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Clínica Universitária de Oftalmologia

Ocular and systemic risk markers for development of Macular Edema and Proliferative Retinopathy in Type 2 Diabetes. A five-year longitudinal study

António Henrique Cunha-Vaz Martinho

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Orientado por:

Dr. David Sousa

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Preâmbulo

Ao longo do curso tive a oportunidade de realizar alguns estágios na área de oftalmologia. Logo no primeiro ano, realizei um estágio no serviço de oftalmologia do Hospital Santa Maria que serviu como primeiro contacto real com a especialidade. Apesar de a minha família já se encontrar ligada à oftalmologia e à visão desde há 3 gerações, esta foi a minha primeira experiência num serviço hospitalar.

Mais tarde, no meu 5º ano do curso, tive a possibilidade de fazer um estágio de 1 mês em oftalmologia no hospital Johns Hopkins em Baltimore, EUA. Foi uma experiência inesquecível. Abriu a minha visão para uma realidade diferente, de uma dimensão enorme, e que funciona de forma excecional. Lá, todas as subespecialidades de oftalmologia estão individualizadas e tive a oportunidade de experiência a realidade de cada uma delas. Pude participar em sessões com internos da especialidade em que foram debatidos casos clínicos, e também assistir e participar em algumas cirurgias. Durante este tempo tive sempre à minha disposição, 24 sobre 24 horas, um laboratório para treino de suturas oftalmológicas bem como um simulador 3D de cirurgia de cataratas. Esta experiência deixou-me muito interessado na área de oftalmologia.

Ao longo destas experiências, fui percebendo que a área da retinopatia diabética era uma área que despertava a minha atenção e entusiasmo, pela sua crescente importância e impacto que tem nas pessoas com diabetes, em que a perda a visão é dos maiores problemas que estas enfrentam.

Sendo eu de Coimbra e já tendo acompanhado alguma da investigação realizada pela AIBILI, um centro de referência internacional em investigação clínica na área da visão, foi-me dada a oportunidade de colaborar num projeto de investigação clínica deste centro podendo conciliá-lo com o meu trabalho final de mestrado.

Foi uma tarefa desafiante. Realizar investigação científica é uma tarefa multidisciplinar que junta médicos, cientistas, engenheiros e técnicos e como tal foi preciso uma comunicação articulada para sincronizar o trabalho, a contribuição e o esforço de todos.

Neste projeto pude acompanhar todo o processo de análise de dados que foi impressionante pela sua dificuldade e pelo rigor que necessita, apresentando apenas o que

é real e com o maior cuidado possível. Sendo este um estudo que analisou uma população ao longo de 5 anos, permitiu-me entender a importância de ter bases de dados corretas, fidedignas e estruturadas da mesma forma, possibilitando a comparação dos dados. Descrever este processo corretamente nos métodos exigiu muita planificação para que ficasse claro. Foi curioso perceber o impacto do tamanho da população em estudo e como isso influencia a significância dos resultados. Também foi interessante fazer previsões do que se esperava observar na população e perceber se se verificavam realmente como era previsto, ou se apareciam novas informações para as quais tínhamos de encontrar possíveis explicações.

Além disso, foram desafiantes as discussões sobre o artigo, a convergência ou divergência de opiniões e perspetivas. Até mesmo a dificuldade de explicar o que se está a pensar, o que se pretende pesquisar nos dados e se é a melhor forma de chegar a conclusões relevantes. Após o registo dos resultados obtidos foram precisas inúmeras versões e revisões para que se conseguisse elaborar um texto que fosse devidamente descritivo, mas ao mesmo tempo apelativo e de fácil leitura pois só assim é que um artigo consegue atingir o seu objetivo final, trazer informação relevante de forma compreensível para a comunidade cientifica.

A discussão, que apesar de ser bastante estimulante, exigiu muito trabalho e necessidade de rigor científico para se poderem tirar as devidas interpretações dos dados sem nunca dizer mais do que o que foi observado.

Finalmente, após o trabalho concluído e com muitas horas de reuniões, esforço e trabalho de equipa o artigo vai ser enviado para a revista científica *DiabetesCare* para ser sujeito a *peer-review* e poder ser publicado.

Resumo

Objetivo: Comparar o valor preditivo relativo de marcadores de risco sistémicos e oculares no desenvolvimento de edema macular diabético (EMD), considerando o edema macular clinicamente significativo (EMCS) e o edema com envolvimento do centro da mácula (EECM), e da retinopatia diabética proliferativa (RDP) em pessoas com diabetes *mellitus* tipo 2 (DM2).

Plano da investigação e métodos: Pacientes com DM2 e retinopatia diabética não proliferativa ligeira foram seguidos prospectivamente durante um período de 5 anos. Realizaram-se exames à data de início, 6 meses após primeira visita e anualmente (melhor acuidade visual corrigida, fotografia do fundo ocular a cores e tomografia de coerência ótica). Progressão foi definida pelo desenvolvimento de um dos subtipos de EMD (EMCS e EECM) ou RDP. Avaliaram-se marcadores de risco sistémicos e oculares.

Resultados: Dos 212 olhos/doentes com DM2 incluídos no estudo, 172 foram seguidas por um período de 5 anos ou até ao desenvolvimento de uma das complicações - 27 desenvolveram EMD ou RDP. Relativamente ao EMCS e à RDP, um valor mais alto de HbA_{1c} foi o fator de risco sistémico mais importante. A análise multivariada incluindo os marcadores de risco oculares e a HbA_{1c} revelou que o *Turnover* de microaneurismas (TAM) (HR:1,03; p<0.018) e a espessura central da retina (ECR) (HR:1,08; p<0,003) estavam associados a um aumento significativo da probabilidade do desenvolvimento de EMCS e/ou RDP, sendo que a ECR foi o único marcador de risco para EECM (HR:1,17; p<0,001).

