Microaneurysm turnover is a predictor of diabetic retinopathy progression

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ABSTRACT

Aim To analyse retinopathy phenotypes and microaneurysm (MA) turnover in mild non-proliferative diabetic retinopathy (NPDR) as predictors of progression to diabetic central-involved macular oedema (CIMO) in patients with type 2 diabetes mellitus (DM) in two different ethnic populations.

Methods 205 patients with type 2 DM and mild NPDR were followed in a prospective observational study for 2 years or until development of CIMO, in two centres from different regions of the world. Ophthalmological examinations, including best-corrected visual acuity (BCVA), fundus photography with RetmarkerDR analysis, and optical coherence tomography (OCT), were performed at baseline and 6, 12 and 24 months.

Results 158 eyes/patients reached either the study endpoint, CIMO (24) or performed the last study visit (24-month visit) without developing CIMO (134). From the eyes/patients in analysis, 27 eyes (17.1%) progressed to more advanced ETDRS (Early Treatment Diabetic Retinopathy Study) levels: 6 progressed to mild NPDR (level 35), 15 progressed to moderate NPDR (level 43), 5 progressed to moderately severe NPDR (level 47) and 1 progressed to high risk PDR (level 71). Worsening in ETDRS level is associated with phenotype C (p=0.005). From the 130 eyes/patients with a low MA turnover, 18 (13.8%) eyes/patients had an increase in ETDRS level, and from the 19 eyes/patients with a high MA turnover, 9 (47.4%) had an increase in ETDRS level (p<0.001).

Conclusion Eyes in the initial stages of diabetic retinopathy show different phenotypes with different risks for progression to CIMO. In phenotype C, MA turnover correlates with ETDRS grading worsening and development of CIMO.

INTRODUCTION

Diabetic retinopathy (DR) is a common and serious condition. It is the leading cause of blindness among working-age adults in the USA.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. The Eye Diseases Prevalence Research Group classified DR into two major outcomes: any DR, as any DR consisting of mild, moderate or severe DR; and vision threatening DR (VTDR), as DR likely to result in vision loss in the absence of treatment, consisting of proliferative DR, clinically significant diabetic macular oedema (CSMO), or both.2 This concept is crucial to address the issue of management of DR in order to prevent vision loss and to identify which patients will progress to VTDR (ie, to CSMO and/or proliferative DR). It is now apparent that systemic markers of diabetes do not identify DR progression to VTDR.3

It is therefore fundamental to identify organ-specific biomarkers such as retinal lesions and their dynamics in the earlier stages of DR and look for their correlation with worsening of any stage of DR to VTDR.4

Previous studies by our group show that some patients progress rapidly to macular oedema in contrast to others that remain stable, even under similar metabolic control. Our group identified three phenotypes with different risks for the development of macular oedema.4

Automated image analysis of microaneurysms (MA) turnover performed on colour fundus photographs contributed to the identification of those eyes that were at risk of developing clinical significant macular oedema.5 In the current prospective study, performed in two centres from different regions of the world, we examine if MA changes occurring in the posterior pole of the eye and detected by automated image analysis are directly correlated with progression of DR represented by worsening in retinopathy severity (Early Treatment Diabetic Retinopathy Study (ETDRS) levels) or development of central-involved macular oedema (CIMO).

METHODS

Details of this study have been previously reported.6 In brief, one eye from 205 subjects with types 2 diabetes, aged over 35 years, mild NPDR (levels 20 to 35, according to the ETDRS diabetic retinopathy severity scale), best-corrected visual acuity (BCVA) >20/25 on the ETDRS chart and glycated haemoglobin (HbA1c) ≤11% were included in a prospective observational study for 2 years or until development of CIMO, at two clinical sites (AIBILI, Coimbra, Portugal; and LV-Prasad Eye Institute (LVPEI), Hyderabad, India). Other inclusion/exclusion criteria were: no previous treatment with laser or anti-vascular endothelial growth factor (anti-VEGF) or steroid intravitreal injections, no other retinal vascular disease or glaucoma, or inadequate ocular media and/or pupil dilatation that did not permit good quality fundus photography. Informed consent was obtained from each patient after explanation of the nature of the study and before any study procedure. The tenets of the Declaration of Helsinki were followed, and approval was obtained from each of the ethics committee (ClinicalTrials. gov number, NCT01607190).
Data were collected in an initial period of three visits, performed at 6-month intervals, followed by another examination 1 year later for a total of 2 years follow-up.

