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Measurement of drusen and their correlation with visual symptoms in patients affected by age-related macular degeneration

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ABSTRACT

Age-related macular degeneration (AMD) is a common retinal disorder, which became more and more prevalent in the last decades. AMD is now the most prevalent cause of blindness in the western world. The disorder is classified into two phenotypes named dry and wet AMD. This is based on the recruitment of novel blood vessels and inflammatory exudates in wet AMD. In both phenotypes, the pathological hallmark is the presence of proteinaceous aggregates called drusen, which mostly accumulate between the choroid and the retinal pigment. Drusen in dry AMD represent the evident pathological finding although they are present, though less defined, in wet AMD. In AMD drusen are supposed to be a pathogenic trigger of the disorder. In fact, drusen may mechanically alter retinal function. A novel hypothesis exists, suggesting that a metabolic defect (systemic or focal within the retinal pigment epithelium) may be the real determinant of visual impairment, while causing the concomitant accumulation of proteinaceous debris and lipids forming the drusen. Here we face such an issue by analyzing the retinal anatomy to correlate visual impairment with the occurrence of drusen number, size and the extent of a drusenoid area in the foveal region. A comparison is made with wet AMD where new vessels and retinal exudates prevail. The study is carried out in 120 patients affected by dry or wet AMD and 21 patients where paradoxical findings are described. The main question consists in inferring whether the occurrence of visual impairment is due, in fact, to a drusen-dependent mechanical damage or drusen just occurs as an independent consequence of an upstream metabolic alteration, which concomitantly impairs the visual process. The present data indicate that, despite a significant difference in visual function between mild and severe AMD patients in the amount of drusen exists, a strong correlation between drusen and visual impairment does not occur. This suggests that drusen and visual deterioration develop as a consequence of similar upstream biochemical alterations but it is likely that drusen do not produce visual deterioration. This is strengthened here by extreme clinical conditions, where visual impairment is severe with a slight alteration in the planar pattern of the retina or, vice versa an extended drusenoid area occurs concomitantly with fair visual acuity, contrast sensitivity and lack of metamorphopsia. A biochemical analysis of key areas in the function of specific domains in the pigment epithelium as described in the accompanying manuscript should help to better disclose the real morpho-functional deficit, which takes place in AMD.

Key words

Age-related macular degeneration • Neurodegeneration • Autophagy • Drusen • Metamorphopsia • Retinal anatomy • Pigment epithelium

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Key words
AMD (age-related macular degeneration) • OCT (optical coherence tomography) • BCVA (best-corrected visual acuity) • RPE (retinal pigment epithelium)

1. Introduction

Age-related macular degeneration (AMD) is an increasingly prevalent disorder, which leads to irreversible blindness in people over the age of 50 in the Western World (Congdon et al., 2004; Pascolini et al., 2004; De Jong, 2006; Jager et al., 2008). Although AMD affects the whole retina, degeneration is typical when invading the macular region. Such an area produces visual acuity and contrast sensitivity, which explains why, in the course of the disease, there is a progressive loss of vision.

It still remains unknown why the disease prevalence among Western Countries is increasing over time with an estimated geometrical growth predicting that by 2040 affected population will approximate 240 million worldwide (Datta et al., 2017). The basic nosography of AMD reports two main variants (phenotypes) which can be grossly defined as "dry" (or atrophic) AMD and "wet" (exudative or neovascular) AMD (Seddon and Chen, 2004; De Jong, 2006; Jager et al., 2008; Wong et al., 2014). The dramatic increase in the incidence of AMD mostly relates to the dry variant, although wet AMD is also more and more prevalent. The classification in two (wet or dry) disease phenotypes reflects the pathology of the macular region, where the absence (dry) or the presence (wet) of ectopic and abundant blood vessels and exudates is described within degenerating areas (Jager et al., 2008). Such a clear-cut distinction, despite being useful during early disease stages and for outcome purposes, does not reflect the course of disease, which may switch from a dry into a wet phenotype or it appears as a mixed degeneration already at onset, with some retinal areas showing abundant neo-vascularization intermingled with dry AMD (Gehrs et al., 2006). The overlapping between phenotypes has been well described by Ambati and Fowler (2012) and it was recently reviewed for pathogenetic mechanisms by Pinelli et al. (2020a). When considering dry AMD phenotypes the best working definition should include those forms which remain as dry with no development of new blood vessels. In this case it was reported that, among patients suffering from AMD, those affected by the dry phenotype are in excess of 80% (Seddon, 2001). Thus, the dry variant is prevalent and it progresses slowly with a loss of visual acuity which is delayed compared with the wet variant (Jager et al., 2008). This does not rule out the occurrence of blindness at later stages, which confirms again a similar nature of both phenotypes (De Jong, 2006; Wong et al., 2008; Girmens et al., 2012). From a pathological standpoint, polymorphous debris named "drusen" occur both in dry and wet phenotypes. These are considered as pathological hallmarks of AMD (De Jong, 2006; Jager et al., 2008).