Conclusões: Neste estudo longitudinal de 5 anos de olhos com retinopatia ligeira em pessoas com DM2, o risco de desenvolver complicações visuais associou-se a marcadores de risco oculares, tais como TAM e ECR, enquanto a HbA_{1c} foi o marcador sistémico identificado mais relevante.

Palavras-chave: Diabetes tipo 2, Retinopatia diabética, Edema macular, Retinopatia proliferativa, Biomarcadores oculares

Abstract

Objective: To compare the relative predictive value of ocular and systemic risk markers of development of diabetic macular edema (DME), considering both clinically significant macular edema (CSME) and center involved macular edema (CIME), and proliferative retinopathy (PDR) in persons with type 2 diabetes (T2D).

Research Design and Methods: Patients with T2D and mild nonproliferative retinopathy (NPDR) were followed prospectively for a 5-year period. Examinations were performed at baseline, 6 months after first visit and annually (best corrected visual acuity, color fundus photography and optical coherence tomography (OCT)). Progression was identified by the development of the two subtypes of DME (CSME and CIME) or PDR. Systemic and ocular risk markers were evaluated.

Results: Of the 212 eyes/patients with T2D included in the study, 172 were followed over a 5-year period or until the development of a study outcome. Twenty-seven developed DME or PDR. Regarding CSME and PDR development, a higher HbA_{1c} was the most important systemic risk factor. A multivariate analysis including ocular risk markers and HbA_{1c} revealed that Microaneurysm Turnover (MAT) (HR:1.03; $p<0.018$) and central retinal thickness (CRT) (HR:1.08; $p<0.003$) were significantly associated with an increased likelihood of development of CSME and/or PDR, with CRT measurements being the only risk marker for CIME (HR:1.17; $p<0.001$).

Conclusions: In a 5-year longitudinal study of eyes with mild diabetic retinopathy, the risk of developing vision-threatening complications was associated with ocular risk markers such as MAT and CRT. HbA_{1c} remained the most relevant systemic marker identified.

Key words: Type 2 Diabetes, Diabetic Retinopathy, Macular Edema, Proliferative Retinopathy, Ocular Biomarkers

ÍNDICE

Introduction

The incidence of diabetes mellitus (DM) is increasing worldwide. Recent estimates suggest that the number of people with diabetes between the ages of 20 and 79 years will increase from 415 million in 2015 to 642 million in 2040 (1). Diabetic retinopathy (DR) is one of the most common complications of diabetes and may lead to blindness through vision-threatening complications, such as diabetic macular edema (DME) and proliferative retinopathy (PDR).

Diabetic macular edema (DME) threatens patient independence and can lead to reduced quality of life. It is characterized by retinal thickening around the fovea, being classified in two sub-types: clinically significant macular edema (CSME), identified as retinal thickening within 500 μm of the center of the fovea or presence of hard exudates (with thickening of the adjacent retina) within 500 μm of the center of the fovea, or thickening of at least 1 disc area located less than 1 disc diameter from the center of the fovea (2), or center-involving macular edema (CIME), defined as central retinal thickness (CRT) ≥ 290 μm in women and ≥ 305 μm in men, for Zeiss Cirrus spectral-domain optical coherence tomography (SD-OCT (3).

Since DME can be present in all DR severity stages and may be unnoticed by the patient until late, early screening and detection is crucial to improve management and visual outcomes. Several studies have established that certain systemic factors have associations with incidence and progression of DR, namely glycemic control, arterial hypertension, high cholesterol and hyperlipidemia, obesity, inflammatory markers, sleep-disordered breathing, and exercise (4). Although the benefits of the control of some of these factors in DR are not completely established, a revision of the literature depicts the benefits for good glycemic and blood pressure control (5), providing a foundation for preventing the vision-threatening complications of DR. Many more associations are present with univariate testing than with multivariate testing, suggesting that the information carried by these associations may be redundant across more than one factor (6). In addition, predictive factors are not always the same for different outcomes. For example, those factors that predict PDR, CSME and CIME may be different.

In 1993, the Diabetes Control and Complications trial (7) concluded that intensive therapy lowered time-averaged blood glucose values measured as hemoglobin A_{1c} (HbA_{1c}) and significantly reduced the risk of sustained retinopathy progression by 73% compared with standard treatment. However, HbA_{1c} and duration of diabetes (glycemic exposure) explain only about 11% of the variation in retinopathy risk for the entire study population, suggesting that the remaining 89% of the variation risk is presumably explained by other factors, independent of HbA_{1c} (8).

In addition to systemic factors, there are ocular factors that should be considered since they may identify the eyes at risk and are essential components of different phenotypes of DR. Our group has reported different ocular markers that seem to be related with different risks for development of CSME in type 2 diabetic (T2D) persons, namely the turnover of microaneurysms (MAT) (9), central retinal thickness (CRT) (10) and the different phenotypes of non-proliferative diabetic retinopathy (NPDR), based on MAT and CRT (11).

In this study, we report a five-year prospective longitudinal analysis of both systemic and ocular factors that may play a role in the development of DME and PDR, the vision-threatening complications of DR.

Methods

This is a 5-year prospective longitudinal observational cohort study that included eyes/patients with mild NPDR - Early Treatment Diabetic Retinopathy Study (ETDRS classification) grades 20 and 35 (12). Patients were followed for a period of 5 years or until the time of development of CIME, CSME or PDR. The tenets of the Declaration of Helsinki were followed, the ethics approval was obtained from the local Institutional Ethical Review Board and each participant signed a written informed consent to participate in the study after all procedures were explained.

