. False colour; B. Gray scale.

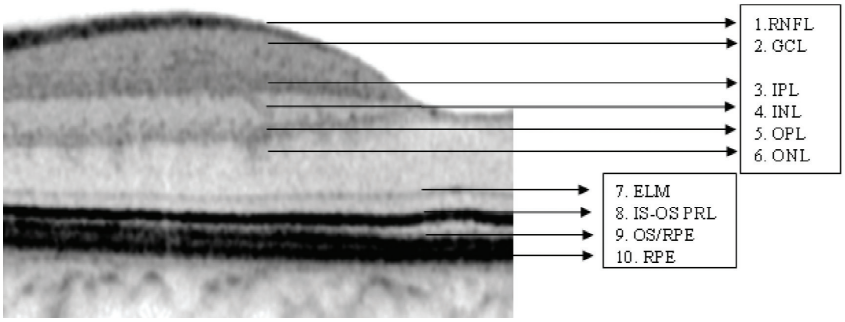

Figure 3.8. Normal cross-sectional macular image (gray scale) and anatomic correlation. 1. RNFL: retinal nerve fiber layer; 2.GCL: ganglion cell layer; 3. IPL: inner plexiform layer; 4. INL: inner nuclear layer; 5. OPL: outer plexiform layer; 6. ONL: outer nuclear layer; 7. ELM: external limiting membrane; 8. IS-OS PRL: inner segment – outer segment photoreceptor layer; 9. OS/RPE junction: outer segment / retinal pigment epithelium; 10. RPE: retinal pigment epithelium

The internal structure of the retina has heterogeneous reflections and distinct bands, and an anatomic correlation with the layers of the human retina has been proposed (Drexler, 2007) (Figure 3.8). Retinal nerve fiber layer is aligned horizontally, demonstrating higher tissue signal strength and appears thicker closer to the optic nerve as expected. Axially aligned cellular layers - ganglion cell layer, inner nuclear layer and outer nuclear layer - have lower tissue signal compared with horizontally aligned layers, internal limiting membrane, retinal nerve fiber layer and plexiform layers, which have higher tissue signal. Typically, nuclear layers appear hyporeflective, while plexiform layers (inner plexiform layer and outer plexiform layer and axonal layers are relatively hyper-reflective.

In the outer retina, different hyperreflective structures (bands) are visualized. TD Stratus OCT image the outer retinal layers as two hyperreflective bands, the photoreceptor's outer segments (inner) and the retinal pigment epithelium / choriocapillaris complex (outer). On the other hand, SD-OCT scans of the outer retina allows the visualization of more bands than the TD-OCT. With this high resolution technology, 3 or 4 distinct strongly reflective bands are apparent, although their histological correlation remains a matter of discussion. According to Pircher et al (2006), the first (inner) band may correspond to the external limiting membrane, the second to the interface of the inner and outer segments of the photoreceptor laye, the third band may represent the outer segment - retinal pigment epithelium junction and the fourth (outer) is assumed to represent the retinal pigment epithelium (Figure 3.8). The analysis of structural changes in the outer retinal layers, particularly affecting photoreceptors and their interface, is now possible, using SD-OCT (Coscas, 2009).

Since the commercialization of OCT systems, several types of software to quantify macular thickness became available. Mean macular retinal thickness is displayed as a 2-dimension false color-coded map, where bright colors (for example red and white) represent thick areas and dark colors (for example, blue and black) represent thin areas, and as a numerical map, for 9 ETDRS-type areas (Figure 3.9). Average retinal thickness is calculated automatically for each of the nine quadrants. Because data point density is greater centrally than peripherally, interpolated thickness measurements of regions farther from the fovea are determined from fewer measurements and thus may be less accurate than those in central regions.

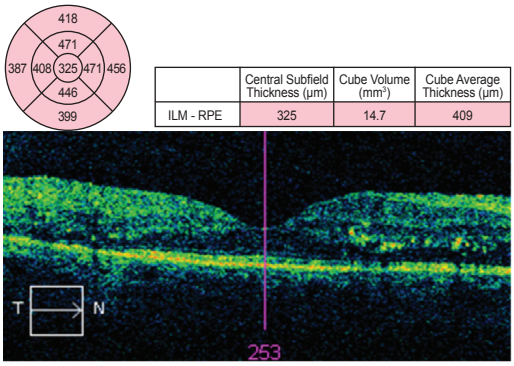

Figure 3.9. Diabetic retina showing increased thickness identifying the presence of retinal edema (OCT examination).

OCT scans should be analyzed in two steps, regarding qualitative and quantitative information. Qualitative assessment relies on the characterization of the reflectivity profiles and morphological properties of the intra-ocular structures, whether normal or abnormal, visualized in the scans; quantitative evaluation refers to the possibility to measure these structures. After this analysis, the data should be integrated and correlated with clinical data (Lang, 2007).

Nowadays, OCT is increasingly used in the early detection of subclinical macular edema. Cross-sectional images of the retinal structures and thickness maps provide an objective and reproducible baseline characterization of the retinal disease. OCT imaging seems to be more sensitive than slit-lamp biomicroscopy to detect small changes in retinal thickness (Hee et al, 1995; Yang et al, 2001; Lang, 2007) and to visualize infraclinical foveolar detachments (Massin et al, 2006). OCT scans also allow an accurate evaluation of disease progression, over time, and particularly after treatment.

OCT images of diabetic macular edema depict the presence of low intraretinal reflectivity, due to fluid accumulation in the extracellular space of the retina. The process begins as a diffuse retinal thickening with sponge-like appearance of the retinal layers, showing increase in the extracellular spaces advancing to the typical image of cystoid spaces (Otani et al, 1999; Alkuraya et al, 2005). The hyporeflective cystoid-like cavities within the neurosensory retina are separated by highly reflective septae bridging retinal layers (Figure 3.10). They can progress to large and confluent hyporeflective

(cystoid) spaces, involving the full thickness of the retina, with atrophy of the surrounding layers. Therefore, in newly developed central macular edema, cystoid spaces are primarily located in the plexiform layer, while in well established central macular edema, cystoid spaces become confluent and large cystoid cavities appear.

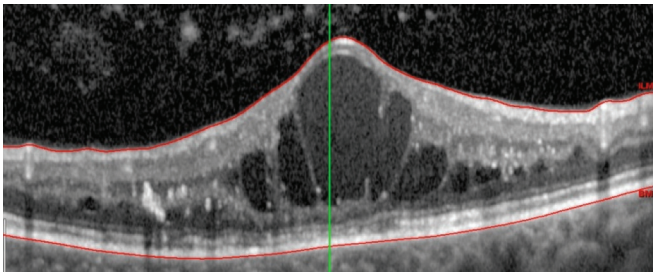

Figure 3.10. Cystoid Macular Edema.

Another advantage of the OCT is the possibility to analyze the vitreomacular interface. It is possible to determine the status of the posterior hyaloids when it is only slightly detached from the macular surface (Gaucher et al, 2005). The concept of vitreoretinal traction is now considered of major relevance in the OCT classification of diabetic macular edema (Panozzo et al, 2003; Kang et al, 2004).

More recently, our group has identified the possibility of using OCT, a non-invasive method, to examine blood-retinal barrier breakdown. In a study performed by our research group OCT images allowed comparisons between different areas of the same retinas that were simultaneously identified to be leaky and non-leaky, i.e., having respectively localized alterations of the blood-retinal barrier and a normal blood-retinal barrier (Bernardes et al, 2011). Areas with intact blood-retinal barrier and areas from localized disrupted blood-retinal barrier were therefore identified in the same retinas and the OCT images compared (Figure 3.11). Clearly local statistical differences in the OCT data were detected between the areas of normal blood-retinal barrier and abnormal blood-retinal barrier (Figure 3.12). These findings showed that differences with regions of the retina receiving the same classification, either intact or disrupted blood-retinal barrier, differed statistically from the

differences between regions receiving classifications of intact or disrupted blood-retinal barrier status. Thus, different optical properties of the human retina were found in relation to the changes in blood-retinal barrier function.

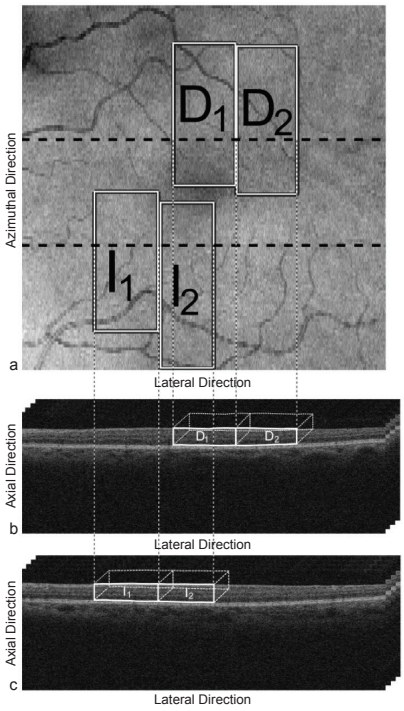

Figure 3.11. OCT fundus reference with delimited areas (intact blood-retinal barrier – I – and disrupted blood-retinal barrier – D). (a). No notorious difference can be seen in the fundus reference and B- scans passing through disrupted (b) and intact areas (c).

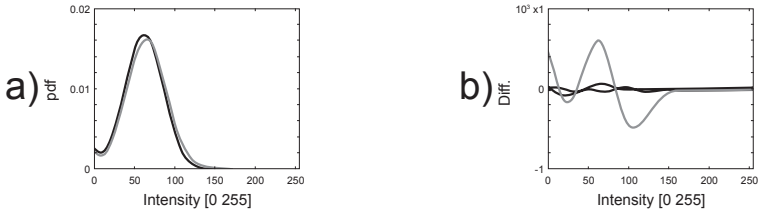

Figure 3.12. Eye with diabetic retinopathy (DR 2; a, b). PDF plot (a) for 4 areas, 2 from intact (black lines) and 2 from disrupted blood-retinal barrier regions (gray lines) (plot of similar regions overlap, hence only 2 lines are noticeable). PDF differences are shown in plot B with black lines representing differences between similar blood-retinal barrier regions (either intact or disrupted blood-retinal barrier) and the gray line representing the differences between dissimilar blood-retinal barrier regions (intact/disrupted blood-retinal barrier regions).

Detection of localized changes in extracellular space of the retina offers a non-invasive method that may be used as a technique to quantify breakdown of the blood-retinal barrier without the use of intravenous injection of fluorescein. The volume of the extracellular compartments of the neurosensory retina in the normal eye is regulated by the retinal capillary endothelial cell tight junctions and retinal pigment epithelium cell tight junctions (i.e., the inner and outer blood-retinal barrier), as well as by the transport function of both endothelial and retinal pigment epithelium cells. This fluid accumulates in the extracellular space of the retina when there is loss of functional integrity of the blood-retinal barrier.

In summary, OCT allows early detectin and objective follow-up of diabetic macular edema and may become a non-invasive method to evaluate the alteration of the blood-retinal barrier. It allows a clear identification of the intraretinal fluid distribution and the presence or absence of vitreous traction. It is an excellent method to document these findings. Furthermore, OCT allows a quantitative diagnosis of macular edema, as it is used to obtain numerical representation of the retinal thickness.

3.5. Electrophysiological testing

Electrophysiological changes in diabetes may have a microvascular origin (Scholl and Zrenner, 2000), or be due to retinal cell dysfunction (Tzekov and Arden, 1999). Despite many psychophysical and electrophysiological methods that have been used to document diabetic retinopathy a consensus on the ideal tool to detect retinal dysfunction in the initial stages of diabetic retinopathy is still missing.

In this respect, it must be kept in mind that assessment of standard visual acuity is not expected to be very rewarding since visual acuity remains stationary until ~50% of the neuroretinal pathways are affected (Frisèn, 1976), and the foveal avascular zone is frequently enlarged in diabetic patients without any sign of change in visual acuity (Arend et al, 1995). This suggests that psychophysical techniques should aim to assess separately distinct functional channels (Bresnick et al, 1985; Green et al, 1985). Evidence for predominant early involvement of the parvocellular pathway suggests that the physiology of the perifoveal area should be under scrutiny

in future studies, since changes in the microvasculature in this region may be predictive of visual outcome.

Diabetic retinopathy is initially focal in its nature, which renders standard electrophysiological methods that measure the global response of retinal photoreceptors, such as the flash electroretinogram (ERG), rather unpromising approaches. For these reason conflicting reports have emerged in the literature, some describing significant differences (Juen and Kieselbach, 1990; Holopigian et al, 1992) and others not (Jenkins and Cartwright, 1990). The reduction of the b wave of the conventional ERG is often reported only for advanced cases, and more sensitive results can only be obtained with the calculation of intensity-response functions, which are of limited clinical applicability.

There is an ongoing search of measures capable of detecting earlier dysfunction. Recent candidates concerning parametric evaluation have included amplitude and delay of oscillatory potentials, pattern-ERG, the scotopic threshold response, and more recently the multifocal ERG.

In summary, despite the mentioned difficulties, it has been shown that even standard ERG methods are often capable of detecting electrophysiological damage well before the detection of any sign of vascular damage.

3.5.1. Oscillatory potentials (OPs)

These are high frequency retinal electrophysiological responses (100-160 Hz) which are superimposed on the ascending limb of the b-wave (Yonemura, 1962; Wachtmeister and Dowling, 1978), and seem to be changed by early retinal dysfunction in diabetes. They are a signature of the involvement of inner retinal layers since they are thought to originate in the inner plexiform layer, namely from inhibitory circuits connecting amacrine and ganglion cells.

Oscillatory potentials are usually taken as good indicators of the extent of retinal ischemia and may be reduced at all stages of diabetic retinopathy, with a good correlation with severity, especially during proliferative stages.

Bresnick and colleagues (1984, 1985, 1987) confirmed and extended these results, by showing that oscillatory potentials amplitude predicts progression (independently from predictors taken from fundus photography

and fluorescein angiography) of eyes with nonproliferative diabetic retinopathy or mild proliferative diabetic retinopathy to severe proliferative diabetic retinopathy. Eyes with abnormal oscillatory potentials amplitudes had a steady rate of progression to severe proliferation (28% after one year, and 52% after two years). Eyes with normal oscillatory potentials had a much lower rate of progression (0 and 7%, respectively).

Peak latencies of oscillatory potentials may represent a better marker of damage than amplitudes, since they are changed earlier. This has been well documented in rats with streptozotocin-induced diabetes, with changes of the latency of the second peak of oscillatory potentials occurring as early as two weeks after induction, which suggests that direct neuropathy may occur, concomitantly to the angiopathy (Shirao and Kawasaki, 1998).

3.5.2. Multifocal electroretinography

Multifocal electroretinography (mfERG) (Sutter and Tran, 1992) provides functional topographic detail that overcomes the disadvantages of conventional electrophysiology. Objective measurement of retinal dysfunction simultaneously at multiple locations, by means of this technique is now being increasingly used in diabetes research.

Electrophysiological local amplitude changes and delays have been found in the retinas of diabetic patients with or without retinopathy, both in regional and local averages (Palmowski et al, 1997; Fortune et al, 1999; Scholl and Zrenner, 2000; Bearse et al, 2004.)

Multiple prospective analyses of local mfERGs identified functional abnormalities in eyes with diabetic retinopathy, both in retinal regions corresponding to retinopathy and in areas without signs of it (Fortune et al, 1999; Bearse et al, 2004). In the same line it has been found that mfERG implicit time delays are associated with retinal locations in which new nonproliferative diabetic retinopathy will develop one year later (Han et al, 2004a). All these promising results suggest that mfERG may become a pivotal technique for the study of neural impairment in the diabetic retina. Apparently, in some eyes, functional abnormalities of the retina and vision can occur before clinical signs of retinopathy vascular damage are visible on ophthalmoscopy (Figure 3.13).

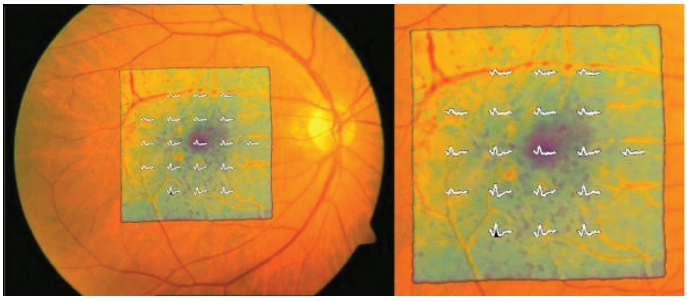

Figure 3.13. Deformable image registration between color fundus photograph (background image), leakage analysis (retinal leakage analyzer) (central squared area) and multifocal electroretinogram (mfERG) (curve responses in white).

Similarly, there are reports indicating that the amplitude of the b-wave of the scotopic full-field (flash) ERG and the implicit time of the oscillatory potentials maybe abnormal in diabetes in the absence of visible fundus signs of retinopathy (Yonemura and Kawasaki, 1978; Coupland, 1987; Shirao et al, 1991).

These findings suggest that in a certain percentage of patents the function of the neuronal components of the middle and inner retinal layers is altered in diabetes prior to the development of retinopathy. Other observations using electrophysiological methods also suggest some degree of photoreceptor damage in some patients (Weiner et al 1997) and studies using the electroretinogram indicate disruption of the outer blood-retinal barrier as an early manifestation of diabetic eye disease.

Given the local nature of early-to-moderate nonproliferative diabetic retinopathy, the association between retinopathy and functional status would be best established using local functional measures. One might expect that global measures can "miss" localized abnormalities because the remaining healthy retina predominates the functional measure. It is well recognized that known risk factors (e.g. metabolic control, disease duration, lipid levels) are not sufficient to make prediction of retinopathy development in specific retinal locations.

There is thus a need to identify a measure that maps local functions and predicts the development of retinopathy at specific locations. The mfERG has been proposed as such.The "standard" visual stimuli are comprised

of an array of 103 hexagonal elements displayed on a monochrome CRT (part of an eye camera / display / refractor unit) at a 75Hz frame rate. Abnormalities of mfERG implicit time are locally associated with retinopathy and appear also to be related to the severity of the retinopathy. It has been reported that early stage retinopathy, approximately 49% of the local mfERG had abnormal implicit times, whereas only 20% of the local implicit time were abnormal in areas that did not have signs of retinopathy (Bearse et al, 2004).

Delayed localized mfERG implicit times often precedes (i.e., predicts) the appearance of diabetic retinopathy in corresponding local retinal patches. In a small study os 11 patients with mild nonproliferative diabetic retinopathy and 11 patients without visible retinopathy resting with mfERG was performed at baseline and then retested at 12 months later (Han et al, 2004a). After one year, two-thirds of the eyes that had some diabetic retinopathy at baseline developed new retinopathy in zones that were free of retinopathy at baseline. In these eyes, abnormal baseline mfERG implicit times (defined as implicit time Z-score ≥ 2) occurred within 35% of the retinal zones that were free of retinopathy at baseline. Of the 63 initially retinopathy-free zones with abnormal baseline implicit times, 22 (35%) developed new diabetic retinopathy at follow-up. In contrast, only 2% of the retinal zones with normal baseline implicit times developed new diabetic retinopathy. Development of new retinopathy within one year was approximately 21 times more likely in retinal zones with abnormal baseline mfERG implicit times than it was in zones with normal baseline implicit times.

Delayed multifocal ERG implicit time is the main outcome in a large clinical trial entitled “EUROCONDOR - European Consortium for the Early Treatment of Diabetic Retinopathy. Neurodegeneration as an early event in the Pathogenesis of Diabetic Retinopathy: A multicentric, prospective, phase II-III, double blind randomized controlled trial to assess the efficacy of neuroprotective drugs administered topically to prevent or arrest Diabetic Retinopathy”, run by the European Vision Institute Clinical Resaerch Network (EVICR.net) which is staring in November 2012 with funding from the European Union Research Programme.

3.6. Multimodal macula mapping

There are currently a variety of diagnostic tools and techniques to examine the macular region and to obtain information on its structure and function. The different methods available offer different perspectives and fragmentary information. Multimodal Macula Mapping, a methodology initiated by our research group, aims to combine different methodologies and to obtain maps of the alterations occurring in the macular region in health and in different stages of disease, and therefore to establish correlation between those fragmentary pieces of information and so to build the complete puzzle (Bernardes et al, 2002).

Detection devices for obtaining information for macula mapping are numerous and varied, often complementing one another with differing degrees of invasiveness, accuracy and object of measurement. Some chart anatomy whereas others measure an aspect of physiology. Together, they can combine structure and function. Our research group has been developing methods to combine and integrate data from fundus photography, angiographic images, maps of fluorescein leakage into the vitreous, maps of retinal thickness and maps of visual function of the macular area to achieve multimodal macula mapping (Lobo et al, 1999; Lobo et al, 2000; Bernardes et al, 2002).

3.7. Summary

One of the major challenges for research on diabetic retinopathy is its early detection and improved characterization of what is still identified as the preclinical stage of the retinopathy.

It is crucial to identify the most initial alterations occurring in the diabetic retina in order to develop prevention strategies and test new drugs that may stop disease progression early in the disease process, thus avoiding development of clinically significant macular edema and proliferative retinopathy, the complications of the retinopathy that are directly associated with vision loss.

The initial alterations of diabetic retinopathy are focal and localized and involve the macular region. They include microaneurysm formation and disappearance, alteration of the blood-retinal barrier with retinal edema, and signs of neuronal and glial dysfunction.

We have been able to develop a new method of automated analysis of digital fundus photographs which allows quantification of microaneurysm turnover, the RetmarkerDR.

Alteration of the blood-retinal barrier was first demonstrated by fluorescein angiography and then quantified by vitreous fluorometry, a method also introduced by our research group. The invasive nature of vitreous fluorophotometry associated with the difficulty in obtaining localized information of the initial retinal changes led to its present use being restricted to experimental research. It is now possible to quantify increased thickening of the retina, demonstrating different degrees and location of subclinical retinal edema using OCT. Our research group has recently shown that OCT, a non-invasive method, may be able to replace vitreous fluorometry to identify localized alterations of the blood-retinal barrier.

Neuronal and glial changes can also be detected non-invasively using OCT. Ganglion cell and nerve fiber loss can be quantified with OCT.

Multifocal ERG is also being tested as an indicator of focal and localized functional alterations in the retina due to diabetes and the initial findings are promising.

It is expected that multimodal macula mapping combining automated analysis of fundus photographies, OCT and multifocal ERG will further contribute to the characterization of the initial changes occurring in the retina in diabetes, identifying at the same time structural and functional changes.

Chapter 4
Pathogenesis

4.1. Retinal microvascular cell changes in diabetes

Clinical diabetic retinopathy is identified on ophthalmoscopic examination by the appearance of retinal microaneurysms at the posterior pole. l had the opportunity of examining the pathology of diabetic retinopathy and other vascular retinopathies using injection methods, a variety of stains, and the digestion technique when working in London (1963-1966) (Cunha-Vaz, 1967). Light microscopic examination of retinal digests is particularly appropriate to study alterations in the retinal vascular bed (Figures 4.1 and 4.2). From this study a clear pattern of disease was observed to occur in the vascular lesions of diabetic retinopathy. The results suggested that vascular changes are initiated in the small vessels in the form of endothelial proliferation, microaneurysms, and signs of impending cellular degeneration in a few vascular branches. These initial lesions are focal and located preferentially at the posterior pole of the retina.

At first the endothelial proliferation and microaneurysms appear to be confined to the venous side of the retinal circulation, whereas at this stage endothelial degeneration changes appear to be limited to capillaries on the arterial side of the circulation.

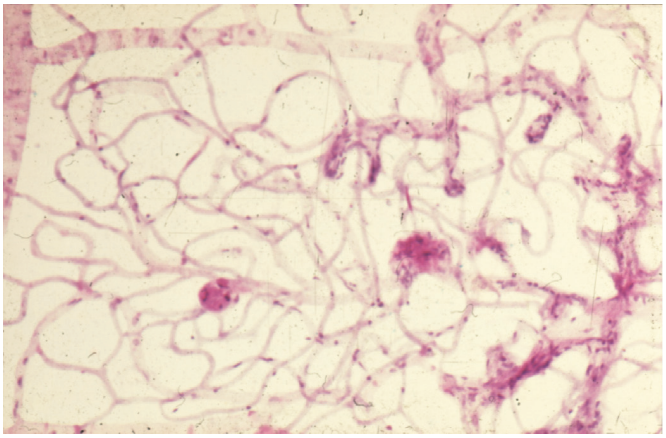

Figure 4.1. Diabetic retinopathy. Retinal digest showing endothelial proliferation and microaneurysm formation preferentially on the venous side with cell loss on the arteriolar side.

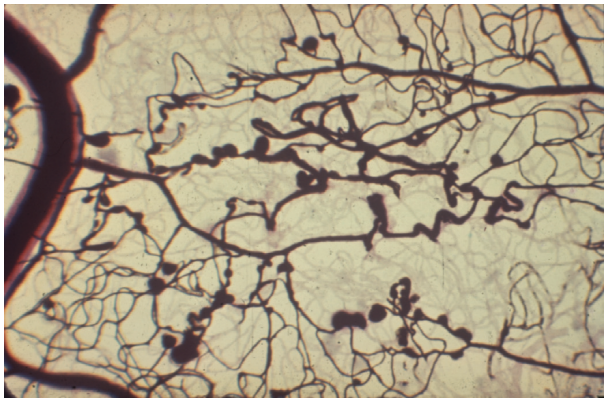

Figure 4.2. Retinal digest of an indraw ink injected diabetic retina. There is widespread capillary closure and many microaneurysmal dilations.

The pericytes, although showing clear signs of disease, are affected in a highly irregular manner.

With progression of the disease the capillaries on the arterial side of the retinal circulation show increased cell loss and closure (Figure 4.2). Simultaneously, on the venous side of the circulation, there is an increase in the number of microaneurysms. As the areas of capillary closure enlarge, they are seen to be traversed by a few enlarged capillaries, which may act as arteriovenous shunts, receiving the blood diverted from the surrounding closed capillary net. These observations are summarized in Table 4.1.

Table 4.1. Evolution of clinical retinopathy in diabetes

Stages	Ophthalmoscopy	Pathology Small vessels		Large vessels
		Venous side	Arterial Side	
1. Initial	Rare microaneurysms Microaneurysms ++	Endoth. Prol. ++ Pericyte death ++	Endoth. Deg. +	Vein ±
		Microaneurysms		
2. Intermediate	Numerous microaneurysms Hemmorhages	Endoth. Prol. ++ Pericyte death ++	Endoth. Deg. ++	Vein ±
	Exudates, IRMAs	Microaneurysms ++	Focal cap. Clos.	
1. Advanced	Same lesions as in stage 2	Endoth. + Pericyte Deg.+++		Vein ++
	+ venous beading	Microaneurysms +++		Artery ++
	+ abnormally dilated vessels	A-V shunts		
		Large area cap. clos.		

