EXTENDED REPORT

Microperimetry and fundus autofluorescence in patients with early age-related macular degeneration

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Background: Early age-related macular degeneration (AMD) has been correlated with different functional alterations, but the exact relationship between fundus lesions and overlying sensitivity is not well known. The aim of this study was to compare fundus-related sensitivity (microperimetry) and fundus autofluorescence (FAF) of the macular area with drusen and pigment abnormalities in early AMD.
Methods: 13 consecutive patients with early AMD and visual acuity of 20/20 were studied by means of microperimetry, which automatically analyses macular light differential threshold and fixation patterns. Fundus colour photo and FAF of the macular area were recorded on the same day. Microperimetry was exactly (topographically) superimposed over FAF images.
Results: Macular sensitivity significantly decreased over large drusen (11.2 $\pm$ 5.6 dB, $p<0.0001$) and over pigment abnormalities (13.1 $\pm$ 3.6 dB, $p<0.0001$). When both characteristics were present the reduction was greater if compared with its absence (9.6 $\pm$ 4.3 versus 15.0 $\pm$ 4.5 dB, $p<0.0001$). Sensitivity reduction was significant in areas with altered FAF when compared with areas with normal FAF ($p<0.0001$).
Conclusions: Increased FAF in early AMD has a functional correlate exactly quantified by microperimetry. In retinal areas affected by early AMD retinal sensitivity deteriorates, despite good visual acuity. Microperimetry may allow the early detection of functional impairment caused by these lesions. Both microperimetry and FAF may be useful to monitor AMD progression.

Age-related macular degeneration (AMD) is a degenerative macular disorder and a major cause of visual impairment in individuals over 50 years of age in industrialized countries.[1 2] AMD can be subdivided into early and late stages. Early AMD is characterized by soft intermediate drusen (larger than 63 μm) and areas of hyperpigmentation or hypopigmentation of the retinal pigment epithelium (RPE).[3] Drusen are attributed to distinct deposits between the inner collagenous layer of Bruch's membrane and the basement membrane of the RPE exceeding those of the normal aging process.[4] Ultrastructural findings of material deposited in the inner Bruch's membrane are in accordance with the assumption that the material predominantly derives from the RPE cells. All human RPE cells show an age-related accumulation of lipofuscin granula within the lysosomal compartment, as a byproduct of the permanent phagocytosis of lipid-rich distal photoreceptor outer segments.[5] Lipofuscin is considered a biomarker for cellular aging and a cumulative index for oxidative damage. Lipofuscin contains the dominant fluorophores responsible for the in-vivo fundus autofluorescence (FAF) phenomenon as determined by fundus spectrophotometric measurements.[6 7]

With the advent of confocal scanning laser ophthalmoscopy it is possible to visualize FAF and its spatial distribution *in vivo* easily, and therefore to evaluate RPE during the aging process and in ocular disease.[6 8–12] It is believed that lipofuscin accumulation precedes photoreceptor degeneration, and is responsible for measurable visual deficits, abnormal retinal sensitivity measurements in the central visual field, and slower rates of dark adaptation associated with age-related maculopathy.[13–17] A novel fundus-related perimetry technique called microperimetry has recently been introduced into clinical practice in order to evaluate macular function by exactly correlating fundus lesions to retinal sensitivity.[18–23]

The aim of this study was to compare macular microperimetry with fundus lesions and FAF (increased or decreased) in patients with early AMD.

MATERIALS AND METHODS

In this cross-sectional study, 13 consecutive and eligible bilateral early AMD patients were enrolled. All patients were recruited from the Medical Retina Clinic at the Department of Ophthalmology, University of Padova. The inclusion criteria were the presence of small drusen ($\leqslant$63 μm) and at least five or more intermediate soft drusen (>63 μm) or large (or confluent) soft drusen (>125 μm) localized within 3000 μm from the centre of the fovea and associated with pigment abnormalities. Visual acuity was 20/20 in all patients. Exclusion criteria were significant media opacities, clinical evidence of geographical atrophy (defined as hypopigmentation $\geqslant$125 μm), choroidal neovascularization, any evidence of diabetic retinopathy, myopia greater than 6 D, glaucoma, and previous macular laser treatment. One eye of each patient, randomly selected, was studied.

This study was conducted in accordance with the tenets of the Declaration of Helsinki, and with the approval of our institutional ethical committee.