Baseline and follow-up examinations included BCVA, colour fundus photography (CFP) analysed by automated MA analysis using RetmarkerDR software, Cirrus HD-OCT (optical coherence tomography) (Carl Zeiss Meditec, Inc, Dublin, CA, USA) for retinal thickness (RT) measurements, blood pressure evaluation, HbA1c, and lipid blood levels.

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 colour fundus images. This software includes a patented co-registration 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 (ie, MA formation rate and MA disappearance rate, respectively). The formation and disappearance rates were calculated for each visit compared with the baseline visit, and the MA turnover was computed as the sum of the MA formation and disappearance rates. Patients were thereafter classified based on the presence of MA formation rate ≥2 and MA formation and disappearance rates. Patients were thereafter classified based on the presence of MA formation rate ≥2 and on the presence of an MA turnover ≥6, according to Nunes et al, given that these cut-off values—to separate different mild NPDR phenotypes—have been proposed as being predictive of progression of diabetic retinopathy.

To identify eyes/patients with increased RT in the central subfield (clinical and subclinical macular oedema) and in the inner and outer rings, the reference values established by DRCR.net were used. 1,9 For clinical macular oedema:

- RT ≥290 µm in women and ≥305 µm in men for Cirrus HD-OCT (Carl Zeiss Meditec, Inc, Dublin, CA).
- For subclinical macular oedema: 10
  - RT ≥260 µm and <290 µm in women and ≥275 µm and <305 µm in men for Cirrus HD-OCT (Carl Zeiss Meditec, Inc, Dublin, CA).

Patients were classified into one of the three phenotypes of DR progression—phenotype A: low MA turnover and normal retinal thickness (MA turnover <6 and central subfield (CSF) RT <260 µm (women) or CSF RT <275 µm (men)); phenotype B: low MA turnover and increased retinal thickness (MA turnover <6 and CSF RT ≥260 µm (women) and CSF RT ≥275 µm (men)); and phenotype C: high MA turnover (MA turnover ≥6) with or without increased retinal thickness.

Statistical analysis

Frequency and percentages are reported for all categorical measures.

Associations between MA formation rate and MA turnover, at 6 and 12 months, changes in ETDRS level and development of CIMO were tested using χ² test. A multivariate logistic regression was computed with development of CIMO as the dependent variable, and ETDRS changes, phenotypes, HbA1c, body mass index, blood pressure and cholesterol variables at baseline as independent variables.

Correlations between the different parameters were tested using the non-parametric Spearman correlation coefficient.

Statistical analyses were performed using the Stata software version 12.1 (StataCorp LP, College Station, TX, USA). Values of p≤0.05 were considered to be statistically significant.

RESULTS

Baseline results for the 205 eyes/patients included in the study have been published previously. From these 205 eyes/patients, only 158 eyes/patients reached either the study endpoint, CIMO (24 eyes/patients) or performed the last study visit (24-month visit) without developing CIMO (134 eyes/patients). There were a total of 47 dropouts from the study (one patient died, 11 withdrew consent, two had health problems and 33 were lost to follow-up). Ethnic origin was significantly different between those patients who completed the study and those who dropped out of the study, with more Asians dropping out of the study. Low-density lipoprotein (LDL) cholesterol and diastolic blood pressure were also significantly different between those patients who completed the study and those who dropped out of the study: LDL cholesterol was higher in the group of patients who completed the study, and diastolic blood pressure was higher in the group of patients who dropped out of the study. 6

Eyes/patients were classified into one of the three phenotypes of diabetic retinopathy progression. Eighty-eight (56.4%) were identified as phenotype A, 49 (31.4%) as phenotype B, and 19 (12.2%) as phenotype C. Comparing both clinical sites, LVPEI had a higher number of patients with phenotype C: in AIBILLI, 44 (46.8%) of the eyes/patients were identified as phenotype A, 44 (46.8%) as phenotype B and only 6 (6.4%) as phenotype C; in LVPEI, 44 (71.0%) of the eyes/patients were identified as phenotype A, 5 (8.0%) as phenotype B and 13 (21.0%) as phenotype C.