Are these lesions specific for diabetes? Examination of other vascular retinopathies emphasizes the probable importance of local factors and that the lesions described in diabetic retinopathy are shared by a wide variety of apparently unrelated diseases.

Endothelial proliferation, prevailing on the venous side of the circulation, is a frequent finding in polycythaemia, leukaemia, myelomatosis, central retinal vein thrombosis, macroglobulinaemia and Eales's disease.

Microaneurysms are probably the commonest and most characteristic retinal lesion in diabetes (Ashton, 1963) but they are also seen, frequently in large numbers, in Eales's disease, leukaemia, myelomatosis, macroglobulinaemia, central retinal vein thrombosis and hypertensive retinopathy.

Areas of capillary closure are seen in scleroderma, hypertensive retinopathy, pernicious anaemia, and central retinal vein thrombosis. Arteriovenous shunts appear in retinal vein thrombosis. Basement membrane thickening and pericyte degeneration are similarly found in other retinopathies, but they clearly have a higher prevalence in diabetic retinopathy.

Finally, are these lesions the only cell changes occurring in diabetic retinopathy? We must keep in mind that these lesions are more easily detected in ophthalmoscopic examination and retinal digests eliminate the neuroglial tissue in the process of digestion.

Basement membrane thickening is believed to play an important role not only in diabetic retinopathy but in diabetic microangiopathy as a whole. Ashton reviewed the subject in detail in 1974, commenting on basement membrane thickening in several non-diabetic conditions, its inconstancy, and its presence in all types of diabetes. Electron microscopical studies of diabetic retinopathy show that basement membrane thickening is associated with a variety of haematogeneous elements, which could have arrived at this situation only through leakage due to breakdown of the endothelial barrier. Leakage of plasma components is especially evident in microaneurysms and is apparently responsible for the greatly thickened wall. Lt is also important to recall that leakage of this kind necessarily implies a preceding endothelial injury.

In his electron micrographs of diabetic basement membranes Ashton (1974) observed a characteristic multi layered appearance with interposed

cellular debris, a finding which Vracko and Benditt (1970) interpreted as due to repeated endothelial cell death with regeneration and replacement, each new generation of cells contributing their own basement laminae and entrapping the cellular debris of the dead cells. This could explain the comparatively healthy appearance of the endothelium even in the presence of gross periendothelial pathology, including degenerate pericytes. Basement membrane thickening appears, therefore, to be a secondary feature of the disease, due in part, possibly, to glycoprotein insudation from an initial endothelial dysfunction , as considered by Williamson and Kilo (1976).

There is also abundant evidence that pericytes are selectively involved in the diabetic process (Cogan and Kuwabara, 1963), the damage taking the form of a peculiar eosinophilic degeneration of the nucleus before the cell disintegrates altogether. Although similar changes can be observed in other retinopathies, they are by no means so common as in diabetes.

It has been shown that there is no one vascular lesion which is absolutely specific for diabetic retinopathy. The most characteristic features of diabetic retinopathy, microaneurysms, capillary closure, basement membrane thickening, and pericyte damage appear to differ from other vascular retinopathies only in their frequency and widespread distribution. While the vascular involvement in other retinopathies is localized initially either to the arterial or to the venous side of the circulation (Cunha-Vaz, 1967; Wise et al, 1971), there are indications that in diabetic retinopathy the entire vascular tree at the posterior pole of the retina is involved from the beginning. The most similar histopathological picture is seen in radiation retinopathy, which is initiated by damage to the endothelial cells of the retinal vessels.

4.1.1. Cell changes relevant to blood-retinal barrier breakdown

Breakdown of the blood-retinal barrier is a hallmark of diabetic retinopathy (Cunha-Vaz et al, 1975a). It has been shown that both inner and outer blood-retinal barrier are affected by diabetes, but the inner retinal vasculature appears to be the primary site of leakage in diabetic humans and rats (Vinores et al, 1989, 1993; Carmo et al, 1998). Most of research in this field has been addressed to the inner blood-retinal barrier, and therefore most data are related with retinal microvasculature and endothelial cells.

The retina is separated from blood circulation by two barriers, the inner blood-retinal barrier, tight junctions confined to endothelial cells from retinal blood vessels, and the outer blood-retinal barrier, located at retinal pigment epithelial cells. The barrier properties are due to the presence of junctional structures between cells (Shakib and Cunha-Vaz, 1966). These structures, named tight junctions, prevent solutes from moving between cells, thus regulating paracellular permeability, and provide the retina with a selective mechanism to regulate its environment (osmotic balance and nutrients) (Figure 4.3).

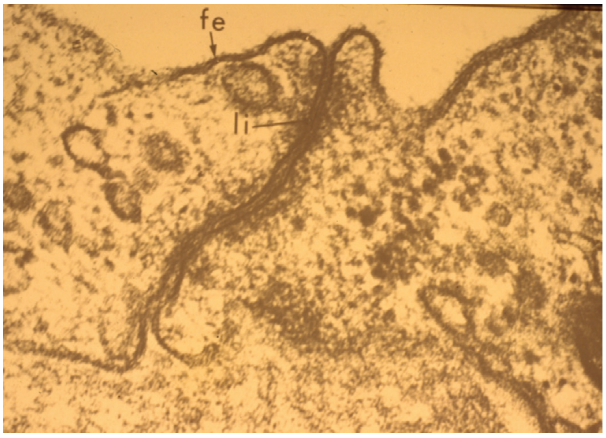

Figure 4.3. Election microscopy image showing a tight junction between adjacent endothelial cells in a human retinal capillary. li. Fusion of the external leaflets of cell membranes; fe. External leaflet.

Regarding the molecular architecture of tight junctions, several structural proteins have been identified in both endothelial and epithelial cells in various tissues. Occludin, a 65 kDa protein, was the first transmembrane protein identified as a component of tight junctions (Furuse et al, 1993). Also, it was shown that a family of multiple integral membrane proteins called claudins, smaller than occludin (about 22 kDa), is also localized in tight junctions (Furuse et al, 1998; Morita et al, 1999). Confocal and immunoelectron microscopy have shown that another integral membrane protein, called junctional adhesion molecule (JAM), co-distributes with tight junction components (Martin-Padura et al, 1998). Intracellular non-transmembrane

proteins on the cytosolic leaflet have been also identified as components of tight junctions: zonula occludens-1 (ZO-1) (Stevenson et al, 1986), ZO-2 (Jesaitis and Goodenough, 1994), ZO-3 (Haskins et al, 1998), cingulin (Citi et al, 1988), 7H6 (Zhong et al, 1993) and symplekin (Keon et al, 1996). ZO-1, ZO-2 and ZO-3 have a high capacity for multiple protein-protein interactions and may function to couple extracellular signaling pathways to the cytoskeleton, thus suggesting a role for cytoskeleton regulation of tight junctions (Wittchen et al, 1999).

The increase in blood-retinal barrier permeability is one of the the first clinically evident sign of diabetic retinopathy (Cunha-Vaz et al, 1975a). Considering that tight junction proteins have a predominant role in regulating blood-retinal barrier permeability, changes in tight junction proteins expression and localization in endothelial or epithelial cells, due to diabetes, may account for increased permeability properties. The first evidence demonstrating that the content of occludin decreases in the retinas of streptozotocin (STZ)-induced diabetic rats in comparison to control animals was reported by Antonetti et al (1998). The immunoreactivity of occludin decreased in the retinas of diabetic animals, as demonstrated by immunoblot analysis and immunohistochemistry. In addition, occludin was differentially distributed in the blood vessels. In diabetic retinas, occludin immunoreactivity decreased in the capillaries and it was redistributed in the arterioles from continuous cell border to punctate immunoreactivity. Treatment with insulin, only for 48 h, reversed the pattern of occludin immunoreactivity in diabetic retinas, clearly demonstrating that this is not an irreversible process (Barber et al, 2000). The reduction of occludin immunoreactivity was not accompanied with a reduction in claudin-5 immunoreactivity, suggesting that occludin might play a prominent role in this process (Barber and Antonetti, 2003). However, recently, two studies have demonstrated that the mRNA expression and protein content of claudin-5 are decreased in the retinas of STZ-induced diabetic rats (Bucolo et al, 2009; Klaassen et al, 2009). Moreover, Leal and colleagues (2007) found that the levels of ZO-1, in addition to occludin levels, are also decreased in diabetic mice retinas.

Vascular endothelial growth factor (VEGF) has been largely implicated in the pathogenesis of diabetic retinopathy and has been shown to have an important role in regulating vessel permeability (Wilkinson-Berka, 2004).

In addition to occludin phosphorylation, VEGF also rapidly increases the phosphorylation of ZO-1 in tyrosine residues, contrary to occludin, which seems to be predominantly phosphorylated in serine or threonine residues. These results indicate that the phosphorylation of tight junction proteins might promote an increase in tight junction permeability or signal a long-term decrease in tight junction proteins content.

The results obtained in experimental models clearly demonstrate that blood-retinal barrier permeability is associated with changes in the expression, phosphorylation and localization of tight junction proteins in endothelial cells, principally occludin and ZO-1, but more recent evidences also point a role for claudin-5. Also, VEGF, a potential key player in the pathogenesis of diabetic retinopathy, was found to regulate the expression and phosphorylation of tight junction proteins, probably through protein kinase C.

The role played by the retinal pigment epithelium in the alteration of the blood-retinal barrier that occurs in diabetes remains controversial. There is evidence of its damage in experimental diabetes (Tso et al, 1980) and involvement of the retinal pigment epithelium has been proposed to occur when macular edema is present (Sander et al, 2007).

4.1.2. Microvascular cell death

Retinal diabetic microangiopathy is characterized as we have indicated previously by blood-retinal barrier breakdown, basement membrane thickening of retinal vessels, formation of microaneurysms and hemorrhages. Another early feature is the presence of capillary obliteration and acellular capillaries, which ultimately lead to retinal ischemia and neovascularization (Cunha-Vaz, 1978b). Some of these events occur because retinal vascular cells, namely pericytes and endothelial cells, die prematurely during diabetes. Microvascular cells may become dysfunctional and undergo apoptosis due to hyperglycemia.

Mizutani et al (1996) have shown, using the TUNEL technique, which detects cells undergoing apoptosis in situ, that diabetes causes accelerated death of both retinal pericytes and endothelial cells in diabetic patients and rats. The early apoptosis in retinal microvascular cells, detected after 6 to

8 months of diabetes, predicts the development of the histologic lesions, characteristic of diabetic retinopathy, which appear several months later (Kern et al, 2000).

Consistent with the fact that apoptosis of pericytes and retinal endothelial cells appears to be the mechanism of retinal capillary cells loss, it was shown that caspase-3, an executioner apoptotic enzyme, is activated in diabetic rat retinas and in retinal capillary cells exposed to high glucose (Kowluru and Koppolu, 2002; Mohr et al, 2002; Busik et al, 2008).

The enzyme poly ADP-ribose polymerase (PARP) also seems to have an important role in the development of diabetic retinopathy and in microvascular cells apoptosis. The activity of PARP is increased in whole retinas, and in endothelial cells and pericytes of diabetic rats. Treatment with a PARP inhibitor inhibited NF-kappaB activation, microvascular cell death and the development of the characteristic histopathologic lesions of diabetic retinas (Zheng et al, 2004).

This group of findings demonstrates that hyperglycemia causes apoptosis in microvascular cells and that retinal pericytes seem to be more susceptible than endothelial cells to stress conditions triggered by diabetes. However, it must be noted that it is easier to identify an apoptotic pericyte in situ than an apoptotic endothelial cell. Pericytes are surrounded by the basement membrane, and therefore it is much more difficult to eliminate its debris, whereas dying endothelial cells are easily removed by blood flow and substituted by another endothelial cell. Anyway, it is obvious that microvascular cell death will have consequences on capillary architecture and function, and will compromise blood-retinal barrier properties.

4.1.3. Microthrombosis

Capillary nonperfusion and eventual obliteration can be the consequence of retinal microthrombosis. Increased platelet adhesiveness and aggregation has been documented in diabetic patients since at least three decades ago (Heath et al, 1971; Bensoussan et al, 1975). In experimental diabetes, it was observed the formation of microthrombi by electron microscopy in retinal vessels obtained from diabetic rats (9 to 12 months). These microthrombi were mainly composed of aggregated platelets and fibrin strands (Ishibashi

et al, 1981). Boeri and colleagues (2001) demonstrated, after isolating the intact vascular network from postmortem retinas obtained from diabetic and nondiabetic donors, and using antibodies to fibrin cross-linking factor XIII and platelet glycoprotein (GP)-IIIa to identify fibrin-platelet thrombi, that diabetic retinas present a greater than normal number and size of platelet-fibrin thrombi in the retinal capillaries. Also, there was a topographical association of microthrombosis with apoptotic cells in both diabetic and nondiabetic vessels.

In some clinical studies the use of an inhibitor of platelet aggregation proved to be useful in the treatment of diabetic retinopathy. Trifusal, a platelet antiaggregant drug, decreased the leakage of fluorescein and the number of microaneurysms, while in the untreated group both parameters were increased (Esmatjes et al, 1989). Also, treatment of diabetic rats with aspirin plus dipyridamole inhibited the production of thromboxane B2 by platelets and decreased the synthesis of prostacyclin (De la Cruz et al, 1997). These observations indicate that platelets are involved in microthrombosis, although leukocytes may be also involved, which seems to play an important role in the pathogenesis of diabetic retinopathy. The molecular mechanisms involved in the formation of microthrombi in diabetes are not yet completely understood, but they deserves a particular attention, since it might be a promising therapeutic target.

4.2. Retinal neuronal and glial changes in diabetes

Diabetic retinopathy has been widely considered a disease that affects principally retinal microvessels because the initial clinical picture is characterized by vascular alterations (Cunha-Vaz, 2001). Several clinical evidences, such as the formation of microaneurysms and cotton-wool spots, hemorrhages, macular edema, and neovascularization, are indicative that retinal microvascular cells are susceptible to hyperglycemia. This interpretation was reinforced by the clinical observations associating the ophthalmoscopic diagnosis of diabetic retinopathy and retinal digest preparations with microvascular changes. However, other retinal cell types, such as neurons, Muller cells, astrocytes and microglial cells appear also to be affected by diabetes. In recent years, a large body of evidence has

clearly demonstrated that other cell types are affected by diabetes in the retina (Gardner et al, 2000; Lorenzi and Gerhardinger, 2001), and alterations occurring in each cell type can potentially affect other cells. However, it is not clear yet which cell types might be affected first.

4.2.1. Neuronal cell death

Changes in the electroretinogram obtained from patients with diabetic retinopathy (Simonsen, 1974; Uccioli et al, 1995), and alterations in color vision and contrast sensitivity (Daley et al, 1987) reveal abnormal function of the visual system. The amplitude and latency of oscillatory potentials is reduced in the retina of diabetic patients or STZ-induced diabetic rats (Simonsen, 1974; Frost-Larsen et al, 1981; Sakai et al, 1995). Interestingly, these alterations in electroretinograms can be reversed by treatment with aldose reductase inhibitors (Funada et al, 1987; Segawa et al, 1988; Lowitt et al, 1993), indicating not only that the increased activity of the polyol pathway is involved in the development of these abnormalities, but also suggesting that the neural function is not irreversibly compromised.

Since these functional changes may be identified before detection of microvascular lesions (Lopes de Faria et al, 2001), it seems that diabetes directly compromises the function of neural retina, even before it might be further affected by the alteration in the permeability of blood-retinal barrier.

Evidence of programmed cell death in retinal ganglion cells (RGC) was found in rat retinas in the initial satges of experimentally induced diabetes. Treatment with nerve growth factor (NGF) prevented apoptosis in these cells, suggesting that neurotrophic factors may be useful therapeutic agents in diabetic retinopathy (Hammes et al, 1995). A very important study, which may have changed the concept of diabetic retinopathy was published by Barber and colleagues (1998) a decade ago. This study was the first quantitative report showing an increase in neuronal cell apoptosis in the diabetic retina. It was demonstrated that the thickness of the inner plexiform and inner nuclear layers are reduced upon 7.5 months of STZ-induced diabetes, and that the number of ganglion cells is also decreased.

4.2.2. Glial cells reactivity and changes

The retina has two types of macroglial cells: Muller cells that are only encountered in the retina and span all retinal layers, and astrocytes that migrate into the retina from the optic nerve, and are located at the nerve fiber layer, being less abundant than Muller cells. Both cell types envelope retinal vessels, the initial segments of ganglion cells axons and neurons as well. These cells have rather important functions in the retina. They provide structural and metabolic support for retinal neurons and blood vessels and are essential to retinal homeostasis (Bringmann and Reichenbach, 2001). Among other important functions they regulate the expression of tight junction proteins and control the permeability of blood-retinal barrier (Tout et al, 1993; Gardner et al, 1997).

Alterations in Muller cells, with possible implications in blood-retinal barrier, have been reported more than two decades ago in STZ-induced diabetic rats (Hori and Mukai, 1980). The accumulation of highly electron-dense bodies, which resembled lysosomes, in the cytoplasm of Muller cells, was correlated with metabolic alterations in the retina. Changes in the nucleus, consistent with apoptotic features, were also observed in Muller cells in the retinas of diabetic rats (Schellini et al, 1995). The pioneer work of Hammes and colleagues (1995) clearly showed that in addition to neurons and microvascular cells, Muller cells exhibit apoptotic features and increase the expression of the intermediate glial fibrillary acidic protein (GFAP).

There are also evidences demonstrating that astrocytes and Muller cells react differently to diabetes (Barber et al, 2000). The immunoreactivity against GFAP is limited to astrocytes in control retinas, and two months after induction of diabetes with STZ, the GFAP immunoreactivity is reduced in astrocytes and increased in Muller cells. After 4 months, the astrocytes do not express GFAP, but Muller cells have intense staining. Similar changes were observed in BB/Wor diabetic rats. Also interesting, is the fact that the expression of occludin in retinal vessels decreases in the same areas where GFAP is reduced. This observation suggests that the alterations in glial cells may contribute to increased permeability of the blood-retinal barrier.

Glutamine synthetase, the enzyme responsible to convert glutamate into glutamine, is only expressed in Muller cells in the retina (Newman and

Reichenbach, 1996). The ability of diabetic rat retinas to convert glutamate into glutamine is decreased when compared to control retinas (Lieth et al, 1998), due to a reduction in the activity and content of glutamine synthetase (Mizutani et al, 1998; Lieth et al, 2000). The oxidation of glutamate is also significantly reduced in diabetic retinas (Lieth et al, 2000). These results indicate that diabetes induces at least two enzymatic abnormalities in the glutamate metabolism pathway: transamination to alpha-ketoglutarate and amination to glutamine. Insulin restored the activity of glutamine synthetase, suggesting that some glial changes may be due to hypoinsulinemia.

The glutamate content increased by 1.6-fold (Lieth et al, 1998) or 40% (Kowluru et al, 2001) in diabetic retinas. Excessive glutamate may cause excitotoxicity, and therefore may be responsible for neural degeneration in the retina during diabetes. The administration of antioxidants inhibited the increase in retinal glutamate content caused by diabetes (Kowluru et al, 2001).

At present, it is not yet clear whether glutamate excitotoxicity is responsible for neural cell apoptosis in diabetic retinas. However, there is no doubt that glutamate metabolism, glutamate levels and the expression of glutamate receptors are altered by diabetes, and at least, this will have consequences at the level of neurotransmission and the retinal function. Whether blood-retinal barrier might be affected by these changes it is not known.

Several evidences have shown that microglial cells might also be involved in the pathophysiology of diabetic retinopathy. The increase in microglial density and the alterations in the shape of microglial cells, as an indication of functional activation, were found in the retinas of diabetic animals (Rungger-Brandle et al, 2000; Gaucher et al, 2007; Yang et al, 2009). Zeng and colleagues (2000) have shown that microglial cells are activated and appear hypertrophic, just 1 month after inducing diabetes in rats. The number of microglial cells was increased, and some cells appeared in the outer plexiform layer at 4 months. At 14 and 16 months, reactive microglial cells were detected in the outer nuclear layer and photoreceptor layer. Also, in retinas of patients with diabetic retinopathy microglia is markedly increased in number and are hypertrophic at different stages of the disease (Zeng et al, 2008).

These changes may be elicited by neural cell death occurring in ganglion cell layer and inner nuclear layer, as well as by some alterations in photoreceptors. Although the evidences in the literature related with the involvement of microglial cells in the pathogenesis of diabetic retinopathy are not abundant, these reports clearly demonstrate that retinal microglial cells are activated by diabetes, and suggests that inflammation has a role in the pathogenesis of diabetic retinopathy.

4.3. Inflammation

The role of inflammation in the development of diabetic retinopathy, and the adhesion of leukocytes to retinal vessels has been linked to the breakdown of blood-retinal barrier and may play an important role in the development of diabetic retinopathy. Leukocytes are large cells, which have high cytoplasmic rigidity and capacity to generate free radicals and proteolytic enzymes. In diabetes, leukocytes are more activated and less deformable, and therefore may be involved in capillary non-perfusion, endothelial cell damage, and vascular leakage in the retinas.

Miyamoto and colleagues (1998) demonstrated for the first time an increase in leukostasis, in vivo, in the retinas of diabetic rats. Retinal leukostasis was correlated with increased expression of intercellular adhesion molecule-1 (ICAM-1) and vascular leakage, since the blockade of ICAM-1 with a monoclonal antibody prevented both leukostasis and vascular leakage (Miyamoto et al, 1999).

The inhibition of beta isoform of protein kinase C (PKC), which has been shown to play a key role in the pathogenesis of diabetic retinopathy (Curtis and Scholfield, 2004), prevented the adhesion of leukocytes to retinal vessels (Nonaka et al, 2000). Advanced-glycation end-products, VEGF, and nitric oxide produced by endothelial nitric oxide synthase (eNOS) also seem to play important roles in the adhesion of leukocytes to retinal vessels, thus suggesting that these players are potentially good targets in the treatment of early diabetic retinopathy (Joussen et al, 2002; Moore et al, 2003; Mamputu and Renier, 2004). More recently, evidences have demonstrated that nitric oxide produced by inducible NOS (iNOS) appears to have a predominant role in leukostasis and blood-retinal barrier breakdown (Leal et al, 2007; Zheng et al, 2007).

This group of results clearly demonstrates that leukocytes have a predominant role in the early stages of diabetic complications in the retina, and diabetic retinopathy can be characterized as a chronic, low-grade, inflammatory process, that may be responsible for vascular lesions occurring in retinal vessels.

Analyzing the profile of gene expression in the retinas of diabetic and normal rats, using cDNA microarrays, it was found that most of the genes that are upregulated are correlated with an inflammatory response (Joussen et al, 2001). Our group has also shown that the levels of the pro-inflammatory cytokine IL-1beta and the activity of NOS are increased in the retinas of STZ-induced diabetic rats (Carmo et al, 1999). The administration of an anti-inflammatory drug, cyclosporin A, inhibited the production of IL-1beta, and the expression of inducible NOS and cyclooxygenase-2 (COX-2). These observations were correlated with a decrease in the permeability of blood-retinal barrier (Carmo et al, 2000).

4.4. Mechanisms of molecular damage to the retina

Hyperglycemia appears to be sufficient to initiate development of diabetic retinopathy as revealed by the development of retinopathy in animals experimentally made hyperglycemic (Engerman and Kern 1984; Kador et al 1990; Kern and Engermann 1996). Consistently, a number of experimental studies have shown that intensive therapy sufficient to minimize hyperglycemia inhibits the development of retinopathy (Engerman et al 1977, 1993). Excessive transport of glucose or concentration of glucose within cells of the retina is a common thread underlying most of the biochemical and molecular mechanisms that have been postulated to play a role in the pathogenesis of diabetic retinopathy.

However, the observation that not all patients with poor metabolic control develop advances stages of diabetic retinopathy suggests that other factors, such as genetic predispositions, are likely to determine individual susceptibility to the disease.

The retina consists of three major types of cells: neurons, glial cells and blood vessels and most, if not all, of these cell types are affected to some degree in diabetic retinopathy.

The retina is primarily a neuronal tissue. Indeed, neurons and glial cells comprise about 95% of the retinal mass. The glial cells of the retina, Muller cells and astrocytes serve as support cells for the neurons and blood vessels. The inner blood-retinal barrier, which is a specific and unique structure in the retina, and is affected early in diabetes, is formed by the retinal neurovascular unit, assembling in a functional unit, the retinal vessels and the glial cells immediately surrounding the retinal vessels. The earliest alterations detected clinically in the retina in diabetes are the breakdown of the blood-retinal barrier and alterations in the neurosensory retinal function. Both these alterations can be detected before ophthalmoscopic signs of diabetic retinopathy are visible, in preclinical retinopathy (Cunha-Vaz et al 1975; Daley et al 1987).

These and other observations indicate that dysfunction of the inner retina may affect neurons and glial cells and induce changes in the neurovascular unit and blood-retinal barrier before obvious signs of vascular lesions.

Oxygen consumption and metabolic activity in the retina is one of the highest in the body. The neuroretina is nourished by transport of glucose across the endothelial cells of capillaries of the inner blood-retinal barrier and from the choroidal vessels across the retinal pigment epithelium of the outer blood-retinal barrier (Kumagai, 1999).

Mechanisms that may concur in diabetic retinopathy to explain toxicity of glucose in diabetes include: activation of protein kinase C (PKC), activation of aldose reductase, formation of advanced glycation end products and the hexosamine pathway.

Moreover, inhibitors of some of these pathways or enzymes were shown to ameliorate, although at times only marginally, many of the modifications associated with diabetes both in cell culture and animal models (for a review: (Brownlee, 2001)). For many years all of these mechanisms were considered independently to explain modifications associated with pathogenesis of diabetic retinopathy independently.

More recently, however, it was realized that a common thread might exist that could account for the hyperglycaemia-induced damage in diabetes. Indeed, over the past four years it has consistently been shown that hyperglycaemia induces overproduction of superoxide by the mitochondrial electron transport chain (Du et al, 2000; Nishikawa et al, 2000; Brownlee,

2001) and that this excess production of reactive oxygen species (ROS) can be the upstream event leading to other mechanisms implicated in endothelial cell damage associated with diabetes.