After a detailed explanation of the purpose of the study, a written consent form was signed by all enrolled patients. The ophthalmological examination consisted of: refraction and best corrected visual acuity determination; anterior segment examination; 90 dioptres lens biomicroscopy; fundus photography; microperimetry; and FAF.

Best corrected distance visual acuity for each eye was measured at 4 m distance using standard early treatment

Abbreviations: AMD, age-related macular degeneration; FAF, fundus autofluorescence; RPE, retinal pigment epithelium

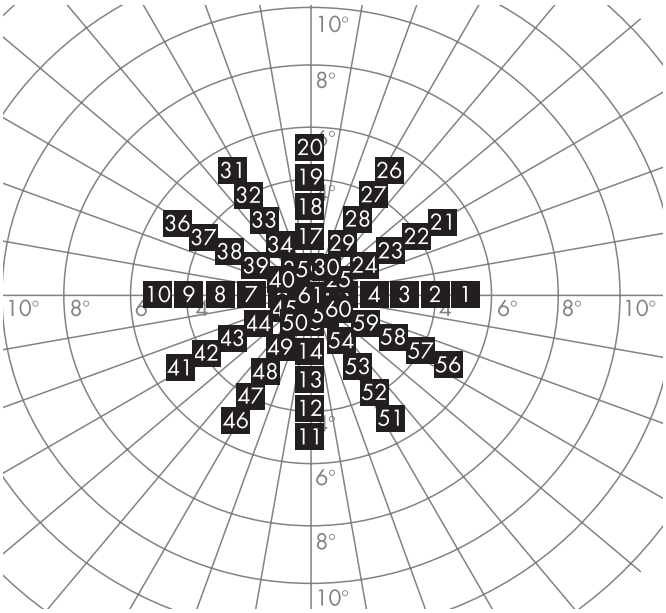

Figure 1 A microperimetric customized radial grid pattern of 61 stimuli covering the central 10° (centred onto the fovea). The stimuli are projected randomly onto the 0°, 30°, 60°, 90°, 120°, 150° axes, 1° apart.

diabetic retinopathy study protocols with a modified early treatment diabetic retinopathy study distance chart transilluminated with a chart illuminator (Precision Vision, Bloomington, Illinois, USA).[24] Visual acuity was scored as the total number of letters read correctly and expressed as the Snellen equivalent.

Microperimetry

All patients underwent at least 5 mm pupil dilation with tropicamide 0.5% and 15 minutes mesopic conditions adaptation before examination.

Microperimetry was performed on all subjects using a new automatic fundus-related perimeter: the MP1 Microperimeter (Nidek Technologies, Padova, Italy). This instrument has previously been described in detail.[18] Briefly, for the purpose of this study, the following parameters were used: a fixation target consisting of a red ring 2° in diameter; white, monochromatic background at 4 asb, stimulus size Goldman III with 200 ms projection time; customized radial grid of 61 stimuli covering the central 10° (centred onto the fovea); stimuli were projected randomly onto the 0°, 30°, 60°, 90°, 120°, 150° axes, 1° apart (fig 1). A 4–2–1 double staircase strategy was used with an automatic eye tracker that compensates for eye movements.[18 25] In order to allow for better clinical correlation between microperimetric data and retinal details, functional results are always displayed onto a colour digital retinograph, acquired by the charge-coupled device colour camera of the MP1.

Autofluorescence

FAF was recorded using a confocal scanning laser ophthalmoscope (Heidelberg Retinal Angiograph, HRA 2; Heidelberg Engineering, Heidelberg, Germany). The optical and technical principles of the HRA have previously been described in detail.[26 27] In order to amplify the autofluorescence signal of the final image, 10 acquired images were aligned and a mean image was calculated from these, after detection and correction of eye movements were performed by image analysis software. Digital images were saved on hard disc for further analysis and processing. FAF images were graded for different patterns (normal, increased and decreased). Increased or decreased FAF in the central retinal 10° were assumed to be present on the basis of the intensity of the background FAF pattern encountered just immediately peripheral to the superior temporal arcade.

Colour photos

Digital colour images were taken by a Topcon TRC 50IA 30 degree fundus camera (Topcon, Tokyo, Japan), on the same day as the MP1 and autofluorescence images were taken and immediately saved in JPEG format.

