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MULTIMODAL IMAGE: ASSESSMENT OF RISK FACTORS FOR THE DEVELOPMENT OF CHOROIDAL NEOVASCULARISATION IN THE FELLOW EYE

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Multimodal image: assessment of risk factors for the development of choroidal neovascularisation in the fellow eye

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AGRADECIMENTOS

ABSTRACT

Objectives

To characterize the progression of eye fundus changes by multimodal imaging and to identify morphological and/or functional predictors of conversion in wet AMD in the fellow eyes of patients with exudative AMD.

Sample Description and Methods

Single-center, prospective, observational, longitudinal 2-year study. Sixty-two patients were enrolled in the study, with diagnosis of neovascular age-related macular degeneration in one eye and age-related maculopathy in the fellow eye. Each patient underwent a detailed ocular and medical history, a complete ophthalmologic examination with color fundus photography, fluorescein angiography, indocyanine green angiography and fundus autofluorescence imaging at baseline and repeated at six-month intervals. CFP images were graded resorting to the RetmarkerAMD (Critical Health SA) software, a computer assisted grading system based on the International Classification Grading System guidelines (Bird, Bressler *et al.*, 1995).

Results

Fifty-three patients completed the two-year follow-up period, eighteen of which converted to the exudative form in the fellow eye during the study period. No measurable evolution was found in consecutive fundus autofluorescence and indocyanine green angiography images. Total drusen area in CFP images presented no significant increase between each visit and the subsequent (p=0.100), but significant increase was found between the first and the last (p=0.048). **IGC images**: 23% of patients presented a normal exam; observations have shown that the hot spots or areas and the hypofluorescent spots or areas where either attributable to drusens or drusenoid pigmentary epitelium dettachments or did not correspond to any lesion visible in the CFP images; the sensitivity of the overall exam result was 88% and the specificity of presence of early hot spots or areas was 97%. **FAF**

images: the distribution of patterns for the cases that converted and those that did not is significantly different. **CFP images**: drusen area was significantly higher in the group of patients which did not convert when considering total area (p=0.012), sub-field 4 (p=0.020) and sub-field 5 (p=0.039); two main groups were identified by hierarchical cluster analysis, with Ward's linkage, when considering total area of drusens of the last visit available; principal component analysis identified total area as the most characterizing feature; no significant differences were found between the groups in what concerns the areas of drusen with specific sizes (C1: p=0.28; C2: p=0.11; C3: p=0.31); no correlation was found between age or total drusen area and conversion, and these two variables are not correlated.

Conclusion

The percentage of patients which converted during the study (34%) exceeds the expected rate for this time period. In what concerns evolution, further study including a longer follow-up period is suggested as it was only apparent for total drusen area calculated in CFP images between the first and last visits available. **FAF images:** FAF may be an interesting exam for predicting AMD progression but further studies are necessary to pinpoint the characterizing patterns. **ICG images**: the most characterizing features for conversion were the overall exam result and the presence of early hot spots or areas. **CFP images:** in this sample conversion does not occur for patients with the higher extension of lesions; no relation was found between conversion and patient's age or drusen of specific sizes.

Key-words: choroidal neovascularization, exudative age-related macular degeneration, age-related maculopathy.

Resumo

Objetivos

Estudar a progressão das alterações do fundo ocular através de imagem multimodal e identificar os fatores de risco para conversão em degenerescência macular relacionada com a idade (DMRI) neovascular no olho contra-lateral de doentes com DMRI exsudativa.

Descrição da Amostra e Métodos

Estudo de centro único, prospetivo, observacional, longitudinal com a duração de dois anos. Foram incluídos no estudo 62 doentes com o diagnóstico de DMRI neovascular num dos olhos e maculopatia relacionada com a idade no outro. Cada doente foi submetido a uma história médica e ocular detalhada, um exame oftalmológico completo com retinografia a cores (CFP), angiografia fluoresceínica (FA), angiografia com verde de indocianina (ICG) e imagem de autofluorescência do fundo na linha de base e repetidos com intervalos de seis meses. As CFP foram classificadas com recurso ao software RetmarkerAMD (Critical Software SA), um sistema de classificação assistida por computador baseado nas linhas guias do sistema de classificação internacional (Bird, Bressler *et al.*, 1995).

Resultados

Cinquenta e três doentes completaram o período de seguimento de dois anos, dezoito dos quais converteram para a forma exsudativa no olho em estudo durante a duração do estudo. Não foi encontrada evolução mensurável em imagens consecutivas de angiografia com verde de indocianina e imagem de autofluorescência do fundo. A área total de drusens nas CFP não revelou aumento significativo entre visitas subsequentes (p=0,100), mas verificou-se a existência de aumento significativo de área entre a primeira e a última (p=0,048). **Imagens de ICG**: 23% dos casos apresentavam um exame normal; as zonas de hiperfluorescência e hipofluorescência foram atribuídas a drusens ou descolamentos drusenoides do epitélio pigmentar da retina ou não correspondiam a nenhuma lesão identificável nas imagens de CFP; a sensibilidade do resultado global do exame é de 88% e a

especificidade da presença de hiperfluorescências é de 97%. **Imagens de FAF**: foram encontradas diferenças significativas entre a distribuição de padrões de cada grupo (doentes que converteram e doentes que não converteram). **Imagens de CFP**: a área de drusens é significativamente maior no grupo de doentes que não converteu quando se considera a área total (p=0,012), sub-área 4 (p=0,020) e sub-área 5 (p=0,039); foram identificados dois grandes grupos através da análise hierárquica de agrupamentos, com ligação de Ward, quando se considera a área total de drusens da última visita disponível; a análise de componentes principais identificou a área total como a variável mais caracterizadora; não foram encontradas diferenças significativas entre os grupos no que diz respeito à área de drusens com tamanhos específicos (C1: p=0,28; C2: p=0,11; C3: p=0,31); não se encontrou correlação entre a idade ou a área total de drusens e a conversão e estas duas variáveis não estão correlacionadas entre si.

Conclusão

A percentagem de doentes que converteu durante o estudo (33%) é superior à esperada para este período de tempo. No que diz respeito à evolução, parece pertinente realizar estudos com período de seguimento mais alargado uma vez que esta só foi manifesta para a área total de drusens calculada através das imagens de CFP entre a primeira e a última visitas disponíveis. **Imagens de FAF**: a imagem de FAF pode ser interessante para prever a progressão da DMRI mas são necessários mais estudos para determinar os padrões caracterizadores. **Imagens de ICG**: os achados mais caracterizadores para a conversão são o resultado global do exame e a presença de hiperfluorescências precoces. **Imagens de CFP**: nesta amostra a conversão não ocorre nos doentes com extensão de lesões superior; não se encontrou relação entre a conversão e a idade ou drusens de tamanhos específicos.