The significance of drusen goes beyond a mere fingerprint for AMD since they are considered the main pathogenic trigger in altering visual acuity in AMD patients. Drusen typically accumulates between the retinal pigment epithelium and Bruch's membrane thus enlarging the space between retina and choroid and progressively increasing their size (Chew et al., 2014; Thompson et al., 2015; Handa et al., 2019, as reported here in Figure 1). In detail, such a presence is postulated to exert a mechanical uncoupling between choroid and pigment epithelium as well as disrupting the retinal planar arrangement as evident by retinal topography according to two different OCT (detected in the horizontal and vertical axis, Figure 2). On this basis, drusen, amongst various visual symptoms are considered to induce more specifically linear image distortion (metamorphopsia) (Algvere et al., 2016). Similarly, the loss of visual acuity and contrast sensitivity is routinely attributed to a mechanical impairment in the harmonic planar adjustment between the choroid and retina (De Jong, 2006; Pollreisz et al., 2021).

This would determine more specific symptoms beyond loss of visual acuity and contrast sensitivity. This is the case of metamorphopsia measured as distortion in the perception of linear images in AMD patients (Jager et al., 2008; Miller, 2013; Spaide et al., 2018; Xu et al., 2018), which strengthens a pathogenic correlation between drusen and visual alterations in AMD. The "core" of drusen is represented by polymorphic extracellular

proteinaceous and lipid aggregates (Mullins et al., 2000; Malek et al., 2003; Luibl et al., 2006; Wang et al., 2010). The proteomic analysis of drusen occurring in AMD reveals that most proteins derive from blood with a significant amount being contributed by the pigment epithelium and a minimal contribution from photoreceptors (Bergen et al., 2019; Pinelli et al., 2020a, 2020b). Apart from their origin, in keeping with classic hypothesis there is no doubt that the physical space filled by drusen may potentially interfere with visual processing. In fact, they enlarge the space between the basal lamina of the retinal pigment epithelium (RPE) and the inner collagenous tier of the Bruch's lamina (BL, so called sub-RPE-BL space, Bergen et al., 2019). Apart from representing a mechanical source of derangement in the finely tuned architecture at the choroid-pigment border, drusen alter the planar distribution of photoreceptors, which may specifically induce lines distortion. However, if drusen are solely responsible to produce typical visual symptoms, then we could not explain the OCT reported in Figure 3, where a patient, who is suffering from metamorphopsia and slight loss of visual acuity does not show any drusen neither in the OCT nor in the retinal topography nor in the bidimensional OCTs (Figure 4). Again, as the opposite paradox, here we report the case of 20 patients with an intact visual functions, including the absence of metamorphopsia, in the presence of severe drusen pathology. Such discrepancies pose the need to analyze further the significance of the drusen in the genesis of visual impairment in AMD. In line with this, one should consider that occurrence of drusen and visual impairment may both depend on upstream biochemical alterations in protein handling around the pigment epithelium (Pinelli et al., 2020a). In fact, this external layer of the retina provides key functions to maintain the homeostasis of photoreceptors. Such a challenging hypothesis leads to interpret drusen mostly as innocent bystanders of a primary metabolic defect, which also produces visual alteration in AMD. This hypothesis, which was pioneerly postulated by Tolentino et al. (1994) is really not bizarre when considering the general significance of protein aggregates in the pathogenesis of most degenerative disorders of the central nervous system. This is the case of Lewy bodies in Parkinson's disease and dementia with Lewy bodies, plaque (drusen) in Alzheimer disease, inclusion bodies in amyotrophic lateral sclerosis (Fornai et al., 2005). We posed this question two decades ago questioning whether neuronal inclusions in neurodegenerative disorders represent static pathogenic features or they rather represent a key to understand the previous dynamic steps generating neurodegeneration itself (Fornai et al., 2002, 2003 a, 2003b, 2005). Such a vision was recently re-introduced as a common pathway for late stages in various form of retinal degeneration (Pfeiffer et al., 2020) and we hypothesized an abnormal exosomal release to spread pathological proteins in late stages of AMD (Pinelli et al., 2020a). These challenging concepts led us to undertake the present study to measure the statistical power of the correlation between drusen and visual symptoms in patients affected by dry AMD. In fact, even assuming the alternative hypothesis as the most correct, one would expect to find drusen in higher amount in patients suffering from AMD compared with normal control. However, if the drusen were the authentic cause of visual dysfunction a correlation between druses and visual symptoms should be significant and very strong. *Vice versa*, if drusen are an accompanying phenomenon they would be expected to occur in a significantly higher amount in AMD patients compared with strong correlation with visual deterioration. The lack of a powerful correlation between the amount (and macular placement) of drusen and visual impairment may suggest alternative hypothesis. According to this, the amount of drusen would be not the direct cause of visual impairment. In line with this, we then expect to document in some patient the occurrence of clinical symptoms typical of AMD in the absence of sub-macular drusen. As a matter of fact, it is a common experience among ophthalmologists to examine people with severe AMD-related visual impairment where drusen are lacking with only slight irregularities of the retinal layer as reported here for the patient in Figures 3 and 4.