Image analysis

For every single retinal point tested with the MP1 on each eye, the presence and type of drusen (small, intermediate, large), pigment (present, absent) and FAF pattern (increased FAF, decreased FAF, normal FAF) were graded. A microperimetry sensitivity map was also overlaid onto the FAF images using dedicated MP1 software. This software allows for the exact superimposition of sensitivity data to different images separately (FAF, fluorescein angiography, indocyanine green). The superimposition is obtained by the semi-automatic detection of three identical anatomical landmarks on both images (fig 2). This software has previously been tested for validity and repeatability.[28] Thereafter, in every single pair of images correlation between the drusen type, RPE pigment and RPE atrophy (smaller than 125 μm) and distinct FAF pattern was evaluated.

Independent, combined grading of colour fundus images and FAF images was carried out by two trained retinal specialists. In the case of disagreement, arbitration grades were awarded by a different retinal specialist and these results were taken as final.

Statistical analysis

All sensitivity data were analysed using an analysis of variance test for repeated measures analysis of variance and a Bonferroni post-hoc test. FAF data were analysed using the chi-square test. To assess interobserver variability, kappa statistics were calculated.[29]

We assumed that data were significant if p was less than 0.05. The SAS statistical package on a personal computer (version 8.4; SAS Institute Inc., Cary, North Carolina, USA) was used for all analyses.

RESULTS

Of the 13 patients examined, three were men and 10 were women, with seven left and six right eyes included. The mean age was 76.2 ± 6.55 years and mean visual acuity was 20/20. Anterior segment examination was unremarkable, with no significant media opacities.

Of 793 total retinal points examined (61 for each patient), 310 points presented with drusen (39.1%), 483 points had no drusen (60.9%), whereas 161 points had pigment alterations (20.3%) and 632 were without pigment alterations (79.7%); 697 points had normal FAF (87.9%), 30 decreased FAF (3.8%) and 66 increased FAF (8.3%) (see table 1).

Retinal sensitivity was significantly influenced by both drusen and RPE pigment abnormalities (analysis of variance $p<0.0001$). In particular, retinal sensitivity was not significantly decreased over small or intermediate drusen ($p>0.05$), whereas it was significantly decreased over large drusen when compared with an absence of drusen (15.0 ± 4.5 versus 11.2 ± 5.6 dB, Bonferroni test $p<0.0001$). Retinal sensitivity was also decreased when pigment abnormalities were found, compared with their absence (15.0 ± 4.5 versus 13.1 ± 3.6 dB, $p<0.0001$). If RPE pigment abnormalities were associated with large soft drusen sensitivity reduction was even

Table 1 Fundus characteristics and fundus autofluorescence data of the studied population

	Pigment		Drusen		Autofluorescence		
Patient ID	No N (%)	Yes N (%)	No N (%)	Yes N (%)	Normal N (%)	Hypo N (%)	Hyper N (%)
1	25 (41.0)	36 (59.0)	26 (42.6)	35 (57.4)	38 (62.3)	8 (13.1)	15 (24.6)
2	33 (54.1)	28 (45.9)	28 (45.9)	33 (54.1)	60 (98.4)	1 (1.6)	0 (0.0)
3	35 (57.4)	26 (42.6)	34 (55.7)	27 (44.3)	48 (78.7)	1 (1.6)	12 (19.7)
4	58 (95.1)	3 (4.9)	51 (83.6)	10 (16.4)	61 (100.0)	0 (0.0)	0 (0.0)
5	29 (47.5)	32 (52.5)	22 (36.1)	39 (63.9)	58 (95.1)	0 (0.0)	3 (4.9)
6	59 (96.7)	2 (3.3)	39 (63.9)	22 (36.1)	61 (100.0)	0 (0.0)	0 (0.0)
7	53 (86.9)	8 (13.1)	44 (72.1)	17 (27.9)	56 (91.8)	2 (3.3)	3 (4.9)
8	57 (93.4)	4 (6.6)	35 (57.4)	26 (42.6)	52 (85.2)	5 (8.2)	4 (6.6)
9	56 (91.8)	5 (8.2)	46 (75.4)	15 (24.6)	52 (85.2)	6 (9.8)	3 (4.9)
10	55 (90.2)	6 (9.8)	25 (41.0)	36 (59.0)	50 (82.0)	1 (1.6)	10 (16.4)
11	61 (100.0)	0 (0.0)	47 (77.0)	14 (23.0)	49 (80.3)	5 (8.2)	7 (11.5)
12	57 (93.4)	4 (6.6)	43 (70.5)	18 (29.5)	53 (86.9)	0 (0.0)	8 (13.1)
13	54 (88.5)	7 (11.5)	43 (70.5)	18 (29.5)	59 (96.7)	1 (1.6)	1 (1.6)
Total	632 (79.7)	161 (20.3)	483 (60.9)	310 (39.1)	697 (87.9)	30 (3.8)	66 (8.3)

Hyper, Increased autofluorescence; Hypo, decreased autofluorescence.

higher if compared with its association with small or intermediate drusen (9.6 ± 4.3 versus 12.3 ± 0.6 dB, $p<0.0001$) (table 2). In all eyes fixation was central and stable.