The mechanism whereby hyperglycemia induces increased production of superoxide by mitochondria appears to be related to the excessive production of oxygen donors from trycarboxylic acid cycle (TCA). Glucose oxidation inside the cells begins with glycolysis in cytosol, which generates NADH and pyruvate. The intracellular pool of pyruvate that is not reduced to lactate is then transported into the mitochondria where it is oxidized producing water, carbon dioxide and reducing potential in the form of NADH and FADH2. Mitochondrial inhibition of glycolysis derived pyruvate transport into mitochondria completely inhibited hyperglycemia-induced ROS production (Nishikawa et al, 2000). These data indicate that the TCA cycle is the source of increased ROS-generating substrate induced by hyperglycemia. All together the increased production of glycolysis intermediates increase flux through TCA cycle and an increased electrochemical potential difference in inner mitochondrial membrane cause a marked increase in the production of superoxide by endothelial cells.

This unifying hypothesis is consistent with the four pathways suggested to be involved in the development of diabetic complications (activation of aldose reductase, increased formation of AGEs, activation of protein kinase C and increased hexosamine pathway flux). This hypothesis further accounts for the relevance of increased production of reactive oxygen species in diabetes and also provides a unifying hypothesis regarding the effects of hyperglycaemia on cellular dysfunction (Nishikawa et al, 2000; Brownlee, 2001).

Furthermore, the activation of the transcription factor NF-kB by superoxide (Nishikawa et al, 2000) provides an extra link between hyperglycemia and the expression of multiple genes related to vascular stress response (Collins, 1993). Significantly, hyperglycemia-induced activation of NF-kB is also prevented by inhibiting mitochondrial superoxide overproduction (Nishikawa et al, 2000).

Increased production of superoxide may further interfere with NO function in the retina and, as mentioned before, may even result in an increased production of superoxide by a mechanism that does not involve

the mitochondria, thus amplifying the production of reactive oxygen species. NO is generated from the metabolism of L-arginine by the enzyme nitric oxide synthase (NOS). There are three known isoforms of the enzyme, the constitutive brain (bNOS) and endothelial (eNOS) isoforms and an inducible isoform (iNOS). A number of stimuli were shown to induce iNOS, including hyperglycaemia (Baek et al, 1993; Leal et al, 2012). Overproduction of superoxide may interfere with NO function at different stages. For example, superoxide may quench NO thus reducing its vasodilator properties and disrupting the general homeostasis of the vasculature (Benz et al, 2002). On the other hand, induction of iNOS in response to hyperglycaaemia and the resulting accumulation of NO may, in the presence of superoxide, lead to formation of peroxynitrite (Beckman and Koppenol, 1996). Peroxynitrate may then oxidize tetrahydrobioptrerin, an iNOS cofactor (Beckman and Koppenol, 1996) rendering iNOS to an uncoupled state which results in electrons being diverted from the original path iNOS reductase domain – oxidase domain, to molecular oxygen leading to formation of more superoxide rather than NO (Cosentino et al, 1997; Brodsky et al, 2002).

4.5. Summary

Diabetes is a complex multifactorial disease which has chronic hyperglycemia as its main abnormality. Excess glucose appears to have a particular important toxic effect on the retinal microvasculature and retinal tissue function by a variety of mechanisms that may work separately or in association in different patients.

In recent years, important steps have been done in the process of improved understanding of the pathogenic mechanisms underlying diabetic retinopathy. Its pathology should no longer be considered only a microvascular disease, although microvascular alterations predominate clinically. Indeed, in addition to alterations in retinal microvessels, essentially due to functional changes or apoptosis of endothelial cells and pericytes, other retinal cells are affected: neurons, Muller cells, astrocytes and microglial cells. In addition, the involvement of leukocytes and platelets should not be forgotten.

It may even be possible that neurons and glial cells might be first affected by hyperglycemia than microvascular cells, but this question remains to be

solved. In fact, in some experimental models the breakdown of blood-retinal barrier occurs very early, and this event maybe still the first event influencing retinal neuroglial damage.

It must be realized that the capillaries of the retina make together with the adjoining glial cells and neurons a true neurovascular unit, whose damage affects both the blood-retinal barrier and neuroglial function.

In conclusion, with the present level of knowledge, diabetic retinopathy should be considered a disease of the retinal microvascular unit, initiated by microvascular and neurodegenerative damage with an inflammatory, reactive, component. These alterations appear to be due to the hyperglycemia of diabetes and resulting chronic glucose toxicity to the retina.

Experimental studies have shown consistently that intensive therapy sufficient to minimize hyperglycemia inhibits the development of retinopathy. Toxicity of excess glucose to the retina include activation of protein kinase C, alteration of aldose reductase, formation of advanced glycated end products, abnormalities in the hexosamine pathway and in association , over production of reactive oxygen species by the mitochondrial election transport chain. There is an association of disease pathways that is highly complex.

All these mechanisms of toxic damage of the retinal microvasculature and retinal neuronal and glial cells may occur in association or separately in different patients contributing to the variability of retinopathy progression in individual patients and different risks for development of the retinopathy complications, clinically significant macular edema and proliferative retinopathy in different patients.

Chapter 5
Biomarkers of diabetic retinopathy progression

Pivotal studies have confirmed the importance of metabolic diabetes control in the prevention, development and progression of diabetic retinopathy (DCCT 1993; Holman et al, 2008). Furthermore, treatments are now available that together with laser photocoagulation have been shown to be effective in delaying vision loss.

It can be difficult, however in clinical practice to predict the clinical course and to identify which eyes/patients will develop vision-threatening retinopathy complications. Duration of diabetes, glycemic and blood pressure control only partially explain the variance in diabetic retinopathy development and progression (Hove et al, 2006; Holman et al, 2008).

For instance, patients with good glycemic control may develop rapidly progressive forms of diabetic retinopathy, whilst other people with relatively poor glycemic control may take several years to manifest signs of diabetic retinopathy.

This discrepancy highlights the need to identify biomarkers of disease progression and the importance of better understanding of the pathogenesis of diabetic retinopathy involved in disease progression of each individual.

Biomarkers are indicators of biological and pathological processes that can be measured objectively. They are clinically useful when the biomarker precedes the development of dreaded outcomes, thus serving as a risk factor, or when the biomarker acts as a surrogate for an outcome that should be avoided.

Identification of biomarkers for diabetic retinopathy progression are expected to contribute in early diagnosis, improved clinical monitoring and better risk stratification among patients with diabetes. Importantly, biomarkers should be an objective measure of disease progress that could be acquired quickly and resource saving without much strain on the patient.

We will review here candidates for retinal biomarkers, genetic factors and circulating biomarkers. Our personal contribution has focused on retinal biomarkers and identification of potential genetic expression profiles. The present available information on candidates for circulating biomarkers will be briefly reviewed.

5.1. Accepted clinical outcomes of retinopathy progression

Validation of retinal biomarkers of diabetic retinopathy progression must involve demonstration that these biomarkers are associated with vision loss,

the most generally accepted clinically outcome. This is a major problem as it is well recognized that vision loss only occurs when approximately 50% of the macula neuronal component is damaged. Vision loss is clearly a late development in retinal disease and what we need is to identify outcomes that can be identified well before vision loss is present.

Vision loss is associated with the two major complications of diabetic retinopathy, clinically significant macular edema and proliferative diabetic retinopathy, and does not occur before these complications develop. Therefore, clinically meaningful outcomes include clinically significant macular edema and proliferative diabetic retinopathy.

The hard clinical endpoints which have been generally accepted in previous studies include:

a) mean difference between groups in visual acuity of at least 3 lines in a ETDRS type chart. i.e., doubling of the visual angle; b) mean difference in visual field of at least 10 decibels; c) reduction in percentage of patients with vitreous hemorrhage; d) reduction in percentage of patients with rubeosis; e) reduction in occurrence of retinal detachments; e) need for photocoagulation treatment according to DRS and ETDRS guidelines for clinically significant macular edema and proliferative diabetic retinopathy (Table 5.1). This last one is of major relevance since it involves the occurrence of the two major complications of diabetic retinopathy, clinically significant macular edema (CSME) and proliferative diabetic retinopathy (PDR), which may occur before vision loss occurs.

Table 5.1. Diabetic Retinopathy Clinical Outcomes

Terminal Outcomes
Mean difference in visual field of at least 10 decibels
Reduction of percentage of patients with vitreous hemorrhage
Reduction in percentage of patients with rubeosis
Reduction in occurrence of retinal detachments
Accepted Clinically Significant Outcomes
Mean difference in visual acuity of 3 lines in ETDRS chart
Need for photocoagulation treatment according to DRS and ETDRS guidelines. ↓ Reducing vision threatening diabetic retinopathy complications: CSME and PDR

All of these are associated with advanced stages of diabetic retinopathy. They have a clear clinical significance but only represent late stages of diabetic retinopathy. There is now a clear need for identifying retinal biomarkers of disease progression, occurring in the earlier stages of the retinal diabetic disease, that predict development of these accepted clinical outcomes.

5.2. Retinal biomarkers of retinopathy progression

Retinal biomarkers have become a crucial target in research, because they can assist in elucidating mechanisms of disease, determine the risk of disease progression and serve as surrogate outcome parameters for clinical studies.

Candidates for retinal biomarkers of retinopathy progression are: microaneurysm turnover values in the central macula, central macular thickness increase, alteration in multifocal ERG implicit times (Table 5.2).

Table 5.2. Candidates for Retinal Biomarkers of Disease Progression

Candidates for Retinal Biomarkers of Disease Progression
Microaneurysm turnover values in the central macula
Central macular thickness increase
Alteration in Multifocal ERG implicit time

5.2.1. Microaneurysm turnover

Microaneurysms and hemorrhages identified as red-dots are the initial changes seen on ophthalmoscopic examination . They may be counted on fundus photography and red-dot counting has been suggested as an appropriate marker of retinopathy progression (Klein et al, 1995a/b).

It must be realized that red-dot formation and disappearance are dynamic processes. During a 2-year follow-up of 24 type 1 diabetics with mild background diabetic retinopathy using fluorescein angiography, Hellstedt and Immonen (1996) observed 395 new microaneurysms and the disappearance of 258 previously identified.

Generally, the disappearance of a microaneurysm is not a reversible process and indicates vessel closure and progressive vascular damage. Therefore, to assess progression of retinopathy, microaneurysm counting should take into account not only every newly developed microaneurysms identified in a new location but also the disappearing ones.

Microaneurysm disappearance is most probably due to thrombotic phenomena leading to subsequent rerouting of capillary blood flow and progressive remodeling of the retinal vasculature in diabetes (Boeri et al, 2001). The presence and number of microaneurysms and their rates of formation and disappearance are, therefore, good candidates as markers of retinal vascular remodeling and may be good indicators of retinopathy progression.

Microaneurysm counting on fundus photographies and microaneurysm counting on fluorescein angiography have been proposed as predictive indicators for progression of diabetic retinopathy (Kohner et al, 1986). A recently developed software, the RetmarkerDR (methodology described in chapter 3), allows the identification of the exact location of each red-dot in successive fundus photographs performed in each eye. Identification of the exact location of an individual red-dot is considered particularly important because a new microaneurysm is considered to develop only once in a specific location, its disappearance being generally associated with capillary closure, leaving in its place mainly remnants of basement membrane (Ashton, 1974; Cunha-Vaz, 1978a).

Our studies have demonstrated a steady turnover of microaneurysms in the diabetic retina, even in the initial stages of retinopathy. In fact, most microaneurysms show a lifetime of less than 1 year, with new ones being formed and disappearing at rates which vary between different patients, confirming previous reports (Kohner and Dollery, 1970).

Using this new methodology, we analyzed data from a group of 113 type 2 diabetic patients with mild-to-moderate nonproliferative diabetic retinopathy, followed up for 2 years as controls in diabetic retinopathy clinical trials, and thereafter by usual care at the same institution (Nunes et al, 2009).

Microaneurysm turnover from the initial 2 years and the occurrence of clinically significant macular edema during the following 8 years were analyzed in this retrospective 10-year follow-up study.

At the end of the 10-year follow-up period, 17 out of the 113 patients developed clinically significant macular edema needing photocoagulation.

At baseline, patients that developed clinically significant macular edema presented HbA1C levels significantly higher (mean ± SD: 8.5 ± 1.2%) than the group of patients that did not develop clinically significant macular edema (mean ± SD: 7.3 ± 1.2%, p = 0.001; table 2). No statistically significant differences were found beween clinically significant macular edema and non-clinically significant macular edema eyes for blood pressure, cholesterol, HDL, LDL and triglyceride levels at baseline.

When counting the total number of microaneurysms over the first 2 years of the follow-up, a significant increase in the number of microaneurysms was found for the clinically significant macular edema eyes (p=0.002), while for the non-clinically significant macular edema eyes the number of microaneurysms remained relatively constant (p=0.647).

A microaneurysm formation rate of at least 2 microaneurysms/year was found in 12 of the 17 eyes that developed clinically significant macular edema (70.6%), whereas this was only found in 8 of the 96 eyes that did not develop clinically significant macular edema during the 10-year follow-up period (8.3%).

This study showed that in the initial stages of diabetic retinopathy higher microaneurysm turnover obtained from color fundus photography is a good indicator of retinopathy activity and development of clinically significant macular edema needing photocoagulation.

Our results have since been confirmed by another research group in Munich using also the RetmarkerDR (M. Ulbig et al, data presented at 11th EURETINA congress, London, UK, May 26-29, 2011). In their study they have analysed a group of 160 eyes that were followed by fundus photography during a period of 5 years (CALDIRET study) (Christos et al, 2009), and were able to compare 49 that did develop clinically significant macular edema over the period of the study with 111 eyes that did not develop clinically significant macular edema. An increased microaneurysm formation rate was confirmed to be clearly associated with development of clinically significant macular edema. Values of microaneurysm formation rate greater than 2 per year in this early stage of retinopathy are present in 71,4% of the eyes that developed clinically significant macular edema. In clear contrast, the eyes

that did not develop clinically significant macular edema during the period of the study showed a microaneurysm formation rate less that 2 per year in 68,5% of the cases. This study, using also the RetmarkerDR software confirms the studies by our group in which 70,6% of the eyes that developed clinically significant macular edema showed a microaneurysm formation rate greater than 2.

Sharp et al (2003) found that the microaneurysm turnover varied widely between eyes of the same retinopathy level. This is also consistent with our findings. Microaneurysm turnover was shown in our study to vary widely between patients that were classified with the same retinopathy level.

Microaneurysm turnover computed from nonivasive color fundus photography appears to be a biomarker to identify eyes/patients at risk of progression to clinically significant macular edema and may serve as a surrogate outcome for development of clinically significant macular edema.

More recently, we performed a prospective, observational study designed to follow eyes/patients with mild nonproliferative diabetic retinopathy (grades 20 and 35) for a period of two years or until the time of development of a vision threatening diabetic retinopathy complication, clinically significant macular edema needing laser photocoagulation (Ribeiro et al, 2012).

Four hundred and ten patients, men and women, with diagnosed adult-onset type 2 diabetes, aged 40 to 75 years, with mild nonproliferative diabetic retinopathy (levels 20 and 35 of ETDRS classification) with best corrected visual acuity ≥ 95 ETDRS letters (20/25), were included. One eye per patient was selected as the study eye. At the three study visits, V0 (entry), V6 (6 months and V24 (2years), the study eye underwent a complete eye examination which included fundus photography and Optical Coherence Tomography (OCT).

The field 2 color fundus images were subjected to automated microaneurysm analysis using the RetmarkerDR (Critical Health SA, Coimbra, Portugal). The RetmarkerDR allows to compute for each eye the number of microaneurysms in each visit, the number of new microaneurysms that appear from one visit to the other (microaneurysm formation rate), the number of microaneurysms that disappeared from one visit to the other (disappearance rate) and the turnover, i.e., the sum of the microaneurysm formation and disappearance rates.

The automated computer-aided diagnostic system, Retmarker DR, consists of software earmarking microaneurysms and vascular lesions; it includes a co-registration algorithm that allows comparison within the same retinal location between different visits for the same eye. Being a deterministic algorithm, its performance is not affected by fatigue, stress, ambient light conditions, or other factors that may influence a human grader. Thus RetmarkerDR brings objectivity to subjective tasks (Figures 3.1 and 3.2).

The algorithm detects the presence of microaneurysms and red-dot like lesions. To detect these pathologies, the images are initially converted to processing size. Next follows contrast normalization and enhancement based on principal component analysis. Then, dark objects of a given size are detected and used as candidates.

For each of these candidates, features such as area, shape, intensity distribution, and gradient magnitude distribution are extracted using a region covariance descriptor. Next, a state of the art classifier, based on support vector machines is used to classify the candidates as true or false. The training of this classifier is done with a dataset in which a different grader was asked to earmark only small lesions that appeared as a round or ovoid red spot of 20 – 200 μm in diameter with regular borders and located within the superior and inferior arcades. Since by ophthalmoscopy or colour fundus photography microaneurysms are identified as deep red dots, sometimes they are difficult to differentiate from punctuate haemorrhages or localized vascular abnormalities. Punctuate haemorrhages typically have irregular borders, if the borders happen to be more regular they may be wrongly classified as microaneurysms (Fleming, 2006; Tuzel et al, 2006; Nunes et al, 2009). The images used for training are not part of the dataset from which the results are generated.

The images from field 2 are co-registered (Bernardes et al, 2005) to indicate disease activity in the central 3000-μm circle of the macula. Image co-registration is achieved by extracting a retinal vascular tree, which is used for landmarks during the registration process. A rigid registration estimates the translation based on fovea displacement. The rotation is estimated using a polar representation of the vascular tree. This

rigid transformation is then adjusted to obtain exact pairings of selected landmarks (Ferreira et al, 2005).

Of the 410 eyes/patients that entered the study, 348 were considered for analysis because they reached either the study endpoint, clinically significant macular edema (CSME) needing laser photocoagulation, or performed the last visit (V24) (Tables 5.3 and 5.4).

Table 5.3. Patient characteristics at baseline comparing the two patient groups, the ones that did not develop CSME and the ones that developed CSME (P value for statistically significant differences between CSME and non-CSME eyes; SD – Standard Deviation; IQR- Inter-Quartile Range – 1st and 3rd quartiles; NS – Not Significant p>0.05)

	Non-CSME (n=322)		CSME (n=26)		P
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
Age [years]	60.8 ± 8.2	*62.0 (55.0-67.0)*	62.3 ± 8.9	*63.0 (54.0-70.0)*	NS
Duration of Diabetes [years]	10.0 ± 5.1	*10.0 (6.0-14.0)*	11.0 ± 4.8	*11.5 (7.0-15.0)*	NS
HbA_{1C} [%]	7.9 ± 1.4	*7.6 (6.9-8.8)*	8.6 ± 1.8	*8.1 (7.3-10.0)*	0.022
Cholesterol [mg/dl]	195.2 ± 39.3	*193.0 (167.0-218.0)*	197.2 ± 38.9	*197.0 (164.0-228.0)*	NS
HDL [mg/dl]	50.9 ± 12.9	*49.0 (42.0-57.0)*	50.1 ± 11.9	*48.0 (44.0-55.0)*	NS
LDL [mg/dl]	126.1 ± 30.0	*124.0 (104.0-146.0)*	129.3 ± 30.2	*121.5 (105.0-150.0)*	NS
Glycose [mg/dl]	175.2 ± 73.3	*162.0 (119.0-225.0)*	180.9 ± 74.5	*171.0 (128.0-239.0)*	NS
Triglycerides[mg/dl]	180.8 ± 123.9	*147.0 (104.0-220.0)*	161.0 ± 77.2	*142.0 (112.0-199.0)*	NS
Systolic Blood Pressure [mmHg]	151.5 ± 21.0	*151.0 (137.0-164.0)*	151.6 ± 20.0	*147.5 (135.0-168.0)*	NS
Diastolic Blood Pressure [mmHg]	75.9 ± 10.9	*76.0 (69.0-82.0)*	74.5 ± 11.5	*71.5 (65.0-84.0)*	NS
BCVA [letters]	98.8 ± 2.1	*100.0 (100.0-100.0)*	98.8 ± 2.1	*100.0 (100.0-100.0)*	NS
Retinal Thickness in the central 500 μm [μm]	181.5 ± 24.8	*179.6 (164.8-199.3)*	193.2 ± 32.6	*186.2 (174.5-206.3)*	0.025
Retinal Thickness in the central 1500 μm [μm]	241.6 ± 21.0	*243.4 (227.6-255.4)*	250.0 ± 22.3	*250.5 (233.5-260.6)*	NS
Number of Microaneurysms	3.3 ± 3.7	*2.0 (1.0-4.0)*	6.2 ± 5.4	*4.5 (2.0-11.0)*	<0.001
Microaneurysm Formation Rate	2.5 ± 3.5	*1.0 (0.0-3.0)*	6.3 ± 8.3	*3.5 (1.0-8.0)*	<0.001
Microaneurysm Disappearance Rate	2.5 ± 2.8	*2.0 (0.0-3.0)*	4.8 ± 4.7	*3 (2.0-7.0)*	<0.001
Microaneurysm turnover	5.0 ± 5.2	*4.0 (1.0-7.0)*	11.2 ± 11.2	*9.0 (3.0-16.0)*	<0.001

Table 5.4. Multivariate Poisson Regression Analysis. Incidence-Rate Ratios (IRR) and 95% Confidence Interval (CI) for the IRR; P value for predictive parameters for CSME eyes

	IRR	95% CI	P
Males / Females	0.601	0.239- 1.511	0.279
Age [years]	1.03	0.976- 1.098	0.251
Duration of Diabetes [years]	1.008	0.931- 1.092	0.842
Patients taking Insulin	0.957	0.355- 2.582	0.932
HbA1C [%]	1.400	1.039- 1.886	0.027
Cholesterol [mg/dl]	0.990	0.949- 1.033	0.651
HDL [mg/dl]	1.002	0.949- 1.058	0.934
LDL [mg/dl]	1.016	0.967- 1.068	0.521
Glycose [mg/dl]	0.996	0.988- 1.004	0.339
Triglycerides [mg/dl]	0.998	0.993- 1.004	0.597
Systolic Blood Pressure [mmHg]	0.996	0.970- 1.022	0.759
Diastolic Blood Pressure [mmHg]	1.007	0.960- 1.056	0.779
BCVA [letters]	1.010	0.816- 1.251	0.924
Retinal Thickness in the central 500 μm [μm]	1.012	0.985- 1.039	0.398
Retinal Thickness in the central 1500 μm [μm]	1.008	0.971- 1.046	0.680
Number of Microaneurysms	1.023	0.908- 1.151	0.709
Microaneurysm turnove	1.085	1.014- 1.160	0.018

Of these 348 eyes/patients, 26 were diagnosed during the two-year period of follow-up as having clinically significant macular edema and were treated with laser photocoagulation. The other 322 eyes/patients completed the last visit of follow-up without developing clinically significant macular edema.

Microaneurysm turnover was 11.2±11.2 is the 26 eyes/patients that developed clinically significant macular edema and 5.0±5.2 in the remaining 322 eyes ($p<0.001$). The microaneurysm turnover showed a higher predictiveness for clinically significant macular edema than the remaining microaneurysm parameters with a ROC area =0.695. for a microaneurysm turnover cut-off of 9 or more, a sensitivity of 57.7% and a specificity of 81.2% was achieved (i.e. 79.4% of the eyes are correctly classified).

Eyes with a microaneurysm turnover higher than 9 during the initial 6 month period showed a higher tisk for clinically significant macular edema development than eyes with a lower microaneurysm turnover (OR=5.886; 95% CE= (2.503-13.844).

The microaneurysm turnover predictive values for clinically significant macular edema development were, for the period of two years of follow-up a positive predictive value of 20%, and a negative predictive value of 96%, showing that a low microaneurysm turnover value predicts slow disease progression and indicates that development of clinically significant macular edema is unlikely.

Furthermore, eyes that developed clinically significant macular edema before the 24 months visit presented a higher microaneurysm turnover (26.6±15.9) when compared to the eyes in which clinically significant macular edema was detected only at month 24 (12.8±3.6 – p=0.018), indicating again that there is correlation between high turnover values and risk for the development of clinically significant macular edema for eyes with same ETDRS retinopathy level.

When considering the systemic parameters only HbA1c values at baseline correlated with the development of clinically significant macular edema. The other systemic parameters examined such as blood pressure (systolic and diastolic) and other blood lipids (triglycerides, cholesterol, HDL and LDL) did not show any correlations with the occurrence of clinically significant macular edema.

Multivariate analysis showed also that microaneurysm turnover is predictive of clinically significant macular edema independently of the HbA1c values.

This two year prospective, longitudinal study of patients with diabetes type 2 and mild nonproliferative diabetic retinopathy (ETDRS levels 20 and 35) showed that microaneurysm turnover in field 2 of the eye fundus is a good biomarker for retinopathy worsening and development of clinically significant macular edema needing photocoagulation.

In conclusion, these results based on precise identification of the location of each red-dot on fundus photograph of diabetic eyes, suggest that microaneurysm formation and disappearance rates may be an appropriate retinal biomarkers of disease activity and retinopathy progression to

clinically significant macular edema, a clinical outcome that is associated with vision loss.

From the data available a microaneurysm formation rate higher than 2/year determined on repeated fundus photography exams predicts that clinically significant macular edema has a higher likelihood of development in a 10-year period in these eyes. Also, eyes that show in a 6-month period a microaneurysm turnover (microaneurysm formation rate + microaneurysm disappearance rate) lower than 9 are highly unlikely to worsen and develop clinically significant macular edema within a 2-year period.

5.2.2. Macular thickening

Clinically significant macular edema has been defined by the Early Treatment Diabetic Retinopathy Study (ETDRS) using the following criteria: a) Thickening of the retina located less than 500 μm from the center of the macula; b) Hard exudates (with thickening of the adjacent retina) located less than 500 μm from the center of the macula; and c) A zone of retinal thickening 1 disk area or larger in size, located less than 1 disk diameter from the center of the macula (ETDRS, 1985).

The clinical evaluation of macular edema is characterized by its difficulty. Direct and indirect ophthalmoscopy may reveal nothing but an alteration of the foveal reflexes. Slit lamp biomicroscopy demonstrate changes in retinal thickness in the macular area but it is dependent on the observer. These parameters are now well identified using OCT. Measurement of retinal thickness by OCT is reliable and an increase in retinal thickness defines macular edema (Hee et al, 1995).

OCT brought new insights about morphological changes of the retina in diabetic retinopathy and diabetic macular edema. It showed that macular edema may assume different morphologic patterns (Yamamoto et al, 2001; Kim et al, 2006). In addition, a quantitative characterization of macular edema became feasible, as determined by measurements of retinal thickness and volume. OCT has been demonstrated to be more sensitive than slit-lamp biomicroscopy in detecting small changes in retinal thickness (Hee et al, 1995; Yang et al, 2001; Massin et al, 2006; Lang, 2007) and is clearly less subjective. In cases of diabetic macular edema, OCT scans may demonstrate

diffuse thickening of the neurosensory retina and loss of the foveal depression; cystoid retinal changes, manifest as areas of low intraretinal reflectivity and serous retinal detachment, alone or combined.