Palavras-chave: neovascularização coroidal, degenerescência macular relacionada com a idade exsudativa, maculopatia relacionada com a idade.

INTRODUCTION

Age-related macular degeneration (AMD) is a degenerative disorder of the macula that affects people over 50 years old (Bird, Bressler *et al.*, 1995). It is the leading cause of irreversible severe loss of sight in elderly populations in the developed world (Coleman, Chan *et al.*, 2008 and references therein).

The pathogenesis of the disease is not currently fully established (Coleman, Chan *et al.*, 2008) but it is believed that it involves aging changes as well as additional pathological changes that result in injury to the retina, choriocapillaris and Bruch's membrane (Zarbin, 2004).

Age, family history and smoking are the most consistent relationships of systemic risk factors to AMD (Jennifer R, 2001; Klein, Peto *et al.*, 2004; Donoso, Kim *et al.*, 2006).

The early manifestations of AMD include hard and soft "drusens", areas of increased pigment or hyperpigmentation (in the outer retina or choroid) and areas of depigmentation or hypopigmentation of the retinal pigment epithelium (RPE).

There are two morphologic forms recognized in the late stages of AMD: (1) the "dry" AMD or geographic atrophy and the (2) "wet", "neovascular" or "exudative" AMD (Bird, Bressler *et al.*, 1995). These morphologic forms of AMD are interrelated (Green and Enger, 1999). It is the exudative form that is responsible for most of the central visual impairment attributed to this disease (Bressler, Bressler *et al.*, 1988).

Approximately 10 to 20% of the patients with early AMD will develop the wet form of the disease (Pauleikhoff, Radermacher *et al.*, 2002 and references therein). The presence of soft drusen, macular pigmentary change, and choroidal neovascularisation in the other eye are accepted as ocular risk factors for the development of exudative AMD in the fellow eye (Jennifer and Shirley, 2000). Large drusen ($\geq125\mu$m), soft indistinct drusen and any pigment

changes were found to be particularly predictive of developing the exudative form (Ferris, Davis *et al.*, 2005; Klein, Klein *et al.*, 2002).

AMD is a bilateral disease and, in patients with unilateral late exudative AMD, the risk of visual loss in the second eye was estimated at 12 to 15% per year (Holz, Wolfensberger *et al.*, 1994) and 42-58% develop bilateral CNV within 5 years (MPSG[1], 1993; MPSG, 1997).

The objective of our study was to characterize the progression of eye fundus changes during a 2 year follow-up period, by multimodal imaging during the two-year follow-up period and to identify morphological and/or functional predictors of conversion in wet AMD in the fellow eyes of patients with exudative AMD.

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[1] Macular Photocoagulation Study Group

SAMPLE DESCRIPTION AND METHODS

This is a single-center, prospective, observational, longitudinal two-year study of 62 patients with neovascular AMD in one eye (the non-study eye) and early ARM in the fellow eye (study eye) at risk for the development of CNV. The visits and image acquisition were held from August, 2006, to December, 2008.

The methodology, as well as inclusion and exclusion criteria, was previously described in Silva, Cachulo *et al.*, 2011 and Cachulo, Silva *et al.*, 2011 and is briefly summarized in what follows. Eligibility included the following criteria: (a) older than 50 years of age, (b) any race and either sex, (c) signed informed consent, (d) ability to attend the required visits, (e) clinical diagnosis of wet AMD in one eye (non-study eye) and the presence of the following characteristics in the second eye (study eye): 1) at least 5 or more intermediate drusen ($\geq$63 μm and <125 μm, and/or confluent drusen, within 3000 μm of the foveal center; 2) with or without pigmentary changes. Criteria for exclusion incuded: (a) other fundus disease like vascular retinopathy, central serous chorioretinopathy, inflammation or non-AMD choroidal neovascularization, (b) current or past history of a medical condition that would preclude scheduled study visits or completion of the study, (c) clinical signs of myopic rethinophaty or refractive power of $\geq$8 diopters or fundoscopic evidence of degenerative myopia, (d) past history of intraocular surgery within 60 days prior to enrolling in the study, and (e) evidence of past or present CNV in the study eye.

A detailed ocular and medical history and a complete ophthalmologic examination were preformed. Each patient underwent a best corrected Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity measurement (BCVA), fluorescein angiography (FA), indocyanine green angiography (ICG), color fundus photograph (CFP) and fundus autofluorescence imaging (FAF) at baseline and at 6-month intervals until a total of 5 visits

(24 months) or up to 6 months after conversion to wet AMD in the study eye. FA images were used as the gold standard exam for defining conversion from early to late exudative AMD. The study design was approved by the ethics committee at AIBILI and informed consent was obtained from all patients before enrollment in the study.

FA and ICG Acquisition and Grading

Simultaneous FA and ICG were acquired using the HRA II (Heidelberg Retina Angiograph – Heidelberg Engineering, Dossenheim, Germany) with the 30° field of view centered on the macula. The ICG images were inspected for hyperfluorescent and hypofluorescent spots and areas at early arterial and late venous phases. Only spots larger than 750 μm (approximately half the diameter of the optical disc) were considered. The findings were then compared to the CFP images and classified accordingly, by translating the hyperfluorescent and hypofluorescent spots and areas found in the ICG to drusen, subfoveal drusenoid pigment epithelial detachment (PED) or other types of lesions found in the CFP.

