

Predicting Progression of Diabetic Retinopathy with the Retmarker

BY JOSÉ CUNHA-VAZ, MD, PhD

Diabetic retinopathy (DR) is a chronic retinal disorder that eventually develops, to some degree, in nearly all patients with diabetes mellitus. DR is characterized by gradually progressive alterations in the retinal microvasculature and is the leading cause of new cases of legal blindness among Americans between the ages of 20 and 74 years of age.¹

It is apparent that, from the available data from a variety of large longitudinal studies and clinical experience, the evolution and progression of DR vary between different individuals, not necessarily progressing to the terminal stage of proliferative diabetic retinopathy (PDR) in all patients.

MONITORING PROGRESSION OF DR

The initial stages of nonproliferative diabetic retinopathy (NPDR) are characterized by the presence of microaneurysms (MA), small hemorrhages, and indirect signs of vascular hyperpermeability and capillary closure, ie, both hard and soft exudates or cotton-wool spots, respectively.

These alterations dominate the initial stages of NPDR and are the only ones used for characterization of levels 20 and 35 of Early Treatment of Diabetic Retinopathy Study (ETDRS) classifications.

An abnormality of the blood-retinal barrier (BRB), demonstrated both by vitreous fluorometry and fluorescein angiography (FA), is also an early finding both in human and experimental diabetes.²⁻⁴ This alteration of the BRB well demonstrated by fluorescein leakage is one of the earliest findings in diabetic retinal disease. It appears to lead directly to macular edema. On ophthalmoscopic examination and fundus photography, the formation of MA, round hemorrhages and hard exudates are the initial changes. They may be counted, and

To assess progression of DR, the MA counting should be considered with every newly developed MA identified in a new location.

retinal MA counting has been suggested as an appropriate marker of retinopathy progression.⁵⁻⁷

It must be realized that MA formation and disappearance are dynamic processes. During 2-year follow-up of 24 type 1 diabetics with mild background DR using FA, Hellstedt and Immonen⁸ observed 395 new MAs and the disappearance of 258 previously identified MAs.

The disappearance of MAs indicates vessel closure and progressive vascular damage. Therefore, to assess progression of DR, the MA counting should be considered with every newly developed MA identified in a new location.

To establish progression it is crucial to be able to compare exams performed at regular intervals. We realized that fundus photography would be the ideal examination to identify changes because of its simplicity and noninvasive nature. In fact, it is possible to identify on fundus digital photography most of the initial changes, such as MA, hemorrhages, and hard exudates.

NEW TOOL TO IDENTIFY CHANGES ON FUNDUS IMAGING

We have developed proprietary software that is able to identify changes in the eye fundus image by comparing successive visits to the reference image (any baseline chosen) based on coregistration and exact colocalization of the changes. This software shows the alterations and identifies their occurrence at each visit.

In a follow-up study with repeated fundus images obtained at regular intervals, MA in the fundus were counted and added as they became visible in new locations in the retina. In this study, 50 eyes of 50 patients with type 2 diabetes mellitus and mild NPDR were prospectively followed.⁹ These were consecutive patients that fulfilled the inclusion criteria and maintained stable metabolic control. Using the software's ability to identify each MA as a single entity in a specific location with identifiable coordinates, the following parameters were assessed: cumulative number of MA, MA formation rate, and MA disappearance rate.

Using the traditional procedure, the total amount of MA detected at every visit remained stable. However, the cumulative number of MA increased from 115 at the first visit to 505 at the last visit, showing a marked increase in new MA. These figures emerged because of the software's potential for counting every MA as a single entity once it was identified by its specific location. It is now obvious that there were many more new MA in the fundus in this 2-year time period than expected using data for each examination separately.

Using the software ability to precisely identify the location of each MA on fundus photographs of diabetic eyes, we looked at MA formation and disappearance rates in 134 patients with type 2 diabetes and NPDR followed up for 2 years as controls of DR clinical trials (81 men and 53 women; range: 41-70 years of age, mean $\pm$ SD: 55.6 $\pm$ 6.3 years) and with a diabetes duration ranging from 1 to 20 years (mean $\pm$ SD: 7.9 $\pm$ 4.4 years). Patients were maintained under acceptable metabolic control during this period and underwent ophthalmologic examinations (including color fundus photography) every 6 months.^{10,11}

MONITORING MA COUNTS AND TURNOVER

At baseline, all patients showed mild to moderate retinopathy and were classified as levels 20 (MA only) or 35 (MA/hemorrhages and/or hard exudates) according to the ETDRS grading scale, based on seven-field stereoscopic fundus photographs. No evidence of clinically