The possibility to quantify retinal thickness by OCT is based in the distance between the anterior and posterior highly reflective boundaries of the retina, using appropriate algorithms (Hee et al, 1998).

OCT images of diabetic macular edema depict the presence of low intraretinal reflectivity, due to fluid accumulation in the extracellular space of the retina. The process begins as increased thickening with sponge-like appearance of the retinal layers, showing increase in the extracellular spaces, later advancing to the typical image of cystoid spaces (Otani et al, 1999; Alkuraya et al, 2005). Our research group has now shown that it is possible to measure the blood-retinal barrier alterations with OCT, a non-invasive procedure without the need for the intravenous injection of fluorescein (Bernardes et al, 2011). The alteration of the blood-retinal barrier can be quantified in the initial stages of edema and is a direct measure of the edema.

In summary, OCT is today the only method that allows an objective follow-up of subclinical diabetic macular edema. It allows a clear identification of the intraretinal fluid distribution and the presence or absence of vitreous traction. Furthermore, OCT allows a quantitative diagnosis of macular edema, as it is used to obtain numerical representation of the retinal thickness (Figure 3.9).

Clinical evaluation of macular edema should include the following parameters: extension of macular edema (i.e. thickened area); location of the edema in the macular area and particularly, central foveal involvement (central area, 500 μm wide); presence or absence of vitreous traction; and chronicity of the edema (i.e. time elapsed since initial diagnosis and response to therapy). These parameters are obtained directly from the OCT images and more detailed maps of retinal thickness can be obtained using appropriate software developed by our research group (Bernardes et al, 2008).

A potential retinal biomarker for clinically significant macular edema is increased macular thickening measured using OCT.

A progressive increase in retinal thickness measured by OCT appears to predict the development of clinically significant macular edema. In a recent prospective study performed by our group, a two-year follow-up study of

eyes with mild nonproliferative diabetic retinopathy ETDRS level of ≤35 from patients with diabetes type 2 performed by our research group, six-month increases in the central retinal thickness area of 10% or more and maximal increases in central retinal thickness values of 5% or more were predictive of development of clinically significant macular edema within the 2-year study period (Ribeiro et al, 2012).

Measurements of changes in retinal thickness have, clearly, the potential to become a valuable surrogate outcome for the development of clinically significant macular edema in studies of drug development for diabetic retinopathy. It must be realized, however, that macular edema does not translate directly into vision loss and the correlation between increased retinal thickness and vision loss has been shown to be weak (Nunes et al, 2010). However, clinically significant macular edema, is by definition, increased retinal thickness involving the central macula and its occurrence is the most frequent vision-threatening diabetic retinopathy complication. Improved understanding between subclinical macular edema as identified bt OCT and its progression to clinically significant macular edema with better definition of threshold values that predict progression to clinically significant macular edema is expected in the near future.

5.2.3. Changes in the implicit time of multifocal ERG

The mfERG has been proposed as capable of detecting early functional changes, to provide an index of retinal status, and to predict not only which eyes but also which retinal locations will develop new retinopathy signs in the near future (Bearse et al, 2006). The implicit time in mfERG (elapsed time from the stimulus to P1 peak) is spatially associated with retinopathy, correlates with retinopathy severity and is a predictor for the development of retinopathy over 1-year (Han et al, 2004a/b), and 3-year period (Ng et al, 2008). In addition, implicit time has greater sensitivity and less inter-subject variability than other mERG functional parameters such as b-wave amplitude and oscillatory potentials. Furthermore, it should be noted that mfERG P1 component is generated primarily by bipolar cells (Hood et al, 2002), which lie within the inner nuclear layer of the retina. Thus, the neurons primarily involved in generating the index of retinal function that

we are investigating lie in the same intraretinal location as the vascular cells that are implicated in diabetic retinopathy lesions. For all this reasons we have selected this parameter as the gold standard measurement of functional changes related to neurodegeneration in the eye of diabetic patients (Simó et al, 2012). An European funded multinational clinical trial, EUROCONDOR, will be looking at the potential role of mfERG as a predictor of diabetic retinopathy progression.

5.3. Genetic Biomarkers

Several studies have provided evidence that good diabetes control is important to prevent progression of diabetic retinopathy, but it is clear that some patients develop a rapidly progressing retinopathy despite good control, while others escape the development of severe retinopathy despite poor control.

The onset, intensity and progression of diabetic complications show large interindividual variations (Lobo et al, 2004). There is evidence from aggregation in families and specific ethnic groups, together with lack of serious complications in some diabetic patients with poor metabolic control that there is a genetic predisposition to develop some diabetic complications such as retinopathy (Warphea and Chakravarthy, 2003).

It is recognized that polymorphic variability in the genetic make-up of an individual can profoundly influence the expression of a gene and its response to environmental factors. As we predict that the impact of single common mutations on diabetic retinopathy development will be modest (increasing relative risk (RR) by 20–40% at most), the main issue of clinical relevance is whether the conferred risk of such a mutation is very much higher in some population subgroups (Humphries et al, 2001).

Such subgroups might be those carrying a second important mutation in another gene and such individuals might be identified using conventional genetic strategies. Alternatively, one might identify individuals exposed to a given environment which amplifies the risk associated with that gene (i.e., gene–environment interaction).

Diabetic retinopathy shows familial aggregation and variation in disease severity which is not explained by environmental, biochemical or biological

risk factors alone. It is important to recall that our work has confirmed the clinical impression that there are substantial variations in onset and severity of retinopathy in different patients which are independent of the duration of diabetes and level of glycemic control.

A relatively large number of candidate genes have been examined in patients with diabetes but clear genotype–phenotype associations have not yet been identified.

One of the major problems is associated with poor characterization of different retinopathy phenotypes. It is fundamental before embarking in a search for candidate genes to define clinical phenotypes characterized by specific patterns of severity and progression of diabetic retinopathy. It is clear that it is necessary to identify first and well the diabetic retinopathy phenotypes that are associated with rapid progression of the retinopathy to severe forms of the disease, such as macular edema and proliferative retinopathy. Only then, studies on candidate genes are worth pursuing, involving appropriately well-defined subgroups of patients (Warpeha and Chakravarthy, 2003). This goal is now becoming possible with the results of our research group published for the first time in 2004 (Lobo et al, 2004).

Variations in the genes expressed in the aldose reductase pathway may influence microvascular susceptibility. Aldose reductase is strongly expressed in retinal pericytes and is also found in the vascular endothelium (Viñores et al, 1993). It has been suggested that 7q35 is a susceptibility region for diabetic retinopathy by virtue of the aldose-reductase gene (AR2) (Patel et al, 1996).

In type 1 diabetes the strongest genetic risk component is localized within the major histocompatibility complex. The HLA region that is located on 6p21, has also been implicated as genomic region of interest for susceptibility to retinopathy in both type 1 (Stewart et al, 1993) and type 2 diabetes (Serrano-Rios et al, 1983).

There are indications that the vascular complications of diabetes are related with the formation of advanced glycated-end products (AGE). The glycation of proteins and lipids is a result of hyperglycemia and this effect of AGEs is mediated the AGE receptor (RAGE) which is regulated by a gene (Hudson et al, 2001).

Angiotensin-converting enzyme (ACE) is another important mediator of vasoconstriction and homeostasis; however, studies to date on genetic markers of members of this signalling pathway (Matsumoto et al, 2000) have not shown definitive evidence of direct genetic risk. It remains, however, an interesting candidate gene to play a role in diabetic retinopathy. Clinical studies have shown that ACE inhibition may play a useful specific role in the management of diabetic retinopathy.

Large interindividual differences in plasma ACE levels exist but are similar within families, suggesting a strong genetic influence. The human ACE gene is found on chromosome 17 and contains a restriction fragment length polymorphism consisting of the presence (insertion, I) or absence (deletion, D) of a 287 base pair ''ALU'' repeat sequence in intron 16 (Rigat et al, 1990). In 1992, the I/D polymorphisms of the ACE gene were reported to be associated with risk of myocardial infarction (Cambien et al, 1992) and this effect, though more modest then originally reported has been confirmed in larger studies (Keavney et al, 2000).

Glucose may itself be the mitigating environmental factor that induces expression of the polymorphism.

Our research group has initiated a research programme trying to associate candidate genes to well defined phenotypes of diabetic retinopathy progression. Preliminary results obtained in 236 patients with diabetes type 2 and categorized in different phenotypes showed promising results, although still waiting for further data collection from larger number of patients (Lobo et al, 2012). 236 patients (147 male and 89 female) with a mean age of 60.8±5.1 years were first classified during a two-year period of follow-up as: Phenotype A (normal retinal thickness and microaneurysm turnover lower than 9), 105 patients (44.5%); Phenotype B (increased retinal thickness and microaneurysm turnover lower than 9), 60 patients (25.4%); and Phenotype C (microaneurysm turnover higher than 9 and variable thickness), 71 patients (30.1%). Development of clinically significant macular edema occurred during a 2-year follow-up period in 9.3% of these patients (22 eyes from the 236 eyes/patients).

The candidate genes tested based on gene organization and Single Nucleotide Polymorphisms (SNPs) density were: ALR (AKR 1B1), VEGF, TNF-a, NOS-1, RAGE, ICAM-1 and ACE. A statistically significant difference between the 3 phenotypes was found for NOS-1 – rs4519169 ($p=0.031$).

Between eyes that developed clinically significant macular edema during the 2-year follow-up period and eyes that did not develop statistically significant differences were also found for AKR1B1 – rs759853 (p=0.034) and ICAM-1 RS 1801714 (p=0.044). These results need now to be continued and extended.

5.4. Circulating biomarkers

It is well known that glycemic control, diabetes duration and blood pressure are risk factors for microvascular complications in diabetes. Hemoglobin A1c is well recognized as the circulating biomarker of relevance of diabetic metabolic control (Hirsch and Brownlee, 2010). More recently, other biomarkers have been investigated. In a cohort of the Diabetes Control and Complications Trial, the severity of retinopathy was associated with increasing triglyceride levels and inversely correlated with HDL levels.

Some authors have suggested that serum apolipoprotein levels may be stronger biomarkers of diabetic retinopathy than traditional cholesterol, or even the LDL-to-HDL ration (Allen et al, 1998). The proteins apoAI (found in HDL) and apoB (found in LDL), and specifically the apoB-to-apoAI ration, were significantly and independently associated with diabetic retinopathy development and severity.

Other studies have suggested a significant association between the plasma plasmin-μ2-antiplasmin complex and severity of diabetic retinopathy. A marked increase was also found in protein oxidation in patients with diabetic microangiopathic complications (including retinopathy) through assessment of advanced oxidation protein products. Elevated levels of cytokines in ocular fluid may reflect disease activity but also reveal mechanisms of disease, such as in diabetic macular edema and age-related macular degeneration. With any diabetic retinopathy specifically associated with type 1 diabetes a significant increase in soluble E-Selectin (p=0.0017) and soluble Vascular Cell Adhesion Molecule -1 (VCAM-1) (p<0.001) was identified compared to diabetic patients without retinopathy (Vestweber, 2007). Among type 2 diabetic patients, no significant differences were found in C-Reactive Protein (CRP) (p=0.61) or vascular endothelial growth factor (VEGF) (p=0.624) between patients with and those without retinopathy. There was also no

significant difference in Endothelin (ET- I) (p= 0.33) levels when compared type 2 diabetic patients with diabetic retinopathy to healthy non-diabetic controls. In conclusion, there is, at the present, no well identified circulating biomarker for diabetic retinopathy progression.

5.5. Summary

Studies such as the Diabetes Control And Complications Trial, the United Kingdom prospective diabetes study, the diabetic retinopathy study research group and the early treatment diabetic retinopathy study validated methods now considered standard in treating diabetic retinopathy when it occurs, i.e., tight control of blood glucose levels to prevent retinopathy and laser photocoagulation to halt progression after development of clinically significant macular edema or proliferative retinopathy. However, despite the aim of tight blood glucose control and the use of retinal photocoagulation, blindness still occurs. Therapies targeted at the earliest stages of retinal disease, involving necessarily the demonstration of efficacy of a new drug are needed and remain a priority for eye research. To achieve this goal is urgent to identify biomarkers of disease progression that can be accepted as surrogates for generally accepted endpoints.

The clinical endpoints that have been accepted in the past only give indications about the late, irreversible, stages of diabetic retinopathy.

Microaneurysm turnover on fundus photographs, taking into account their exact, specific location in the eye fundus has the potential to become an extremely valuable biomarker of the overall progression of diabetic retinal vascular disease. Microaneurysm turnover rate appears to be a direct indication of the progression of retinal vascular damage and activity of disease.

Reduction in macular thickening by measuring the changes in retinal thickness with dedicated instrumentation, is another promising alternative. The measurements are reliable, and changes in retinal thickness are a direct indication of macular edema and breakdown of the blood-retinal barrier. Another promising examination procedure is multifocal ERG. Testing of these potential biomarkers and their final validation is expected to contribute decisively to design clinical trials capable of evaluating the efficacy of new drugs capable to halt diabetic retinopathy in the initial stages of the disease.

Chapter 6
Phenotypes of diabetic retinopathy

It is well recognized that the duration of diabetes and the level of metabolic control acondition the development of diabetic retinopathy.

However, these risk factors do not explain the great variability that characterizes the evolution and rate of progression of the retinopathy in different diabetic individuals. There is clearly great individual variation in the presentation and course of diabetic retinopathy. There are many diabetic patients who after many years with diabetes never develop sight-threatening retinal changes, maintaining good visual acuity. However, there are also other patients that even after only a few years of diabetes show a retinopathy that progresses rapidly and may not even respond to available treatments.

6.1. Phenotypes of nonproliferative diabetic retinopathy

In a prospective 3-year follow-up study of the macular region in 14 patients with type 2 diabetes mellitus and mild nonproliferative retinopathy, using multimodal macula mapping we found that there is marked individual variations in the progression of diabetic retinopathy and activity of the retinal disease (Lobo et al, 2004).

In a span of 3 years, eyes with minimal changes at the start of the study (levels 20 and 35 of the Early Treatment Diabetic Retinopathy Study-Wisconsin grading) were followed at 6-month intervals in order to monitor progression of the retinal changes. The most frequent alterations observed, by decreasing order of frequency, were leaking sites (Lobo et al, 1999), areas of increased retinal thickness and microaneurysms / hemorrhages.

Leaking sites were a very frequent finding and reached very high blood-retinal barrier permeability values in some eyes. These sites of alteration in the blood-retinal barrier, well identified in leakage maps using the Retinal Leakage Analyzer, maintained, in most cases, the same location on successive examinations, but the blood-retinal barrier permeability values fluctuated greatly between examinations, indicating reversibility of this alteration.

Areas of increased retinal thickness were another frequent finding. They were present in every eye at some time during the follow-up and were absent, at baseline, in only 2 of the 14 eyes. This confirms previous observations by our group (Lobo et al, 2004) and by others (Fritsche et al, 2002).

The number of microaneurysms and small hemorrhages increased in most eyes during the 3-year follow-up period. This was particularly well demonstrated when the location of each microaneurysm was taken into consideration. This increase in the number of microaneuryms was considered to have the potential to be the most reliable indicator of retinal vascular damage and remodeling of the retinal circulation, particularly in the initial stages of diabetic retinopathy.

Increased rates of microaneurysm accumulation were registered in eyes that had more microaneurysms at baseline and higher values of blood-retinal barrier permeability during the study. In summary, in this study, the rate of microaneurysm formation appeared to have the potential to be a good indicator of retinopathy progression. We realized that by combining different imaging techniques, multimodal imaging of the macula made apparent three major patterns of disease progression occurring during the follow-up period of 3 years. Pattern A included eyes with reversible and relatively little abnormal fluorescein leakage, a slow rate of microaneurysm formation and a normal foveal avascular zone. This group appeared to represent eyes presenting slowly progressing retinal disease. Pattern B included eyes with persistently high leakage values, indicating a dominant alteration in the blood-retinal barrier, high rates of microaneurysm accumulation and a normal foveal avascular zone. All these features suggested a rapid and progressive form of the disease. This group may identify a ‘wet’ form of diabetic retinopathy. Pattern C included eyes with variable and reversible leakage, increased microaneurysm formation and an abnormal foveal avascular zone. This group is less well characterized considering the small number of eyes that showed a n abnormal foveal avascular zone. It may be that abnormalities of the foveal avascular zone may occur as a late development of groups A and B or progress rapidly as a specific ‘ischemic’ form (Table 6.1; Figure 6.1).

Table 6.1. Evolution of diabetic retinopathy

	VF/RLA	Microaneurysm formation rate	FA/FAZ	Phenotype
Pattern A (62%)	<4 ng/ml	<3/year	Normal	Slow progression
Pattern B (20%)	>4 ng/ml	>3/year	Normal	Leaky
Pattern C (18%)	<4 ng/ml	>3/year	Abnormal	Ischemic

FA=Fluorescein Angiography; RLA=Retinal Leakage Analyzer; VF= Vitreous Fluorometry; FAZ=Foveal Avascular Zone

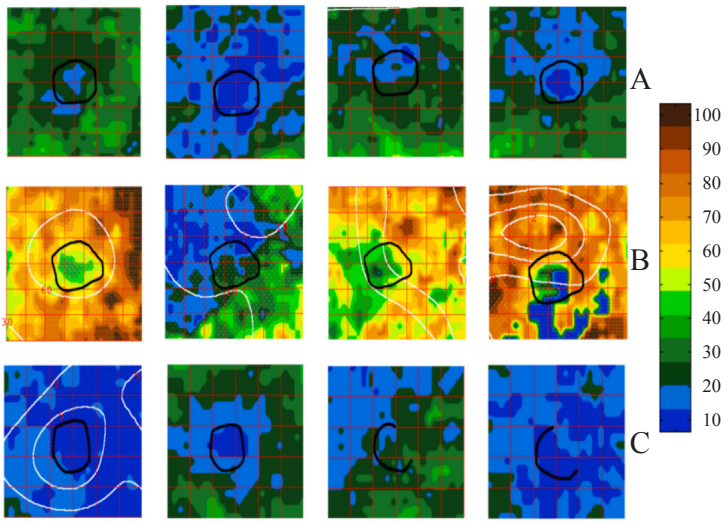

Figure 6.1. Multimodal images taken at 0, 12, 24 and 36 months visits (left to right) showing for each visit the foveal avascular zone – black contour – retinal leakage analyzer results and retinal thickness analyzer results. The retinal leakage analyzer color-coded maps of the blood-retinal barrier permeability indexes are shown; retinal thickness analyzer views show white dot density maps of the percentage increases in retinal thickness. Top row: pattern A. Note the little amount of retinal leakage over the 4 represented visits and the normal foveal avascular zone contour. This patient showed a slow rate of mictroaneurysm formation. Middle row: pattern B. Note the high retinal leakage showing a certain degree of reversibility and the normal foveal avascular zone contour. This patient showed a high rate of microaneurysm accumulation over the 3-year follow-up period. Bottom row: pattern C. Note the revesible retinal leakage and the development of an abnormal foveal avascular zone contour. This patient showed a high rate of microaneurysms formation.

We then extended our observations by following 57 patients with type 2 diabetes for 7 years; at the time of enrollment, all eyes presented mild nonproliferative diabetic retinopathy.

In this larger study, with longer follow-up, the three different phenotypes were again clearly identified after an initial 2-year follow-up period. The discriminative markers of these phenotypes were: microaneurysm formation rate, measurements of fluorescein leakage, and signs of capillary closure in the capillaries surrounding the foveal avascular zone.

After an average of 7 years of follow-up, 10 of these 57 eyes had developed clinically significant macular edema with clear indication for photocoagulation treatment. In this series of patients, after the initial 2-year follow-up period, 35 eyes (61% of the total) were identified as showing the characteristics of pattern A, i.e. slow progression, 12 (21 %) were classified as presenting pattern B, and the other 10 (18%) had the characteristics of pattern C.

Severe macular edema needing laser photocoagulation developed after 7 years of follow-up only in those eyes classified as belonging to patterns B and C. Of the 12 eyes classified as having pattern B, 5 (42%) developed severe macular edema. Similarly, of the 10 eyes identified with pattern C, 5 (50%) developed severe macular edema.

None of the eyes classified as belonging to pattern A developed severe macular edema in the 7-year follow-up period.

In summary, the slow progression type, pattern A, takes longer than 7 years to develop severe macular edema, one of the main complications of diabetic retinopathy, confirming that this subtype of diabetic retinopathy has a good prognosis.

On the other hand, both other types of diabetic retinopathy progression, the leaky type, or pattern B, characterized initially by particularly high levels of leakage, i.e. alteration in the blood-retinal barrier, and the ischemic type, or pattern C, characterized by signs of capillary closure, much more frequently lead to the development of severe macular edema needing photocoagulation, with incidences at 7 years of 42% and 50%, respectively.

If diabetic retinopathy is a multifactorial disease- in the sense that different factors or different pathways may predominate in different groups of cases with diabetic retinopathy - then it is crucial that these differences and the possible different phenotypes be identified (Grange, 1995). The characterization of three different phenotypes of diabetic retinopathy, with different progression patterns, opens particularly interesting perspectives

to gain more insight into the understanding and management of diabetic retinopathy (Table 6.2; Cunha-Vaz and Bernardes, 2005; Cunha-Vaz, 2007).

Table 6.2. Characterization of three different phenotypes of diabetic retinopathy

Phenotype A	• Slow progression (<2 red dots/year) • Accelerated ageing process (diabetes)
Phenotype B	• Rapid progression (>2 red dots/year) • Increased flow • Alterations of blood-retinal barrier – leakage • Increased retinal thickness – edema • Haemodynamic changes predominate
Phenotype C	• Rapid progression (>2 red dots/year) • Decreased flow • Foveal avascular zone outline changes • Thrombotic changes predominate

6.2. Identification of phenotypes of diabetic retinopathy progression using non-invasive procedures

The initial identification of 3 major patterns of progression of nonproliferative diabetic retinopathy was made using elaborated multimodal imaging methodology involving new examination technologies, some still in the research domain. Furthermore, the sample size was small.

In another study by our group we looked into a much larger population of eyes/patients with well characterized mild nonproliferative retinopathy in patients with diabetes type 2, in an effort to verify if similar phenotypes of progression of mild nonproliferative diabetic retinopathy could be identified using only non-invasive procedures such as digital color fundus photography and OCT.

A two-year prospective, observational study was designed to follow eyes/patients with mild nonproliferative diabetic retinopathy (grades 20 and 35) for a period of two years or at the time of development of clinically significant macular edema (clinically significant macular edema) needing laser photocoagulation.

Four hundred ten patients (410) were included, men and women with diagnosed adult-onset type 2 diabetes, age 40 to 75 years, mild nonproliferative diabetic retinopathy (20 and 35 of ETDRS classification), best corrected visual acuity ≥95 ETDRS letters (20/25).

Metabolic control was also assessed by measuring in the plasma concentrations of HbA1C and lipid fractionation identifying total cholesterol, HDL, LDL and triglycerides.

One eye per patient was selected the physician at baseline as the study eye based on the inclusion/exclusion criteria. When both eyes fulfil the same criteria one of the eyes was selected by choosing sequentially the right or the left eye.

At the three study visits, V0, V6 and V24 (or pre-treatment visit), the study eyes underwent a complete eye examination, which included best corrected visual acuity, as tested in the Early Treatment Diabetic Retinopathy Study (ETDRS), slit-lamp examination, intraocular pressure measurements, fundus photography and OCT.

Color fundus photography was performed according to the ETDRS protocol. The automated computer-aided diagnostic system, RetmarkerDR (Critical Health SA, Coimbra, Portugal), was used to automatically detect microaneurysms on the field-2 color fundus images.

OCT scans were performed using the Stratus OCT (Carl Zeiss Meditec, Dublin, USA).

To identify phenotypes of progression of mild nonproliferative diabetic retinopathy to clinically significant macular edema using non-invasive procedures a cluster analysis was performed based on the following parameters, at baseline: mean retinal thickness in the central 1000 μm in diameter macular area at baseline, increase retinal thickness in the central 1000 μm in diameter macular area at baseline from baseline to month-6 (V6) , number of microaneurysms and microaneurysm turnover (computed for the first 6 months of follow-up).

Cluster analyses are unsupervised segmentation techniques which builds models of the observed data in order to identify and create homogeneous groups (Everitt, 1995). These techniques groups data sharing some similarity measure or feature (Kaufman and Rouseeuw, 1990), being it either of the hierarchical or non-hierarchical type, respectively when the number of

underlying clusters is unknown or known a priori. In this work the first type was used as an exploratory tool to establish the number of homogeneous groups underlying (clusters) in the data set.

The Ward's method was used for the hierarchical clustering. This method creates clusters by agglomeration, i.e. starting by considering initially the existence of as many clusters as cases and keeping agglomerating clusters until it achieves a single one enclosing all of the cases. The Ward's method process promotes the minimization of the within-cluster variance using the square of the Euclidian distance as the similarity metric. It has been proposed in areas such as public health or medicine (Mathers and Dongseok, 2004; Rosenberg et al, 2004). Along the agglomerative process the dissimilarity measure between the grouped clusters. The analysis of these coefficients along the agglomeration process allows the number of homogeneous clusters in the data set to be identified. Moreover, to determine the best number of clusters the Calinsky-Harabasz pseudo-F statistics was also computed (Milligan and Cooper, 1985).

To perform cluster analysis on the collected data, a data normalization was performed since the range of values for different variables had one order of magnitude. In this way, the normalization was achieved through a Z-distribution, thus having a zero mean and unitary standard deviation (Z-score values).

To compare risk for clinically significant macular edema development between the identified phenotypes of mild nonproliferative diabetic retinopathy, Odds Ratio (OR) were computed.

From the 410 eyes/patients included in the study, 376 completed the first 6-months of follow-up from which only 348 eyes/patients reached either the study endpoint, clinically significant macular edema needing laser photocoagulation, or performed the last study visit, 24-month visit (Figure 6.2).

For cluster analysis, only the 376 eyes/patients that performed the 6-month visit and that have the turnover computed were considered.