CFP Acquisition and Grading

A digital color fundus photography was performed using a Zeiss FF450 fundus camera with a Nikon high-resolution camera to produce a 30° field of view, centered both on the macula and optic disk. The grading of CFP images was done resorting to the software RetmarkerAMD (Critical Health SA), a computer assisted grading system based on the International Classification Grading System guidelines (Bird, Bressler *et al.*, 1995). This software allows to: i) track graded/non-graded images; ii) display and grade images in full screen view; iii) draw free forms of all the lesions' types over the images; iv) draw preset circular objects of different color codes and sizes (ranging from 63 μm to 500 μm) to represent various lesions (namely drusen, hyperpigmentation and hypopigmentation); v) mark

drusens that are confluent; vi) zoom in/out the image or display an image area with a higher or lower degree of magnification; vii) measure distances in the image; viii) control RGB channels as well as brightness and contrast of the images; ix) superimpose a standard grid to identify eye sub-fields; x) manually enter data for study protocol questionnaire. Before grading each image was calibrated within RetmarkerAMD itself by using the optic disc diameter (which corresponds roughly to 1.5 mm) as a reference. By manually referencing the fovea the software can then overlay the reference grid that allows identify the eye sub-fields. This grid (Figure 1) consists of three concentric circles and a right angled cross at 45° and 135° to the horizontal. The central, inner and outer circles are respectively 1000 μm, 3000 μm and 6000 μm in diameter and represent the central, middle and outer subfields. The specifications for this grid are identical to those described by the International Classification System (Bird, Bressler *et al.*, 1995). Using this semiautomated grading system some results are displayed onscreen in real time (Figure 2), namely: i) the number of drusen of each size; ii) a semi quantitative indication of the total area occupied by drusen and hyperpigmentation and/or hypopigmentation of the retinal pigment epithelium (for central, inner and outer circle separately); iii) the cumulative real area by drusen and area in each subfield; iv) a semi quantitative indication of the degree of confluence of drusen; v) the presence of atrophic or neovascular AMD. The final characterization of the predominant type of drusen (hard; soft distinct; soft indistinct: semisolid, serogranular, crystalline) is then adjusted manually.

Preliminary results from comparative tests have shown that RetmarkerAMD is more effective than 35-mm film manual grading allowing significant time savings (overall, it is 35% faster) and is more accurate (improved accuracy: overall 32% more lesions identified) (Silva, 2010).

The use of digital images implies that, depending of the screen resolution, a pixel may be equivalent to several micrometers which means that, when converting between these units,

approximations must be made. However, the data obtained in testing indicates that these deviations are less than 0.1%. In this grading process we used a screen resolution of 1920 x 1080 pixels in a poorly lit room.

Data was then exported, compiled in a datasheet and analyzed.
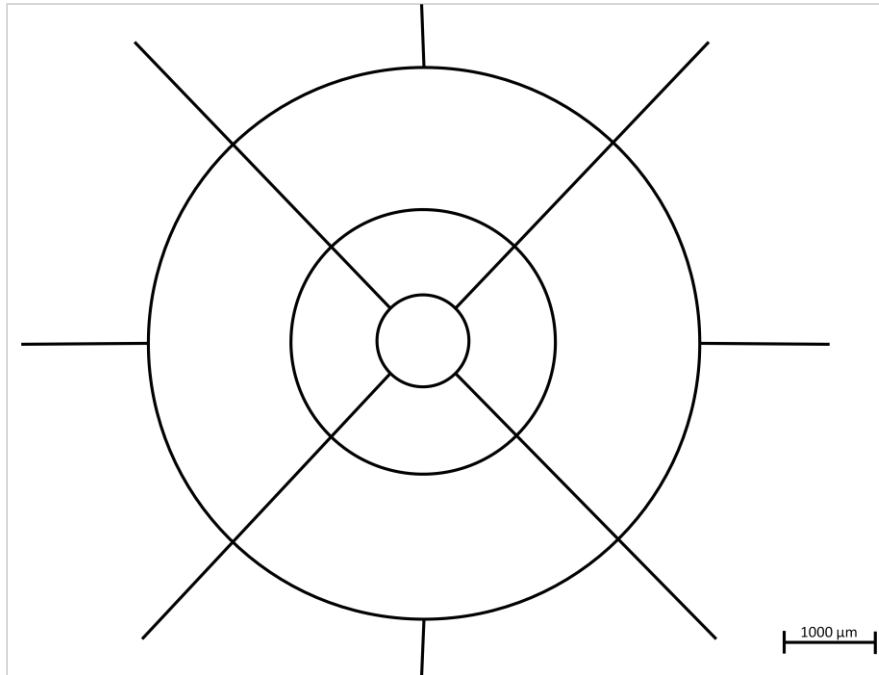
Figure 1: *Representation of the overlaid reference grid that defines the eye sub-fields*

Figure 2: *Screenshot of the results displayed onscreen in real time.*

FAF Acquisition and Grading

FAF imaging was performed with the same retina angiograph scanning laser ophthalmoscope following a standard protocol. For the acquisition of the FAF film, the ametropic corrector of the angiograph was first adjusted to focus on the macula and sensitivity was altered until clear images of the macula and optic nerve were obtained. An argon laser (488 nm) was used to excite and a barrier filter enabled the detection of light above 500 nm. Multiple single 30° autofluorescence images were acquired, aligned and averaged. The composite images obtained were then graded for their predominant FAF pattern according to the Fundus Autofluorescence Classification Group (Bindewald, Bird *et al.*, 2005).

Statistical Analysis

Paired t-tests, one-tailed, were used to assess the evolution of the total drusen area, from each visit to the subsequent one, and also from the first visit to the last.

Mean comparisons for two samples were performed between the area of drusens for converted and non-converted patients. These comparisons included total area, and sub-fields 1-5, for the last recorded visit. Each of the two samples was firstly subjected to a two-tailed F-test, to assess homo- or heterocedasticity, followed by a corresponding one-tailed t-test with the purpose of finding whether the means can be considered equal or if the mean area corresponding to the non-converted patients exceeds that of the converted.

Data structure analysis resorted to standard multivariate techniques, including hierarchical cluster analysis (HCA), with Ward's linkage, for exploratory assessment of the data structure (Ward, 1963) and principal component analysis (PCA), for data visualization and variable selection, on the variance-covariance matrix (Brereton, 2003). The software code was developed in Octave.

The main goal of clustering algorithms is to group the data into a number of sensible clusters according to their similarities. In the exploratory data analysis, hierarchical cluster analysis (HCA) was used, for which a recent review can be found in reference Almeida, Barbosa *et al.*, 2007. Ward's method is a standard procedure for such analysis, and will be the one selected for the present study. In this method the distance between objects or groups is established based on minimizing the within cluster variance, when each new cluster is constructed (Ward, 1963).

The HCA procedure is graphically represented by a dendrogram, which consists of a cluster structure and illustrates the fusions or divisions made at each successive stage of the analysis. It allows inspecting the overall structure of the data, and estimate the number of clusters.

In turn, PCA allows compressing the data by reducing the number of dimensions, with a minimized loss of information. The most influential variables in the system are highlighted, and underlying factors may be identified.

