Cluster Analysis Identification Of Early Diabetic Retinopathy Phenotypes

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Purpose

To identify different phenotypes in mild non-proliferative diabetic retinopathy (NPDR) in type-2 diabetic patients based on non-invasive ophthalmological examinations using cluster analysis.

Methods

Study Design
412 type-2 diabetic patients with mild NPDR were included in a 2-years observational, prospective study with visits at 0, 6 and 24 months, to establish the association between phenotypes and genotypes in NPDR.

371 patients completed the first 6-month follow-up period (32 dropped-out; 3 were treated before month-6).
Vital signs and blood tests were assessed (Systolic and Diastolic Blood Pressure; HbA_{1C}; Cholesterol; HDL; LDL and; Triglycerides). Additionally, at the end of the study, a genotype analysis of the genes ARL, RAGE, VEGF, ICAM-1, TNF-α, ACE and NOS-1 was performed.

Ophthalmic Examination - Non-Invasive Imaging Procedures
- Color Fundus Photography (CFP) was performed for automatic microaneurysm turnover assessment (formation and disappearance rates - MAFR and MADR, respectively) (RetmarkerDR, Critical Health SA).
- Optical Coherence Tomography (Stratus OCT, Carl Zeiss Meditech Inc.) was performed to compute Retinal Thickness (RT) maps using proprietary software allowing to compute the central macular RT values (central 500 µm and 1500 µm in diameter areas).

Phenotypes of DR
To identify DR phenotypes a cluster analysis was performed based on MAFR and RT (central 500 µm) using a hierarchical clustering process with Ward's Method.

Statistical Analysis
Statistically significant differences between clusters (phenotypes) were tested using ANOVA tests with Bonferroni correction.

Phenotypes were correlated with the progression of DR to clinically significant macular edema (CSME) using the χ^2 test.

Results

Phenotypes of DR

The clustering solution suggests the existence of 3 different phenotypes of DR (Figures 1 and 2; Table 1):

- Cluster 1 (Phenotype 1) – composed by 122 patients/eyes (32.9%): characterized by normal values on both the MAFR and the central RT;
- Cluster 2 (Phenotype 2) - composed by 182 patients/eyes (49.0%): characterized by a high RT;
- Cluster 3 (Phenotype 3) - composed by 67 patients/eyes (18.1%): characterized by a high MAFR. Phenotype 3 presents also a higher MA turnover (MAFR + MADR) when compared to Phenotypes 1 and 2.

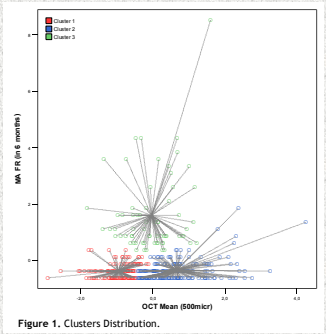

Figure 1. Clusters Distribution.

Table 1. Phenotypes characteristics (mean ± SD).