In a way, this investigation re-purposes the ancient question: "what does come first the egg or the chicken?", which always remains difficult to solve in humans. Thus, despite data reported in this investigation are quite significant, further experimental studies are needed to ascertain the causal association between drusen and AMD.

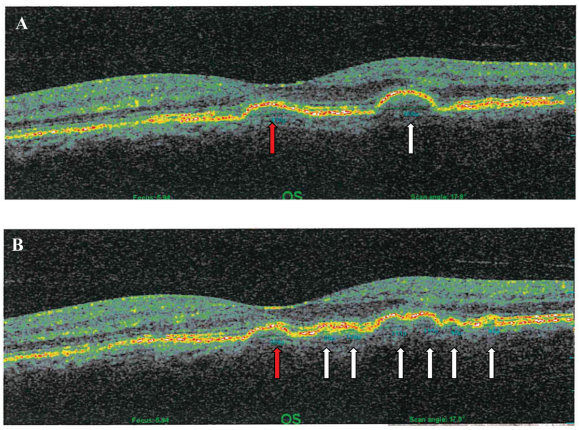

Fig. 1. - Representative OCT scans in dry AMD showing altered macular profiles. The OCT scan in (A) reports two drusen one (red arrow) is present in the foveal region while the other is placed in extra-foveal position (white arrow). In (B) the OCT scan shows several confluent drusen in the extra-foveal region (white arrows), which reach the edge of the fovea one foveal drusen is evident (red arrow).

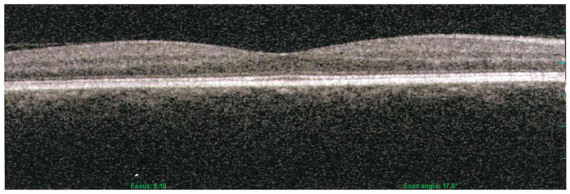

Fig. 3. - Representative OCT scan in a paradox patient. The OCT scan reveals a slight irregularity of the retinal layer without any foveal and extra-foveal drusen in a patient suffering from metamorphopsia and loss of visual acuity.