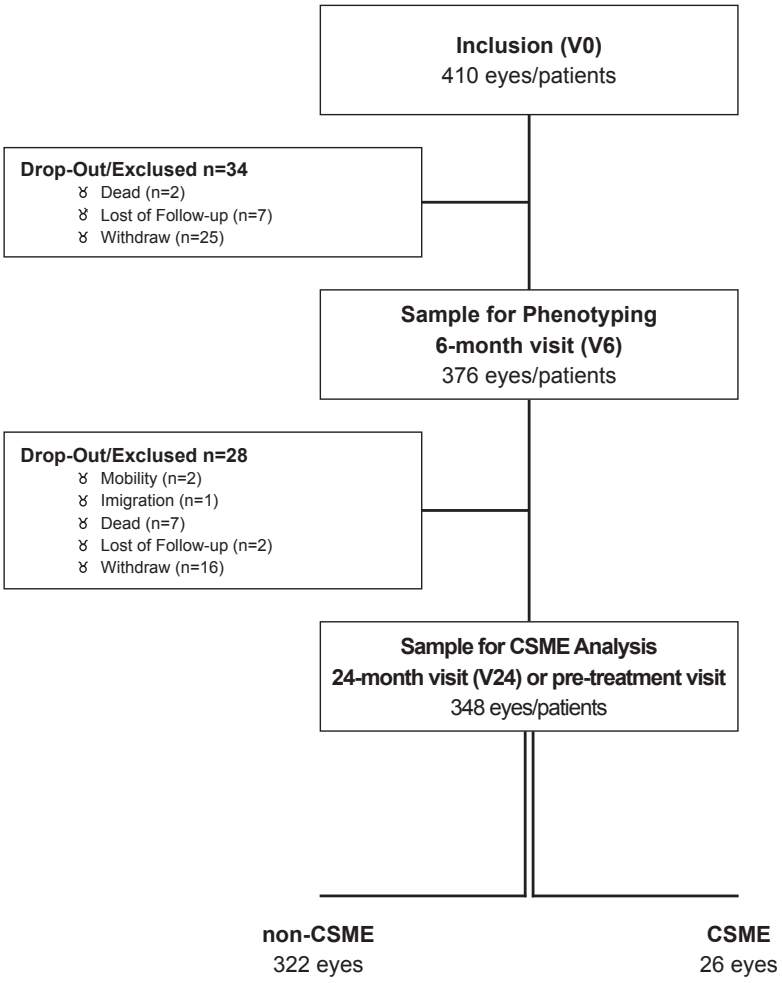

Figure 6.2. CONSORT flowchart (NPDR: Nonproliferative diabetic retinopathy; CSME: Clinically Significant Macular Edema needing photocoagulation treatment).

6.2.1. Clusters of mild nonproliferative diabetic retinopathy

The existence of 3 clusters was identified by the hierarchical clustering (Ward's method) since the dissimilarity measure for 3 clusters corresponding to the higher Calinsky-Harabasz pseudo-F statistics (for the 3 clusters solution the Calinsky-Harabasz pseudo-F statistics is 91.9 while for the 4 clusters solution the Calinsky-Harabasz pseudo-F statistics is 69.7) (Figure 6.3).

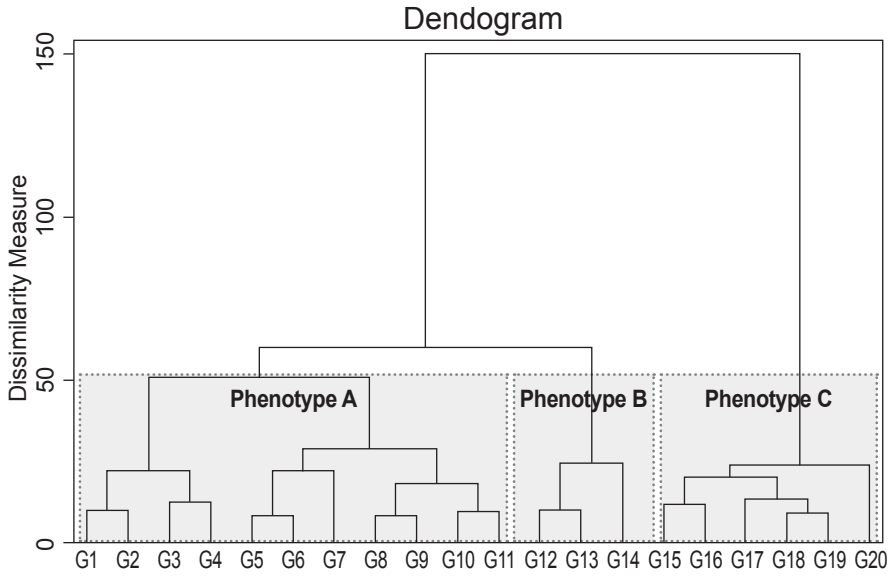

Figure 6.3. Dendogram and dissimilarity measures for the Ward's clustering (G represent groups of eyes/patients that were grouped).

The separation in 3 clusters results from the statistically significant differences for the microaneurysms and the retinal thickness parameters ($P<0.001$, Table 6.3). Statistically significant differences between clusters were also found for sex ($P=0.028$), age ($P<0.001$), HbA1C levels ($P=0.050$), LDL ($P=0.049$) and for BCVA ($P=0.001$) (Figure 6.4).

Table 6.3 – Clusters characteristics (n=376) (median value, inter-quartile range – IQR and P values for the 3 clusters – statically significant level adjusted to 0.02)

Median (IQR)	**Cluster C1 (n=181)**	**Cluster C2 (n=87)**	**Cluster C3 (n=108)**	**P**
Males / Females - Frequency (%)	*101 (55.8%) / 80 (44.2%)*	*62 (71.3%) / 25 (28.7%)*	*72 (66.7%) / 36 (33.3%)*	0.028
Taking Insulin (Yes / No) - Frequency (%)	*50 (27.6%) /*		*50 (27.6%) /*	
Age [years]	*62 (54-68)*	*64 (59-70)*	*59 (53-65)*	<0.001
Duration of Diabetes [years]	*10 (6-13)*	*10 (6-15)*	*10 (6-14)*	0.645
Creatinine [mg/dl]	*0.89 (0.78-1.01)*	*0.90 (0.80-1.03)*	*0.87 (0.76-1.01)*	0.279
HbA_{1C} [%]	*7.7 (6.7-8.8)*	*7.6 (6.9-9.0)*	*7.9 (7.0-9.1)*	0.050
Cholesterol [mg/dl]	*196 (169-218)*	*200 (177-222)*	*182 (161-215)*	0.058
HDL [mg/dl]	*49 (42-57)*	*50 (41-58)*	*48 (42-56)*	0.909
LDL [mg/dl]	*127 (107-148)*	*128 (110-149)*	*116 (101-136)*	0.049
Glycose [mg/dl]	*165 (118-226)*	*166 (110-219)*	*162 (127-226)*	0.742
Triglycerides [mg/dl]	*157 (109-236)*	*145 (106-208)*	*138 (99-189)*	0.088
Systolic Blood Pressure [mmHg]	*153 (136-166)*	*149 (141-165)*	*148 (134-159)*	0.159
Diastolic Blood Pressure [mmHg]	*75 (68-82)*	*75 (70-82)*	*76 (69-83)*	0.783
BCVA [letters]	*85 (83-89)*	*84 (82-88)*	*87 (84-89)*	0.001
Central Subfield Retinal Thickness (central 1000 µm) [µm]	*204 (192-212)*	*234 (227-245)*	*218 (198-235)*	<0.001
Central Subfield Retinal Thickness at month-6 (central 1000 µm) [µm]	*204 (190-219)*	*235 (222-247)*	*216 (202-231)*	<0.001
Number of microaneurysms [#]	*3 (1-4)*	*1 (1-2)*	*9 (6-12)*	<0.001
Microaneurysm Turnover [# per 6 months]	*3 (1-5)*	*1 (0-2)*	*9 (7-14)*	<0.001

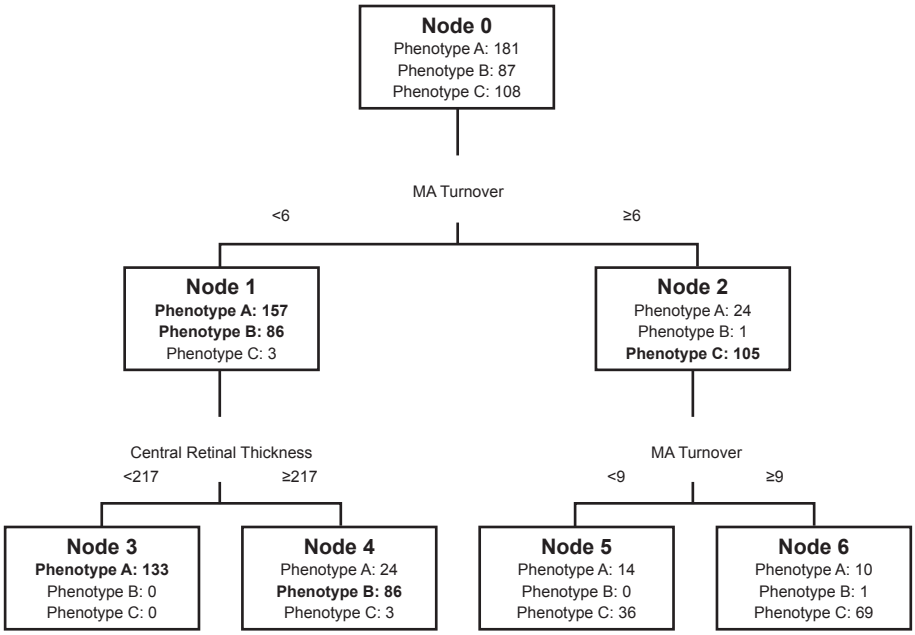

Figure 6.4. Decision and classification tree for the 3 clusters (CART).

Identification of threshold values for phenotypes characterization

Based on the three clusters, threshold values were identified using a classification tree (Figure 6.4). Eyes/patients in cluster 1 were characterized by a microaneurysm turnover < 6 and a mean retinal thickness in the central 1000 μm in diameter macular area at baseline < 217 μm; eyes/patients in cluster 2 were characterized by a microaneurysm turnover <6 and mean retinal thickness in the central 1000 μm in diameter macular area at baseline ≥ 217 μm; and, eyes/patients in cluster 3 were characterized by a microaneurysm turnover ≥ 6.

Considering these thresholds, and based on the clinically meaningful parameters, i.e. microaneurysm turnover and presence of edema (i.e., mean retinal thickness in the central 1000 μm in diameter macular area increased over the normal reference value, 201.1±18.9 μm), we were able to define 3

groups of eyes/patients (phenotypes). As a result, the following phenotypes of nonproliferative diabetic retinopathy are identified:

- Phenotype A: microaneurysm turnover < 6 and normal retinal thickeness values (i.e., mean retinal thickness in the central 1000 µm in diameter macular area at baseline < 220 µm, normal mean + 1 SD). (Figure 6.5);
- Phenotype B: microaneurysm turnover < 6 and increased retinal thickeness values (i.e., mean retinal thickness in the central 1000 µm in diameter macular area at baseline ≥ 220 µm). (Figure 6.6);
- Phenotype C: microaneurysm turnover ≥ 6. (Figure 6.7)

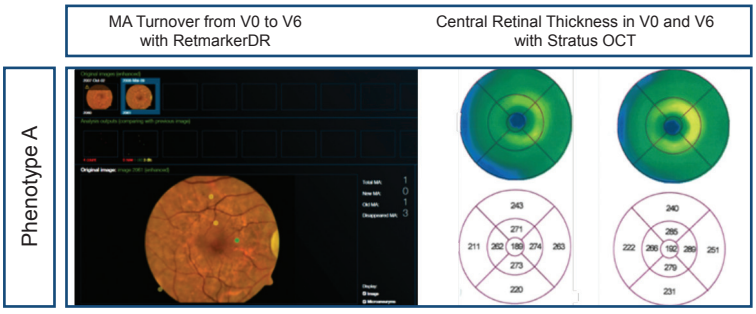

Figure 6.5. Phenotype A - microaneurysm turnover < 6 and mean retinal thickness in the central 1000 µm in diameter macular area at baseline < 220 µm.

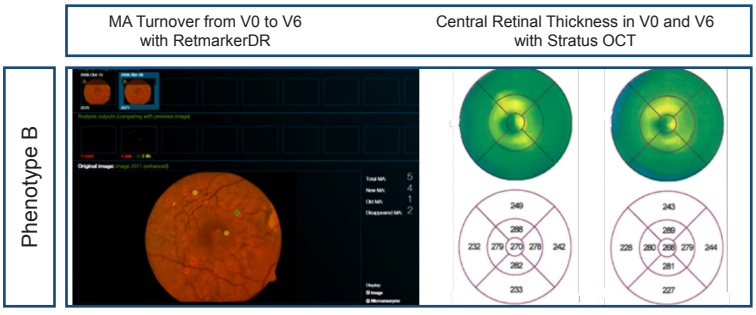

Figure 6.6. Phenotype B - microaneurysm turnover < 6 and mean retinal thickness in the central 1000 µm in diameter macular area at baseline ≥ 220 µm.

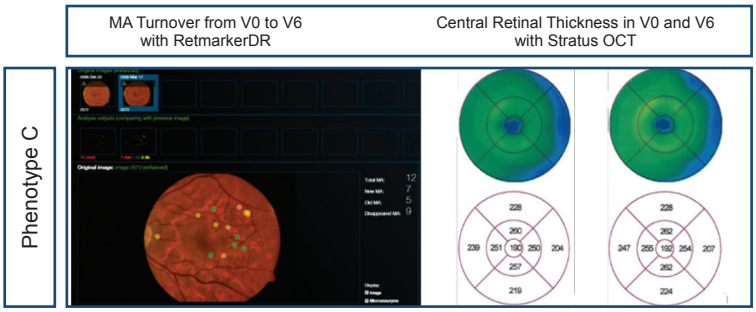

Figure 6.7. Phenotype C - microaneurysm turnover > 6 and variable retinal thickness.

6.2.2. Phenotypes of mild nonproliferative diabetic retinopathy and risk for clinically significant macular edema

From the 348 eyes/patients that reach the study end-point or that completed the 24-month visit, 26 developed clinically significant macular edema needing laser photocoagulation, 1 from phenotype A (i.e., 0.7%), 6 from phenotype B (i.e., 8.5%) and 17 from phenotype C (i.e., 14.5%) (Figure 6.8).

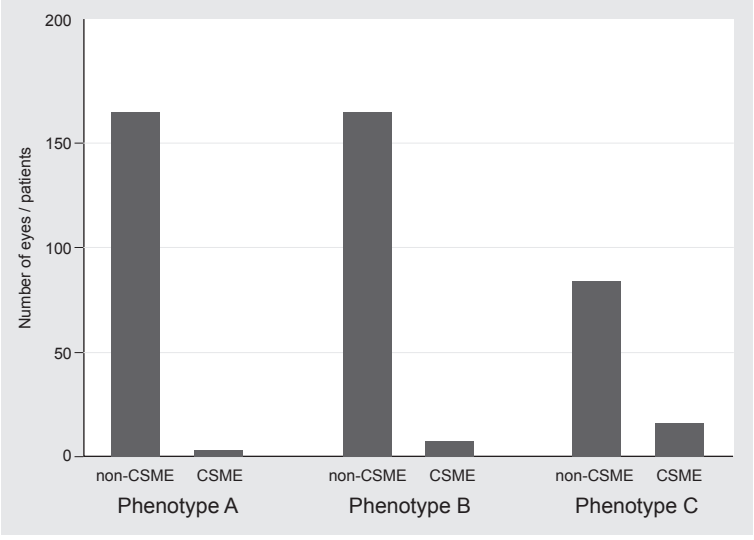

Figure 6.8. Phenotypes distribution by non-clinically significant macular edema (non-CSME) and clinically significant macular edema (CSME) eyes.

Eyes/patients from phenotypes C and B showed a higher risk for clinically significant macular edema than eyes/patients from phenotype A (for phenotype C the OR are 3.536, 95%CI (1.917-6.524); P<0.001; and for phenotype B the OR are 2.802, 95%CI (1.445-5.434); P=0.002). Phenotype C shows also a higher risk for clinically significant macular edema when compared to phenotype B (the OR are 1.994, 95%CI (1.144-3.477); P=0.015) (Figure 6.8).

This study shows that using the mathematical method of cluster analysis and only non-invasive procedures (color fundus photography and OCT), three different phenotypes of nonproliferative diabetic retinopathy can be identified, which show different risks of progression to clinically significant macular edema.

These three phenotypes are in agreement with the number of phenotypes of diabetic retinopathy progression proposed previously by Lobo et al. (2004) in a different and smaller sample. In this original study three phenotypes of mild nonproliferative diabetic retinopathy were characterized, Phenotype A as slow progression, Phenotype B as "leaky" and Phenotype C as ischemic. Similar categorization was found in this study as a result of cluster analysis, cluster 1 corresponding to phenotype A, cluster 2 to phenotype B with predominance of edema, and cluster 3 to phenotype C with predominance of microaneurysm turnover, i.e. with increased rates of microaneurysm formation and disappearance.

The main initial alterations occurring in the early stages of nonproliferative diabetic retinopathy are microaneurysm formation and disappearance, capillary closure and alteration of the blood-retinal barrier with associated retinal edema (Cunha-Vaz et al, 2005).

Digital color fundus photography and OCT alone, both non-invasive procedures, appear to be adequate to document these initial alterations. Microaneurysms and small hemorrhages can be detected by color fundus photography and its turnover (i.e., disease activity), quantified using a novel software for automatic analysis of the fundus images, the RetmarkerDR. Microaneurysm turnover is a composite of microaneurysm formation rate, i.e. number of new microaneurysms per time interval and microaneurysm disappearance rate, i.e. number of disappearing microaneurysms per time interval. Both parameters represent microvascular disease activity and more

specifically, the microaneurysm disappearance rate is considered a sign of capillary closure.

On the other hand, increased retinal thickness quantified by OCT identifies the presence of edema, which is a direct result of the alteration of the blood-retinal barrier.

It was striking to find that 348 eyes/patients with similar levels of ETDRS classification (20-35) show such a large inter-quartile range (IQR) both in microaneurysm turnover and retinal thickness values demonstrating the wide range of values for each of these alterations. Cluster analysis showed that these initial changes occur in different levels of intensity in different groups of patients and that different groups of patients can be characterized by levels of intensity of the retinal changes.

Our findings suggest that increased activity of the microvascular disease in the macular region (field-2) well demonstrated by increased rates of microaneurysm turnover which characterize phenotype C is associated with higher risk for development of clinically significant macular edema in the relatively short period of two years. This phenotype represents approximately 20% of the patients in the study.

Of relevance is also the finding that on the other hand, phenotype A, which is characterized by low microaneurysm turnover and also no signs of the blood-retinal barrier alteration (representing approximately 50% of the total number of patients), has the lowest risk for development of clinically significant macular edema. These observations have clear implications for management of diabetic retinopathy and allocation of health resources. Furthermore, this observation indicates that a large proportion of the eyes with mild nonproliferative diabetic retinopathy will progress very slowly suggesting that these eyes should not be included in clinical trials because of their slow rates of progression.

Various systemic risk factors have been proposed to influence progression of diabetic retinopathy. Our study shows that progression to clinically significant macular edema from the initial stages of the retinopathy is apparently only correlated with HbA1C and LDL levels, i.e., mainly glycemic metabolic control. Blood pressure measurements did not show any correlation with the risk for development of clinically significant macular edema. It appears that these risks factors may play a role only in more

advanced stages of the disease when vision threatening complications such as clinically significant macular edema and proliferative retinopathy are present.

The identification of different retinopathy phenotypes characterized by different rates of progression and different dominant retinal alterations opens new perspectives for personalized management of diabetic retinopathy. If the patients with the greatest risk of progression and with the greatest potential to benefit from treatment can be identified, fewer patients will need to be treated to prevent one case of blindness. This is of extreme importance at a time where scarce resources must be focused and concentrated on the individual cases that need close follow-up and timely treatment.

Several pathways of hyperglicemic damage have been incriminated in the process of triggering diabetic retinal disease. One possibility that must be considered is that they may play different and specific roles in the development of specific retinopathy phenotypes.

Similarly, major mechanisms of diabetic retinal disease such as accelerated aging, hemodynamic factors, microthrombosis and inflammation may play complementary roles and may be present in differenty degrees in different patients.

One major limitation of this study is the relatively short duration of the follow-up period, only two years.

In summary, eyes in the initial stages of diabetic retinopathy show three different phenotypes which can be identified by non-invasive procedures and which have different risks for short-term development of clinically significant macular edema.

6.3. Suggested dominant pathophysiological mechanisms in different phenotypes

6.3.1. Accelerated aging phenomena

Retina tissue like the brain consists predominantly of neurons and neuroglia. Neurons are post-mitotic cells, which are unable to divide in response to damage or to replace abnormal or dead neurons. The total number of neurons gradually declines with age. Furthermore, some of the

commonest causes of neuronal damage are associated with defects in the vascular supply. Although the human brain is about 2% of the total body weight, it uses about 20% of the total oxygen supply.

The retina can be regarded as an extension of the brain. The two types of photoreceptor cells (cones and rods) transmit signals to the bipolar cells and thence to the glanglion cells with axons that extend to the brain. Oxygen is supplied by an extensive capillary network. Several features of the retina illustrate, indeed, the inevitability of age-related changes.

Retinal diabetic microangiopathy is characterized by blood-retinal barrier breakdown, basement membrane thickening of retinal vessels, microaneurysms, hemorrhages, cotton-wool spots, capillary obliteration and acellular capillaries, which may ultimately lead to retinal ischemia and neovascularization. Some of these events occur because retinal vascular cells, namely pericytes and endothelial cells, die prematurely during diabetes (Mizutani et al, 1996).

In summary, there is a wealth of evidence indicating that the chronic hyperglycemia of diabetes induces apoptosis in all retinal cells, microvascular, neuronal and glial creating a situation that may be identified with accelerated aging. This pathophysiological mechanism of retinal damage may occur across the different phenotypes, dominating in the most common and least severe phenotype, +henotype A (Table 6.2).

6.3.2. Hemodynamic phenomena

It is a well recognized "clinical impression" that one of earliest alterations observed in the retinal circulation in diabetes is dilatation of the retinal capillary network in the posterior pole. This increased visibility of the retinal capillary network is probably associated with an increase in retinal capillary blood flow.

In a study performed by our research group, when comparing the retinal capillary blood flow measurements obtained from the papillomacular area using the Heidelberg Retinal Flowmeter in diabetic eyes with preclinical retinopathy and in healthy control eyes, we found an overall statistical significant increase in retinal blood flow in the diabetic eyes. However, it was particularly interesting to find that when analyzing the results obtained

in each eye that this increase in retinal capillary blood flow varied markedly between them. The increases were clearly much higher in five of the ten patients studied. Five of the ten showed clearly abnormal increases, with four of them presenting values higher than the mean +2SD of the values registered in the normal control group. Of interest also was the finding that the other five diabetic patients in this study has retinal capillary blood flow values within the normal control range (Ludovico et al, 2003).

These results may have particular relevance. They explain the conflicting results in the literature regarding retinal capillary blood flow values in the initial stages of diabetic retinal disease. They also demonstrate that changes in retinal capillary blood flow are an early alteration in the diabetic retina, but not in the same degree or at the same time in every diabetic retina.

We have data on the initial stages of diabetic retinal disease showing that eyes that have an increased retinal capillary blood flow have also more marked alterations of the blood-retinal barrier and increased values of retinal thickness in the macular area (Ribeiro et al, 2003).

Our results show that hemodynamic alterations are directly associated with more marked alterations of the blood-retinal barrier (Lobo et al, 2004). This association appears to be predominant in the eyes showing particularly increased retinal thickness measurements, indicating retinal edema and leakage. They appear to belong to a group where vascular leakage is dominant such as phenotype B (Table 6.2).

6.3.3. Thrombotic phenomena

Capillary nonperfusion and capillary closure occur frequently in the diabetic retina and are a characteristic feature in the more advanced stages of diabetic retinopathy They are indicators of retinopathy progression and direct signs of presence of ischemia.

Both capillary nonperfusion and capillary closure are, most probably, the result of retinal microthrombosis. Increased platelet adhesiveness and aggregation has been documented in diabetic patients since at least three decades (Heath et al, 1971). Microthrombosis have been demonstrated by election microscopy in experimental diabetes, these microthrombi are mainly composed of aggregated platelets and fibrin strands (Ishibashi et al, 1981).

Recently, Boeri and colleagues (2001) demonstrated in post-mortem specimens from diabetic and nondiabetic donors, that diabetic retinas present an increased number of platelet-fibrin thrombi in the retinal capillaries. Our results using the RetmarkerDR and evaluating microaneurysm turnover suggest increased microvascular disease activity and remodelling of the retinal circulation. Disappearing microaneurysms appear to indicate increased microthrombotic activity. These changes characterize and predominate in phenotype C.

Thrombosis is a dynamic process which is initiated when the hemostatic system is perturbed by injury to the vessel wall, activation of coagulation, and/or flow disturbance. Alterations in endothelial integrity, disturbances in laminar blood flow, thrombin generation, and inhibition of endogenous fibrinolytic activities contribute to thrombus formation. This pathological mechanism appears to be the predominant one in phenotype C (Table 6.2).

6.3.4. Adjuvant phenomena

There has been, recently, renewed interest on the role of inflammation as a relevant factor in the development and progression of the vascular dysfunction of diabetic retinopathy Gardner and Aiello (2000) and Adamis (2002) have called upon a number of arguments in favour of this hypothesis.

At microscopic level, inflammation is associated with vascular dilatation, altered flow, exudation of fluids and leukocyte accumulation and migration. All these features can be identified in the pathological picture of diabetic retinal disease.

It has been shown that within one week of experimental diabetes, leukocytes adhere to and accumulate within the vasculature of the retina (Miyamoto et al, 1999). The leukocytes attach to the endothelial cell lining via classic adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) on the vasculature and B-integrins in the leukocytes. The expression levels for these adhesion molecules increase in early diabetes.

When experimental techniques are applied to detect the earliest changes occurring in the diabetic retina, the initial alterations are the breakdown of the blood-retinal barrier, endothelial cell injury and death and capillary ischemia. Leukocytes may be involved in these changes. At what point is their role relevant is, however, not clear yet.

It must be kept in mind that a variety of clinical studies have shown that anti-inflammatory drugs have a beneficial effect on the alterations of diabetic retinopathy. Macular edema may be improved by local administration of steroids and there are a few reports suggesting that this is so.

The available data is not conclusive but at least an adjuvant role may be played by a coexistent low grade inflammatory status in the development and progression of diabetic retinopathy.

Abnormalities in the leukocytes and platelets may facilitate the conditions for more rapid progression of the retinopathy and correction of these abnormalities may help stabilize diabetic retinopathy.