The association between the FAF pattern (normal FAF, decreased FAF and increased FAF) and the presence of drusen was significant ($p<0.0001$). Increased and decreased FAF was found in only 27 of the examined points without drusen (6.4%), one of the examined points with small and intermediate drusen (1.7%) and in 32 of the examined points associated with large soft drusen (20.4%). Of 67 hyperpigmented points without drusen, seven (10.4%) presented with decreased FAF, four (6.0%) with increased FAF and 56 (83.6%) with a normal FAF pattern. When large soft drusen were associated with hyperpigmentation, 24 (26.4%) presented with increased FAF, one (1.1%) with decreased FAF and 66 (72.5%) with a normal FAF pattern. There were no points with small drusen and pigment abnormalities, and there were only three points with intermediate drusen associated with pigment abnormalities characterized by normal FAF (table 3).

The mean sensitivity was significantly decreased over areas with altered FAF (increased and decreased) when compared with areas with normal FAF (9.7 ± 5.5 and 10.2 ± 6.4 dB versus 14 ± 4.7 dB, $p<0.0001$).

The overall interobserver variability resulted in a kappa of 0.74 (95% confidence interval 0.64–0.84).

DISCUSSION

In the past, macular function in early AMD was studied by means of visual acuity, macular recovery function, central visual field sensitivity and spatiotemporal contrast sensitivity determinantion.[30] More recent studies used short wavelength automated perimetry for the study of cone sensitivity, and a photopic and scotopic fine matrix mapping technique for the study of cone and rod sensitivity.[31 32] Retinal sensitivity loss was documented regardless of good visual acuity in patients with early AMD. The fine matrix mapping technique detected more rod than cone sensitivity decreases.[32] Both studies agreed that functional alterations correlate with funduscopic abnormalities (large soft drusen and pigmentary RPE changes).[31 32] Conventional (automatic) perimetry has been used in the past to study early AMD, but the quantification of retinal thresholds over small and descrete retinal lesions were beyond the possibilities of the technique.[33] Microperimetry, also known as fundus-related perimetry, allows for a detailed examination of macular function, giving a highly reproducible evaluation of retinal sensitivity at selected points, by a continuous eye tracking system.[18] The semiautomatic MP1 software developed for this study allowed us an exact, point-to-point correlation between fundus lesions and FAF and sensitivity values. We found a significant sensitivity reduction over the areas characterized by large soft drusen using microperimetry,

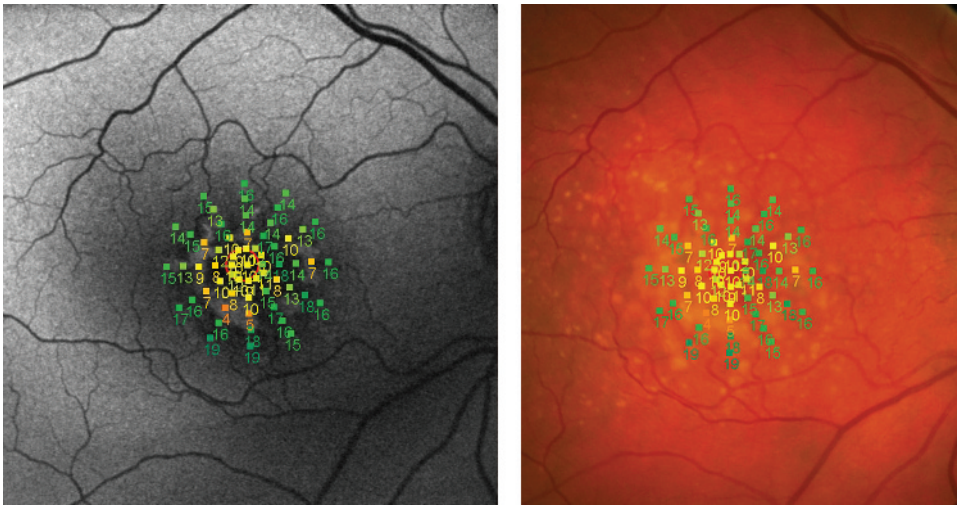

Figure 2 Fundus autofluorescence and colour images of the same patient with large soft drusen and pigment abnormalities with the overlapped sensitivity map. The numerical map (values of sensitivity expressed in decibels) shows the sensitivity decrease over areas with increased fundus autofluorescence (on the left) that correspond to drusen and pigment (on the right).