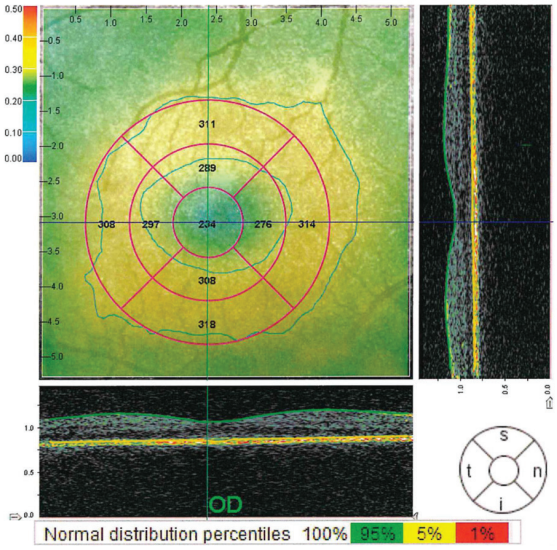

Fig. 4. - Representative retinal topography with map diagram in a paradox patient. The retinal topography reveals regular macular thickness in all regions according to two different OCT detected in the horizontal and vertical axis. Such an absence of drusen and frank alterations in retinal thickness is paradoxically concomitant with metamorphopsia and loss of visual acuity.

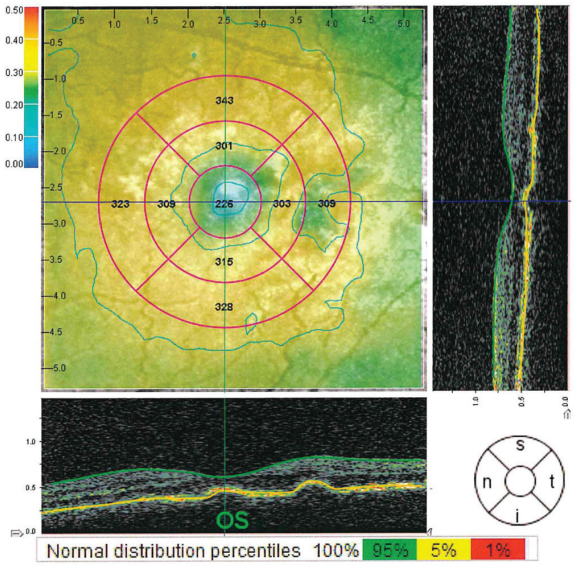

Fig. 2. - Representative retinal topography with map diagram from a dry AMD patient. The alteration of the retinal planar arrangement induced by the drusen is evident in the retinal topography obtained by combining OCT detection both on the horizontal and vertical axis.

Methods

Patients

The present study was carried out in a total of 141 patients who gave their informed consent to the study. Of these, n = 120 patients were affected by AMD. In this group, 60 patients suffered from dry AMD, while other 60 patients were affected by wet AMD. In addition, we selected a group of patients (n = 20) in which drusen were evident in a way which is reminiscent of dry AMD although visual impairment was absent. An additional (n = 1) patient is reported to strengthen the paradox: the patient is suffering from a loss of visual acuity and metamorphopsia in the absence of drusen.

Dry AMD patients were 23 males and 37 females, with an age of 69.42 ± 1.33. The wet AMD patients were 15 males and 45 females with an age of 72.88 ± 1.41.

The non-AMD patients included 9 males and 11 females with an age of 63.55 ± 1.71. The single patient with a loss of visual acuity and metamorphopsia in the absence of drusen was a 71 years old male.

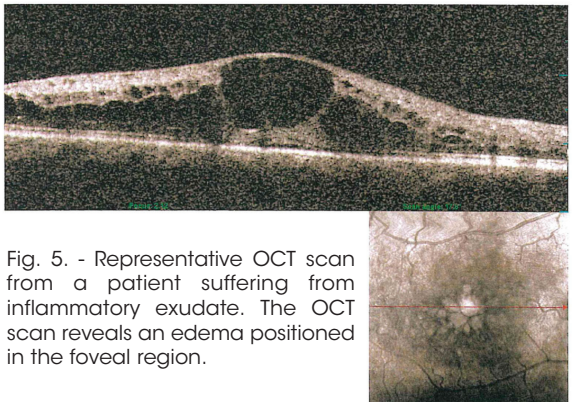

Fig. 5. - Representative OCT scan from a patient suffering from inflammatory exudate. The OCT scan reveals an edema positioned in the foveal region.

