Diabetic Retinopathy Phenotypes of Progression to Macular Edema: Pooled Analysis From Independent Longitudinal Studies of up to 2 Years’ Duration

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Submitted: March 1, 2017
Accepted: June 9, 2017
Citation: Cunha-Vaz J, Ribeiro L, Costa M, Simó R. Diabetic retinopathy phenotypes of progression to macular edema: pooled analysis from independent longitudinal studies of up to 2 years’ duration. Invest Ophthalmol Vis Sci 2017;58:BIO206–BIO210. DOI:10.1167/iovs.17-21780

PURPOSE. To test the risk of progression to macular edema (ME) of different phenotypes of mild nonproliferative diabetic retinopathy (NPDR).

METHODS. Data from 882 patients with mild NPDR, Early Treatment Diabetic Retinopathy Study grades 20 and 35, with no prior laser treatment, enrolled in four separate longitudinal studies during 2007–2015 using the same reading center and with the same inclusion criteria and were pooled for analysis. One eye per patient was followed for up to 2 years until development of ME. Ophthalmological examinations included best corrected visual acuity, color fundus photography (CFP), and optical coherence tomography (OCT). They were performed at baseline and 6 months, with the last visit at 12 or 24 months, depending on the study. The eyes/patients were classified as belonging to phenotypes A, B, and C on the basis of OCT central subfield thickness and microaneurysm activity.

RESULTS. A total of 882 eyes/patients performed the 12- or 24-month visit or developed ME. Of these 882 eyes/patients that completed the studies, 103 developed ME, 14 from phenotype A (14 of 466: 3.0%), 48 from phenotype B (48 of 164: 18.6%), and 41 from phenotype C (41 of 252: 16.3%). Eyes/patients from phenotypes B and C showed much higher risks for ME development compared with phenotype A: odds ratio (OR) 95% confidence interval (CI): 13.30 (7.09–24.97) P < 0.001; OR (CI): 6.32 (3.36–11.90) P < 0.001, respectively.

CONCLUSIONS. NPDR phenotypes based on microaneurysm turnover and central macular thickness OCT at the 6-month visit using CFP and OCT, both noninvasive examinations, identified the eyes at increased risk of developing ME.

Keywords: diabetic retinopathy, phenotypes, macular edema, microaneurysms, biomarkers

Diabetic retinopathy (DR) is a common and serious condition. It is the leading cause of blindness among working-age adults in the United States.1 Vision loss related to eye disease among people with diabetes is an important disability that threatens independence and can lead to depression, reduced mobility, and reduced quality of life.2

The Eye Diseases Prevalence Research Group classified DR into two major outcomes: any DR, or any DR consisting of mild, moderate, or severe DR, and vision-threatening DR (VTDR), as DR likely to result in vision loss on the absence of treatment, consisting of proliferative DR, clinically significant diabetic macular edema (CSME), or both.3 This concept is crucial because it addresses the need to identify the eyes that are more likely to progress to VTDR (i.e., to CSME and/or proliferative DR), realizing that VTDR is currently the only stage of DR for which there is treatment available.

It is well recognized that the duration of diabetes and the level of metabolic control condition the development of the retinopathy, but these risk factors do not explain the great variability that characterizes the evolution and rate of progression of the retinopathy in different diabetic patients.4–6 Our group has proposed three phenotypes of mild nonproliferative diabetic retinopathy (NPDR) progression with different risks for development of CSME based on the microaneurysm (MA) turnover rate (TR) and central retinal thickness (CRT) measurements: phenotype A, patients with TR <6 µm and CRT <260 µm (females) or CRT <275 µm (males); phenotype B, patients with TR <6 µm and CRT ≥260 µm (females) or CRT ≥275 µm (males); and phenotype C, patients with TR ≥6. These three different phenotypes were shown to be associated with different risks for the development of macular edema.7

For this study, we pooled the data from four independent longitudinal studies, NCT00763802, NCT01145599, NCT01607190, and NCT01726075, compiling a dataset of 882 NPDR patients with up to 2 years of follow-up, to test the risk of progression to macular edema (ME) of different mild NPDR phenotypes,7 which is, to the best of our knowledge, the largest pooled database of longitudinal noninterventional follow-up of NPDR patients.