From the eyes/patients analysed, 27 eyes (17.1%) progressed to more advanced ETDRS levels: six progressed to mild NPDR (level 35), 15 progressed to moderate NPDR (level 43), five progressed to moderately severe NPDR (level 47) and one progressed to high risk PDR (level 71) (table 1). The majority of eyes/patients who progressed were from LVPEI. In fact, of the 92 eyes/patients from AIBILLI only three eyes (3.3%) progressed to mild NPDR (level 35), while from the 57 eyes/patients from LVPEI 24 eyes (42.1%) progressed to more advanced ETDRS levels: three progressed to mild NPDR (level 35), 15 progressed to moderate NPDR (level 43), five progressed to moderately severe NPDR (level 47) and one progressed to high risk PDR (level 71).

| Table 1 | Eyes/patients with ETDRS changes from baseline to month-24 |
|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| ETDRS level at baseline       | ETDRS level at month-24       | ETDRS level at month-24       | ETDRS level at month-24       | ETDRS level at month-24       | ETDRS level at month-24       | ETDRS level at month-24       | ETDRS level at month-24       | ETDRS level at month-24       |
| 20                            | 10                            | 12                            | 14                            | 20                            | 35                            | 43                            | 47                            | 71                            |
| Total                         | 16                            | 5                             | 1                             | 21                            | 65                            | 35                            | 71                            | 15                            | 5                             | 1                             | 49                            |

ETDRS, Early Treatment Diabetic Retinopathy Study.
Changes in ETDRS level from baseline to month 24, by phenotype, are shown in table 2. Phenotype C is associated with a two-step worsening in ETDRS level. Of the phenotype C patients, 41.2% experienced at least a worsening one-step in ETDRS level from baseline to month 24, whereas an improvement was observed in just 11.8% of these patients. The remaining 47.1% remained stable and experienced no change in ETDRS level from baseline to month 24 (table 2).

Phenotype A, although showing a similar percentage of eyes/patients without change in ETDRS level, presented ETDRS level worsening in only 19.5% of the eyes versus the ETDRS level worsening of 41.2% registered in eyes/patients with phenotype C.

From the 107 eyes/patients with an MA formation rate at month 6 <2, only 13 (12.1%) eyes/patients had an increase in ETDRS level; and from the 42 eyes/patients with an MA formation rate ≥2, 14 (33.3%) eyes/patients had an increase in ETDRS level (p=0.003). From the 130 eyes/patients with an MA turnover at month 6 <6, 18 (13.8%) eyes/patients had an increase in ETDRS level; and from the 19 eyes/patients with an MA turnover ≥6, nine (47.4%) eyes/patients had an increase in ETDRS level (p<0.001).

At month 12, from the 99 eyes/patients with an MA formation rate <2, 12 (12.1%) eyes/patients had an increase in ETDRS level; and from the 50 eyes/patients with an MA formation rate ≥2, 15 (30%) eyes/patients had an increase in ETDRS level (p=0.007). From the 119 eyes/patients with an MA turnover at month 6 <6, 18 (15.2%) eyes/patients had an increase in ETDRS level; and from the 19 eyes/patients with an MA turnover ≥6, nine (47.4%) eyes/patients had an increase in ETDRS level (p<0.001) (table 3).

A significant association between MA parameters and ETDRS level change was found in LVPEI eyes/patients for MA turnover ≥6, 12 (33.3%) eyes/patients had an increase in ETDRS level; and from the 30 eyes/patients with an MA turnover ≥6, 12 (40.0%) eyes/patients had an increase in ETDRS level (p<0.001) (table 3).

In a multivariate logistic regression, considering phenotypes, metabolic control and cardiovascular risk variables as predictors to analyse risk of developing CIMO, eyes/patients from phenotype B, 13 eyes (26.5%) developed CIMO, of which eight (61.5%) presented no change in ETDRS level. For eyes/patients identified as phenotype C, five eyes (26.3%) developed CIMO, none of which improved on the ETDRS severity scale (table 4).