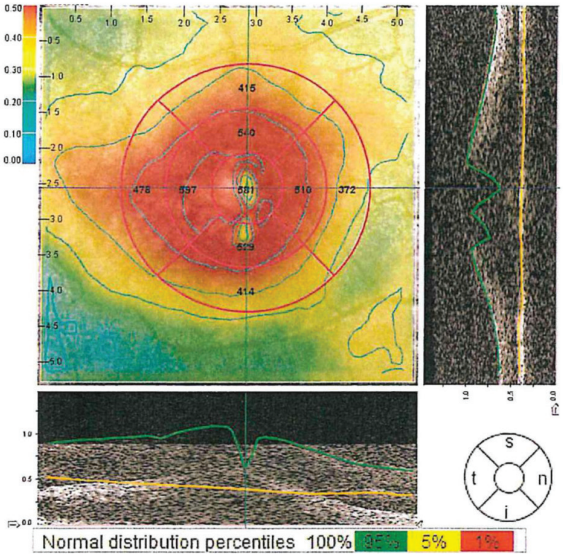

Fig. 6. - Representative retinal topography with map diagram of exudate. The retinal map diagram reveals an exceeding foveal thickness according to two OCT detected in the horizontal and vertical axis. This patient possesses inflammatory exudate.

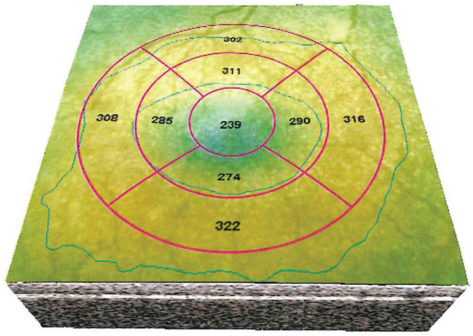

Fig. 7. - Representative 3D retinal topography with map diagram. The 3D retinal topography was obtained by scanning of 1 mm^3 of the macular region in each spatial plane (the "macular cube"). The 3D retinal topography is used to obtain the macular cube, which corresponds to the volume of the macular region assuming a constant length of 1 mm for the three spatial axis within the fovea.

Experimental design

These patients were evaluated concerning optical coherency tomography (OCT), retinal topography and visual symptoms according to various tests. These were classic test routinely used to detect and score specific symptoms of AMD: near and far visual acuity, contrast sensitivity, and metamorphopsia.

In each patient, the severity of visual symptoms was related to the amount of drusen detected by OCT, which were scored according to various criteria, since the aim of the study was to detect the role of drusen in producing AMD-related visual symptoms. The 60 patients suffering from dry AMD represented the core group of the study. In this group of patients it was possible to make a number of correlations between drusen and visual loss. In these patients, a variety of drusen measurements including a novel quantitative evaluation of the real foveal area filled with drusenoid material (the "drusenoid area") in each macular region was applied. This extensive quantification of drusen allowed an in depth analysis of their role, and mostly their association, with visual symptoms. For each drusen measurement (1-drusen number, 2-mean drusen diameter, 3-classic drusen scoring system, and 4- a novel quantitative evaluation measuring the drusenoid area) we carried out statistical analysis to infer the power of correlation in the significance between drusen pathology and the severity of visual symptoms. Most importantly, in order to infer whether the severity of visual symptoms was depending on the amount of drusenoid material, we draw various regression curves between drusen severity and the severity of visual loss. The addition of the 60 patients suffering from wet AMD was carried out to further question the significance of a generic (non-drusenoid) mechanical derangement between the choroid and pigment epithelium in determining the loss of visual function. Thus, in this group of patients suffering from wet AMD, the correlation between pathology and visual loss was only based on the classic scoring system used to assess the amount of vessels or simply the amount of inflammatory exudate in wet AMD. In fact, in this condition drusen were not detectable as clearly distinct anatomical structures (Figure 5 and Figure 6). Therefore, in these patients we did not apply