METHODS

Study Design

Patients enrolled in four independent, institutional review board–approved, longitudinal studies of mild NPDR from 26
European centers were pooled, yielding a total of 882 eyes/patients enrolled and followed between 2007 and 2016. Eligible patients had type 2 diabetes mellitus with mild NPDR, with Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale grading of 35 or less, classified by the same reading center, the Coimbra Ophthalmology Reading Centre (CORC), and diabetes duration of at least 5 years. Exclusion criteria included previous laser photocoagulation, refractive error greater than or equal to ±5 diopters, and eyes with hazy ocular media or inadequate pupil dilatation. Only one eye per patient was included in the analysis. The duration of the follow-up was 2 years in three of the four studies and only 1 year in one study. For each study, the endpoint was macular edema identified either as CSME, according to ETDRS classification, as center-involving macular edema (CIME) as defined by the Diabetic Retinopathy Clinical Research Network. All four studies adhered to the tenets of the Declaration of Helsinki and informed consent was obtained from the subjects before performing the study procedure; where applicable, the research was approved by the relevant review board for each institution.

Follow-up

Ocular coherence tomography (OCT), best corrected visual acuity (BCVA), and color fundus photography (CFP) were obtained at baseline, 6 months, and with the last examination at 12 or 24 months, depending on the study. CFP was performed according to the ETDRS protocol. An automated computer-aided diagnostic system (RetmarkerDR; Retmarker SA, Coimbra, Portugal) was used to detect MAs automatically on the field-2 color fundus images. This software includes a patented coregistration algorithm that allows comparison within the same retinal location between different visits for the same eye. The RetmarkerDR computes for each eye/patient the number of MAs at each visit and 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 is computed as the sum of the MA formation and disappearance rates. MA turnover less than six was identified as a threshold to separate different mild NPDR phenotypes. All OCT measurements were converted to ZEISS Cirrus scale by using published correction factors for ZEISS Stratus, Heidelberg Spectralis, and Topcon 3D-OCT. Eyes/patients were classified as belonging to phenotypes A, B, and C on the basis of OCT central subfield thickness and MA activity following previous studies. The cases identified as subclinical macular edema did not show major cysts or structural retinal abnormalities such as cysts or disorganization of inner retinal layers. Statistical analysis was performed with Stata 12.1 (Stata Corp. LP, College Station, TX, USA) and P values ≤ 0.05 were considered statistically significant results unless otherwise specified.

Statistical Analysis

The significance of differences between patients who experienced the study endpoint and those who did not was inferred with independent samples t-test, and the significance of differences between phenotypes was inferred with 1-way ANOVA with post hoc Bonferroni corrections. Odds ratios (ORs) were calculated with 95% confidence intervals (CIs), and a multivariate logistic regression was employed to assess the OR of the occurrence of the endpoint per phenotype, with HbA1C as a covariate. A factorial ANOVA model was used to identify significant predictors for the development of ME, with mild NPDR progression phenotypes, HbA1C, and an interaction term as independent variables.

RESULTS

One hundred and three patients of the 882 included in this pool (11.7%) achieved the study endpoint before the completion of the follow-up period. The mean age was 60.9 ± 8.4
years, 65.8% of the patients were males, and the mean HbA1c was 7.8% ± 1.5%. The characterization of the patients according to the mild NPDR phenotypes is summarized in Table 1.

HbA1c presented statistically significant differences, being consistently higher on patients with phenotype C, regardless of the development of ME (Table 2), corroborated by a factorial ANOVA model in which the mild NPDR progression phenotypes is the only significant term (P < 0.001).

Patients with mild NPDR progression phenotype B present the highest OR for progression to ME (OR: 13.30, 95% CI: 7.09–24.95) when compared with phenotype A (Table 3). However, when splitting phenotype C in patients with normal retinal thickness (RT), phenotype C1, and increased RT (central RT [CRT] ≥ 260 μm in females and CRT ≥ 275 μm in males), phenotype C2, the latter presented a greater OR of progression to ME (OR: 29.02, 95% CI: 13.56–62.12) when compared with phenotype A (Table 3).

Microvascular parameters alone present lower ORs, with MA formation rate (FR) at 6 months ≥ 2 and microaneurysm turnover at 6 months ≥ 6, presenting ORs of 1.55 (95% CI: 1.01–2.40; P = 0.035) and 1.78 (95% CI: 1.13–2.77; P = 0.007), respectively (Table 4).