Table 2 Microperimetry data by drusen size and presence of pigment

Mean sensitivity in decibels ± SD (n)			
Drusen size	**Pigment**	**No pigment**	**p Value**
No drusen	13.1 ± 3.6 (67)	15.0 ± 4.5 (416)	0.0012
Small (<63 μ)	0.0 (0)	15.6 ± 3.3 (32)	na
Intermediate (63–125 μ)	12.3 ± 0.6 (3)	15.3 ± 4.9 (27)	na
Large (>125 μ)	9.6 ± 4.3 (91)	11.2 ± 5.6 (157)	0.0231

na, Not applicable as a result of lack of data.

Table 3 Classification of retinal points by pigment, drusen size and autofluorescence pattern

		Autofluorescence, number (%)		
Pigment	**Drusen size**	**Normal**	**Hypo**	**Hyper**
Presence*	No drusen	56 (83.6)	7 (10.4)	4 (6.0)
	Intermediate	3 (100.0)	0 (0.0)	0 (0.0)
	Large	66 (72.5)	1 (1.1)	24 (26.4)
Absence†	No drusen	389 (93.5)	11 (2.6)	16 (3.8)
	Small	32 (100.0)	0 (0.0)	0 (0.0)
	Intermediate	26 (96.3)	0 (0.0)	1 (3.7)
	Large	125 (79.6)	11 (7.0)	21 (13.4)

*None of the points with pigment had small drusen; p=0.0015; †p<0.0001.
Small drusen <63 μ; intermediate drusen 63–125 μ; large drusen >125 μ.
Hyper, Increased autofluorescence; Hypo, decreased autofluorescence.

although this is not in accordance with previously published data obtained with traditional perimetry.[34] Sensitivity deterioration was greater when pigment abnormalities were associated with drusen. Large soft drusen and RPE pigment alterations were mutually independent, and large soft drusen had a greater impact on retinal sensitivity than RPE pigment abnormalities alone. This fact can be explained by RPE and photoreceptor degeneration as a result of the presence of drusen. Johnson *et al.*[35] reported that both inner and outer rod and cone segments overlying drusen were physically deflected. Outer photoreceptor segments overlying drusen were shorter than nearby outer segments not overlying drusen. This was correlated with drusen size.[35] Johnson *et al.*[36] also observed a consistent reduction in the density of photoreceptors over drusen, with an approximate reduction of 30% in photoreceptor numbers. These retinal abnormalities are correlated with drusen size and can be functionally documented with microperimetry. We found a more relevant decrease of macular sensitivity over large soft drusen compared with small or intermediate drusen, despite normal visual acuity.

We found that the FAF pattern was altered (increased or decreased) in only 20.4% of the points examined that were associated with large soft drusen. Previous studies have shown that not all types of drusen have the same FAF pattern, which might be explained by different fluorophores contained by the lipofuscin and consequent different stages of RPE degeneration.[37] The material deposited might also be at different levels in the retina and therefore not always detectable by means of FAF.[38]

Our data show a significant and inverse correlation between retinal sensitivity and an increased FAF pattern, confirming that lipofuscin accumulation in the RPE cells has a functional correlate in patients with early AMD.[39] Both clinical fundus FAF and experimental findings show that lipofuscin contains toxic biomolecules that may interact with normal cell function. The major retinoid fluorophore found in lipofuscin is A2E, which is responsible for the inhibition of lysosomal enzymes, detergent and phototoxic properties within the RPE cells.[4] All these molecular pathophysiological effects increase apoptotic mechanisms with the consequent death of RPE cells. Clinical entities characterized by the accumulation of lipofuscin thus point to RPE cell dysfunction with secondary functional correlates. Therefore, comparing fundus morphological features with functional data may allow for a better understanding of the mechanisms underlying this pathological process, and may suggest a more appropriate follow-up for patients.