A total of 212 patients were included, men and women with diagnosed adult-onset T2D and aged 42 to 82 years, with a maximum HbA_{1c} value of 10%. Exclusion criteria included any previous laser treatment or intravitreal injections, presence of age-related macular degeneration, glaucoma, vitreomacular disease, high ametropia (spherical equivalent greater than -6 and +2 diopters) or any other comorbidity that could affect the retina. Excluded were also subjects with uncontrolled systemic hypertension above 210 mmHg and history of ischemic heart disease. Eyes with baseline central thickening identifying CIME (13) were also excluded.

At baseline visit (V0), the following data was collected for each participant: age, duration of diabetes, body weight, height, blood pressure, concomitant medications and lipidic and HbA_{1c} levels. Best-corrected visual acuity (BCVA) was measured for each eye using the ETDRS protocol and Precision Vision charts at 4 m. Baseline demographics and clinical characteristics of study patients, including those who were lost to follow-up (n=40) and those that remained in the study (n=172) were previously described (12).

The DR severity level was assessed as previously described elsewhere (14), based on the 7-field protocol using the ETDRS classification, being the study eye selected at baseline. If both eyes fulfilled the inclusion criteria, the eye showing the more advanced ETDRS grading in any given patient was chosen to be the study eye.

Study follow-up visits were performed at 6 months (V1), 12 months (V2), 24 months (V3), 36 months (V4), 48 months (V5) and 60 months (V6) or last visit before treatment (in the eyes that developed either CSME or PDR). The patients underwent a complete eye

examination, which included BCVA, slit-lamp examination, intraocular pressure measurement, digital seven-field color fundus photography (CFP) and optical coherence tomography (OCT).

The outcomes considered were CIME, CSME, and PDR. CIME is defined as CRT ≥ 290 μm in women and ≥ 305 μm in men (Zeiss Cirrus SD-OCT), according to pre-defined OCT values (13); CSME is identified on clinical examination as defined by the ETDRS group as retinal thickening within 500 μm of the center of the fovea or presence of hard exudates (with thickening of the adjacent retina) within 500 μm of the center of the fovea, or thickening of at least 1 disc area located less than 1 disc diameter from the center of the fovea (2). Finally, PDR is identified by the presence of abnormal new vessels in the retina.

Laboratory analyses included creatinine, glucose and HbA_{1c} concentration, red blood cell count, white blood cell count, platelet amount, and hematocrit. Metabolic control was also assessed by measuring the plasma concentrations of lipid fractionation identifying total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides.

Color Fundus Photography and Microaneurysm quantification by RetmarkerDR

Color fundus photography (CFP) was performed according to the ETDRS protocol. The seven-fields photographs were obtained at 30/35º, using a Topcon TRC 50DX camera (Topcon Medical Systems, Japan) for ETDRS DR classification according to the ETDRS grading scale (14).

Additionally, 45/50º field-2 images were obtained and subjected to automated microaneurysm (MA) analyses using the RetmarkerDR (Retmarker SA, Coimbra, Portugal). This automated computer-aided diagnostic system consists of software earmarking MA and red dot like vascular lesions in the macula (all referred to as MA); it includes a co-registration algorithm that allows comparison within the same retina location between different visits for the same eye (15,16). Briefly, the algorithm computes for each eye the number of MAs in each visit, the number of MAs that appear and/or

disappear from one visit to the other, allowing calculation of the number of MAs appearing and/or disappearing per time interval (i.e., the MA formation rate and the MA disappearance rate, respectively). The MA turnover (MAT) is computed as the sum of the MA formation and disappearance rates.

Optical Coherence Tomography

Thinning and thickening of the retina layers (Neurodegeneration and Edema)

OCT was performed using the Cirrus Zeiss 5000 (Carl Zeiss Meditec, Dublin, CA).

The Macular Cube 512x128 acquisition protocol, consisting of 128 B-scans with 512 A-scans each, was used to assess the subjects' CRT and the average thickness value of the ganglion cell layer inner plexiform layer (GCL+IPL), collected from the standard Cirrus examination reports. Retinal layers segmentation for layer thickness calculation was performed on OCT using the segmentation software implemented by AIBILI, as previously described (12,17). Automated analysis results were reviewed by a masked grader.

Eyes with CIME were identified following the reference values established by the DRCR.net for Cirrus SD-OCT (3). GCL+IPL thickness decreases were considered to identify neurodegeneration (18) whereas full CRT increases were considered to identify edema (19), comparing to a healthy control population (17,20).

Characterization of retinopathy phenotypes

The three different DR phenotypes for NPDR, A, B and C, previously described by our group (11,21) were identified according to the following rules - Phenotype A: MAT < 6 and normal CRT values (CRT < 220 μm, i.e., normal mean ±1 SD); Phenotype B: MAT < 6 and increased CRT values (CRT ≥ 220 μm); Phenotype C: MAT ≥ 6, with or without increased CRT.

Statistical Analysis

The collected data on each eye is synthesized as means and corresponding standard deviations for continuous variables or absolute and relative frequencies for categorical and ordinal variables. Comparison of baseline characteristics of patients that did not develop outcome and those who developed CSME, CIME or PDR was performed using Mann-Whitney test (due to violation of assumption of normality) or the Chi-square test with Monte-Carlo correction.

The Cox proportional hazards model was used to assess the risk of development of CSME and CIME associated with each ocular marker evaluated at the baseline or 6 months appointment – MAT, MA formation rate, MA disappearance rate, CRT, GCL+IPL thickness, GCL+IPL InRing thickness – both individually (univariate analysis) or adjusted for systemic confounders (multivariate analysis). Results were presented as hazard ratios (HR) and corresponding 95% confidence intervals.

Additionally, to assess the capability of the ocular markers on the prediction of development of CSME or CIME, MAT, CRT, GCL+IPL thickness, GCL+IPL InRing thickness were introduced as independent predictors in a binary logistic regression. The obtained predicted probabilities were then tested for the discriminatory performance using ROC curves.