Based on an orthogonal linear transformation, PCA defines a lower dimensionality system, such that the highest variance of the data comes projected on the first principal component (PC1), the second largest on the second coordinate (PC2), and so on (Brereton, 2003). PCA requires the solution of an eigenvalue problem, either based on the correlation or variance/covariance matrices of the original variables. In this work we present results based on the variance/covariance approach.
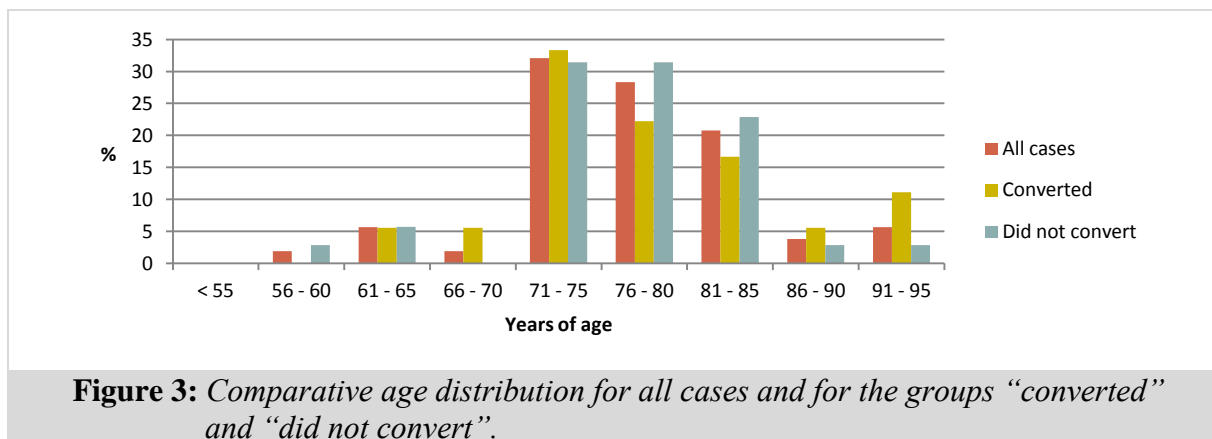
RESULTS

Characterization of the Sample

Sixty-two patients were initially enrolled in the study but only fifty-three patients (24 men and 29 women) completed the two-year study follow-up. The mean age of the patients included in the study was 77, ranging from 56 to 93 years.

The drop-outs from the study were due to death (4 cases), withdrawal of informed consent (4 cases) and worsening of health conditions (1 case).

Of the total fifty-three patients, eighteen converted during the two-year follow-up period. The comparative age distribution in all cases and for the groups of patients which converted and which did not convert is represented in Figure 3, presented in percentage relative to the total of cases in each group. The mean age of patients which converted was 78 and that of the patients which did not convert was 77. The difference between these two averages is not significant (p=0.50).

Figure 3: *Comparative age distribution for all cases and for the groups "converted" and "did not convert".*

We now present the results obtained from the CFP, FAF and ICG images. Figure 4 presents an example of each.
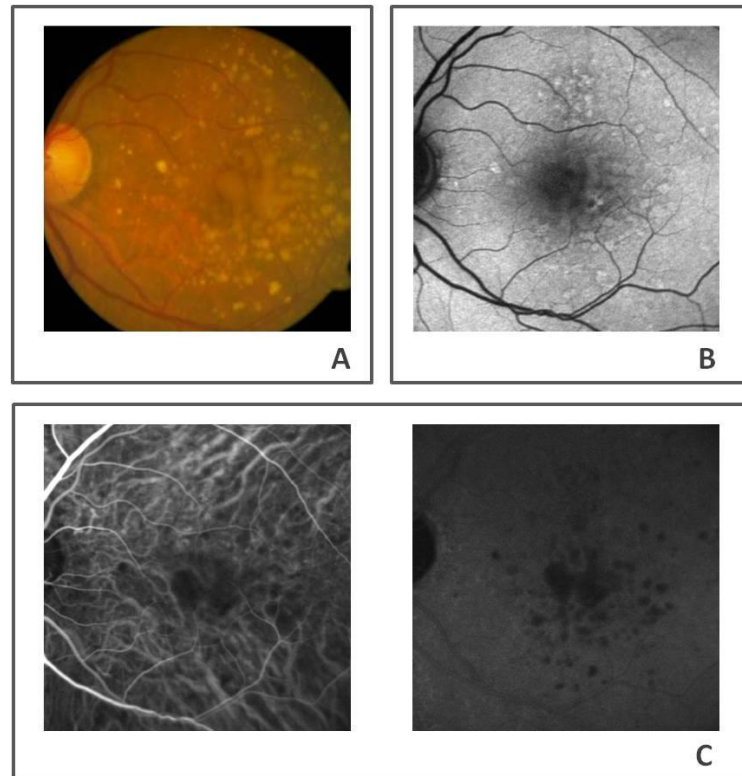
Figure 4: *Examples of images obtained by the different techniques.* **A:** CFP; **B:** FAF; **C:** early (left) and late phases (right) of ICG.

ICG Findings

Data Overview

We were able to obtain ICG images for all fifty-three patients. Only 23% of all patients had a normal ICG exam (no early or late hot spots/areas or hypofluorescent spots/areas).

The hot spots/areas, when coincident with lesions visible in CFP images, were attributed to either drusen or subfoveal drusenoid PED. In 50% of the cases no correspondent lesion was found in the CFP image. A similar distribution was found regarding the hypofluorescent spots/areas, in which 38% in early phase and 47% in late phase did not correspond to any lesion visible in CFP images, as can be seen in Figure 5.