Further prospective studies on larger samples are needed in order to obtain a better understanding of the evolution of large soft drusen and pigment abnormalities characterized by increased FAF and decreased sensitivity, and whether patients with these characteristics are at greater risk of developing advanced stages of AMD when compared with areas with clinical features of early AMD and normal FAF.

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Competing interests: None.

REFERENCES

1 **Klein R**, Klein BEK, Linton KLP. Prevalence of age related maculopathy. The Beaver Dam Eye Study. *Ophthalmology* 1992;**99**:933–43.
2 **Vingerling JR**, Dielemans I, Hofman A, *et al.* The prevalence of age-related maculopathy in the Rotterdam Study. *Ophthalmology* 1995;**102**:205–10.
3 **Bird AC**, Bressler NM, Bressler SB, *et al.* An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol* 1995;**39**:367–74.
4 **Pauleikhoff D**, Hermans P, Holz FG, *et al.* Histopathology. In: Holz FG, Pauleikhoff D, Spaide RF, Bird AC, eds. *Age-related macular degeneration*. New York, NY: Springer-Verlag, 2004:47–67.
5 **Weiter JJ**, Delori FC, Wing GL, *et al.* Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. *Invest Ophthalmol Vis Sci* 1986;**27**:145–52.
6 **Delori CD**, Dorey CK, Staurenghi G, *et al.* In vivo fluorescence of the ocular fundus exhibits retinal pigment epithelium lipofuscin characteristics. *Invest Ophthalmol Vis Sci* 1995;**36**:718–29.
7 **Delori CD**, Staurenghi G, Arend O, *et al.* In vivo measurement of lipofuscin in Stargardt's disease–fundus flavimaculatus. *Invest Ophthalmol Vis Sci* 1995;**36**:2327–31.