Although there were differences in baseline characteristics between the eyes/patients in each clinical site—for example, patients from LVPEI were younger, had poorer metabolic control and cardiovascular risk factors. In a multivariate logistic regression, considering phenotypes, metabolic control and cardiovascular risk variables as predictors to analyse risk of developing CIMO, eyes/patients from phenotype C showed a higher risk of developing CIMO than eyes/patients from phenotype A (OR 44.8, 95% CI 6.8 to 293.8; p<0.001) and eyes/patients from phenotype B showed a higher chance of developing CIMO than eyes/patients from phenotype A (OR 31.4, 95% CI 5.4 to 183.3; p<0.001).

### Table 2 Changes between baseline and month 24 in ETDRS level, by phenotype

| Phenotype | DR worsening | DR improving |
|-----------|--------------|--------------|
|           | ≥3 steps     | 2 steps      | 1 step       | No change | 1 step      | 2 steps     | ≥3 steps |
| A, n (%)  | 0 (0.0)      | 4 (4.6)      | 13 (14.9)    | 40 (46.0) | 13 (14.9)   | 1 (1.2)     | 16 (18.4) |
| B, n (%)  | 1 (2.2)      | 0 (0.0)      | 3 (6.7)      | 30 (66.7) | 7 (15.6)    | 0 (0.0)     | 4 (8.9)   |
| C, n (%)  | 2 (11.8)     | 4 (23.5)     | 1 (5.9)      | 8 (47.1)  | 1 (5.9)     | 0 (0.0)     | 1 (5.9)   |
| Total     | 3 (2.0)      | 8 (5.4)      | 17 (11.4)    | 78 (52.4) | 21 (14.1)   | 1 (0.7)     | 21 (14.1) |

DR, diabetic retinopathy; ETDRS, Early Treatment Diabetic Retinopathy Study.

### Table 3 MA formation rate and MA turnover, at months 6 and 12, correlated with changes in ETDRS level

| ETDRS level | MA formation rate | MA turnover |
|-------------|------------------|-------------|
|             | Month 6          | Month 12     | Month 6 | Month 12 |
|             | <2               | ≥2           | <6      | ≥6      | <6      | ≥6      |
| Remain or decrease, n (%) | 94 (87.9) | 28 (66.7) | 87 (87.9) | 35 (70.0) | 112 (86.2) | 10 (52.6) | 104 (87.4) | 18 (60.0) |
| Increase, n (%) | 13 (12.1) | 14 (33.3) | 12 (12.1) | 15 (30.0) | 18 (13.8) | 9 (47.4) | 15 (12.6) | 12 (40.0) |

P values* 0.003 0.007 <0.001 <0.001

*χ2 test.

ETDRS, Early Treatment Diabetic Retinopathy Study; MA, microaneurysm.
This 2-year prospective, longitudinal study of patients with type 2 diabetes and mild NPDR (ETDRS levels 20 and 35, at baseline) shows that MA turnover in field 2 is a good predictor of retinopathy worsening, as demonstrated by step-changes in ETDRS grading and development of macular oedema. In previous studies demonstration of MA formation rate and development of macular oedema, that is, central-involved macular oedema, was mainly in the placebo group of a clinical trial. Therefore, we should use automated analysis of non-invasive colour fundus photographs and based solely on field 2 images may help to identify the eyes/patients at risk for worsening of their diabetic retinal disease.

**DISCUSSION**

This 2-year prospective, longitudinal study of patients with type 2 diabetes and mild NPDR (ETDRS levels 20 and 35, at baseline) shows that MA turnover in field 2 is a good predictor of retinopathy worsening, as demonstrated by step-changes in ETDRS grading and development of macular oedema.

In previous studies demonstration of MA formation rate and development of macular oedema, that is, central-involved macular oedema, was mainly in the placebo group of a clinical trial. Therefore, we should use automated analysis of non-invasive colour fundus photographs and based solely on field 2 images may help to identify the eyes/patients at risk for worsening of their diabetic retinal disease.

Recent studies have shown the relevance of retinopathy severity improvement based on ETDRS grading levels as a clinically important outcome. In eyes treated with anti-VEGF agents or with corticosteroids, greater degrees of improvement in ETDRS grading levels correlate with greater magnitudes of functional and anatomic improvement.