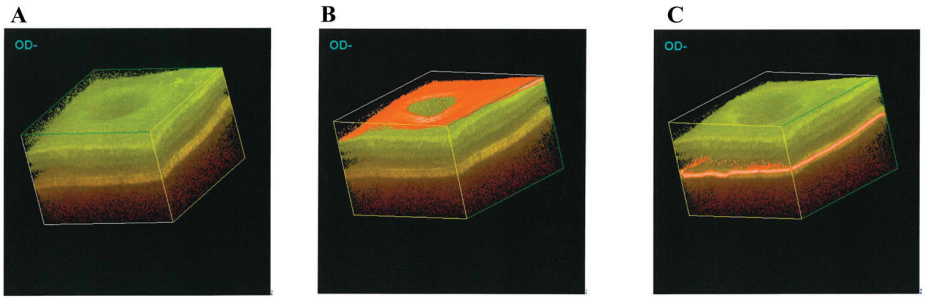

Fig. 8. - Representative computer-aided manipulations of the macular cube. (A) The macular cube corresponds to the volume of the macular region that equals 1 mm^3. In the macular cube it is possible to count drusen based on a single plane obtained by OCT, which transforms the region of interest from a volume into an area. The inner limitans membrane (B) and the retinal pigment epithelium (C) are highlighted in red by computerized processing of the macular cube.

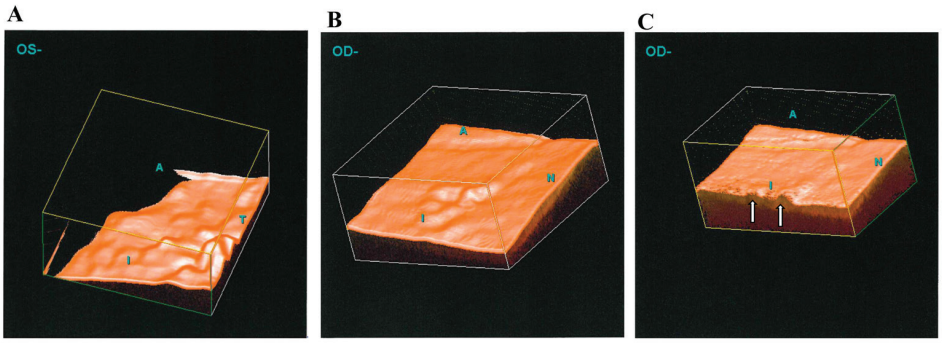

Fig. 9. - Representative reconstruction of the retinal pigment epithelium in the presence of drusen. (A) Drusen spreading along the posterior-anterior axis above the retinal pigment epithelium are shown as fractals alterations of the planar arrangement of the retinal pigment epithelium. The drusen size and shape towards the choroid and beneath the retinal pigment epithelium towards the medio-lateral (B) and dorso-ventral extent of the retina (C) are highlighted. The white arrows points to drusen (C).

any further advancement in the measurement of the anatomical abnormality produced by fluid accumulation. In these wet AMD patients we wish to quickly analyze any rough discrepancy between the correlation of visual symptoms with anatomical abnormality in wet AMD. The additional non-classified patients (n = 21) were reported as paradox clinical conditions to state and certify the potential conclusions drawn by measurements carried out in dry AMD patients (i.e. no correlation between drusen severity and symptoms severity). In fact, we report 20 patients with evident drusen accumulation in the absence of visual symptoms and one patient where AMD-related visual symptoms were severe with no foveal deposition of drusen or liquids below the pigment epithelium owing a magnificent planar architecture of the retina.

Optical coherence tomography

The optical coherence tomography (OCT) is a gold standard exam used in the diagnosis of AMD, which provides a direct visualization and measurement of drusen number and size as well as altered flatness of the retinal surface and derangements of specific layers (as reported for a number of foveal and extra-foveal drusen in Figure 1). OCT consists of a non-invasive imaging procedure based on visible light waves, which are reflected from different layers of the retina and adjacent choroid structures. This method also allows to measure the amount of altered flatness produced by inflammatory exudate and newly formed blood vessels.