Figure 5: *Hot spots/areas and hypofluorescent spots/areas in ICG and the correspondent lesions found in CFP images.*
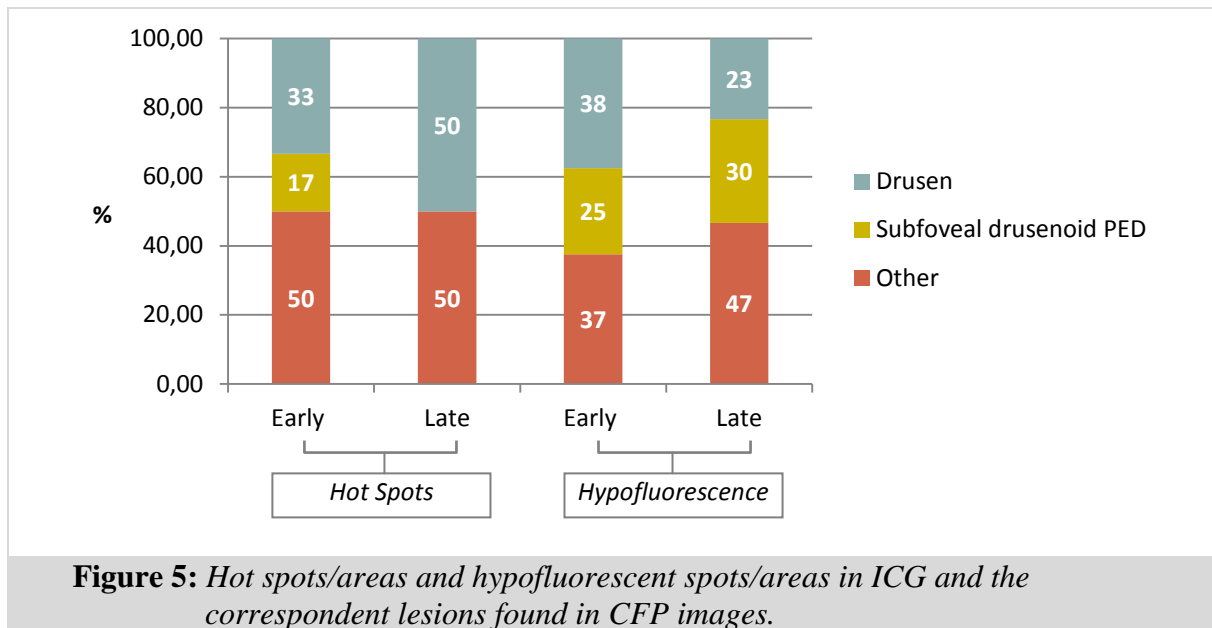
Table I shows the statistical measures sensitivity, specificity, positive predictive value and negative predictive value for presence/absence of certain findings in ICG images. In the first row we consider the exam negative when there are no hot spots/areas or hypofluorescent spots/areas on both early and late phases.

Table I – *Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for ICG findings.*

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Overall	88	29	38	83
Hot Spots/areas	35	86	55	73
Early Hot Spots/areas	29	97	83	74
Late Hot Spots/areas	23	89	50	70
Hypofluorescence	76	34	36	75
Early Hypofluorescence	59	37	31	65
Late Hypofluorescence	71	49	40	77

Evolution

In what concerns the ICG images, no evolution was found regarding the existence or absence of either early or late hot spots/areas and hypofluorescence areas, their correspondent lesions (as observed in the CFP images) or predominant location.

FAF Findings

Data Overview

Of the total fifty-three patients we obtained gradable FAF images for forty-six. The number of patients who displayed each overall pattern is shown in Table II.

	Normal	Minimal Change	Focal Increase	Patchy	Linear	Lacelike	Reticular	Speckled
Did not convert	0	4	1	1	0	0	6	1
Converted	1	12	2	6	0	0	11	1
All cases	1	16	3	7	0	0	17	2

Table II – *Number of cases that presented each pattern at the last available visit.*

When comparing the distributions of the patterns displayed by the group of cases which converted and those which did not convert, the chi-squared test shows that there is a significant difference between the groups (Chi Square value determined = 3,54; table value for Chi Square= 0,62 for a 95% confidence level with 5 degrees of freedom).

Evolution

In all but four cases the overall pattern observed was the same throughout the visits in the 2-year follow-up period. In two of the patients which did not convert and in one which did convert the initial minimal change pattern was replaced by a reticular pattern. In another patient, which converted, the initial minimal change pattern was replaced by a focal increased pattern.

It also appears interesting to point out that approximately 35% of the eyes that presented reticular pattern at some point during the two-year follow-up period, converted. This compares to a conversion rate of 20% for those that displayed minimal change throughout the study. However, due to the size of the sample, definite conclusions cannot be drawn.

CFP Findings

Data Overview

Table III – *Summary of results pertaining to average drusen area and number.*

	Total Number	Total Area (μm^2)	Subfield 1 (μm^2)	Subfield 2 (μm^2)	Subfield 3 (μm^2)	Subfield 4 (μm^2)	Subfield 5 (μm^2)
Did not convert	150	3028678	119863	173671	174920	259098	260573
Converted	103	1217022	50499	101005	5791	102351	125914

Table III shows the average total area of drusen and number of drusens specifying for the former values pertaining to subfields 1 to 5. A test conducted on combination of drusen area with age, illustrated in Figure 6, has indicated that these variables possess no discriminantion power between cases that converted and those that did not.
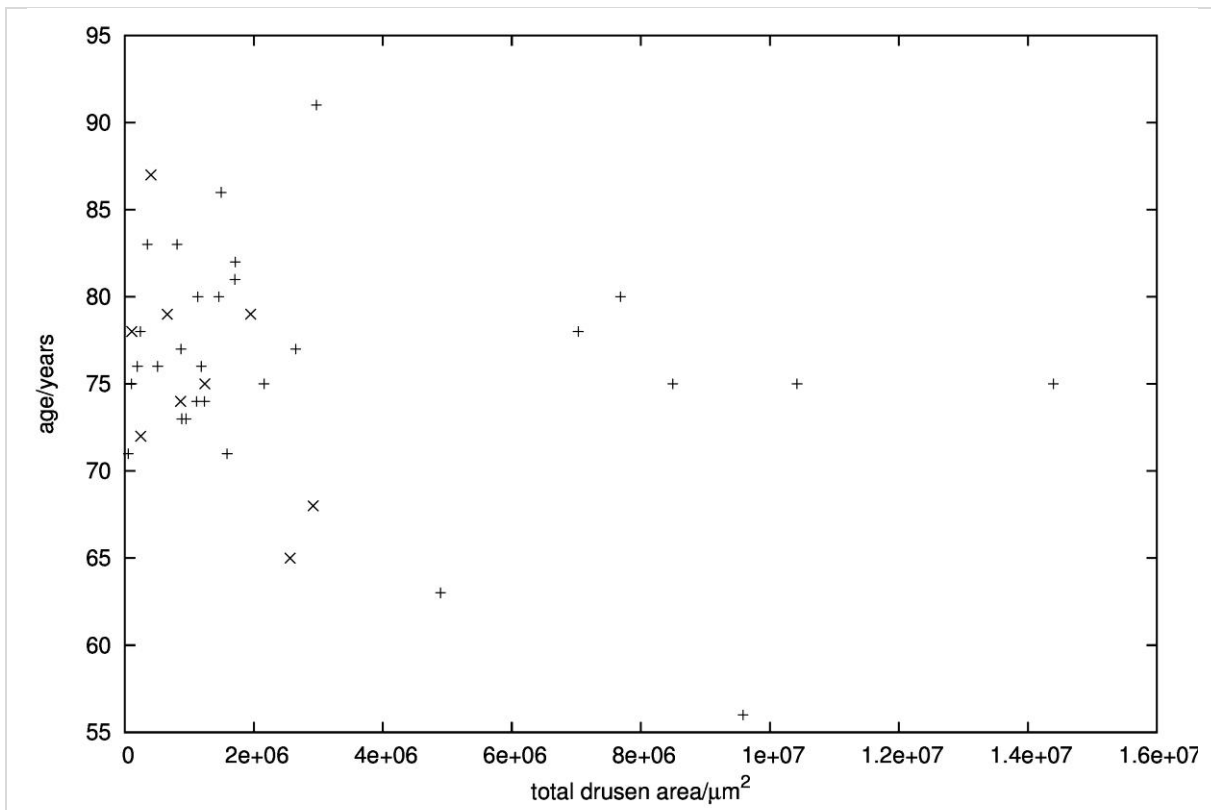
Figure 6: *Representation in the plane age/total drusen area of the cases that converted* (x) *and those that did not convert* (+)