This study shows that automated analysis of MA turnover correlates well with changes in severity of ETDRS grading levels, validating its use as a simple to use biomarker of DR progression. Automated analysis techniques offer advantages of repeatability and consistency.

It is also relevant that MA turnover calculated by the Retmarker DR (Retmarker SA) is much less time consuming than ETDRS grading and MA counting by expert graders.

This study confirmed the previously identified distribution of three different phenotypes of DR progression with different risks for the development of diabetic macular oedema (DMO).

**Table 4** Changes between baseline and month 24 in ETDRS level, by study endpoint

| Phenotype | Endpoint | # Patients | DR worsening | DR improving |
|-----------|----------|------------|--------------|--------------|
|           |          |            | All          | ≥3 steps     | Two steps    | One step     | All          | One step     | Two steps    | ≥3 steps     | No change    |
| A (n=87)  | No CIMO  | 86 (96.9%) | 17 (19.8)    | 4            | 13           | 30 (34.9)    | 13           | 1            | 16           | 39 (45.3)    |
|           | CIMO     | 1 (1.1%)   | 0 (0.0)      | 0            | 0            | 0 (0.0)      | 0            | 0            | 0            | 1 (100.0)    |
| B (n=45)  | No CIMO  | 32 (71.1%) | 2 (6.3)      | 0            | 0            | 2 (8.25)     | 6            | 0            | 2            | 22 (68.8)    |
|           | CIMO     | 13 (28.9%) | 2 (15.4)     | 1            | 1            | 3 (23.1)     | 1            | 0            | 2            | 8 (61.5)     |
| C (n=17)  | No CIMO  | 12 (70.6)  | 4 (33.3)     | 0            | 3            | 1 (16.7)     | 1            | 0            | 1            | 6 (50.0)     |
|           | CIMO     | 5 (29.4%)  | 3 (60.0)     | 2            | 1            | 0 (0.0)      | 0            | 0            | 0            | 2 (40.0)     |

CIMO, central-involved macular oedema; DR, diabetic retinopathy; ETDRS, Early Treatment Diabetic Retinopathy Study.

**Phenotype A** (50% of the eyes with mild NPDR) shows a very low risk for the development of DMO in contrast to phenotypes B and C that show a much higher risk for progression to DMO. Within phenotype C there is a good correlation between MA turnover, progression in ETDRS levels and development of DMO. However, this correlation is not present in phenotype B. In phenotype B, DMO may occur without ETDRS level changes. The ETDRS severity scale does not take into account the presence or absence of macular oedema, and macular oedema, that is, central-involved macular oedema, may be present in eyes without any or minimal microvascular changes. In order to evaluate progression of DR to VTDR it is necessary to evaluate not only DR worsening by ETDRS scale standards but also retinal thinning measured by OCT.

The majority of eyes/patients who progressed during the study were from LVPEI, in India, where we found a higher number of patients from phenotype C (68.4%). It is of interest that even in this group, phenotype C from the India centre, metabolic control and cardiovascular risk variables did not reach statistical significance. There is previous evidence from aggregation in families and specific ethnic groups that there is a genetic predisposition to develop some diabetic complications such as retinopathy. Heritability has been estimated to be as high as 27% for DR and 52% for proliferative DR. It is noteworthy that our research group performed a case-control study and found a statistically significant association between different phenotypes of DR progression as described here and different gene variants.

The major limitation of this study is its relatively short duration (2 years) and the fact that phenotype C, associated with a higher number of MAs and increased MA turnover, was mainly present in the clinical site from India. Finally, the results of this prospective study confirm, in a relatively large number of eyes/patients, that MA turnover values obtained from automated analysis of non-invasive colour fundus photographs and based solely on field 2 images may help to identify the eyes/patients at risk for worsening of their diabetic retinal disease.
Ethics approval Approval was obtained from the ethics committee from each site (L.V. Prasad Eye Institute: LEC 11-241; Association for Innovation and Biomedical Research on Light and Image: 038/2018/AIBLI/CE).

Provenance and peer review Not commissioned; externally peer reviewed.

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