All analyses were performed with SPSS (IBM SPSS Statistics version 24), and a p-value <0.05 was considered statistically significant.

Results

Of the 212 eyes included in the study, 172 eyes of persons with T2D, one eye per person, were followed in a prospective longitudinal study for a period of 5 years or until development of vision-threatening complications such as DME, identified as CSME or CIME, or PDR. Fourteen eyes developed CSME (8%) and ten developed CIME (6%), whereas four eyes developed PDR (2%) with one of these eyes showing both CSME and PDR.

Baseline characteristics of the different eyes, considering the different outcomes, are presented in table 1. Patients developing CSME were younger ($p=0.002$) and showed increased levels of triglycerides ($p=0.044$) (Table 1). Either for CIME or PDR no systemic marker could be identified as related (Table 1). However, the analysis of descriptive data of demographic and systemic characteristics determined that patients who developed CSME and/or PDR (supplementary table 1) had lower age ($p<0.001$), lower BMI ($p<0.032$) and higher HbA_{1c} values ($p<0.015$).

Regarding ocular characteristics and their relationship with vision-threatening outcomes, it was possible to identify statistically higher values of MAT in patients that developed CSME ($p=0.001$) or PDR ($p=0.007$), when compared to those who did not develop any of these outcomes. These findings were corroborated by the two components of MAT, i.e. MA formation rate and MA disappearance rate (table 1). Noteworthy, no statistically significant differences could be found between mean MAT values of CIME and no outcome patients ($p=0.846$).

Regarding CRT, which increase indicates the presence of edema, the highest values were, as expected, detected in patients that developed CIME (297.6 ± 9.3 µm), followed by CSME (282.4 ± 16.3 µm), both statistically different ($p<0.001$) from the values of patients with no outcome (263.2 ± 20.4 µm). Retinal thickness of PDR patients did not differ from those who did not develop any outcome ($p=0.466$).

Less thinning of the GCL+IPL was observed in eyes that developed CIME and CSME ($p<0.001$ and $p=0.002$, respectively, table 1). No statistically significant differences were found for PDR patients comparing to no outcome patients ($p=0.860$). In fact, baseline

data showed less thinning of the GCL+IPL in eyes that developed CSME and CIME even when the thickness of the GCL+IPL was normalized and corrected for the thickness value of the full retina (Ratio GCL+IPL / RT), both statistically higher than the ratio of the no outcome patients (p=0.006 and p=0.001, respectively). Patients with PDR had a similar baseline GCL+IPL / RT ratio to patients not developing outcome (p=0.897) (table 1).

In a univariate Cox proportional hazards regression for the systemic markers, younger age (HR: 0.87, 95%CI 0.81-095, p<0.001), an increased HbA_{1c} (HR: 1.58, 95%CI 1.05-2.39, p=0.030), higher LDL (HR: 1.02, 95%CI 1.00-1.03, P=0.041) and lower BMI (HR: 0.90; 95%CI 0.81-0.99, P=0.040) were associated with an increased likelihood of developing CSME (table 2). For CIME, only a lower systolic blood pressure showed risk association (HR: 0.96; 95%CI 0.92-1.00, P=0.044), whereas no association was found for systemic markers and PDR.

The Cox hazard regression confirmed the importance of the ocular markers in the risk of development of CSME (table 3). After adjustment for the systemic characteristics age, duration of diabetes, gender, HbA_{1c}, total cholesterol, HDL, LDL, triglycerides, systolic blood pressure and BMI, only the baseline value of GCL+IPL InRing was not significantly associated with CSME risk. Contrarily, MAT presented an HR of 1.03 (95% CI: 1.01-1.06; p=0.018), indicating that per unit increase in MAT, the risk of development of CSME increased 3%. Retinal thickness presented an HR of 1.08 (95% CI: 1.03-1.14; p=0.003) and GCL+IPL thickness presented an HR of 1.13 (95% CI: 1.04-1.22; p=0.002) corresponding, respectively 8% and 13% increase in the risk of development of CSME per unit increase in the values of CRT and GCL+IPL thickness. Among the systemic factors used for adjustment of the risk of each ocular marker, age was consistently a significant confounder, with risk reduction of 11-17% per unit increase (hazard ratios varying from 0.83 to 0.89 described in table 3), that is, the older the patient, the lower the risk of developing CSME. BMI was also associated with risk reduction in association with MAT and GCL+IPL thinning. For CIME, only the baseline retinal thickness and GCL+IPL thickness were associated with risk increase (table 3). No significant systemic confounders were found except systolic BP associated with GCL+IPL thickness.

The ROC curve (figure 1A) indicates that MAT, retinal thickness, GCL+IPL CSF thickness and GCL+IPL InRing are good predictors of the development of CSME, with an AUC of 0.87 (0.75 – 0.98), with 85.7% sensitivity and 83.4% specificity at the cutoff value. For CIME, the predictive value of these markers is higher (figure 1B), with an AUC of 0.97 (0.92 – 1.00), 90.0% sensitivity and 91.7% specificity.

When considering together the two vision-threatening complications, CSME + PDR (supplementary table 2), the ocular markers remain determinant in the risk of development of outcome, namely MAT (HR=1.04, 95% CI: 1.02 – 1.07; p=0.001), CRT (HR 1.06, 95% CI: 1.02 – 1.10; p=0.001) and GCL+IPL thickness with HR= 1.10 (95% CI: 1.03 – 1.17; p=0.005). Younger age was the most frequent systemic factor associated with the vision-threatening complications, together with lower BMI and increased HbA_{1c} values. HbA_{1c}, in particular, is associated with a high increase in the risk of development CSME and/or PDR with consistent hazard ratios varying from 1.54 to 1.59, indicating approximately 50% increase in the risk of having a vision-threatening complication per unit increase in the HbA_{1c} levels.