The situation on a complex and multifactorial disease such as diabetes favors the presence of gene–environment interactions. A key factor in the identification and study of gene–environment interaction is that an individual carrying such a mutation will develop the phenotype only if and when they enter the high risk environment. Thus, the mutation will cause a specific retinal vascular alteration, i.e., alteration of blood-retinal barrier or blood-flow changes in the presence of a specific environmental challenge. This classical ‘‘lack of penetrance’’ of a mutation will cause analytical problems and mis-phenotyping which will be particularly problematic with some sampling analytical designs. This ‘‘content dependency’’ of a mutation (i.e., gene X environment effect) must be taken into consideration when analyzing associations between a candidate gene polymorphism and intermediate phenotypes.

Most of the results published indicate the presence of genetic determinants for resistance or susceptibility to vascular complications. However, there is evidence of problems in replicating results suggesting that the studies performed have been plagued with confounding factors.

The results of our research group on the characterization of different phenotypes of diabetic retinopathy confirm that there are distinct morphological manifestations in diabetic retinopathy with different subjects presenting different rates of progression and different evolution patterns (Lobo et al, 2004). There is, now, evidence indicating that susceptibility to the late vascular complications of diabetes, such as retinopathy, depend, at least partly, on genetic factors (Wasgenknecht et al, 2001).

The risk of severe diabetic retinopathy in the siblings of affected individuals is substantially increased (Leslie and Pyke, 1982). It is possible

that the problems associated with identifying susceptibility genes for diabetic retinopathy is due mainly to the still accepted view that diabetic retinopathy is one uniform and homogenous disease. Specific types of more severe retinopathy may need to be identified before progress is achieved in this area of research.

Another factor that must be taken into consideration is duration of diabetes. Problems encountered may be minimized by selecting case subjects with short diabetes duration and control subjects with larger duration, or by adjusting to duration during analysis.

It is clear that future studies should focus on the need to characterize more accurately different phenotypes with respect to retinopathy status. We agree entirely with Wharpea and Chakravarthy (2003) when they state that agreed international standards for data collection, particularly agreement on a minimum data set for the phenotyping of retinopathy in subjects with diabetes, would permit the pooling of data from the many studies with enhanced power to detect associations.

A classification of diabetic retinopathy based on both relevant genotypes and disease phenotypes is an ambitious goal. We believe that this route may help identify the particular form that threatens an individual patient and consequently offer an opportunity for specific and more effective therapies.

6.4. Summary

Duration of diabetes and level of metabolic control do not explain the great variability that characterize the evolution and rate of progression of the retinopathy in different diabetic individuals. There is great individual variation in the presentation and course of diabetic retinopathy. There are many diabetic patients who after many years with diabetes never develop sight- threatening retinal changes, maintaining good visual acuity. There are also other patients that even after only a few years of diabetes show a retinopathy that progresses rapidly and may not even respond to available treatment.

We were able to identify three major patterns of diabetic retinopathy progression during a follow-up period of 3 years: Pattern A including eyes with reversible and relatively little abnormal fluorescein leakage, a slow rate

of microaneurysm formation and a normal foveal avascular zone. This group appeared to represent eyes presenting slowly progressing retinal disease. Pattern B including eyes with persistently high leakage values, indicating an important alteration of the blood-retinal barrier and the presence of focal edema. This group was identified as a ‘‘wet’’ form of diabetic retinopathy. Pattern C including eyes with variable and reversible leakage and active remodelling of the retinal microcirculation with signs of capillary closure.

We have now extended our observations with two studies. In a longer follow-up study these three different phenotypes were again clearly identified after an initial two year period of follow-up with repeated examinations at 6 month intervals. After an average 7 years of follow-up, ten of these 57 eyes had developed clinically significant macular edema with clear indication for photocoagulation treatment. The slow progression type, pattern A, did not progress to severe macular edema, during the seven-year period of follow-up confirming that this subtype of diabetic retinopathy has good prognosis. On the other hand, both other diabetic retinopathy subtypes, the leaky type, or pattern B, characterized initially by particularly high levels of leakage, i.e., alteration of the blood-retinal barrier, and the ischemic type, or pattern C, characterized by signs of capillary closure, lead much more frequently to the development of severe macular edema, with incidences at seven years of 41% and 50%, respectively.

In a more recent two-year prospective, observational study of eyes/patients with mild nonproliferative diabetic retinopathy (ETDRS grades 20 and 35) using cluster analysis and non-invasive examination procedures, color fundus photography and OCT, and involving 376 eyes/patients we were again able to confirm three major phenotypes of retinopathy progression. These phenotypes were very similar to the ones previously identified, a slow progression phenotype (54%), a “wet” phenotype (25%) and a third phenotype (21%) identified by high microaneurysm turnover. The development of clinically significant macular edema occurred in the period of two years of the study follow-up mainly in phenotypes B and C, with higher incidence on phenotype C.

The characterization of three different phenotypes of diabetic retinopathy, with different progression patterns opens new and particularly interesting perspectives for personalized management of diabetic retinopathy.

Chapter 7
Present status of the management of diabetic retinopathy

Laser photocoagulation and/or intravitreal injections of steroids or anti-VEGF agents are the tested methods for treating diabetic retinopathy complications. However, these treatments are advised only for patients with advanced retinal disease, either high-risk proliferative retinopathy or clinically significant macular edema. Vitrectomy has also been shown to be beneficial in even more advanced stages of retinopathy. These statements summarize the guidelines of 'preferred practice pattern' issued by the American Academy of Ophthalmology (AAO Retina Panel, 2010).

It is clear that the ophthalmologist views the accepted treatment of diabetic retinopathy to include mainly surgical and ablative procedures. In general, these forms of treatment are given independent of the diabetes disease itself and metabolic status. However, appropriate management of diabetic retinopathy should be particularly useful and effective in the earliest stages of retinal disease, when the disease process may still be reversible and before visual loss is already present.

7.1. Therapeutic strategies

Medical therapy may be targeted to control diabetic retinopathy in the early stages of diabetic retinopathy addressing three different levels. The first level should be directed at achieving near physiological levels of glycemia as soon as the diagnosis of diabetes mellitus is made. This approach is particularly effective if applied when retinopathy is in its initial stages (Skyler, 1996). The second and third levels are still at the developmental stage (Cunha-Vaz and Lobo, 1998). Their potential, however, is tremendous and the ophthalmologist must be aware of this and its rapid recent development.

The second level includes drugs aimed at controlling the biochemical events occurring in the retina as a result of the excessive availability of glucose: good candidates include aldose reductase inhibitors (Kinoshita, 1986), inhibitors of protein kinase C activation (Aiello et al, 1997) and inhibitors of advanced glycation end-products (Corbett et al, 1992). Evidence, mostly experimental but also clinical, is accumulating of their potentially favorable effects on the stabilization of the early stages of diabetic retinal disease.

Finally, the third level at which medical therapy may be targeted in order to halt the progression of diabetic retinal disease is at the lesion site, both at the neuronal and vascular levels. Treatment of the initial sites of vasogenic retinal edema may be considered an appropriate goal, because there are now techniques available to monitor these initial retinal changes clinically (Hee et al, 1998; Lobo et al, 2001).

Neuroglial damage may be potentially addressed using neuroprotective agents such as calcium somatostatin inhibitors, antioxidants and glutamate receptor antagonists. The field of neuroprotective drugs is very active and drugs specifically developed for retinal neuroprotection are now starting to be tested for diabetic retinopathy (EUROCONDOR project).

Vasogenic retinal edema is directly associated with breakdown of the blood-retinal barrier (Cunha-Vaz, 1998). Medical therapy of vasogenic retinal edema may be targeted to act directly on blood-retinal barrier function using corticoids or anti-VEGF agents.

Finally, antihypertensive therapy should be considered at least when there is recurring vasogenic retinal edema with clear breakdown of the blood-retinal barrier. Angiotensin-converting enzyme inhibitors, like lisinopril, have been shown to act favorably on the evolution of the retinopathy (Chatuverdi et al, 1998). Calcium-channel blockers should also be considered because of their multiple effect: antihypertensive, neuroprotective, and correction of ATP synthesis.

In the near future, medical therapy for diabetic retinopathy will probably involve the association of drugs acting at all three levels. Therapy for diabetic retinopathy should therefore include medications necessary for the best euglycemic control (insulin or oral antidiabetic drugs), a second drug given to correct the altered metabolism of the retina associated with excess glucose availability (aldose reductase or protein kinase C inhibitors, or antioxidants) and, finally, a third drug acting as a neuroprotective or vasoprotective agent. The choice of the third drug may be made based the phenotype of progression identified and predominant disease mechanism involved.

The major concern, however, will be safety. Medical therapy of diabetic retinopathy may be initiated immediately after the diagnosis of diabetes, and certainly before there is visual loss and the retinal lesions are irreversible. Therefore, it is long-term therapy. Side effects should be minimal and the medication must have a good benefit to risk ratio to be accepted.

At present it must be realized that medical management for diabetic retinopathy is largely that of prevention.

Controlled clinical trials have demonstrated that aggressive glycemic control reduces the risk of retinopathy. As a consequence, current recommendations for glycemic control are to aim for a fasting plasma glucose of <110 mg/dl and HbA1C of <7% (normal range about 3.0–6.0%). Controlled clinical trials have also shown that aggressive blood pressure control reduces the risk of retinopathy. Current recommendations for blood pressure control are to aim for a systolic blood pressure of <130 mm Hg and a diastolic blood pressure of <85 mm Hg in adults with diabetes.

7.2. Primary interventions

7.2.1. Glycemic control

Analyses from a number of epidemiologic studies and randomized controlled clinical trials suggest a significant relationship between glycemia and retinopathy. In these studies, integrated glycemic control is measured by glycosylated hemoglobin – either HbA1C or HbA1 (which includes HbA1C as well as HbA1a and HbA1b) or total glycosylated hemoglobin. At both 4- and 10-year follow-up in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (Klein et al, 1988 and 1994), there was a statically significant relationship between baseline HbA1 and the incidence of retinopathy, progression of retinopathy by two or more steps on a modified scale developed by the Early Treatment Diabetic Retinopathy Study (ETDRS), and progression to proliferative diabetic retinopathy.

The Diabetes Control and Complications Trial (DCCT) (DCCT, 1993), a randomized, multicenter, controlled clinical trial, demonstrated that intensive treatment of type-1 diabetes with the goal of meticulous glycemic control decreased the frequency and severity of retinopathy, nephropathy, and neuropathy.

The intensive-therapy group achieved a median HbA1C of 7.2% versus 9.1% in the conventional group. This separation in median glycemic values between the 2 groups was maintained for 4–9 years, with mean duration of follow-up 6.5 years for a total of approximately 9,300 patient-years of observation.

Within each treatment group, the mean HbA1C during the trial was the dominant predictor of retinopathy progression. The most important risk factors for early worsening were higher levels of HbA1C at screening and rapid reduction in HbA1C in the first 6 months.

The current glycemic recommendations of the American Diabetes Association (ADA) appear in their 'Standards of Medical Care in Diabetes' (ADA, 1998, 2003 and 2011). The goal is, ideally, for a fasting plasma glucose of <110 mg/dl and a HbA1C of <7% (normal range about 3.0–6.0%). The ADA uses the term 'action suggested' to define another category which might also be defined as 'unacceptable glycemic control', that is a fasting plasma glucose of >140 mg/dl and a HbA1C of >8%.

Contemporary diabetes management is based on the concept of 'targeted glycemic control'. Therapy, based on glycemic goals, utilizes progressive, stepwise additions of whatever treatment modality is necessary to achieve glycemic goals. Medical nutritional therapy and promotion of physical activity are fundamental and needed for all patients, as well as basic diabetes education.

Intensive insulin therapy is mandatory in type-1 diabetes. This is accomplished, as in the DCCT, with insulin administered either as a continuous subcutaneous infusion with a pump or by multiple daily injections; frequent self-monitoring of blood glucose, and meticulous attention to balancing the insulin dose, food intake, and energy expenditure.

In type-2 diabetes progressive pharmacologic therapy is required, the specific choice is based on disease severity and glycemic targets, and should include insulin secretagogues (e.g. sulfonylureas and repaglinide) which stimulate insulin production.

Insulin sensitizers (e.g. biguanides and thiazolidinediones) which enhance muscle glucose uptake and decrease hepatic glucose production, α-glucosidase inhibitors which retard carbohydrate absorption, and, finally, when necessary replacement of insulin deficiency with insulin or insulin analogs.

7.2.2. When vision threatening complications are already present

Blood Pressure Control

For a long time epidemiologic studies have suggested a relationship between blood pressure elevation and progression of retinopathy.

The Hypertension in Diabetes Study (HDS) was embedded in the UKPDS by using a factorial design (UKPDS, 1998a/b). The HDS was conducted in 20 centers with 1,148 patients who had type-2 diabetes and coexisting hypertension. The design was a randomized controlled trial comparing 'tight' blood pressure control aiming for a blood pressure of <150/85 mm Hg with the use of an angiotensin-converting enzyme inhibitor (captopril) or a β-blocker (atenolol) as the main treatment, and 'less-tight' control aiming for a blood pressure of <180/105 mm Hg. Median follow-up was 8.4 years. The tight control group achieved a mean blood pressure of 144/82 versus 154/87 mm Hg in the less-tight control group ($p < 0.0001$). Patients assigned to the tight control group had a significant 37% risk reduction in microvascular end points compared with the less-tight control group.

Blood pressure lowering with captopril or atenolol was similarly effective in reducing the incidence of diabetic complications. There was no evidence that either drug had any specific beneficial or deleterious effect, suggesting that blood pressure reduction in itself may be more important.

In patients with diabetes, current blood pressure recommendations of the ADA appear in their 'Standards of Medical Care for Patients with Diabetes Mellitus' and in a consensus statement on 'Treatment of Hypertension in Diabetes'. Similar recommendations are contained in 'The 6th Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure' and elsewhere (JNC VI, 1997).

The primary goal of therapy for (non-pregnant) adults (>18 years of age) with diabetes is to decrease blood pressure to, and maintain it at, <130 mm Hg systolic and <85 mm Hg diastolic. For patients with an isolated systolic hypertension of >180 mm Hg, the initial goal of treatment is a reduction of 20 mm Hg. If these goals are achieved and well tolerated, further lowering to <140 mm Hg may be appropriate.

Dyslipidemic Control

Diabetic dyslipidemia, particularly in patients with poor glycemic control, is characterized by increased levels of total cholesterol, low-density lipoproteins (LDLs), and triglycerides and by decreased levels of high-density lipoproteins (HDLs). Data from the ETDRS showed that elevated total serum cholesterol and LDL cholesterol is associated with a significant increase in the

presence of retinal lipid exudates (Chew et al, 1996). ETDRS patients with total cholesterol levels of >240 mg/dl were twice as likely to have retinal lipid exudates than were those patients with serum cholesterol levels of <200 mg/dl.

7.3. Secondary interventions

7.3.1. Medical interventions

Antiplatelet Agents

The ETDRS showed that aspirin (650 mg/d) had no beneficial effect on diabetic retinopathy progression or loss of visual acuity in patients with diabetic macular edema or severe non proliferative diabetic retinopathy during 9 years of follow-up (ETDRS, 1991a; Chew et al, 1995)

Protein Kinase C Inhibitors

Hyperglycemia induces synthesis of diacylglycerol in vascular cells, leading to activation of protein kinase C (PKC) isozymes. Excessive PKC activation may be involved in the pathophysiology of diabetic retinopathy. Ruboxistaurin, an orally active PKC inhibitor, was evaluated in the Protein Kinase C Diabetic Retinopathy Study (PKC-DRS) (PKC-DRS, 2005) which randomized 252 patients with moderate to severe nonproliferative diabetic retinopathy to receive ruboxistaurin (8, 16, or 32 mg) or placebo. No significant difference in diabetic retinopathy progression was observed after 36 months of follow-up, al though patients treated with 32 mg of ruboxistaurin had a significant reduction in the risk of moderate visual loss. Treatment was well tolerated with few adverse events, largely mild gastrointestinal symptoms. A larger study, the PKC-DRS2, which randomized 685 patients, showed similar results (Aiello et al, 2006).

The PKC-DMES Study reported no significant reduction in progression of diabetic retinopathy or incidence of diabetic macular edema in 686 patients with mild to moderate nonproliferative diabetic retinopathy and no prior laser therapy (Aiello et al, 2005 and 2007). There was a trend for a reduction in clinically significant diabetic macular edema among patients treated with 32 mg of ruboxistaurin (P= .04), with a larger effect when patients with HbA1c levels of 10% or greater were excluded (P= .02).

Aldose Reductase Inhibitors

Aldose reductase is the rate-controlling enzyme n the polyol pathway of glucose meholism and is involved in pathogenesis of diabetic retinopathy. Two aldose reductase inhibitors, sorbinil (Pfizer, New York, New York) and tolrestat (Wyeth Ayerst, St Davids, Pennsylvania), showed no clinically significant effect in reducing diabetic retinopathy incidence or progression in randomized clinical trials of 3 to 5 years' duration (Sorbinil Trial Research Group, 1990).

Growth Hormone/ Insulinlike Growth Factor Inhibitors

Observations of improvements in diabetic retinopathy following surgical hypophysectomy (Ray et al, 1968; Hardy and Cine, 1968) and of increased serum and ocular levels of insulinlike growth factor in patients with severe diabetic retinopathy led to studies investigating the use of agents inhibiting the growth hormone/ insulinlike growth factor pathway for prevention of diabetic retinopathy (Sonksen et al, 1993). A small randomized clinical trial conducted over 15 months among 23 patients reported reduction in retinopathy severity with octreotide, a synthetic analogue of somatostatin that blocks growth hormone (Grant et al, 2000), but another randomized clinical trial conducted over 1 year among 20 patients (Kirkegaard et al, 1990) evaluating continuous subcutaneous infusion of octreotide found no significant benefits. Two larger randomized clinical trials currently evaluating long-actingrelease octreotide injection (NCT00248157 and NCT00248131, http://clinicaltrials.gov) have reported inconclusive preliminary results (Grant, 2006), with significant adverse effects (eg, diarrhea, cholelithiasis, hypoglycemic episodes).

7.3.2. Laser and surgical interventions for severe nonproliferative and proliferative diabetic retinopathy

Pan-retinal laser photocoagulation

Pan-retinal laser photocoagulation, in which laser burns are placed over the entire retina, sparing the central macula, is an established technique for treating proliferative diabetic retinopathy (DRS, 1978).

The strongest evidence comes from 2 related randomized clinical trials in the 1970s and 1980s, the Diabetic Retinopathy Study (DRS) (DRS, 1978 and 1981)

and the ETDRS (ETDRS, 1991b). The DRS randomized 1758 patients with proliferative diabetic retinopathy in least 1 eye or bilateral severe nonproliferative diabetic retinopathy to receive Pan-retinal laser photocoagulation or no treatment. At 2 years, severe visual loss (visual acuity <5/200 on 2 successive visits) was observed in 6.4% of treated vs 15.9% of untreated eyes, with the greatest benefit in eyes with high-risk characteristics (new vessels at the optic disc or vitreous hemorrhage with new vessels elsewhere, in which the risk of severe visual loss was reduced by 50% (DRS, 1978).

Surgical vitrectomy for vitreous hemorrhage and proliferative diabetic retinopathy

Vitrectomy has been used for treatment of eyes with advanced diabetic retinopathy, including proliferative diabetic retinopathy with nonclearing vitreous hemorrhage or fibrosis, areas of traction involving or threatening the macula, and, more recently, persistent diabetic macular edema with vitreous traction (Ho et al, 1992). The Diabetic Retinopathy Vitrectomy Study (DRVS) randomized 616 eyes with recent vitreous hemorrhage and visual acuity of 5/200 or less for at least 1 month to undergo early vitrectomy within 6 months of observation (DRVS, 1985, 1988a/b and 1990). After 2 years' followup, 25% of the early vitrectomy group vs 15% of the observation group had 20/40 or greater vision, with the benefits maintained at 4 years and longer in individuals with type 1 diabetes.

7.3.3. Laser and surgical interventions for diabetic macular edema

Focal laser treatment

Like pan-retinal laser photocoagulation, there is good evidence that focal laser treatment pre serves vision in eyes with diabetic macular edema. The ETDRS randomized 1490 eyes with diabetic macular edema to receive focal laser treatment or observation. At 3 years, treatment significantly reduced moderate visual loss as compared with observation (ETDRS, 1985) with the greatest benefits in eyes with clinically significant diabetic macular edema (ETDRS, 1987). Adverse effects include inadvertent foveal burn, central visual field defect, color vision abnormalities, retinal fibrosis, and spread of laser scars (ETDRS, 1995; Aiello, 2003).

Focal laser applied directly to localized microvascular alterations such as microaneurysms and intraretinal vascular abnormalities has been shown to be effective, particularly if there is a good correlation between the leaking vessels and the macular edema.

Sub-threshold laser

The ETDRS demonstrated that laser photocoagulation applied to patients with clinically significant macular edema reduced the incidence of visual loss by approximately 50% at 3 years follow-up (ETDRS, 1985). The conventional green laser treatment is applied in a focal grid pattern and produces a visible burn in the retina. There have been reports demonstrating the enlargement of laser scars after treatment (Morgan and Schatz, 1989).

Recently, sub-threshold micropulse diode laser was shown to be effective in the treatment of clinically significant macular edema and seems to have a theoretical advantage, since the laser burns will affect deeper layers with relative sparing of the inner neurosensory retina, reducing the scars and the complaints of paracentral scotomas pots-treatment (Akduman and Olk, 1999).

We conducted a prospective randomized double masked interventional controlled trial at two-centers to compare the efficacy and side effects of these two types of laser treatment for diabetic clinically significant macular edema (Figueira et al, 2008).

This study included 84 eyes from 53 type 2 diabetic patients with clinically significant macular edema (as defined by ETDRS study) in two centers: Coimbra with 21 patients (42 eyes) and London with 32 patients (42 eyes).

Our study population included patients less than 80 years old, with type 2 diabetes with both eyes fulfilling the ETDRS criteria for clinical significant macular edema based on stereo fundus photography and best-corrected visual acuity of more than or equal to 55 letters on the modified ETDRS chart (equivalent to 20/80 or better).

We randomized the eyes into two treatment groups, according to a randomization table. A total of 40 eyes received conventional green laser and 44 received the sub-threshold micropulse diode laser. Patients with bilateral

clinically significant macular edema were randomized in order to receive conventional green laser in one eye and sub-threshold micropulse diode laser in the other eye.

Patients were examined at baseline (V0), month 4 (V4) and month 12 (V12) visits.

Of the 53 patients, 32 were men and 21 were women and all of them completed the 12 months of follow-up. The mean (SD) age was 60.5 (9.8) (range: 34 to 78) years. The mean duration of diabetes was 12.4 (5.56) years. The mean value of hemoglobin A1c at baseline was 9.0% (1.5).

No statistical significant differences between the 2 laser groups were found between parameters at baseline (p>0.10) (Table 7.1). The non-statistically significant difference between groups was also verified within the two centers (p=0.067).

Table 7.1. Baseline clinical characteristics of the two groups (mean ±SD)

	CGL Group (n=40)		MPDL Group (n=44)	
Age	61.1	±9.9	59.8	±9.9
Diabetes Duration	12.4	±5.7	12.4	±5.6
HbA1c	9.1	±1.6	9.0	±1.6
Systolic Blood Pressure	136.1	±26.4	134.3	±26.1
Diastolic Blood Pressure	78.1	±8.9	77.4	±9.1
Best-Corrected Visual Acuity	78.0	±7.8	78.4	±8.1
Contrast Sensitivity	31.2	±3.4	31.1	±3.1
Central Retinal Thickness	255.0	±61.9	248.9	±58.7

CGL: conventional green laser; MPDL: micropulse diode laser; SD: standard deviation.

No statistical significant differences were found at V0, V4 and V12 between the conventional green laser and micropulse diode laser groups considering the mean visuaol acuity value (p=0.81). There was also no statistical difference in change in best-corrected visual acuity at 12 months between the two laser groups (-7.3 ETDRS letter in conventional green laser group compared to -6.6 letters in micropulse diode laser group; p=0.88).

From baseline to V12 there was an increase in central macular thickness of 28.7μm (105.3) and 41.9μm (103.8), for the conventional green laser and micropulse diode laser group respectively. No statistical significant difference was found between the 2 groups, regarding the changes from V0 to V4 and from V0 to V12 (p=0.81).

No statistical significant differences were found in all of the visits, between the conventional green laser and micropulse diode laser groups considering the mean contrast vision value (p=0.87).

Fundus photos of good quality could be obtained only from 82 eyes at V12. The masked grader detected laser scars in 6 (13.9%) of the 43 eyes from micropulse diode laser group compared to 23 (59.0%) of the 39 eyes from conventional green laser group. This difference is statistically significant (p=0.001).

The risk for developing laser scars is 8.9 times higher in one eye treated with conventional green laser, compared with one eye treated with micropulse diode laser (95% CI=[3.0 to 26.0]).

The results of this prospective randomized study show that sub-threshold micropulse diode photocoagulation for diabetic clinically significant macular edema compares well with conventional green laser photocoagulation. Although the difference did not reach significant levels, it showed a trend of better vision at 12 months in the micropulse treated eyes. There was no significant difference in contrast sensitivity and central retinal thickness between the two types of laser modality either at 0, 4, and 12 months suggesting similar effect. We also noted less scarring in the micropulse diode laser group compared to conventional green laser group. This is an important finding as spread of retinal atrophy around conventional laser scars occur over years and is considered as a complication especially for macular laser (Maeshima et al, 2004). It would be useful to observe whether these differences in retinal scarring are maintained in the long-term.

Surgical vitrectomy for diabetic macular edema

Widespread or diffuse diabetic macular edema that is nonresponsive to focal laser treatment may benefit from vitrectomy (Yang, 2000; La Heij et al, 2001; Dilinger and Mester, 2004; Kralinger et al, 2006). However, the few randomized clinical trials to date have had small sample sizes and

short follow- up, with inconsistent results. The presence of vitreous traction in macular edema, now readily documented with Optical Coherence Tomography (OCT), in association with visual impairment is currently a common indication for vitrectomy.