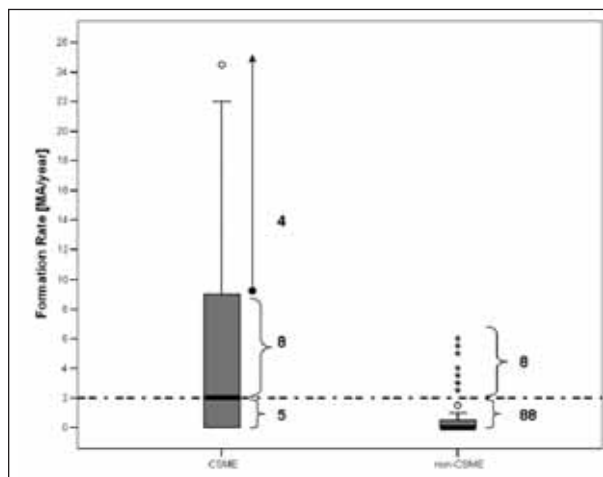


Figure 1. An MA turnover of at least 2 MA/year was found in 12 of the 17 eyes that developed CSME (70.6%), whereas this was only found in 8 of the 96 eyes that did not develop CSME during the 10-year follow-up period (8.3%).

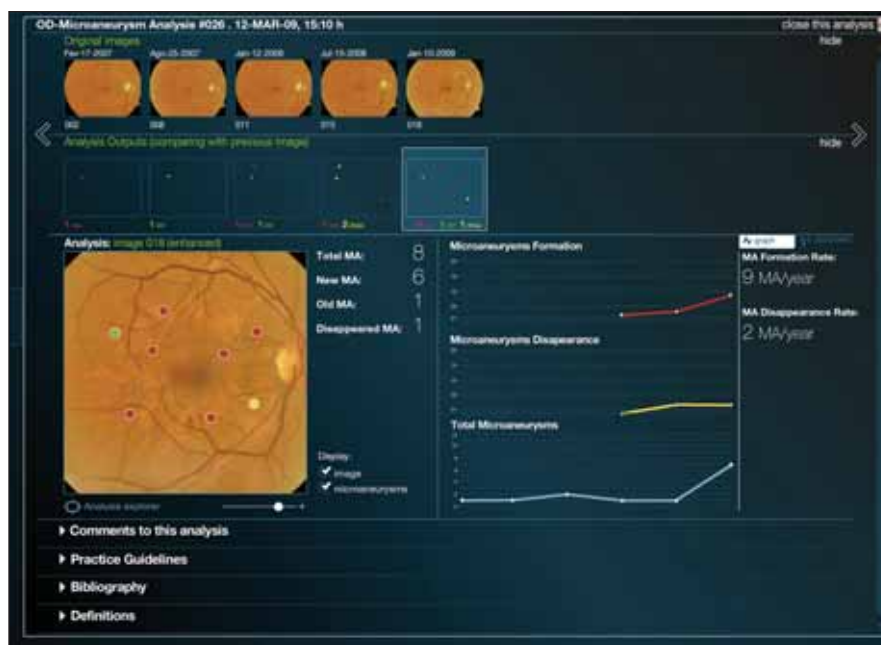


Figure 2. A screen shot of the Retmarker software.

significant macular edema (CSME) was found. Best corrected visual acuity was 20/20 on the ETDRS visual acuity chart in every enrolled eye.

These patients were followed in the same unit by conventional general and ophthalmologic care.

Ophthalmologic and systemic data were collected retrospectively and MA turnover for the 2 first years of follow-up was computed. Only one eye per patient was used for the final data analysis: either the treated eye for patients that developed CSME needing photocoagula-

tion or, alternatively, either the right or the left eye.

During the second period of follow-up (8 years), three patients died and 10 were lost to follow-up. Another eight patients had missing data, leaving 113 eyes to be analyzed from the initial cohort of 134 patients.

At the end of the 10-year follow-up period, 17 out of the 113 patients developed CSME needing photocoagulation.

The first patient who developed CSME needing photocoagulation developed CSME at year 4, while the last patient developed CSME at year 10, respectively 2 and 8 years after the initial follow-up period.

When counting the total number of MA over the first 2 years of the follow-up, a significant increase in the number of MA was found for the CSME eyes ($P=.002$), while for the non-CSME eyes the number of MA remained relatively constant ($P=.647$).

When computing the MA turnover for the same period of time, a higher MA turnover was found in the group of eyes that developed CSME (higher MA formation and disappearance rates). Formation and disappearance rates of 9.2 ± 18.2 and 7.5 ± 16.6 MA/year, respectively, were found for the eyes that developed CSME, while rates of 0.5 ± 1.2 and 0.5 ± 1.2 MA/year were found for the non-CSME eyes ($P<.001$).

An MA turnover of at least 2 MA/year was found in 12 of the 17 eyes that developed CSME (70.6%), whereas this was found in only eight of the 96 eyes that did not develop CSME during the 10-year follow-up period (8.3%; Figure 1).