Discussion

This longitudinal follow-up study of persons with T2D and mild NPDR (ETDRS grades 20 and 35) was designed to evaluate the development of vision-threatening complications, either CSME or CIME, and PDR in a period of 5 years, and to identify the systemic and ocular risk markers associated with the development of these complications.

Our results confirm that development of macular edema, either CSME or CIME, and PDR are associated with ocular risk markers of progression to vison-threatening complications, such as baseline MAT and CRT metrics that predict the development of complications in a more consistent manner than systemic markers of metabolic control. Vision-threatening complications appear to occur in an eye with T2D in association with specific ocular characteristics independent of the diabetic disease, suggesting that local, possibly genetic factors, may underlie DR progression to vision loss (21,22).

Of the major vision-threatening DR complications – PDR, is well defined and well characterized by fundus photography, slit-lamp examination, OCT and OCT-angiography being identified by the presence of abnormal new vessels growing on the surface of the retina and into the vitreous. However, the identification of DME is more controversial and associated with difficulty to reach agreement between observers. CSME is characterized by the ETDRS definition identified by fundus photography or slit-lamp examination. In the DRS and ETDRS studies there was a lack of concordance between the professional graders and clinicians in determining macular edema with the two groups agreeing only 55% of the time on the diagnosis after taking into account the agreement due to chance (22). There is, therefore, clear subjective variability when identifying CSME in the clinical examination, which also makes CRT measurements so attractive. Indeed, presence of edema can be defined objectively as an abnormal increase in CRT and this can now be objectively measured using OCT (23). There has been, therefore, and attempt to objectively identify DME and consider that both CSME and CIME represent basically the same disease process, edema resulting from excessive accumulation of extracellular fluid in the retina (24).

Our findings in this 5-year study suggest that different disease processes may be involved in the development of CSME and CIME, indicating that these outcomes should not be analyzed together. CIME and CSME are associated with different retinopathy phenotypes, B and C, with CSME occurring mainly in eyes with increased MAT which indicated the presence of ischemic changes (12).

Retinal thickness measurements, indicating the presence of edema, is the only risk marker that is present in both CIME and CSME, confirming previous observations that they may be a predictor for development of macular edema (10).

Our study shows, once again, the limited role of systemic risk markers in the development of vision-threatening complications of DR in T2D. Age, BMI and HbA_{1c} are the systemic markers associated with CSME and/or PDR. Among these, only HbA_{1c} is correlated specifically with phenotype C (12).

The ocular risk markers examined, MAT, CRT and GCL+IPL thinning, offer very important information. This study shows that eyes with mild retinopathy in persons with T2D, identified as phenotypes A and B, with MAT lower than 6 (calculated on CFP, 2 exams with 6 months interval) and with HbA_{1c} measurements around 7%, had a very low likelihood of developing vision-threatening complications, CSME or PDR, in a period of 5 years. Most eyes/patients included in this study were identified as phenotypes A and B (70%).

On the other hand, an eye with mild retinopathy in a patient with T2D, with MAT equal or higher than 6, demonstrating increased microvascular disease, show high likelihood of development of vision-threatening complications such as CSME and PDR.

These findings have major relevance for DR management and open doors for a more efficient follow-up of these patients, particularly when realizing that a major risk factor for vision loss is the presence of any retinopathy (25). It is of particular interest to note that increased GCP+IPL thinning is observed in the eyes that did not develop outcomes, a finding that needs further research.

Limitations of this study include the fact that the study population is a relatively well-controlled group which was selected based on exclusion criteria such as excessive HbA_{1c} levels and uncontrolled blood pressure. However, the use of these criteria guaranteed a relatively homogenous sample, eliminating outliers that could introduce bias to the results. Furthermore, the population included in the study follows closely the usual criteria for inclusion and exclusion in DR clinical trials. Of major relevance is the fact that it is a 5-year prospective, longitudinal study with yearly examinations in eyes/patients with T2D using regular non-invasive ophthalmological examinations.

In conclusion, ocular risk markers seem to be more informative than systemic risk markers to predict development of vision-threatening complications of T2D, CIME, CSME and PDR. Microaneurysm turnover and retinal thickness measurements obtained using noninvasive examinations are able to predict the development of CSME and PDR in a 5-year period and, of particular value, to identify, in eyes already with retinopathy, which ones are at a very low risk of vision loss.

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Quadros e Figuras

Table 1. Baseline characteristics of the patients according to each endpoint.

Values represent Mean ± SD (Median) except where stated. p-values for the comparison of patients with CSME, CIME or PDR and patients with no outcome