8 **von Ruckmann A**, Fitzke FW, Bird AC. Distribution of fundus autofluorescence with a scanning laser ophthalmoscope. *Br J Ophthalmol* 1995;**79**:407–12.
9 **Bellmann C**, Holz FG, Schapp O, *et al.* Topography of fundus autofluorescence with a new confocal scanning laser ophthalmoscope. *Ophthalmologe* 1997;**94**:385–91.
10 **Holz FG**, Bellmann C, Margaritidis M, *et al.* Patterns of increased in vivo fundus autofluorescence in the junctional zone of geographic atrophy of the retinal pigment epithelium associated with age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol* 1999;**237**:145–52.
11 **Bindewald A**, Jorzik JJ, Loesch A, *et al.* Visualization of retinal pigment epithelial cells in vivo using digital high-resolution confocal scanning laser ophthalmoscopy. *Am J Ophthalmol* 2004;**137**:556–8.
12 **Delori FC**, Fleckner MR, Goger DG, *et al.* Autofluorescence distribution associated with drusen in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2000;**41**:496–504.
13 **Smith VC**, Pokorny J, Diddie KR. Color matching and the Stiles–Crawford effect in observers with early age-related macular changes. *J Opt Soc Am A* 1988;**5**:2113–21.
14 **Owsley C**, Jackson GR, Cideciyan AV, *et al.* Psychophysical evidence for rod vulnerability in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2000;**41**:267–73.
15 **Midena E**, Segato T, Blarzino MC, *et al.* Macular drusen and the sensitivity of the central visual field. *Doc Ophthalmol* 1994;**88**:179–85.
16 **Eisner A**, Stoumbos VD, Klein ML, *et al.* Relations between fundus appearance and function. Eyes whose fellow eye has exudative age-related macular degeneration. *Invest Ophthalmol Vis Sci* 1991;**32**:8–20.
17 **Steinmetz RL**, Haimovici R, Jubb C, *et al.* Symptomatic abnormalities of dark adaptation in patients with age-related Bruch's membrane change. *Br J Ophthalmol* 1993;**77**:549–54.
18 **Midena E**, Radin PP, Pilotto E, *et al.* Fixation pattern and macular sensitivity in eyes with subfoveal choroidal neovascularization secondary to age-related macular degeneration. A microperimetry study. *Semin Ophthalmol* 2004;**19**:55–61.
19 **Springer C**, Volcker HE, Rohrschneider K. Central serous chorioretinopathy – retinal function and morphology: microperimetry and optical coherence tomography. *Ophthalmologe* 2006;**103**:791–7.
20 **Sjaarda RN**, Frank DA, Glaser BM, *et al.* Assessment of vision in idiopathic macular holes with macular microperimetry using the scanning laser ophthalmoscope. *Ophthalmology* 1993;**100**:1513–18.
21 **Rohrschneider K**, Gluck R, Blankenagel A, *et al.* Fixation behavior in Stargardt disease. Fundus-controlled studies. *Ophthalmologe* 1997;**94**:624–8.
22 **Vujosevic S**, Midena E, Pilotto E, *et al.* Diabetic macular edema: correlation between microperimetry and optical coherence tomography findings. *Invest Ophthalmol Vis Sci* 2006;**47**:3044–51.
23 **Battaglia Parodi M**, Pedio M, Midena E. Fundus autofluorescence and microperimetry. In: Midena E, ed. *Perimetry and the fundus: an introduction to microperimetry*, Slack Incorporated. NJ: Thorofare, 2006:53–8.
24 **Ferris FL III**, Kassoff A, Bresnick GH, *et al.* New visual acuity charts for clinical research. *Am J Ophthalmol* 1982;**94**:91–6.
25 **Rohrschneider K**, Sprinter C, Bultmann S, *et al.* Microperimetry – comparison between the micro perimeter 1 and scanning laser ophthalmoscope fundus perimetry. *Am J Ophthalmol* 2005;**139**:125–34.
26 **Bartsch DU**, Weinreb RN, Zinser G, *et al.* Confocal scanning infrared laser ophthalmoscopy for indocyanine green angiography. *Am J Ophthalmol* 1995;**120**:642–51.
27 **Holz FG**, Bellmann C, Margaritidis M, *et al.* Patterns of increased in vivo fundus autofluorescence in the junctional zone of geographic atrophy of the retinal pigment epithelium associated with age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol* 1999;**237**:145–52.
28 **Pilotto E**, Midena E, Vujosevic S, *et al.* Fundus autofluorescence and fundus perimetry in patients with age-related macular degeneration. *Invest Ophthalmol Vis Sci*, 2006;47:E-abstract, 2099.
29 **Altman D**. *Practical statistics for medical research*, London: Chapman and Hall 1991;304.
30 **Midena E**, Degli Angeli C, Blarzino MC, *et al.* Macular function impairment in eyes with early age-related macular degeneration. *Invest Ophthalmol Vis Sci* 1997;**38**:469–77.
31 **Remky A**, Lichtenberg K, Elsner AE, *et al.* Short wavelength automated perimetry in age related maculopathy. *Br J Ophthalmol* 2001;**85**:1432–6.
32 **Scholl HP**, Bellmann C, Dandekar SS, *et al.* Photopic and scotopic fine matrix mapping of retinal areas of increased fundus autofluorescence in patients with age-related maculopathy. *Invest Ophthalmol Vis Sci* 2004;**45**:574–83.
33 **Sunness JS**, Schuchard RA, Shen N, *et al.* Landmark-driven fundus perimetry using the scanning laser ophthalmoscope. *Invest Ophthalmol Vis Sci* 1995;**36**:1863–74.
34 **Sunness JS**, Johnson MA, Massof RW, *et al.* Retinal sensitivity over drusen and nondrusen areas. A study using fundus perimetry. *Arch Ophthalmol* 1988;**106**:1081–4.
35 **Johnson PT**, Lewis GP, Talaga KC, *et al.* Drusen-associated degeneration in the retina. *Invest Ophthalmol Vis Sci* 2003;**44**:4481–8.
36 **Johnson PT**, Brown MN, Pulliam BC, *et al.* Synaptic pathology, altered gene expression, and degeneration in photoreceptors impacted by drusen. *Invest Ophthalmol Vis Sci* 2005;**46**:4788–95.
37 **Einbock W**, Moessner A, Schnurrbusch UE, *et al.* Changes in fundus autofluorescence in patients with age-related maculopathy. Correlation to visual function: a prospective study. *Graefes Arch Clin Exp Ophthalmol* 2005;**243**:300–5.
38 **Marmorstein AD**, Marmorstein LY, Sakaguchi H, *et al.* Spectral profiling of autofluorescence associated with lipofuscin, Bruch's membrane, and sub-RPE deposits in normal and AMD eyes. *Invest Ophthalmol Vis Sci* 2002;**43**:2435–41.
39 **Smith RT**, Chan JK, Busuoic M, *et al.* Autofluorescence characteristics of early, atrophic, and high-risk fellow eyes in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2006;**47**:5495–504.