7.3.4. Intravitreal corticosteroids

Corticosteroids have potent antiinflammatory and antiangiogenesis effects. Intravitreal triamcinolone ie, injection of triamcinolone acetonide into the vitreous cavity (Sobrin and D'Amico, 2005), has been used for treatment of diabetic macular edema (Jonas and Sofker, 2001; Martidis et al, 2002) with a number of small clinical trials demonstrating improvements in diabetic macular edema and visual acuity (Massin et al, 2004; Jonas et al, 2006a/b). In the largest randomized clinical trial having the longest follow-up yet reported, eyes with persistent diabetic macular edema were randomized to receive 4 mg of intravitreal triamcinolone or sham injection (saline injection into the subconjunctival space) (Gillies et al, 2006). After 2 years, 19 of 34 intravitreal triamcinolone-treated eyes (56%) had a visual acuity improvement of 5 letters or more compared with 9 of 35 placebo treated eyes (26%) (P= .007). Overall, intravitreal triamcinolone-treated eyes had twice the chance of improved visual acuity and half the risk of further loss. However, many eyes required repeated injections (mean, 2.2), and there was significant intraocular pressure elevation (5 mm Hg in 68% of treated eyes vs 10% of controls). Cataract surgery was required in 55% of intravitreal triamcinolone-treated eyes. Thus, while this study demonstrated significant efficacy of intravitreal triamcinolone in persistent diabetic macular edema, larger randomized clinical trials are needed to provide further data on long-term benefits and safety. Additionally, the ideal dose of triamcinolone remains unclear (Spandau et al, 2005).

More recently, intravitreal or retinal implants have been developed, allowing extended drug delivery. An injectable, biodegradable intravitreal dexamethasone extendedrelease implant (Posurdex; Allergan, lrvine, California) was evaluated in an randomized clinical trial, with reported improvements in visual acuity and macular thickness (Kuppermann et al, 2003). A larger randomized clinical trial of Posurdex for diabetic macular edema is currently under way.

The Iluvien (fluocinolone acetonide implant, Alimera) is a small, nonbioerodable device that is injected in an office setting through a self-sealing wound with a 25-gauge inserter. The phase 3 FAME trial compared this device, formulated in two doses (0.2µg/day and 0.5µg/day), to standard of care, which could include laser or anti-VEGF injection, in 956 patients (Campochiaro, 2011).

Almost 30% of eyes receiving either the low dose or high-dose formulation of the drug achieved three lines or more improvement of visual acuity, compared with 16% of controls, in the 3-year results of the study (Campochiaro, 2011). Subgroup analysis showed that patients with diabetic macular edema duration of more than 3 years experienced better results that those with diabetic macular edema of less than three years duration (Antoszyk et al, 2011).

7.3.5. Intravitreal antiangiogenesis agents

Several randomized clinical trials are currently evaluating agents that suppress vascular endothelial growth factor (VEGF) for treatment of diabetic macular edema.

Ranibizumab (Lucentis, Genentech and Novartis) is an anti-VEGF agent used for treatment of neovascular age-related macular degeneration (Brown et al, 2006; Rosenfeld et al, 2006) and may also be useful for diabetic retinopathy and diabetic macular edema (Chun et al, 2006). Randomized clinical trials (the RESOLVE and RESTORE studies) have demonstrated beneficial effect of intravitreal administration of ranibizumab in diabetic macular edema. It is able in the short-term to improve visual acuity when some degree of vision loss associated with clinically significant macular edema is present. The DRCRnet work confirmed and extended these findings. Intravitreal injection of ranibizumab is now approved for clinical use in diabetic macular edema (Elman et al, 2010).

Bevacizumab (Avastin, Genentech) is an anti-VEGF agent similar to ranibizumab that is approved for the treatment of disseminated colorectal cancer and not licensed for intraocular use. In a number of small studies and in noninferiority trials, bevacizumab appeared to show similar efficacy for treatment of neovascular age-related macular degeneration and may also be

effective for diabetic macular edema (Avery, 2006; Avery et al, 2006; Spaide and Fisher, 2006; Rosenfeld, 2006). Bevacizumab has attracted interest because of its low cost, but local and systemic safety is a concern (Gillies, 2006; Carneiro et al, 2011).

Aflibercept (VEGF-Trap Eye, Bayer) is a recombinant fusion protein, consisting of portions of human VEGF receptor 1 and 2 extracellular domains fused to Fc portion of IgG1 and formulated as an iso-osmotic solution for intravitreal administration (Ohr and Kaiser, 2012).

The diabetic macular edema and VEGF Trap-Eye: Investigation of Clinical Impact (DA VINCI) study was a multicenter, randomized, double-masked phase II trial that compared various dosing regimens of aflibercept to macular grid photocoagulation. Compared to baseline, more patients treated with aflibercept than laser gained 0+, 10+ and 15+ letters of vision (93%, 64% and 34% vs. 68%, 32% and 21%). Reductions in central retinal thickness were greater in patients receiving aflicercept than laser (-127.3μm to -194.5 μm vs. -67.9 μm; $p<0.0066$ for each aflibercept group compared to laser) (Steward, 2012).

7.4. Review of approaches for the treatment of diabetic macular edema

Diabetic macular edema is a leading cause of visual impairment in people with diabetes mellitus (Aroca et al, 2004) and, if left untreated, >50% of patients lose >2 lines of visual acuity within 2 years (Ferris and Patz, 1984). Diabetic macular edema mostly affects the working-age population, imposing a significant burden both on society and on individual patients (Chen et al, 2010) – a burden that is expected to increase with the rising prevalence of diabetes (Wild et al, 2004; Massin et al, 2010; Mitchell et al, 2011).

The standard therapy for visual impairment due to diabetic macular edema – focal and/or grid laser photocoagulation (The Royal College of Ophthalmology, 2005; American Academy of Ophthalmology, 2010) – is mostly only able to stabilise vision. In ETDRS in patients with visual impairment due to diabetic macular edema, laser therapy reduced the relative risk of losing ≥15 letters of visual acuity by 50% compared with

deferring treatment (ETDRS, 1985). More recent trials reported gains of only 0.9 letters (Mitchell et al, 2011) and three letters (Elman et al, 2010) for patients receiving laser monotherapy according to ETDRS guidelines. Nevertheless, a small group of laser-treated patients (21%) in the DRCR. net protocol I study did achieve a 15-letter improvement in visual acuity at 2 years, suggesting a delayed benefit (Elman et al, 2011). Notwithstanding the importance of preventing further vision loss, there was until recently an unmet medical need for therapies that could restore visual acuity in patients with diabetic macular edema who had visual impairment.

Although not fully elucidated, advances in understanding diabetic macular edema pathophysiology have launched the investigation of various pharmacological therapies, including those targeting VEGF, which is upregulated in eyes with diabetic macular edema (Funatsu et al, 2002 and 2003) and is a major mediator of increased retinal permeability (Bhagat et al, 2009). Investigational anti-VEGF therapies include aflibercept, pegaptanib and bevacizumab (although bevacizumab is unlicensed for intraocular use).

We will focus on the most robust evidence for ranibizumab in patients with diabetic macular edema (prospective, randomised clinical trials with at least 6 months' follow-up). To date, two phase II studies (RESOLVE (Massin et al, 2010) and READ-2 (Nguyen et al, 2009 and 2010)) and two phase III studies (RESTORE (Mitchell et al, 2011) and DRCR.net protocol (Elman et al, 2010) have been completed using ranibizumab – a total of 1313 patients with diabetic macular edema.

7.4.1. Recommendations for the treatment of diabetic macular edema

The goal of treatment with laser photocoagulation was mostly visual acuity stabilization. With the approval of ranibizumab for the treatment of visual impairment due to diabetic macular edema, the goal of therapy should now primarily be the improvement or restoration of visual acuity, with stabilization of vision and prevention of further vision loss as a key secondary goal.

Treatment recommendations for diabetic macular edema are based on involvement of the centre of the macula (Figure 7.1). No new recommendations for the treatment of diabetic macular edema without centre involvement,

or for diabetic macular edema with centre involvement but without vision loss, are required as current ETDRS guidelines remain appropriate (ETDRS, 1987) Ranibizumab monotherapy is now recommended for the treatment of diabetic macular edema with centre involvement, with vision loss considered due to diabetic macular edema. In that respect, it is important to exclude other potential causes of vision loss such as epiretinal membrane, vitreomacular traction or macular ischaemia and other conditions such as cataract or glaucoma.

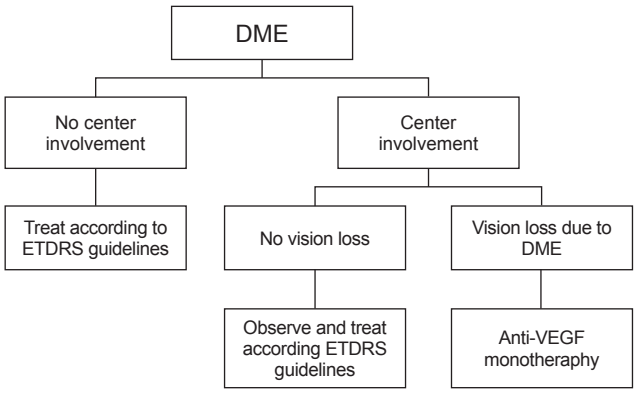

Figure 7.1. Treatment algorithm for diabetic macular edema.

Systemic factors are key to the management of diabetes, and patients should attempt to achieve optimal control of haemoglobin A1c, lipid levels and especially blood pressure (Cheung et al, 2010). Intensive control of blood glucose and hypertension reduces development of clinically significant macular edema (UKPDS, 1998a/c). Achieving control of systemic factors reduces retinal thickness and improves visual acuity to some extent in patients with very mild diabetic macular edema in the absence of other interventions (Singh et al, 2006).

Surgery should be considered in patients with diabetic macular edema due to a significant epiretinal membrane or demonstrated vitreomacular traction.

Proliferative diabetic retinopathy does not fall under the scope of these recommendations; however, caution with anti-VEGF agents in patients who have diabetic macular edema with co-existing proliferative diabetic retinopathy is necessary to avoid accelerated fibrosis and scarring due to the angio-fibrotic switch (Kuiper et al, 2008) Such damage can lead to rapid formation of tractional retinal detachment (Kuiper et al, 2008; Oshima et al, 2009).

7.4.2. Characterization of responders to treatment

In characterizing responders to therapy for diabetic macular edema, it is important to keep in mind the predominant disease mechanisms in diabetic retinopathy: alteration of the blood-retinal barrier, inflammation, and ischemia. Alteration of the blood-retinal barrier can be evaluated with fluorescein angiography or with OCT analysis indicating the presence of edema. Inflammation is a response to disease activity. Microaneurysm turnover (appearance and resolution of microaneurysms) identifies developing ischemia which creates conditions for diabetic macular edema.

The major pathways of progression in diabetic retinopathy are characterized by the leakage (alreattions of the blood-retinal barrier), microaneurysms, inflammation, and ischemia. The therapies that we employ for treatment of diabetic macular edema must act on one or more of these pathways. The rationale for use of vascular endothelial growth factor (VEGF) inhibitors in diabetic macular edema is the association of the presence of VEGF with vascular leakage; VEGF increases leakage, and anti-VEGF action can control leakage. Anti-VEGF therapy may also have an effect on ischemia, depending on the level of ischemia. Steroids act on both leakage and, especially, inflammation. Although we do not fully understand the mechanism of action of laser, we observe that it stabilizes disease activity in diabetic macular edema.

Response to anti-VEGF treatment in diabetic macular edema is generally better than response to any other means of treatment. A randomized controlled trial by the Diabetic Retinopathy Clinical Research Network showed that intravitreal ranibizumab (Lucentis, Genentech) plus prompt or deferred laser resulted in greater visual acuity gain than treatment with either intravitreal triamcinolone acetonide plus laser or laser alone (Elman et al,

2010). However, in clinical trials we are always looking at the mean results of a number of patients. In any trial of a proposed diabetic macular edema therapy, there will be good responders who achieve decreased thickness and increased visual acuity in a relatively short period after the initial injections, but there will also be poor responders and nonresponders. It would be helpful to know more about the nonresponders in order to choose alternative treatments to which they might respond better.

To evaluate response to treatment in diabetic macular edema, retinal thickness measurement with OCT is crucial to evaluate structural changes – decreases in intraretinal or subretinal fluid as markers for reduction of vascular leakage. Visual acuity is also fundamental to evaluate the response to treatment because it determines whether or not we repeat treatments. It does not make much sense to keep injecting a patient whose visual acuity is not improving. Visual acuity also gives clues to the photoreceptor status, which determines the patient's potential for recovery.

For patients with diabetic macular edema who do not respond or respond poorly to anti-VEGF therapy, combination treatments may offer an additional benefit. Applying laser immediately after the first or second injection in the initial stages of anti-VEGF treatment may reduce the number of injections needed and/or improve response. Adding steroid injection or an extended release steroid implant may improve the disease course in patients who do not respond or respond poorly to anti-VEGF monotherapy.

It is crucial to identify responders and nonresponders to therapy for diabetic macular edema. If we can develop mechanisms to recognize early those patients who are not responding to therapy and devise alternative treatment approaches for them, we can be more sure that we are getting the right treatments to the right patients at the right time.

7.4.3. Emerging new treatments

The pharmacological products now in phase II / III clinical trials include corticosteroids using a variety of delivery systems, VEGF inhibitors of which the best tested are ranibizumab and aflibercept, PKC-β inhibitors such as ruboxistaurin, drugs for enzymatic vitreolisis, ACE inhibitors such as candesartan, fenofibrate and other drugs such as sirolimus.

Other drugs that have shown potential but are still in a developmental stage and need further evaluation include: rosiglitazone, anti-sense inhibitors, pigment-epithelium-derived growth factor, novel aldose reductase inhibitors, etc.

7.5. Role of early screening for diabetic retinopathy in patients with diabetes mellitus

The major risk factors for developing diabetic retinopathy are duration of diabetes (Leske et al, 2010; Elshafei et al, 2010) and severity of hyperglycemia (DCCT, 1995; Elshafei et al, 2010).

Timely intervention by laser photocoagulation can reduce severe visual loss by 90% according to ETDRS (ETDRS, 1991c) and diabetic retinopathy study (DRS) (DRS, 1981a). Intravitreal anti-VEGF injections are offering new treatment alternatives and offer for the first time vision recovery. In any case, early detection of diabetic retinopathy vision threatening complications and timely treatment of these patients remains a major challenge for health care providers.

Screening is a process by which unrecognized diseases or defects are identified by means of rapidly applied tests in apparently healthy individuals.

The four cardinal principles for screening recommended by the WHO (World Health Organization, 2001) are as follows:

1. The condition should be an important health problem with a recognizable presymptomatic state.
2. An appropriate screening procedure which is acceptable both to the public and health care professionals should be available.
3. Treatment for patients with recognizable disease should be safe, effective and universally agreeable.
4. The economic cost of early diagnosis and treatment should be considered in relation to total expenditure on health care, including the consequences for leaving the disease untreated.

Diabetic retinopathy conforms well to these principles. In diabetic retinopathy, early detection and treatment is of vital importance as it may prevent vision loss and blindness.

Diabetic retinopathy is a chronic disease with a long latent phase. Among the diabetics, 10-15% constitute type 1 diabetics and the remainder are type 2 diabetics. In about 10 years, diabetic retinopathy develops in 71-90% patients

with type I diabetes and this incidence rises to 95% in 20-30 years. [3] Out of these, 30-50% patients have proliferative diabetic retinopathy (Klein et al, 1984). In type II diabetes, 67% patients develop diabetic retinopathy after 10 years, (Klein et al, 1991) with 10% patients showing features of proliferative diabetic retinopathy. Up to a fifth of newly diagnosed diabetics have some form of retinopathy. Therefore, screening will prove to be beneficial at any stage of this long latent phase of the disease and will also be helpful in avoiding blindness among 90% patients (Ferris, 1994).

Screening for diabetic retinopathy is cost-effective when compared with disability loss for people going blind in the absence of a screening program. The compliance for the screening program should be more than 80% for more gains (Dasbach et al, 1991). The funds invested to increase compliance are small but a vital component of the costs of a screening program.

At a primary level, emphasis should be on early identification of diabetic patients from the community and an efficient referral system so that all the diagnosed cases of diabetes may be referred for timely treatment of diabetic retinopathy complications. It is suggested that patients with type I diabetes should be screened annually for retinopathy, 5 years after the onset of diabetes. Patients with type II diabetes should have an initial examination for retinopathy shortly after the diagnosis and the examination should be repeated annually. Pregnant women with diabetes should have a comprehensive eye examination in first trimester and close follow-up throughout pregnancy.

7.5.1. Criteria for review and referral

1. Annual review without referral:
   a. Normal fundus
   b. Background diabetic retinopathy with small hemorrhages and/or small hard exudes more than one disc diameter distant from the fovea
2. Early referral to ophthalmologist for treatment:
   a. Maculopathy
3. Urgent referral to ophthalmologist for treatment:
   a. Proliferative diabetic retinopathy
   b. Vitreous hemorrhage
   c. Retinal detachment

7.5.2. Screening Tests for diabetic retinopathy

Many different modalities of screening are in use depending on the availability of local facilities. These include number of available ophthalmologists, other trained healthcare professionals, and equipment and resources available for screening. However, whichever method is used, it should have sufficient sensitivity (>80%) and specificity (>80%) for a single modality screening process. The minimum sensitivity for any method to be effective if it is repeated at the recommended interval is 60%. This level of sensitivity can be achieved with ophthalmoscopy through dilated pupils (Sensitivity = 65.7% and Specificity = 93.8%) (Owens et al, 1998) by suitably trained observers (principally ophthalmologists, optometrists, general practitioners, or physicians) or with non-mydriatic photography (Sensitivity = 87.3% and Specificity = 84.8%) (Moss et al, 1985; Owens et al, 1998; Benbassat and Polak, 2009). The sensitivity of detecting diabetic retinopathy by retinal photography has been reported to be higher than that of direct ophthalmoscopy (64% versus 41%; 95% confidence interval of difference, 1.2%- 44.3%) (Siu et al, 1998). Specificities of retinal photography and direct ophthalmoscopy have been reported to be 90% (95% confidence interval, 84%-96%) and 93% (95% confidence interval, 88%-97%), respectively (Siu et al, 1998). Single field fundus photography with interpretation by trained readers could serve as a screening tool to identify patients of diabetic retinopathy. Combining two modalities of screening (e.g. direct ophthalmoscopy in conjunction with retinal photography) provides excellent sensitivity(87.3%) (Benbassat and Polak, 2009), but increases the cost per case screened and is often only possible in a hospital-based setting. Screening involves measurement of visual acuity for both distance and near vision using ETDRS chart.

Tele-medical screening may be undertaken to screen patients with diabetic retinopathy. A major advantage of digital technologies is the ability to transmit images to a centralized reading center for grading. This involves a remote imaging system, a centralized grading center and a data storage system. A significant increase in rate of diabetic retinopathy surveillance and in the rate of laser treatment for diabetic retinopathy may be achieved by implementing retinal image technology in the primary care setting.

The use of the non-mydriatic camera (Sensitivity = 97.7% and Specificity = 84.0%) (Boucher et al, 2003) empowers an additional cadre of health professionals who can participate in screening programs. Screening of diabetics by ophthalmic technicians increases the outreach to the periphery with sufficient sensitivity and specificity and is cost-effective (Wilson et al, 2010).

7.5.3. Automated computer-aided analysis of fundus digital photographs in diabetic retinopathy screening

The development of systematic programmes of screening for retinopathy has been identified as an urgent health care need. Indeed, studies have indicated that the severity of vision loss due to diabetes is caused largely by lack of screening (Oliveira et al, 2011).

We have developed and evaluated a novel two-step approach that automatically screens color fundus photographs in patients with the use of sequential examinations from the same patient to analyse the evolution of the disease in that patient. The automated grading system, RetmarkerSR, consists of software earmarking microaneurysms and "red-dot like" vascular lesions. It includes a co-registration algorithm that allows comparison within the same retinal location between different visits for the same eye. The system generates in a first-step single analysis one of two possible outputs, "disease" or "no disease". "Disease" category comprehends thus those images where vascular lesions are found in the central macula corresponding to level 20 and above of the ETDRS scale, therefore including mild retinopathy, maculopathy, advanced nonproliferative retinopathy and proliferative retinopathy. In the one-step analysis, the algorithm detects the presence of red-dot like lesions in fields 1 and 2 (field 1 is centred on the optic disc, and field 2 is centred on the fovea). We combine this initial analysis (first step) with a second analysis that compares two different and consecutive examinations from the same patient from two successive screenings with approximately one-year interval. The images from the field centered on the macula are co-registered to complete a difference analysis which will indicate disease activity in the central 3000μm circle of the macula. The results show a

clear improvement over available fully automated screening algorithms with a sensitivity of 95.8% and a specificity of 63.2%. The RetmarkerSR has shown that it can perform an useful role by eliminating 50% or more of the screening population who have no retinopathy. Furthermore, it did not miss urgent cases for referral, allowing, therefore, an important reduction in the burden of manual grading with less costs. This two-step analysis shows a clear improvement in specificity over other available automated systems and its integration in a yearly screening program now in progress is expected to bring progressive decrease in the burden of human grading by safely decreasing the number of false positive results to be submitted to human grading, with economic advantage making diabetic retinopathy screening more feasible.

7.5.4. Role of telemedicine in eye care in diabetes

The standard of care for diabetic patients in as annual fundus exam by a qualified eye care provider (Williams et al, 2004; American Academy of Ophthalmology, 2010). With early detection and treatment of diabetic eye disease, vision loss can be mitigated (National Eye Institute, 1993). Unfortunately, only 30% to 60% of individuals with eye disease receive a yearly eye exam (Lee et al 2003; Varma et al, 2008). Telemedicine has the potential to increase the number of patients being screened for eye disease; it has been shown to provide cost-effectiveness and total savings in terms of public health spending (Javitt and Aiello, 1996).

Telemedicine provides a reliable, cost-effective means of screening diabetic patients for retinopathy, which can lead to blindness. Since the number of diabetics is growing fast, but the supply of eye-care practitioners is not, healthcare resources are strained and becoming more so.

Certainly, not every diabetic now receives the standard of care, an annual eye exam, but that situation will worsen unless alternative healthcare delivery systems are employed to address it. With new, easy-to-use, non-mydriatic cameras, nurses and medical assistants without any ophthalmological training can learn to take excellent fundus photographs. These images can be transmitted to a reading center where they can be expertly assessed.

7.6. Summary

Diabetic retinopathy follows a variable time course with different rates of progression between different individuals even with similar metabolic control and duration of disease.

It is clear that a major objective when treating a patient with diabetic retinopathy should be, first of all, to achieve a good metabolic control with hemoglobin A1c levels around 7. Blood pressure and lipid levels should also be monitored and controlled, particularly if the sight-threatening complications of diabetic retinopathy, clinically significant macular edema and/or proliferative retinopathy are present.

The recommended treatment algorithm for clinically significant macular edema has been described and includes: focal laser treatment for eyes with CSME but minimal vision loss; anti-VEGF intravitreal injections if vision loss is clearly present; intravitreal steroid administrations if there is poor response to anti-VEGF treatments; and vitrectomy if there is evidence of vitreoretinal traction. Subthreshold laser remains a very promising alternative, particularly because of the lack of side effects, as shown by our research group.

The recommended treatment for proliferative retinopathy is, at present, panretinal laser photocoagulation.

We have, therefore, treatments with tested efficacy available only for the complications of diabetic retinopathy, clinically significant macular edema and proliferative diabetic retinopathy. These treatments must be applied as soon as they are indicated and timely intervention is crucial for its success. No other treatment has yet demonstrated to be beneficial before the development of these sight-threatening complications.

Meanwhile, it is extremely important to have in place an early intervention program that includes regular eye screening of every patient that has diagnosed diabetes. This program of regular screening will identify the eyes for which effective treatment is already available. Automated analysis of fundus photographs reduce the human burden of human screening programs making them more viable. Present evidence shows that only eyes with diabetic retinopathy complications, clinically significant macular edema and proliferative retinopathy, have been shown to benefit from timely treatment, and these are the eyes that need to be referred.

The most updated management of diabetic retinopathy should include regular screening to identify the development of its complications such as clinically significant macular edema and proliferative retinopathy for which there is treatment available. Before the development of these complications it is mandatory to follow regularly the patients and maintain low hemoglobin A1c levels through appropriate metabolic supervision and dietary recommendations. Prediction of different rates of retinopathy progression in different individuals is now available offering the opportunity of treating the complications timely and more efficiently. Characterization of the different phenotypes of retinopathy progression will also contribute to closer follow-up for some patients and less frequent follow-up for others. Individualized understanding of the specific type of disease activity in a specific patient should now be considered the goal of appropriate management of diabetic retinopathy.

Chapter 8
A step forward to personalized management of diabetic retinopathy

8.1. A new paradigm of personalized management of diabetic retinopathy

The initial stages of nonproliferative diabetic retinopathy are characterized by the presence of microaneurysms and/or hemorhages in the eye fundus and signs of vascular hypermeability and capillary closure. These initial clinical changes are characteristically located in the small retinal vessels of the posterior pole of the retina, that is, in the macular area. The structural changes in the small vessels include endothelial cell, pericyte damage and thickening of basement membrane (Cunha-Vaz, 1978a).

The simplest paradigm that explains capillary permeability and capillary closure centers on the vascular endothelium. In the retina, endothelial cells are the site of the blood-retinal barrier, a specific blood-tissue barrier, and, as in all vessels, provide a nonthrombogenic surface for blood flow. Both these factors are eventually compromised by diabetes.

On the other hand, diabetes also affects the neural and glial cells of the retina. Before or after the microvascular changes?

It must be kept in mind that nonproliferative background diabetic retinopathy progresses over time with very little vision loss. Vision loss occurs late in the disease process and in direct association with the development of two major complications, clinically significant macular edema and proliferative retinopathy.

The challenge is to treat and stop disease progression before these complications develop.

The course of retinopathy is not linear and the progression of diabetic retinopathy varies in different individuals with the time necessary for the development of sight-threatening complications taking much less time in some patients than others. Activity of disease and its progression appears to vary from patient to patient.

We have shown that it is possible to use microaneurysm turnover computed from non-invasive color fundus photographs as a biomarker to identify eyes/patients at risk of progression to clinically significant macular edema (Nunes et al, 2009; Ribeiro et al, 2012).

Microaneurysm counting on fundus photography is particularly atractive because fundus photography is non-invasive and well accepted by the patients, particularly when involving repeated examinations.

Our results, based on precise identification of the location of each microaneurysm on fundus photographs of diabetic eyes, suggest that microaneurysm formation and disappearance rates may be appropriate indicators of retinopathy progression, identifying in this simple way eyes that are at more risk for progression and may represent specific retinopathy phenotypes.

The advent of fluorescein angiography demonstrated, in a clinical setting, the existence of focal breakdowns of the blood-retinal barrier. In 1975, vitreous fluorometry, a clinical quantitative method for the study of the blood-retinal barrier, was introduced by our group (Cunha-Vaz et al, 1975a), showing that an alteration of the blood-retinal barrier could be detected and measured in some diabetic eyes with apparently normal fundi. Thereafter, many experimental and clinical studies have examined the alteration of the blood-retinal barrier in diabetes showing in general that an alteration of the blood-retinal barrier is present in the diabetic retina and may have an important role in its development and progression (Cunha-Vaz, 2000a/b).