	No outcome (n=145)	CSME (n = 14)	p-value	CIME (n = 10)	p-value	PDR (n=4)	p-value
Demographics							
Age, y	63.4 ± 6.9 (63)	56.2 ± 8.5 (54.5)	**0.002***	53.8 ± 6.1 (64.0)	0.890	59.3 ± 5.4 (57.5)	0.210
Duration of diabetes, y	14.5 ± 7.7 (14.0)	12.4 ± 6.2 (12.5)	0.377	12.4 ± 4.3 (13.0)	0.521	11.3 ± 5.5 (10.0)	0.387
Males/Females, frequency (%)	101/44 (69.7/30.3)	8/6 (57.1/42.9)	0.372	8/2 (80.0/20.0)	0.263	1/3 (25.0/75.0)	0.093
Systemic characteristics							
HbA_{1C}, %	7.5 ± 1.4 (7.3)	8.2 ± 1.6 (8.4)	0.117	7.0 ± 1.6 (7.5)	0.318	8.8 ± 1.7 (9.4)	0.095
Total cholesterol, mg/Dl	184.0 ± 37.9 (180.0)	202.7 ± 41.1 (193.0)	0.112	163.6 ± 39.8 (162.0)	0.141	180.0 ± 48.5 (190.5)	0.896
HDL, mg/Dl	47.5 ± 11.4 (46.0)	41.6 ± 6.9 (43.0)	0.078	49.2 ± 6.8 (48.5)	0.313	54.5 ± 20.7 (62.5)	0.205
LDL, mg/dL	122.3 ± 32.1 (118.0)	140.8 ± 30.2 (134.5)	**0.044***	105.0 ± 33.4 (99.0)	0.138	110.8 ± 49.0 (112.5)	0.619
Triglycerides, mg/Dl	165.2 ± 91.6 (143.0)	212.2 ± 119.4 (160.0)	0.192	140.1 ± 71.4 (117.5)	0.408	185.3 ± 181.6 (109.0)	0.464
Systolic blood pressure, mm Hg	138.7 ± 15.7 (139.0)	136.1 ± 14.5 (140.0)	0.588	128.4 ± 19.9 (130.5)	0.105	145.5 ± 4.5 (145.5)	0.262
Diastolic blood pressure, mm Hg	72.3 ± 8.9 (72.0)	70.5 ± 8.1 (69.5)	0.416	68.8 ± 11.8 (66.0)	0.074	68.0 ± 0.8 (69.0)	0.407
BMI, kg/m^2	30.6 ± 5.8 (30.1)	27.3 ± 6.5 (26.9)	0.069	27.7 ± 4.1 (28.7)	0.134	27.9 ± 8.2 (25.1)	0.260
Ocular characteristics							
BCVA, letters	85.7 ± 4.1 (85.0)	86.2 ± 3.0 (85.5)	0.802	84.1 ± 2.7 (85.0)	0.151	84.8 ± 3.6 (83.5)	0.464
MA turnover, no. per 6 months	5.4 ± 7.6 (2.1)	24.8 ± 32.0 (10.9)	**0.001***	3.8 ± 3.2 (3.0)	0.846	27.8 ± 29.3 (17.1)	**0.007***
MA formation rate, no. per 6 months	2.3 ± 3.8 (0.0)	12.3 ± 16.7 (5.7)	**<0.001***	0.6 ± 1.0 (0.0)	0.180	15.2 ± 18.5 (8.0)	**0.006***
MA disappearance rate, no. per 6 months	3.1 ± 4.6 (2.0)	12.5 ± 16.0 (5.5)	**0.001***	3.2 ± 2.6 (2.0)	0.225	12.6 ± 10.8 (9.1)	**0.006***
Central subfield RT, central 1000µm, µm	263.2 ± 20.4 (264.0)	282.4 ± 16.3 (286.5)	**<0.001***	297.6 ± 9.3 (302.0)	**<0.001***	270.8 ± 26.0 (276.0)	0.466
GCL+IPL CSF thickness, µm	37.7 ± 8.6 (38.2)	47.0 ± 10.1 (49.1)	**0.002***	50.2 ± 9.9 (51.1)	**<0.001***	38.1 ± 9.3 (35.1)	0.860
GCL+IPL InRing thickness, µm	90.0 ± 8.9 (90.3)	97.3 ± 9.1 (95.6)	**0.010***	93.4 ± 19.5 (97.4)	**0.012***	95.8 ± 14.9 (94.8)	0.442
Ratio GCL + IPL / RT (CSF)	0.14 ± 0.03 (0.14)	0.17 ± 0.03 (0.17)	**0.006***	0.17 ± 0.03 (0.17)	**0.001***	0.14 ± 0.03 (0.14)	0.897
ETDRS 20/35, frequency (%)	42/103 (29.0/71.0)	2/12 (14.3/85.7)	0.353	4/6 (40.0/60.0)	0.468	0/4 (0.0/100)	0.330
Phenotype A/B/C, frequency (%)	66/40/39 (45.5/27.6/26.9)	0/3/11 (0.0/21.4/78.6)	**<0.001***	0/7/3 (0.0/70.0/30.0)	**0.004***	0/0/4 (0.0/0.0/100)	**0.013***

Table 2: Univariate Cox Proportional Hazards Regression of Progression to any outcome by different types of systemic markers

	CSME (n = 14)		CIME (n = 10)		PDR (n=4)	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Systemic markers						
Age	0.87 (0.81 - 0.95)	**0.001***	1.01 (0.92 - 1.11)	0.822	0.92 (0.79 - 1.06)	0.250
Duration of diabetes	0.96 (0.89 - 1.04)	0.326	0.96 (0.88 - 1.06)	0.405	0.93 (0.80 - 1.09)	0.403
Gender (female)	1.69 (0.59 - 4.89)	0.329	0.59 (0.12 - 2.75)	0.497	6.49 (0.67 - 62.36)	0.105
HbA_{1C}	1.58 (1.05 - 2.39)	**0.030***	0.71 (0.41 - 1.25)	0.235	2.36 (0.99 - 5.63)	0.053
Total cholesterol	1.01 (1.00 - 1.03)	0.084	0.99 (0.97 - 1.00)	0.097	1.00 (0.97 - 1.02)	0.824
HDL	0.94 (0.89 - 1.00)	0.058	1.01 (0.96 - 1.07)	0.652	1.04 (0.97 - 1.11)	0.251
LDL	1.02 (1.00 - 1.03)	**0.041***	0.98 (0.97 - 1.00)	0.086	0.99 (0.96 - 1.02)	0.469
Triglycerides, mg/dL	1.00 (1.00 - 1.01)	0.083	1.00 (0.99 - 1.01)	0.396	1.00 (0.99 - 1.01)	0.661
Systolic blood pressure	0.99 (0.96 - 1.02)	0.494	0.96 (0.92 - 1.00)	**0.044***	1.03 (0.97 - 1.09)	0.406
Diastolic blood pressure	0.97 (0.91 - 1.04)	0.391	0.95 (0.88 - 1.03)	0.218	1.95 (0.84 - 1.08)	0.446
BMI	0.90 (0.81 - 0.99)	**0.040***	0.91 (0.80 - 1.02)	0.115	0.92 (0.76 - 1.11)	0.369