Retinal topography

It allows to measure the thickness of the retinal layer including the inner choroid along the four quadrant of the retina including the macular region. It is useful to detect the specific site where an alteration of the planar arrangement is produced under the mechanical pressure of underlying structures (drusen in dry AMD, exudates and vessels in wet AMD). It is obtained by combining OCT technique in different axis, as reported in Figure 2.

Drusen counts and morphometry

In classic OCT morphometry of AMD, drusen are counted specifically referring to the macular region. The shape of such a region was derived from the concept of the macular cube (Figure 7 and Figure 8). In detail, according to Figure 7 which corresponds to the scanning of 1 mm^3 of the macular region in each spatial plane, we focused on the so-called "macular cube", which corresponds to the volume of the macular region assuming a constant length of 1 mm for the three spatial axis within the fovea. In this volume we counted drusen based on a single plane obtained by OCT, which transforms the region of interest from a volume into an area. Thus, the number of drusen are expressed as the amount of drusen counted within an area of 1 mm^2 (see statistics). By computerized processing of the macular cube it is possible to highlight the inner limitans membrane (Figure 8B) or specifically the retinal pigment epithelium (Figure 8C). Moreover, the specific upload of the retinal pigment epithelium allows a sort of fractal image (Figure 9). From this image, when needed, the underlying choroid layers can be included to document at best the drusen-induced alterations of the planar arrangement of the RPE (Figure 9A, showing drusen spreading along the posterior-anterior axis just above the RPE). Additionally, the drusen size and shape towards the choroid and beneath the RPE (Figure 9B and 9C, respectively towards the medio-lateral and dorso-ventral extent of the retina) can be visualized.

In the present study, drusen were quantified according to classic and additional criteria. In detail, the number of drusen was calculated by counting the number of drusen (independently by their size) occurring in the macular area. The size of the drusen was expressed according to a classic criterium in which the mean diameter of all drusen counted in each macula is reported. A combined criterion is routinely used which combines the number of drusen with the average diameter.

The classic score for this combined criterion clusters drusen severity into three groups. **A** moderate (1 to 3 small drusen up to 60 μm diameter), **B** mild (3 to 6 small drusen or 1 to 3 medium drusen from 60 μm to 120 μm diameter), **C** severe (more than 6 small drusen or more than 3 medium drusen or ≥ 1 large drusen more than 120 μm). This classic combination of drusen number and diameter is far from providing a quantitative measurement of the foveal area actually filled by drusen. Even an esteem of such a measure can be far from the real one in several cases when such a method is applied. Again, this classic combined criterion provides scores and does not allow to deal with real numbers provided by drusen-related quantitative measurements. Therefore, in order to obtain a real measurement of the area/volume of the macular region filled with drusen a more specific measurement is needed. This was prompted in the present manuscript by counting the total size of the macular surface (1 mm^2) which was filled with drusen. In order to produce a number, which was predictable of the real anatomy of the macular region we assumed that the shape of the drusen was spheroidal with a trend towards a spheric size. In this way, the diameter of each drusen counted in a planar view could be inferred to the diameter, which remained not explored (assuming the same diameters in all planes).

Thus, assuming a spheroidal shape for the drusen, we counted the planar (bidimensional) size assuming the size from the posterior to the anterior edge of the drusen to be similar to others (the medio-lateral and dorso-ventral sizes). In this way, the number of drusen and the drusenoid space were used as morphometric measurement to express fully the quantitative measurement of drusen in the macular region. For each eye we specify the number of drusen, the average diameter of drusen, the overall area of drusen given by the sum of the areas of the drusen. Each drusen has an area equal to π times the radius^2 ($A = \pi r^2$). Once calculated the sum of the areas of drusen, we related it to the total macular area in order to obtain the density of drusenoid surface. This concept should be considered and self-limited according to the best practice which follows

in the note concerning the chaos theory (Losa and Nonnenmacher,1996; Mandelbrot, 1998).