**DISCUSSION**

The results of this pooled analysis of four different follow-up studies of mild NPDR in diabetes type 2 confirm previous studies distinguishing three different DR phenotypes of disease progression to development of macular edema, the most frequent vision-threatening complication of DR. The number of eyes/patients analyzed gives further strength to results obtained in isolated studies. This pooled analysis of 882 patients enabled the collection of 103 progression events, a number sufficiently high to allow the application of parametric statistics, an objective difficult to achieve considering the slow rate of DR progression and consequent low number of events. It is noteworthy that this pooled analysis was based on four different studies involving mild NPDR eyes but having the same inclusion criteria, using the same methodology, and having the image analysis performed by the same reading center.

Using only noninvasive procedures, easy to use repeatedly in the clinical practice, the study shows that characterization of mild NPDR phenotypes indicate that the chance of developing macular edema within 2 years is 7.09 to 24.97 times higher if the patients have increased CRT (phenotypes B and C2) when comparing with phenotype A patients and 13.56 to 62.12 times higher chance if the patients have increased CRT measurements and MA turnover greater or equal to six in a period of 6 months.

The main initial alterations occurring in the early stages of NPDR are MA formation and disappearance, capillary closure, and alteration of the blood-retinal barrier with associated retinal edema.13,14 Digital CFP and OCT alone, both noninvasive procedures, appear to be adequate to document most of these initial alterations. MA and small hemorrhages can be detected by CFP, and their turnover (i.e., disease activity) can be quantified by means of software for automatic analysis of the fundus images, the RetmarkerDR.15 On the other hand, increased RT quantified by OCT identifies the presence of edema, which is a direct result of the alteration of the blood-retinal barrier.

In our first report of characterization of phenotypes of NPDR progression, we found that increased activity of microvascular disease in the macular region, demonstrated by increased rates of MA turnover that characterize phenotype C, was associated with higher risk for development of macular edema. In the present analysis, when pooling the data from four independent studies, we found that phenotype B and

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**Table 2.** HbA1c by Demographic and Clinical Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall Mean ± SD</th>
<th>P</th>
<th>Endpoint Mean ± SD</th>
<th>P</th>
<th>No Endpoint Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7.7 ± 1.4</td>
<td>0.005</td>
<td>7.7 ± 1.4</td>
<td>0.333</td>
<td>7.7 ± 1.4</td>
<td>0.008</td>
</tr>
<tr>
<td>Female</td>
<td>8.0 ± 1.5</td>
<td></td>
<td>8.0 ± 1.7</td>
<td></td>
<td>8.0 ± 1.5</td>
<td></td>
</tr>
<tr>
<td><strong>Mild NPDR progression phenotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenotype A</td>
<td>7.7 ± 1.3</td>
<td>&lt;0.001</td>
<td>8.0 ± 1.5</td>
<td>0.022</td>
<td>7.7 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phenotype B</td>
<td>7.6 ± 1.4</td>
<td></td>
<td>7.4 ± 1.3</td>
<td></td>
<td>7.7 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Phenotype C</td>
<td>8.2 ± 1.6*</td>
<td></td>
<td>8.2 ± 1.7</td>
<td></td>
<td>8.2 ± 1.6*</td>
<td></td>
</tr>
</tbody>
</table>

* Differences are statistically significant from phenotype A on ANOVA with Bonferroni post hoc analysis.
† Differences are statistically significant from phenotype A and B on ANOVA with Bonferroni post hoc analysis.

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**Table 3.** OR of Progression to ME by Phenotypes of Mild NPDR Progression on a Logistic Regression Adjusted for HbA1c Values

<table>
<thead>
<tr>
<th>Phenotypes of Mild NPDR Progression</th>
<th>N</th>
<th>Endpoint, OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotype A</td>
<td>466</td>
<td>14 (3.0) Reference</td>
<td></td>
</tr>
<tr>
<td>Phenotype B</td>
<td>164</td>
<td>48 (29.3)</td>
<td>13.30 (7.09–24.97)</td>
</tr>
<tr>
<td>Phenotype C</td>
<td>252</td>
<td>41 (16.3)</td>
<td>6.32 (3.36–11.90)</td>
</tr>
<tr>
<td>Phenotype C1</td>
<td>199</td>
<td>16 (8.0)</td>
<td>2.84 (1.35–5.96)</td>
</tr>
<tr>
<td>Phenotype C2</td>
<td>53</td>
<td>25 (47.2)</td>
<td>29.02 (13.56–62.12)</td>
</tr>
</tbody>
</table>