Table 3: Univariate and Multivariate Cox Proportional Hazards Regression of Progression to CSME and CIME by different types of ocular markers

CSME					
Ocular markers	**Univariate**		**Multivariate***		
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**Significant confounders**
MA turnover	1.04 (1.02 - 1.05)	**<0.001***	1.03 (1.01 - 1.06)	**0.018***	Age HR=0.88 (0.79 - 0.97, p=0.015); BMI HR=0.84 (0.73 - 0.97, p=0.015)
MA formation rate	1.08 (1.05 - 1.11)	**<0.001***	1.06 (1.01 - 1.12)	**0.018***	Age HR=0.87 (0.79 - 0.97, p=0.009); BMI HR=0.84 (0.73 - 0.97, p=0.015)
MA disappearance rate	1.07 (1.04 - 1.11)	**<0.001***	1.06 (1.01 - 1.12)	**0.027***	Age HR=0.88 (0.79 - 0.97, p=0.014); BMI HR=0.85 (0.74 - 0.97, p=0.017)
Retinal thickness	1.06 (1.03 - 1.10)	**0.001***	1.08 (1.03 - 1.14)	**0.003***	Age HR=0.89 (0.80 - 0.98, p=0.023)
ΔRT V1_Vlast	1.03 (1.02 - 1.04)	**<0.001***	1.08 (1.04 - 1.11)	**<0.001***	Age HR=0.83 (0.73 - 0.94, p=0.004); HDL HR=0.83 (0.73 - 0.94, p=0.003)
GCL + IPL thickness	1.12 (1.05 - 1.11)	**<0.001***	1.13 (1.04 - 1.22)	0.002*	Age HR=0.88 (0.79 - 0.98, p=0.022); BMI HR=0.85 (0.74 - 0.98, p=0.028)
ΔGCL + IPL V1_Vlast	0.99 (0.90 - 1.09)	0.864	1.01 (0.91 - 1.12)	0.886	Age HR=0.85 (0.77 - 0.94, p=0.001); HDL HR=0.88 (0.79 - 0.97, p=0.013); BMI HR=0.86 (0.76 - 0.98, p=0.024)
GCL + IPL InRing	1.11 (1.04 – 1.18)	**0.001***	1.05 (0.97 – 1.12)	0.230	Age HR=0.86 (0.78 - 0.95, p=0.003); HDL HR=0.89 (0.80 - 0.99, p=0.029; BMI HR=0.85 (0.76 - 0.96, p=0.006)
ΔGCL + IPL InRing V1_Vlast	1.05 (0.92 – 1.19)	0.483	1.07 (0.93 – 1.24)	0.340	Age HR=0.85 (0.77 - 0.94, p=0.001); HDL HR=0.87 (0.79 - 0.97, p=0.015); BMI HR=0.86 (0.75 - 0.98, p=0.025)

CIME					
Ocular markers	**Univariate**		**Multivariate***		
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**Significant confounders**
MA turnover	0.96 (0.85 - 1.08)	0.529	0.99 (0.87 - 1.12)	0.827	
MA formation rate	0.71 (0.43 - 1.17)	0.177	0.82 (0.52 - 1.30)	0.398	
MA disappearance rate	1.01 (0.88 - 1.15)	0.934	1.07 (0.87 - 1.19)	0.844	
Retinal thickness	1.17 (1.08 - 1.27)	**<0.001***	1.04 (1.02 - 1.07)	**<0.001***	
ΔRT V1_Vlast	1.02 (1.00 - 1.04)	**0.047***	1.03 (1.01 - 1.07)	0.020*	
GCL + IPL thickness	1.18 (1.09 - 1.28)	**<0.001***	1.27 (1.11 - 1.46)	**<0.001***	Systolic BP HR=0.95 (0.91 - 1.00, p=0.038)
ΔCGL + IPL V1_Vlast	0.97 (0.87 - 1.07)	0.525	0.96 (0.85 - 1.09)	0.511	
CGL + IPL InRing	1.05 (0.97 – 1.14)	0.257	1.03 (0.96 – 1.12)	0.393	
ΔCGL + IPL InRing V1_Vlast	1.05 (1.02 – 1.08)	**0.002***	1.05 (1.01 – 1.10)	**0.018***	

**Multivariate analysis adjusted for Age, Duration of diabetes, Gender, HbA_{1c}, total cholesterol, HDL, LDL, Triglycerides, Systolic Blood Pressure, BMI*