Evolution

We now remark on the evolution of the lesions, as assessed by the CFP measurements over the sequence of visits. It is seen that, for the whole set of visits in contiguous and subsequent time points, 49% correspond to a decrease in the total area of drusen and 51% to an increase in area. In the same way, if only the first and last visit is considered for each patient, drusen area decreased in 43% of the cases and increased in 57%.

Paired t-tests, one tailed, indicate that there is no significant increase in the drusen area between each visit and the subsequent (p= 0.100), while this may be present between the first and the last one (p=0.048).

Group Comparison

The comparisons of the means of the drusen area between converted and non-converted patients indicate that the drusen areas are similar for sub-fields 1 (p=0.065), 2 (p=0.266) and 3 (p=0.067).

On the contrary, we found that the drusen area of the non-converted patients probably exceeds that of the converted for total area (p=0.012) and sub-fields 4 (p=0.020) and 5 (p=0.039).

Table IV – *Summary of results pertaining to average drusen area of specific sizes. Values are indicated in μm^2.*

	Did not convert	Converted
C1: ≥63 μm^2, <125 μm^2	378582	321903
C2: ≥125 μm^2, <175 μm^2	269079	166709
C3: ≥175 μm^2, <250 μm^2	174920	122573

Table IV displays the average area of drusens with specific sizes which were found to be similar between these groups of cases (C1: p= p=0.28; C2: p=0.11; C3: p=0.31).

We also found that the number of drusens is not significantly higher for the group of patients which converted during the two-year follow-up (p=0.112).

Structure of the Data

In Figure 7 we depict the dendrogram obtained from Ward's linkage using the CFP results for the last visit. Each case was described by the total area and all the subareas relative to the last visit.
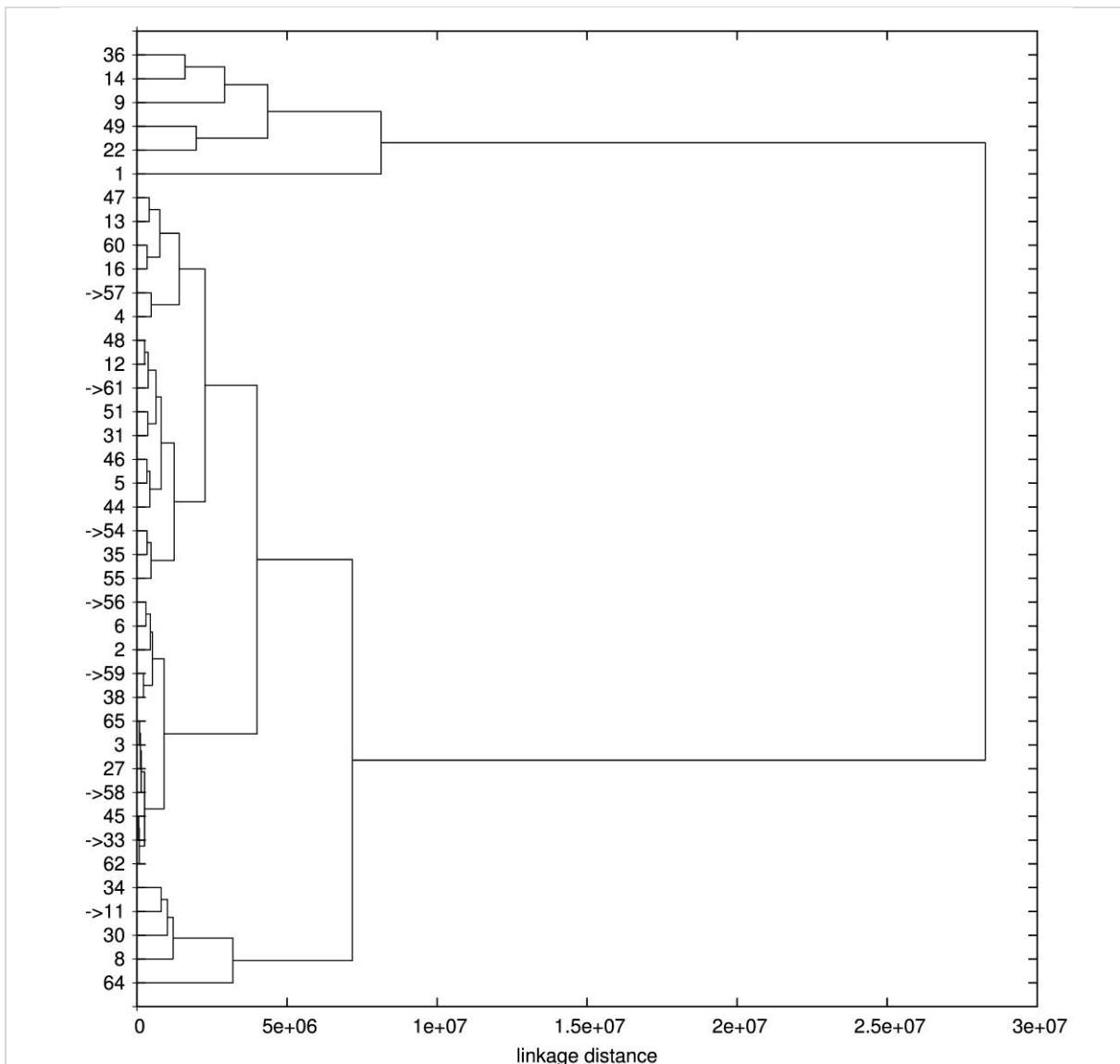
Figure 7: *Dendrogram obtained from Ward's linkage on the areas, total and relative to each subsection, found for each patient on the last considered visit. The cases undergoing conversion are duly indicated.*

It is seen that the data is composed of two main groups, one found to the top of the dendrogram, the other in its lower part. The latter contains all the cases that have undergone conversion. These two groups are well separated as can be seen by the high value of the linkage distance that connects them.