This study shows that in the initial stages of DR, higher MA counts and MA turnover obtained from color fundus photography are good indicators of DR progression and development of CSME needing photocoagulation.

CORRELATION BETWEEN MA TURNOVER AND CSME

It appears that it is possible to use MA turnover computed from noninvasive color fundus photographs as a biomarker to identify eyes at risk of progression to CSME. An MA formation rate of at least two MA per year in eyes with mild NPDR in patients with type 2 diabetes appears to predict eyes at risk of progression to CSME. In our study, with a 10-year follow-up of 113 patients, the percentage of false negatives (eyes that developed CSME with a low MA formation rate) was 29.4% (5/17) and the percentage of false positives (eyes that did not develop CSME with a high MA formation rate) was 8.3% (8/96), resulting in a sensitivity in predicting CSME development of 70.6% and a specificity of 91.7%. The high negative predictive value for CSME (94.6%, 88/93) indicates that a low MA turnover, ie,

fewer than two MA per year, identifies particularly well the eyes that are not expected to progress to CSME within a 10-year period.

AUTOMATED SOFTWARE PROGRAM

The Retmarker (Critical Health, Coimbra, Portugal; www.retmarker.com) software became available in spring of 2009 and is now fully automatic.

Digital fundus imaging is ideally suited to quality assurance and will allow more efficient utilization in widespread screening programs. Automated analysis techniques offer the advantages of repeatability and consistency, and, although not necessarily better than individual graders or ophthalmologists in absolute terms, they avoid the variability inherent to individual human graders who have their own varying internal reference standards.

Our studies show that MA turnover obtained from field 2 fundus photographs can predict progression of DR.

The Retmarker compares to baseline and identifies automatically changes occurring in the retinal lesions (Figure 2). The changes detected and calculations of MA Turnover allow one to establish a risk profile based on ocular and nonocular risk factors, especially MA formation rates, exudate increase, HgA1, and blood pressure levels. ■

José Cunha-Vaz, MD, PhD, is President of the Board of Administration of AIBILI (Association for Biomedical Research and Innovation on Light and Image) in Coimbra, Portugal. He is a member of the advisory board for Allergan, Inc., Merck, Notal Vision, Novartis, and Pfizer. Dr. Cunha-Vaz can be reached at +351 239480100; fax: +351 239480117; or via e-mail at cunhavaz@aibili.pt.



1. Aiello LP, Gardner TW, King GL, et al. Diabetic retinopathy. *Diabetes Care*. 1998;21:143–156.
2. Cunha-Vaz JG, Faria de Abreu JR, Campos AJ, Figo G. Early breakdown of the blood-retinal barrier in diabetes. *Br J Ophthalmol*. 1975;59:649–656.
3. Waltman SR, Krupin T, Hanish S, Oestrich C, Becker B. Alteration of the blood-retinal barrier in experimental diabetes mellitus. *Arch Ophthalmol*. 1978;96:878–879.
4. Waltman SR, Oestrich C, Krupin T, et al. Quantitative vitreous fluorophotometry: a sensitive technique for measuring early breakdown of the blood-retinal barrier in young diabetic patients. *Diabetes*. 1978;27:85–87.
5. Klein R, Meuer SM, Moss SE, Klein BEK. Retinal microaneurysms counts and 10-year progression of diabetic retinopathy. *Arch Ophthalmol*. 1995;113:1386–1391.
6. Klein R, Klein BEK, Moss SE, Cruikshanks KJ. The Wisconsin epidemiologic study of diabetic retinopathy. XV the long-term incidence of macular edema. *Ophthalmology*. 1995a;102:7–16.
7. Csaky KG, Richman EA, Ferris FL 3rd. Report from the NEI/FDA Ophthalmic Clinical 6 Trial Design and Endpoints Symposium. *Invest Ophthalmol Vis Sci*. 2008;49(2):479–489.
8. Hellstedt T, Immonen I. Disappearance and formation rates of microaneurysms in early diabetic retinopathy. *Br J Ophthalmol*. 1996;80:135–139.
9. Cunha-Vaz JG, Bernardes R. Nonproliferative retinopathy in diabetes type 2. Initial stages and characterization of phenotypes. *Progr Retinal Eye Res*. 2005;24:355–377.
10. Bernardes R, Nunes S, Pereira I, et al. Computer-assisted microaneurysm turnover in the early stages of diabetic retinopathy. *Ophthalmologica*. 2009;223:284–291.
11. Nunes S, Pires I, Rosa A, Duarte L, Bernardes R, Cunha-Vaz J. Microaneurysm turnover is a biomarker for diabetic retinopathy progression to clinically significant macular edema: findings for type 2 diabetics with nonproliferative retinopathy. *Ophthalmologica*. 2009;223:292–297.