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**Table 4.** Frequencies, Percentages, and ORs of MA FR and TR at 6 Months

<table>
<thead>
<tr>
<th>ME</th>
<th>MA FR, n (%)</th>
<th>MA TR, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>50 (9.8)</td>
<td>62 (9.8)</td>
</tr>
<tr>
<td>≥2</td>
<td>53 (14.4)</td>
<td>41 (16.3)</td>
</tr>
<tr>
<td>&lt;6</td>
<td>62 (9.8)</td>
<td>211 (83.7)</td>
</tr>
<tr>
<td>≥6</td>
<td>41 (16.3)</td>
<td>568 (90.2)</td>
</tr>
</tbody>
</table>

* Differences are statistically significant from phenotype A on ANOVA with Bonferroni post hoc analysis.
† Differences are statistically significant from phenotype A and B on ANOVA with Bonferroni post hoc analysis.

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**Table 2.** Diabetic Retinopathy Phenotypes and Macular Edema

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Patients</th>
<th>Percentages</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotype A</td>
<td>7.7</td>
<td>1.5%</td>
<td>1.55 (1.01–2.40)</td>
</tr>
<tr>
<td>Phenotype B</td>
<td>7.6</td>
<td>1.6%</td>
<td>1.78 (1.13–2.77)</td>
</tr>
<tr>
<td>Phenotype C</td>
<td>8.2</td>
<td>1.7%</td>
<td>2.82 (1.35–5.96)</td>
</tr>
</tbody>
</table>
subclinical macular edema indicate the higher risk for development of macular edema, although phenotype C is also associated with increased risk. In this context, our study suggests that increased RT detected by OCT is the most reliable predictor of macular edema development in eyes with mild NPDR.

Of great relevance is the finding that phenotype A, which is characterized by low MA turnover and no signs of increased RT, representing approximately 50% of the mild NPDR patient population, shows a negative predictive value of 97% for the development of macular edema. This observation has important implications for the management of DR, indicating that a large proportion of eyes presenting initial stages of retinal vascular disease will progress very slowly, and those eyes are not likely to develop macular edema for a period of at least 2 years. This subtype of NPDR should, therefore, be excluded from clinical trials evaluating new therapies for DR because of its slow rate of progression.

Various systemic risk factors have been considered to influence progression of DR. Our study confirms our previous findings, showing that progression to macular edema from the initial stages of the retinopathy is apparently correlated only with HbA1C (i.e., glycemic metabolic control). This correlation is found only in phenotype C, characterized by increased microvascular activity, indicating that glycemic control is particularly associated with microvascular disease progression.

MAs and hemorrhages identified as red dots counted on fundus photography and red-dot counting have been previously suggested as appropriate markers of retinopathy progression.16–18 Our observations also confirm previous studies suggesting that subclinical macular edema is a prognostic factor for the progression to macular edema.19,20 The main limitation of this study is the relatively short period of follow-up of 2 years.

This pooled analysis of four studies confirms three major phenotypes of progression of mild NPDR with clearly different risks for development of macular edema, and one of the phenotypes representing 50% of patients with type 2 diabetes and NPDR will most probably not develop macular edema in a 2-year period.

The observation here reported showing different DR phenotypes with different risks for progression to macular edema offers promising perspectives for improved personalized management of DR.

Screening for DR using CFP (two fields) and RetmarkerDR identifies the eyes that are free from microvascular disease and those that have some form of vascular retinopathy. Subsequent analysis of these eyes by OCT will then identify the eyes at risk for development of macular edema, creating the conditions for timely and effective treatment.

Acknowledgments

Supported by Fundação para a Ciência e Tecnologia, Portugal (Study NCT00763802; Coimbra Predictive Model [CPM]) under the research project PTDC/SAU-OSM/72635/2006. Study NCT01145599 (ECR-RET-2010-02) was an investigator-initiated study supported by EVICR.net. Study NCT010607190 (C-TRACER Project 1) was supported by the Champalimaud Foundation. Study NCT01726075 (EUROCONDOR) was supported by the European Union Seventh Framework Program (FP7/2007–2013) under Grant Agreement no. 278040. Study groups available at https://cloud.aibili.pt/cloud/index.php/s/aQPjQc1MDBVpV8.

Disclosure: J. Cunha-Vaz, None; L. Ribeiro, None; M. Costa, None; R. Simó, None

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