Measurements of retinal thickness using OCT offer a non-invasive evaluation of retinal edema and show that localized areas of retinal edema are a frequent finding in the diabetic retina in the initial stages of nonproliferative retinopathy in subjects with diabetes type 2. In one study published in 2000 (Lobo et al, 2000) we demonstrated that localized sites of increased fluorescein leakage and zones of increased retinal thickness were found in association. Increased retinal fluorescein leakage identifies an alteration of the blood-retinal barrier and increased retinal thickness characterizes de presence of retinal edema.

The blood-retinal barrier damage modulates and plays a major role in the development of retinal tissue thickening (edema of the retina). We have also shown that measurements of edema obtained with OCT may offer an indirect measure of blood-retinal barrier breakdown (Bernardes et al, 2011).

Macular edema, in diabetes, presents itself as a continuum as the diabetic retinal disease progresses and may represent also a good marker of disease progression, like microaneurysm turnover.

In diabetes increases in retinal thickness may constitute an appropriate target for treatment. A treatment goal could be to arrest, delay, or limit progression of abnormal macular thickness to clinically significant macular edema.

Although the extent of abnormalities in the neural retinal cells in human diabetes appears to be less conspicuous, there is also clear evidence of their occurrence (Lorenzi and Gerhardinger, 2001). The focal increases in retinal thickness due to the alteration of the blood-retinal barrier observed in the initial stages of diabetic retinal disease may, indeed, mask the neuronal and glial cell damage.

Studies such as the Diabetes Control And Complications Trial (DCCT, 2002), the United Kingdom Prospective Diabetes Study (UKPDS, 1998a), the Diabetic Retinopathy Study Research Group (DRS, 1981b) and the Early Treatment Diabetic Retinopathy Study (ETDRS, 1991d) validated methods now considered standard in treating diabetic retinopathy when it occurs, i.e., tight control of blood glucose levels to prevent retinopathy and laser photocoagulation to halt progression after development of clinically significant macular edema or proliferative retinopathy. However, despite the aim of tight blood glucose control and the use of retinal photocoagulation, blindness still occurs. Therapies targeted at the earliest stages of retinal disease, involving necessarily the demonstration of efficacy of a new drug are needed and remain a priority for eye research. To achieve this goal is urgent to identify biomarkers of disease progression that can be accepted as surrogates for generally accepted endpoints.

The clinical endpoints that have been accepted in the past are: mean difference between groups in visual acuity of at least 3 lines in a ETDRS-Type chart,; mean difference in visual field of at least 10 dB; reduction in percentage of patients with vitreous hemorrhage; reduction in percentage of patients with rubeosis; reduction in occurrence of retinal detachments, and need for photocoagulation treatment according to DRS and ETDRS guidelines (DRS, 1981b; ETDRS, 1991d). All of these are what may be called terminal endpoints. They only give indications about the late, irreversible, stages of diabetic retinopathy.

Microaneurysm turnover on fundus photographs, taking into account their exact, specific location in the eye fundus using the RetmarkerDR has the potential to become an extremely valuable biomarker of the overall progression of diabetic retinal vascular disease. Microaneurysm turnover rate appears to be a direct indication of the progression of retinal vascular damage and activity of disease.

Reduction in macular thickening by measuring the changes in retinal thickness with dedicated instrumentation, is another promising alternative. The measurements are reliable, and changes in retinal thickness are a direct indication of macular edema and breakdown of the blood-retinal barrier (Hee et al, 1998; Bernardes et al, 2011). Testing these potential biomarkers and their final validation is expected to contribute decisively to design clinical trials to evaluate the efficacy of new drugs capable of halting diabetic retinal disease in the initial stages of the disease.

We have stated repeatedly that it is well recognized that duration of diabetes and level of metabolic control are major risk factors for development of diabetic retinopathy. However, these risk factors do not explain the great variability that characterize the evolution and rate of progression of the retinopathy in different diabetic individuals. There is great individual variation in the presentation and course of diabetic retinopathy. There are many diabetic patients who after many years with diabetes never develop sight- threatening retinal changes, maintaining good visual acuity. There are also other patients that even after only a few years of diabetes show a retinopathy that progresses rapidly and may not even respond to available treatment.

We were able to identify three major patterns of diabetic retinopathy progression during a follow-up period of 3 years: Pattern A including eyes with reversible and relatively little abnormal fluorescein leakage, a slow rate of microaneurysm formation and a normal foveal avascular zone. This group appeared to represent eyes presenting slowly progressing retinal disease. Pattern B including eyes with persistently high leakage values, indicating an important alteration of the blood-retinal barrier and the presence of focal edema. This group was identified as a ‘‘wet’’ form of diabetic retinopathy. Pattern C including eyes with variable and reversible leakage and active remodelling of the retinal microcirculation with signs of capillary closure identified as an ischemic form (Lobo et al, 2004).

We have now extended our observations by following for seven years 57 patients with diabetes type 2 with all eyes presenting at the time of enrollment mild nonproliferative diabetic retinopathy. In this longer follow-up study these three different phenotypes were again clearly identified after an initial two year period of follow-up with repeated examinations at 6 month intervals.

After an average 7 years of follow-up, ten of these 57 eyes had developed clinically significant macular edema with clear indication for photocoagulation treatment.

None of the 35 eyes identified initially as pattern A, developed after 7 years of follow-up severe macular edema needing laser photocoagulation. This was in clear contrast with the findings registered in the other two retinopathy subtypes, B and C. Five of the 12 eyes classified as having pattern B, (41%) developed severe macular edema needing photocoagulation. Similarly, five of the 10 eyes identified has having pattern C, (50%) developed severe macular edema needing photocoagulation during the seven-year period of follow-up.

In summary, the slow progression type, pattern A, did not progress to severe macular edema, during the seven-year period of follow-up confirming that this subtype of diabetic retinopathy has good prognosis.

On the other hand, both other diabetic retinopathy subtypes, the leaky type, or pattern B, characterized initially by particularly high levels of leakage, i.e., alteration of the blood-retinal barrier, and the ischemic type, or pattern C, characterized by signs of capillary closure, lead much more frequently to the development of severe macular edema, with incidences at seven years of 41% and 50%, respectively.

In a more recent two-year prospective, observational study of eyes/patients with mild nonproliferative diabetic retinopathy (ETDRS grades 20 and 35) using non-invasive examination procedures, color fundus photography and OCT, and involving 376 eyes/patients we have been able to confirm three major phenotypes of retinopathy progression. These phenotypes were very similar to the ones previously identified, showing also similar distribution, a slow progression phenotype (54%), a "wet" phenotype (25%) and a third phenotype (21%) characterized by high microaneurysm turnover. The development of clinically significant macular edema occurred in the period of two years of the study follow-up mainly in phenotypes B and C, with higher incidence on phenotype C.

The characterization of three different phenotypes of diabetic retinopathy, with different progression patterns opens particularly interesting perspectives to gain more insight into the understanding and management of diabetic retinopathy.

Diabetes mellitus is a familial metabolic disorder with strong genetic and environmental etiology. Presence or absence of genetic factors may play a fundamental role in determining specific pathways of vascular disease and, as a consequence, different progression patterns of diabetic retinal disease. It could be that certain polymorphisms would make the retinal circulation more susceptible to an early breakdown of the blood-retinal barrier (type B) or microthrombosis and capillary closure (type C). The absence of these specific genetic polymorphisms would lead to an evolving pattern of type A.

An interesting perspective of our observations, analyzed under the light of available literature, depicts diabetic retinopathy as a microvascular complication of diabetes mellitus conditioned in its progression and prognosis by a variety of different genetic polymorphisms, and modulated in its evolution by HbA1C levels, partly genetically determined and partly dependent on individual lifestyle and environment. The interplay of these multiple factors and the duration of this interplay would finally characterize different clinical pictures or phenotypes of diabetic retinopathy.

The next goal, therefore, should be the characterization of relationships between genetic factors (represented by distinct genotypes) and their clinical expression (distinct diabetic retinopathy phenotypes). It is possible that the phenotype identified in a given patient may change due to environmental and life style changes. This could give major relevance to need of looking to the patient as a person with many conditioning variables.

Another consequence of the characterization of different phenotypes of diabetic retinal disease is its role in the design of future clinical trials. Clinical trials, to be able to show differences between new treatments and placebo, in periods of follow-up of only two or three years, should, indeed, enroll only groups of patients characterized by their homogeneity and level of disease activity: patients presenting a specific retinopathy phenotype characterized by rapid progression (wet/leaky or ischemic), with similar duration of diabetes and at similar levels of blood pressure and metabolic control (HbA1C values).

It is accepted that in the initial stages of diabetic retinopathy when the fundus alterations detected by ophthalmoscopy or slit-lamp

examination are limited to microaneurysms and hard or soft exudates, i.e., mild nonproliferative diabetic retinopathy, an annual examination is indicated to every patient with five or more years of duration of their diabetes.

This is the recommendation of the American Academy of Ophthalmology Guidelines for Diabetic Retinopathy. Our observations and the identification of different diabetic retinopathy phenotypes in the initial stages of diabetic retinopathy, i.e., mild or moderate nonproliferative diabetic retinopathy, characterized by different rates of progression of the retinopathy suggest that specific approaches should be used when managing these different retinopathy phenotypes (Table 8.1).

Table 8.1. Management recommendations for nonproliferative diabetic retinopathy in the absence of clinically significant macular edema

Phenotypes	**A**	**B**	**C**
Metabolic Control	Regular	Tight HbA1C < 7%	Progressive Tighter
Blood Pressure		As low as acceptable	Progressively Lower
Follow-up intervals	1-3 years	6 months	6 months

A patient with mild or moderate nonproliferative diabetic retinopathy, presenting retinopathy phenotype B (wet/leaky), characterized by marked breakdown of the blood-retinal barrier, registered during a period of 1–2 years of follow-up, indicating rapid retinopathy progression, should be watched more closely and examined at least at 6 months intervals.

Furthermore, blood pressure values and metabolic control should be closely monitored at least at 3-month intervals and medication given to keep HbA1C levels at < 7.1%, systolic blood pressure at <140mmHg and diastolic blood pressure at <85mmHg. Communication channels should be rapidly established between ophthalmologist and their diabetologist, internist or general health care provider. Information should be given indicating that the chances of rapid retinopathy progression to more advanced stages of disease are in these patients relatively high, calling for immediate tighter control of both glycemia and blood pressure.

A patient with mild or moderate nonproliferative diabetic retinopathy presenting retinopathy phenotype C, with ischemic characteristics identified by high microaneurysm formation rates would similarly indicate the need for shorter observation intervals than one year with particular attention for other systemic signs of microthrombosis. Here, however, control of hyperglycemia and blood pressure must be addressed with some degree of caution. Improved metabolic and blood pressure control must be progressive and less aggressive than with phenotype B. It is realized that the ischemia that characterizes phenotype C may become even more apparent in eyes submitted to rapid changes in metabolic control and lowering rapidly the blood pressure may increase the retinal damage associated with ischemia. Finally, a patient with mild or moderate nonproliferative diabetic retinopathy, presenting phenotype A, identified by low levels of alteration of blood-retinal barrier, no signs of capillary closure, low microaneurysm formation rates and with a diabetes duration of more than 10 years, all signs indicating a slowly progression subtype of diabetic retinopathy may be followed at intervals longer than one year. If the examination performed at two years intervals confirms the initial phenotype characterization, the patient and his diabetologist, internist or general health care provider should be informed of the good prognosis associated with this retinopathy phenotype.

It would be of great benefit to have a drug available which would prevent the development of complications and vision loss. So many diabetic patients are not well controlled, they do not come to the doctor often, and they are going blind because they do not get medical attention in time for photocoagulation. The major large clinical trials have shown that tight glycemic control slows the development and progression of diabetic retinopathy. The constantly increasing incidence of type 2 diabetes and the evidence that retinal damage begins early on underscores the need for a medical treatment that is targeted to the initial retinal alterations and to specific phenotypes of the retinal diabetic disease. Several key pathways of hyperglicemic damage have been incriminated in the process of triggering diabetic retinal disease and they may play specific roles in the development of specific retinopathy phenotypes.

A role for inflammation has also been proposed, and inflammation mediators have been suggested to be responsible for the increased leakage

observed in the initial stages of diabetes, by causing alterations in the tight junctions of the retinal vessels (Antonetti et aL, 1998).

It is possible that all these different mechanisms of disease play complementary roles in the progression of diabetic retinal disease. The identification of different retinopathy phenotypes characterized by different rates of progression and different dominant retinal alterations, suggest that different disease processes predominate in specific retinopathy phenotypes.

It is also clear now that only a subset of patients with diabetes who develop some form of retinopathy is expected to loose functional vision during their lifetime.

Identification of risk factors to progression to visual loss, precise calculations of risk for progression to visual impairment in individual patients over given time periods appear, finally, to be crucial steps in the decision process of whom to treat, when to initiate treatment and how rigorously.

Diabetic retinopathy is a complication of diabetes mellitus that presents to the practitioner at various stages of a continuum that is characterized by accelerated retinal vascular changes involving also the neuronal and glial retinal tissue with eventual development of complications such as severe macular edema and/or abnormal retinal or optic disk neovascularization leading to irreversible functional visual loss.

The initial changes in the retina are often asymptomatic and undetectable with existing diagnostic tests. This suggests that awaiting overt signs of disease involves accepting some irreversible damage and probable progression. Since patients should be examined in the early stages of the disease, the goal of treatment must be to arrest, delay, or limit progression of predisposing retinal vascular damage to significant visual impairment.

The continuum of diabetic retinopathy progression may be represented as depicted in figure 8.1, taking into consideration the three proposed diabetic retinopathy phenotypes: slow progression, “wet”/leaky and ischemic. Different diabetic patients with retinal disease have clearly different rates of progression and these must be identified and taken into account.

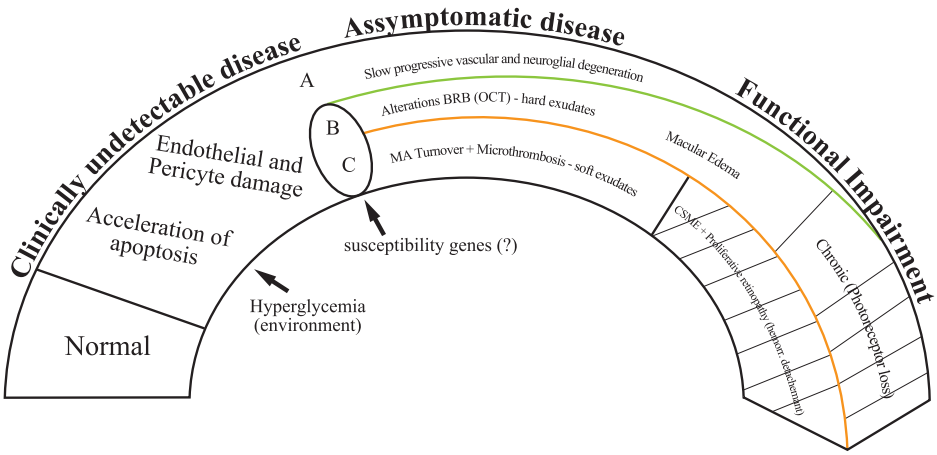

Figure 8.1. Continuum of the progression of diabetic retinopathy.

Consolidation and analysis of cardiovascular risk factors from large patient data sets have led to the development of predictive algorithms that allow physicians to estimate individual patient risk of suffering an atherosclerotic cardiovascular event . Similarly, an algorithm that would allow ophthalmologists to take into account a patient's microaneurysm turnover, retinal thickness progression and HbA1C levels, that is, a so-called "risk calculator", to estimate the risk of visual impairment for a specific diabetic patient would certainly help in determining appropriate treatment for individual patients.

Finally, it is clear that identifying individual variations in disease progression by characterizing the diabetic retinopathy phenotype of each patient and the modulating risk factors such as HbA1C levels and other life style habits may open completely new perspectives for the management of diabetic retinal disease. If the patients with the greatest risk of progression and with the greatest potential to benefit from treatment can be identified by multivariate risk assessment, fewer patients will need to be treated to prevent one case of blindness. This is of extreme importance at a time where scarce resources must be focused and concentrated on the individual cases that need close follow-up and timely treatment.

The proposed approach would allow for individual patient's needs, preferences, and tolerances and consider that the range of patients with diabetes differs substantially in terms of age, disease progression and comorbidities. The patient with diabetic retinopathy must be treated as an individual and it is important to identify his phenotype of progression in order to develop a management plan adequate for him which should be "made to measure".

8.2. Roadmap for improved diabetic retinopathy management

The aim of DIAMAP, a project funded by the European Commission (FP7 HEALTH 2007) was to chart the future of diabetes research in Europe for the benefit of the person with diabetes (www.diamap.eu).

The DIAMAP developed strategic maps from different sub-groups to help guide research in Europe in diabetes for the period of 2010-2019. This was the first attempt to prepare a road map for diabetes research in Europe. However, DIAMAP is set in the context of previous road maps charted in the United States.

The analysis of these roadmaps is of particular interest because it allows to identify and compare its various proposed steps and directions in the area of diabetic retinopathy with the ongoing work of our research group.

I will be including the two roadmaps of special interest to diabetic retinopathy research signalling the steps in which our work is presently focusing and making contributions (Figures 8.2 and 8.3).

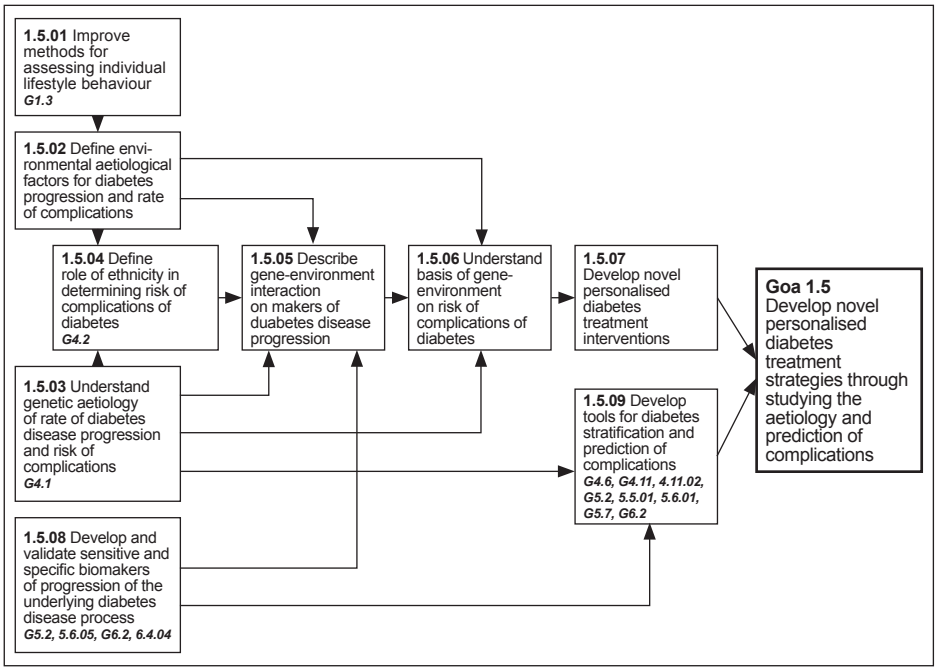

Figure 8.2. DIAMAP – Goal 1.5. Develop novel personalised diabetes treatment strategies through studying the aetiology and prediction of complications.
Short Guide to road map 1.5: There is heterogeneity in the rate of change in the underlying pathophysiological processes that are driving hyperglycaemia among people with established diabetes. This heterogeneity may be explained by genetic, developmental and environmental factors. Understanding these factors could lead to targeting of therapy at these specific pathways, to individualised treatment and improved health outcomes.

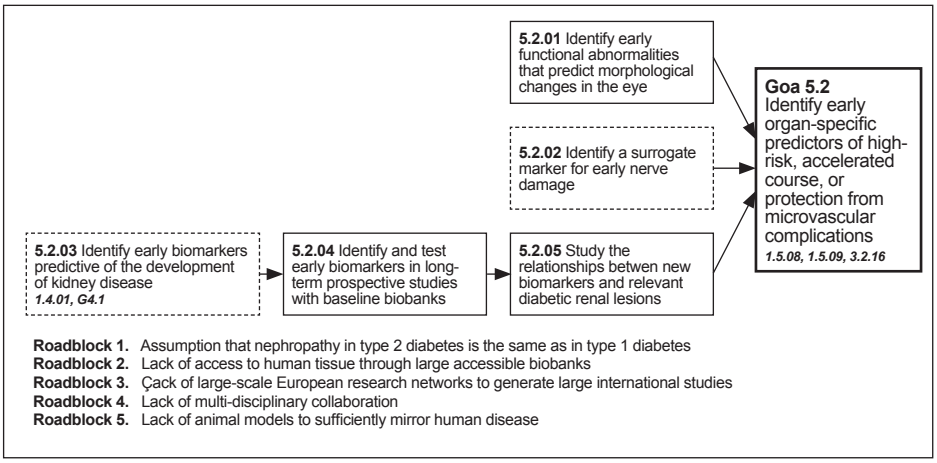

Figure 8.3. DIAMAP – Goal 5.2. Identify early organ-specific predictors of high-risk, accelerated course, or protection from microvascular complications.
Short Guide to road map 5.2: It is crucial to develop early markers able to identify people with diabetes at high risk of developing microvascular complications, given that the best results are obtained when the complications are treated at very early stages of disease. The availability of early markers/predictors would allow the high-risk patients to be treated intensively with currently available therapies as well as with new agents. When the comçlications are established and advanced, current strategies can only partially impact on the progression towards end-organ failure.

On goal 1.5 (Figure 8.2), our ongoing research work is directed to steps 1.5.03, 1.5.07 and 1.5.08 with special relevance to 1.5.08, i.e., to the development of biomarkers of retinopathy progression to vision loss and identification of different phenotypes of retinopathy progression.

On goal 5.2 (Figure 8.3), our wok fits particularly well in steps 5.2.01 and 5.2.02 structural abnormalities in the retina are being identified by the rate of microaneurysm turnover using the RetmarkerDR and layer by layer changes in retinal thickness. Functional alterations such as alterations of the blood-retinal barrier are now being identified non-invasively by Optical Coherence Tomography. The new EUROCONDOR project funded by the European Union aims to identify early functional changes in the neurons and glia of the retina by looking for changes in the implicit times of the multifocal electroretinogram in the early stages of the retinopathy. The multifocal electroretinogram results may help identify early nerve damage in the retina which is understood as a relevant step in the final goal of identifying organ-specific predictors of high-risk, accelerated course, or protection from microvascular complications of diabetes such as diabetic retinopathy.

8.3. Prospective care and personalized health planning in diabetic retinopathy

The great medical advances that have been made in the last fifty years must be used to create and validate new models of prospective healthcare that determine the risk for individuals to develop specific diseases, detect the diseases earliest onset, and prevent or intervene early enough to provide maximum benefits (Snyderman and Williams, 2003).

In our present knowledge of diabetic retinopathy portrayed in the recent developments described in the previous chapters there are the bases necessary to implement this approach. We need to address disease prevention and early personalized management of disease progression. It is now possible to consider for each individual a plan formulated to keep his health, i.e., deal with his diabetic retinopathy and minimize the potential problems associated with progression of the retinopathy to vision loss.

We may now initiate this process by assembling a risk analysis, taking into account the risk-assessment tools already available. Very early in the disease process is now possible to establish a regular program of microaneurysm assessment, based on non-invasive and simple to use fundus photography examinations. Assessment of the retinal disease progression and phenotype identification may easily be refined by adding regular examinations with OCT, another non-invasive methodology.

Characterization of the phenotype of progression, may in the near future be evaluated in the context of other risk factors such genetic, environmental and lifestyle considerations. Further genomic and epidemiological research will contribute because it will benefit from the identification of different phenotypes of progression of the retinopathy, here highlighted.

The present level of knowledge of diabetic retinopathy allows us to envision a personalized health plan for each diabetic patient as regards this complication of diabetes. This personalized health plan will include a good characterization of its diabetes status, a description and characterization of the specific diabetic retinopathy phenotype, a health risk analysis, including whenever possible, genetic, environmental and lifestyle aspects, and even a list of potential countermeasures to be employed to prevent further disease progression.

The challenge of developing a personalized healthcare plan for each diabetic patient must be addressed when the first microaneurysms are identified by fundus photography screening. Only if this is done may this challenge be met with success. Appropriate prevention of diabetic retinopathy progression to its dreaded sight-threatening complications, clinically significant macular edema and proliferative retinopathy is possible and has already been demonstrated to be associated with important cost savings.

Diabetic retinopathy is a chronic disease resulting from diabetes, also a chronic disease. In diabetic retinopathy we have in the pre-symptomatic period a window of opportunity to delay or prevent the sight-threatening complications. We have now many of the risk-assessment tools necessary to assign to our patients as well as planned personalized management program.

Healthcare systems must now take advantage of specific situations such as diabetic retinopathy to implement prospective healthcare by testing novel ways of healthcare delivery.

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Prémio Bial de Medicina Clínica 2012

Instituído em 1984 pela FUNDAÇÃO BIAL, o PRÉMIO BIAL é considerado um dos maiores prémios na área da Saúde em toda a Europa, distinguindo profissionais de referência mundial nas suas áreas de investigação.

A obra "Translating Discoveries in Basic Molecular Biology, Cell Biology, and Molecular Genetics into Transformative Approaches to the Diagnosis and Treatment of Currently Incurable Neurodegenerative Dementias", de autoria de Peter St. George Hyslop, Diretor do Centro para a Investigação de Doenças Neurodegenerativas da Universidade de Toronto e Professor de Neurociências Experimentais na Universidade de Cambridge, foi galardoada com o GRANDE PRÉMIO BIAL DE MEDICINA.

O PRÉMIO BIAL DE MEDICINA CLÍNICA distinguiu o trabalho "Diabetic Retinopathy. New Perspectives for Personalized Management" de José Cunha-Vaz, professor emérito da Universidade de Coimbra e presidente da AIBILI - Associação para a Investigação Biomédica e Inovação em Luz e Imagem.

Na décima quinta edição do PRÉMIO BIAL foram também premiadas duas obras com Menções Honrosas.

O júri da edição PRÉMIO BIAL 2012 foi presidido por António de Sousa Pereira e constituído por Miguel Castelo-Branco, Maria João Marques Gomes, Adelino Leite Moreira, António Martins da Silva, Luís Providência, Nuno Sousa e Rui Victorino.

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