Further insight in the data can be obtained by PCA. Only one axis, PC1, recovers most of the information but, for clarity, Figure 8 provides a PC1/PC2 two-dimensional picture.
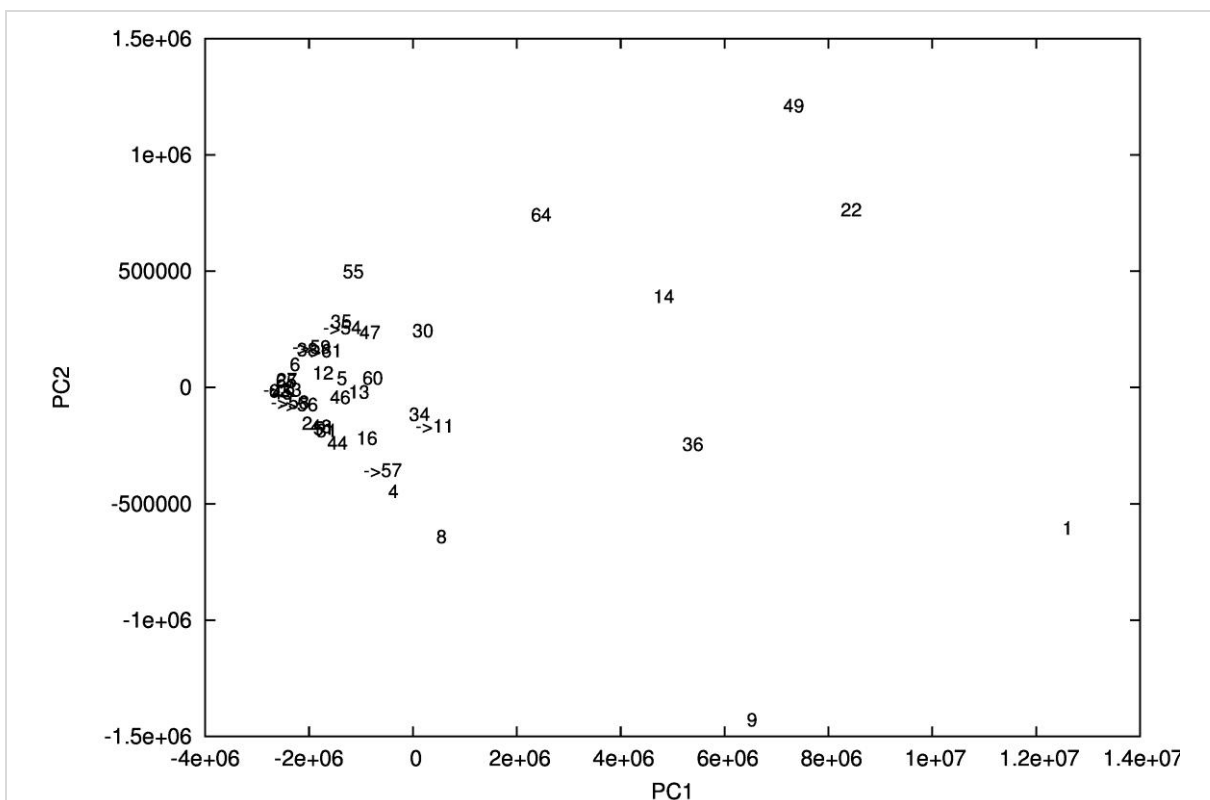
Figure 8: *Representation of the cases, as observed in the last visit, in the PC1/PC2 plane. Conversions are indicated by arrows.*

A compact group, to the left, gives way to gradually more distant objects as we advance towards the right side of the image. The PC1 axis is directly linked to the extent of the drusens: the compact group that contains most of the cases is, thus, characterized by less extensive drusen areas.

It should be noted that results obtained removing the total area from the description of each case, and using only the subareas, are very similar to those described above.

In Figure 9 is represented the total area of drusen, along the PC1 axis.