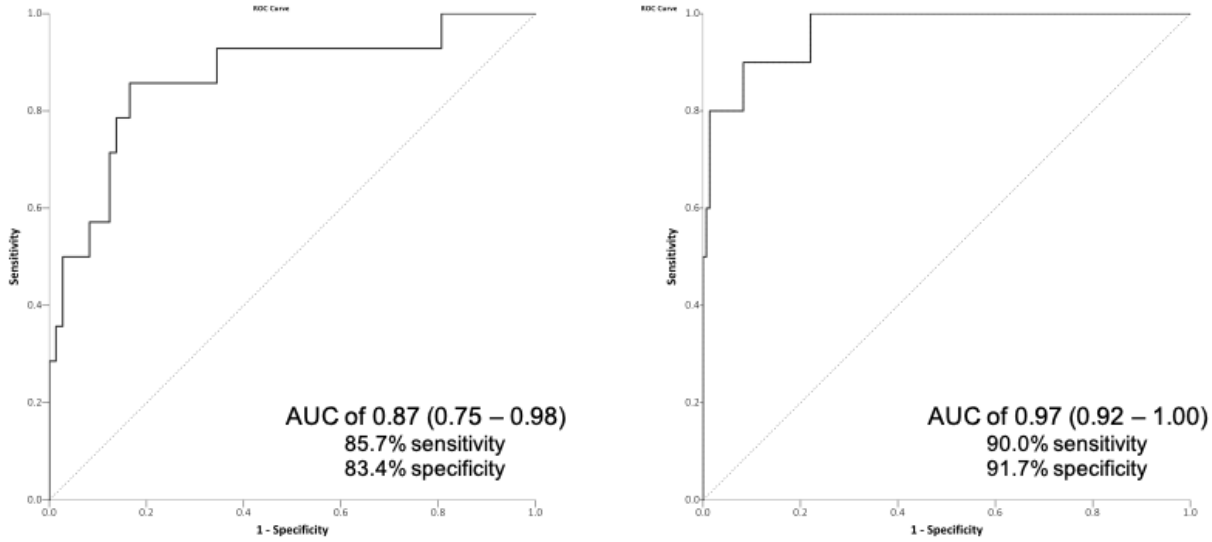

Figure 1 - ROC curve for ocular markers (MAT, retinal thickness, GCL+IPL CSF thickness and GCL+IPL InRing) on the prediction of CSME **(1A)** and of CIME **(1B)**, including sensitivity and specificity at the cut-off value

Supplementary Table 1: Univariate Cox Proportional Hazards Regression of Progression to any outcome by different types of systemic markers

	CSME and/or PDR	
	HR (95% CI)	**p-value**
Systemic markers		
Age	0.89 (0.83 - 0.95)	0.001*
Diabetes duration	0.96 (0.89 - 1.03)	0.255
Gender (Female)	2.43 (0.94 - 6.31)	0.067
BMI	0.90 (0.82 - 0.99)	0.032*
HbA_{1c}	1.60 (1.10 - 2.33)	0.015*
Total cholesterol	1.01 (0.99 - 1.02)	0.230
HDL	0.99 (0.94 - 1.03)	0.506
LDL	1.01 (0.99 - 1.02)	0.240
Triglycerides	1.00 (1.00 - 1.01)	0.280
Systolic blood pressure	1.00 (0.97 - 1.03)	0.790
Diastolic blood pressure	0.97 (0.91 - 1.03)	0.303

Supplementary Table 2: Univariate and Multivariate Cox Proportional Hazards Regression of Progression to CSME and/or PDR by different types of ocular markers

	Univariate		Multivariate*		
	HR (95% CI)	p-value	HR (95% CI)	p-value	Significant confounders
Ocular markers					
MA turnover	1.04 (1.02 - 1.05)	<0.001*	1.04 (1.02 - 1.07)	**0.001***	Age HR=0.92 (0.85 - 1.00, p=0.044); BMI HR=0.88 (0.78 - 0.99, p=0.032)
MA formation rate	1.08 (1.05 - 1.11)	<0.001*	1.08 (1.03 - 1.13)	**0.001***	Age HR=0.92 (0.85 - 0.99, p=0.032); BMI HR=0.88 (0.78 - 0.99, p=0.031)
MA disappearance rate	1.07 (1.04 - 1.11)	<0.001*	1.07 (1.02 - 1.13)	**0.003***	Age HR=0.92 (0.85 - 0.99, p=0.033); BMI HR=0.89 (0.79 - 0.99, p=0.041)
Retinal thickness	1.05 (1.02 - 1.08)	0.003*	1.06 (1.02 - 1.10)	**0.001***	Age HR=0.91 (0.84 - 0.98, p=0.029); Female HR=4.31 (1.09 - 17.00, p=0.037); HbA1c HR=1.59 (1.04 - 2.44, p=0.031)
ΔRT V1_Vlast	1.02 (1.01 - 1.04)	<0.001*	1.04 (1.02 - 1.07)	**0.001***	Age HR=0.88 (0.81 - 0.96, p=0.004)
INL thickness	1.02 (0.95 - 1.11)	0.549	1.05 (0.96 - 1.15)	0.299	Age HR=0.89 (0.82 - 0.96, p=0.003); HbA1c HR=1.54 (1.01 - 2.35, p=0.046)
ΔINL V1_Vlast	1.20 (1.09 - 1.32)	<0.001*	1.22 (1.11 - 1.33)	**<0.001***	Age HR=0.86 (0.78 - 0.94, p=0.001); BMI HR=0.88 (0.78 - 0.98, p=0.024)
GCL + IPL thickness	1.09 (1.03 - 1.15)	0.002*	1.10 (1.03 - 1.17)	**0.005***	Age HR=0.91 (0.84 - 0.99, p=0.032);
ΔCGL + IPL V1_Vlast	1.00 (0.92 - 1.09)	0.989	1.03 (0.94 - 1.13)	0.528	Age HR=0.88 (0.81 - 0.96, p=0.002); HbA1c HR=1.54 (1.01 - 2.34, p=0.043)
GCL + IPL InRIng thickness	1.09 (1.03 - 1.15)	0.005*	1.05 (0.98 - 1.12)	0.177	Age HR=0.90 (0.83 - 0.97, p=0.010); HbA1c HR=1.55 (1.01 - 2.37, p=0.043)
ΔCGL + IPL InRing V1_Vlast	1.07 (0.95 - 1.19)	0.282	1.08 (0.95 - 1.22)	0.255	Age HR=0.89 (0.82 - 0.96, p=0.002); HbA1c HR=1.54 (1.01 - 2.35, p=0.046)

*Multivariate analysis adjusted for Age, Duration of diabetes, Sex, HbA1C, total cholesterol, HDL, LDL, Triglycerides, Systolic Blood Pressure, BMI