			Phenotype 1 (n = 122)	Phenotype 2 (n = 182)	Phenotype 3 (n = 67)	P value
Systemic	Age [Years]		61.2 ± 8.3	62.2 ± 8.0	57.8 ± 9.0	Phenotype 3 - Phenotype 1 (P=0.024) Phenotype 3 - Phenotype 2 (P=0.001)
	Diabetes Duration [Years]		9.9 ± 5.0	10.2 ± 5.1	10.3 ± 5.1	NS (P=0.877)
	Blood Pressure [mmHg]	Systolic	152.2 ± 23.2	151.0 ± 20.3	150.7 ± 19.5	NS (P=0.863)
		Diastolic	75.7 ± 10.6	75.6 ± 11.0	76.6 ± 10.2	NS (P=0.809)
	HbA_{1C} [%]		7.7 ± 1.5	8.0 ± 1.5	8.3 ± 1.6	Phenotype 3 - Phenotype 1 (P=0.020)
	Cholesterol [mg/dl]		195.6 ± 43.3	195.8 ± 39.6	197.9 ± 40.4	NS (P=0.926)
	HDL [mg/dl]		53.0 ± 13.9	49.9 ± 12.4	49.5 ± 11.6	NS (P=0.085)
	LDL [mg/dl]		125.9 ± 32.4	127.6 ± 31.6	127.8 ± 30.4	NS (P=0.877)
	Triglycerides [mg/dl]		178.2 ± 122.7	173.6 ± 102.7	190.5 ± 154.2	NS (P=0.615)
Retinal Thickness	Mean (500 µm) [µm]		158.2 ± 12.1	199.3 ± 20.4	181.7 ± 17.9	P<0.001
	Mean (1500 µm) [µm]		225.0 ± 16.3	253.7 ± 16.8	243.4 ± 18.1	P<0.001
Ophthalmic	Area with Increase [%]		1.8 ± 9.3	12.9 ± 22.6	11.2 ± 21.0	Phenotype 1 - Phenotype 2 (P<0.001) Phenotype 1 - Phenotype 3 (P=0.003)
	Area with Decrease [%]		11.6 ± 23.0	2.9 ± 10.8	5.1 ± 16.2	Phenotype 1 - Phenotype 2 (P<0.001) Phenotype 1 - Phenotype 3 (P=0.033)
	MA (in 6 months)	Number	2.1 ± 2.6	2.0 ± 2.0	4.7 ± 5.2	Phenotype 3 - Phenotype 1 (P<0.001) Phenotype 3 - Phenotype 2 (P<0.001)
		FR	1.0 ± 1.1	1.1 ± 1.5	8.9 ± 5.6	Phenotype 3 - Phenotype 1 (P<0.001) Phenotype 3 - Phenotype 2 (P<0.001)
		DR	1.8 ± 1.9	1.6 ± 2.1	4.5 ± 4.2	Phenotype 3 - Phenotype 1 (P<0.001) Phenotype 3 - Phenotype 2 (P<0.001)
		Turnover [FR+DR]	2.9 ± 2.4	2.8 ± 2.7	13.5 ± 8.2	Phenotype 3 - Phenotype 1 (P<0.001) Phenotype 3 - Phenotype 2 (P<0.001)

Statistically significant differences were found between clusters (phenotypes) for age and HbA_{1C} levels.

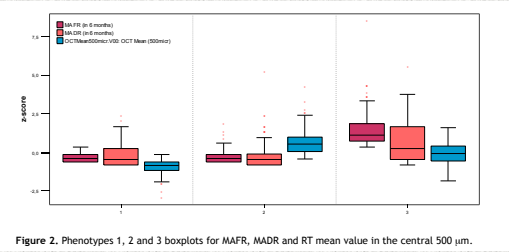

Figure 2. Phenotypes 1, 2 and 3 boxplots for MAFR, MADR and RT mean value in the central 500 µm.

Phenotypes vs. CSME

16 patients (4.3%) developed CSME after the 6-month period:
- Phenotype 1: No patients
- Phenotype 2: 10 patients (5.5%)
- Phenotype 3: 6 patients (8.9%)

Patients from phenotypes 2 and 3 presents a higher risk for DR progression to CSME (RR=1.069; 95%CI=[1.034; 1.104], P=0.008).

Phenotypes vs. Genotypes

46 patients performed the genotypic analysis (Biocant - DNA sequencing Unit, Cantanhede, Portugal). Preliminary results indicate the existence of different genotypes for RAGE, ICAM-1 and NOS-1.

Conclusions

Cluster analysis based on non-invasive techniques (CFP and OCT) identified 3 different phenotypes of early DR, confirming the previous findings from our research group in a different study/group of patients. Patients with high RT and/ or high MAFR present a higher risk for DR progression to CSME (RR=1.069; 95%CI=[1.034; 1.104]).

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