Figure 9: *Total drusen area in the last visit considered, for cases in the order they appear along the PC1 axis. Cases that converted are indicated by an "x".*
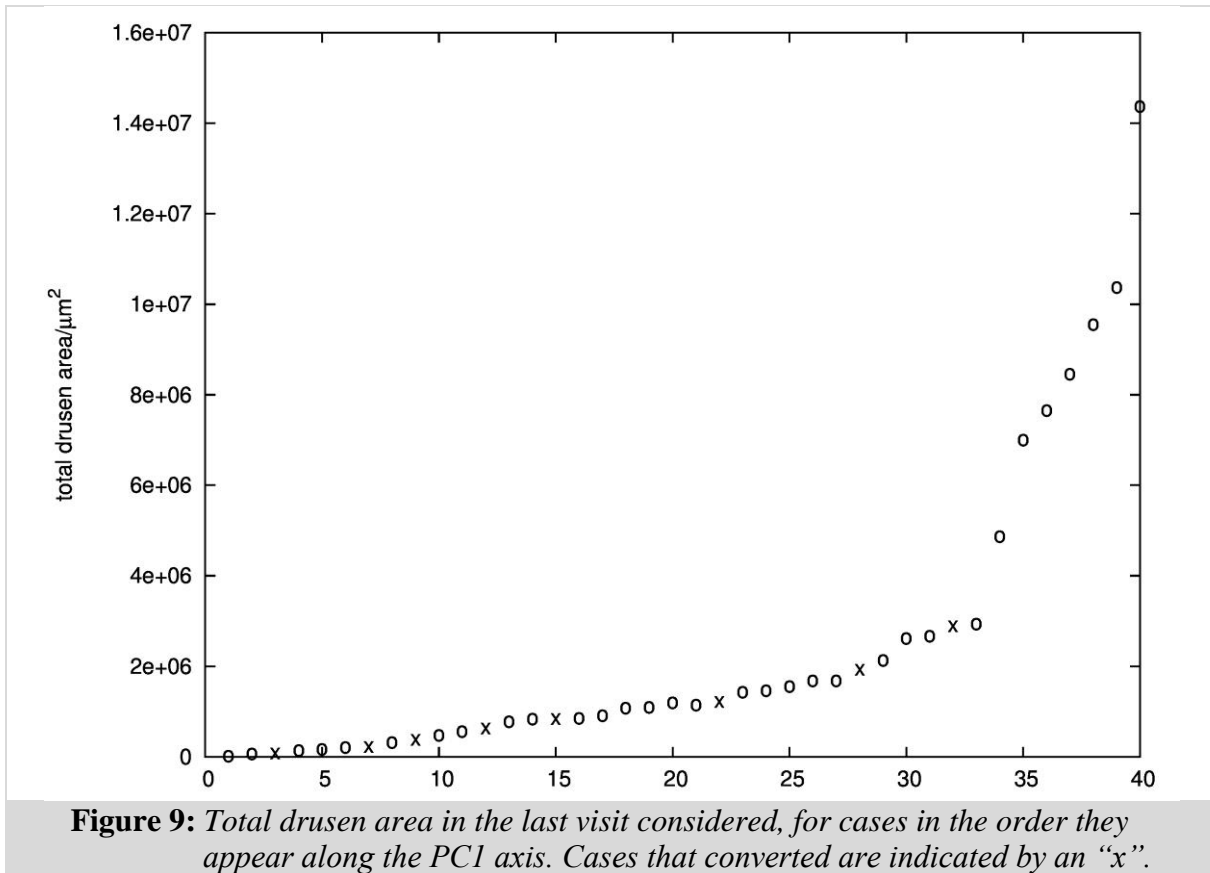
As previously indicated, this area grows along the axis. The main feature in this Figure seems to be the slope change, close to the 35th case as arranged in increasing order, after which the area increases drastically. The cases found for the highest area correspond to those in the more disperse group in the PC1/PC2 plane. It is also interesting to note that *all* the patients that displayed conversion are found in the low area region.

DISCUSSION

In this study we define two groups: the group of patients which did not convert to the exsudative form during the two-year follow-up period and the group of patients that converted. A total of eighteen patients converted, representing 34% of the total enrolled in the study, which exceeds the rate previously suggested (MPSG, 1993; Holz, Wolfensberger *et al.*, 1994; MPSG, 1997). The age distribution between the groups is not significantly different, which fails to support the generally accepted notion of age as a major risk factor for conversion (Jennifer R, 2001; Klein, Peto *et al.*, 2004).

We now remark on the evolution, as perceived by the different exams, comparing the results obtained in successive visits separated by a six-month interval. The ICG and FAF observations indicate very little evolution, not only between visits 6 months apart but also when we consider the whole 2-year follow-up period. The fact that the results were obtained in the form of qualitative or, at most, semi-quantitave data may be part of the explanation for this lack of perceivable evolution. Also, the small sample size and the short follow-up period may also have contributed. In what concerns the CFP findings, no evolution of drusen area was found between successive visits. However, it was possible to observe an increase in average drusen area between the first and last visits, for each case. A possible explanation is that a six-month interval is not long enough to allow a significant evolution, considering possible operator-dependant and/or method-dependant grading fluctuations. It is, however, interesting to point out that positive and negative evolutions of the area of drusens are similar in number. This also indicates that the follow-up period should be increased.

For the ICG findings, our option was to study the importance of hot spots/areas and hypofluorescent spots/areas as possible predictors of conversion, and to evaluate whether these findings can be attributed to drusens or other lesions. We found that the hot spots/areas as well as the hypofluorescent spots/areas were either related to drusens or subfoveal

drusenoid PED, or they did not correspond to any detectable anomaly observed in the ICG images. The limited number of patients enrolled in the study conditions our ability to further detect trends concerning the type of lesions related to ICG finding. Nonetheless, when focusing on the ICG findings alone, we were able to draw some conclusions. Eyes with no ICG abnormality were significantly less prone to develop the exudative form during the period of study. This translates into a high sensitivity (88%) and high negative predictive value (83%). However, a significant number of eyes possessed ICG abnormalities but did not convert, which explains the very low values of the statistical measures specificity (29%) and positive predictive value (38%). If only the hot spots/areas are considered, different information is obtained. Hot spots/areas, particularly early hot spots/areas, were very specific predictors of conversion (achieving specificity above 97%). This is consistent with the previous findings that related the presence of ICG hyperfluorescent drusens to a higher chance of developing exsudative changes (Hanutsaha, Guyer *et al.*, 1998). As for the hypofluorescent spots/areas, they appear not to add any valuable information.

As for the FAF results, the pattern distribution between the patients that converted and those that did not was found to be, overall, different. A previous study by Einbock, Moessner *et al.*, 2005 suggested that the patchy pattern was associated with a relatively high risk of progression to the exudative form, observation that was not confirmed in our sample.. Another study by Smith, Chan *et al.*, 2006 indicated that the reticular pattern, an indicator of widespread inflammatory damage to the RPE, was strongly associated with the development of the exsudative form. In spite of the fact that it is difficult to pinpoint the factors responsible for the difference between the groups, the high rate of conversion observed in patients displaying the reticular pattern is probably one of them. Further inspection is advisable.

The CFP results have shown that the cases which did not convert present a wide range of drusen area. They appear to be organized in two groups, one of which is characterized a

much lower average area occupied by drusens. The drusen areas of the cases which converted all fall within the range presented by this group with lower average area. Statistical tests reveal that the average area occupied by drusens of the cases which did not convert is essentially higher than that of the cases which converted, observations compatible with the aforementioned hierarchical structure of the data. The generally accepted notion that macular area covered by drusen and presence of large sized drusen are predictive of conversion was not verified in the sample we studied, even when the possible confounding factor of patient's age was taken into account (Jennifer and Shirley, 2000; Klein, Klein *et al.*, 2002; Ferris, Davis *et al.*, 2005). No correlation was found between age and total drusen area, and these variable are not related to conversion. All these unexpected findings may be explained both by the small sample size and the short follow-up period.

In conclusion, this study has shown that evolution of lesions is not perceivable between FAF, ICG and CFP images acquired at six-month intervals but it was found in CFP images between the first and last visits available. It also indicated that the presence of early hot spots/areas in ICG imaging has a high specificity for predicting conversion. Conversion occurred independently from patient's age and area of drusen with specific sizes (as measured by CFP imaging).

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