[NOTE The chaos governing enlargement of drusen can be analyzed as many other biological structures undergoing multi variable-induced morphological changes by using the fractal theory. Thus, the dynamics of the drusen would be in fact a sphere according to a Euclidean geometry within a inert context. This is not fully correct due to multiple elements which correct the size and shape changes once the drusen enlarge. One of these innumerous determinants can be easily expected along the anterior-posterior axis. In fact the drusen grows easily towards the inner retinal surface where less resistance is provided compared with the external choroid surface where the Bruch's lamina provides a solid barrier. This is expected to drive the growth and shape of the drusen itself. Even in keeping with the medio-lateral and dorso-ventral axis, the theoretical homogeneity of the RPE-Bruch's border can vary depending on the occurrence of other drusen, which alters the spontaneous growth. Again, the drusen composition may enable specific chemical binds with surrounding structures, which are expected to alter the geometry along the various axis. The fractal theory becomes more and more relevant when deciphering the drusen geometry at higher level of magnification. This explains why adding the posterior-anterior axis and fully considering the macular cube instead of the macular square (which rules out the posterior-anterior size) the representative high magnification of the pigment epithelium under the strength of the underlying growing drusen provides a fractal image which is shown here in Figure 9. This also explicates how gross the level of analysis should be hold, when assuming the drusen shape to be a spheroid, and how unpredictable (under Euclidean principles) may be slight shape skipping, when considered at sub-microscopical level. At this level, the size, and mostly the shape could be better defined by stochastic approaches of relativistic mechanics. Thus, drusen recapitulate the occurrence of morphogenetic dynamics, the emergence of complex patterns, and the architectural organization of active tissues and progressively growing biological systems, which may be driven by mechanisms related to fractal principles considering timing and spacing. This may explains why, in clinical practice we prefer to express the macular region by referring to a macular square, rather than a macular cube. In this way, the undetermined variable are much lower, by neglecting the growth along the posterior-anterior axis. In this way, considering the macular square, and keeping the reference point to a macro/sub-macroscopic level, the roundish shape in the macular square can be predictable according to Euclidean morphometry measurements. The macular topography measuring the macular square also provides the thickness by expressing appropriate numbers, which avoid to loose information along the posterior-anterior axis].

In this way, it was possible to make specific quantitative correlations between drusen and visual impairment. In fact, if the number of drusen correlates with visual impairment more than the drusenoid area, then it is likely that the drusen *per se* do not alter the visual processing. In contrast, the classic view that drusen do impair vision would be confirmed by measuring a powerful correlation between the drusenoid area and the visual impairment with a negligible effect considering the number of drusen especially when several small drusen fill a small macular area.

To test the specific pathogenic effects of the drusen in producing visual impairment when placed in a specific quadrant, measurements of visual function should be also correlated to the specific placement of the drusen in each quadrant of the macula by measuring drusen placement by retinal topography. Such an issue was not specifically measured in the present study, nonetheless is should be taken into account in future investigations. In fact, it is well know that specific disorders produce a specific topography of symptoms (temporal vs. nasal). It remains to be clearly established whether such a distinction can be carried out also for AMD or specific AMD phenotypes.

In keeping with measurement provided in this study a further correlation could be made by comparing the mean drusen diameter with the range of diameters. This value expresses the coherency of drusen size, which may be either homogeneous or highly dispersed. Such a coherency value may correlate specifically with selective disease phenotypes and providing additional information about the significance of the drusen in the pathogenesis and symptoms of AMD. This latter investigation deserves further analysis.

Exudates morphometry

In wet AMD drusen cannot be measured reliably. Therefore, the measurement of anatomical alterations better relies on the thickness provided by the inflammatory exudate or blood vessels. Such a thickness can be used in wet AMD to make correlative studies with visual impairment. In fact, we adopted the simplified classification of wet AMD by scoring the presence of exudates as mild, moderate or severe, based on the ripples of the RPE and the volume of the intra-retinal liquid. In order to have a sharp reference and to replicate other studies, we scored exudates as follow: **mild** (foveal thickness from 150 ± 20 μm to 250 ± 20 μm, macular thickness from 250 ± 50 to 350 ± 50 μm); **moderate** (foveal thickness from 250 ± 2 0 μm to 350 ± 20 μm, macular thickness from 350 ± 50 μm to 450 ± 50 μm); **severe** (foveal thickness ≥ 350 ± 20 μm, macular thickness ≥ 450 ± 50 μm).

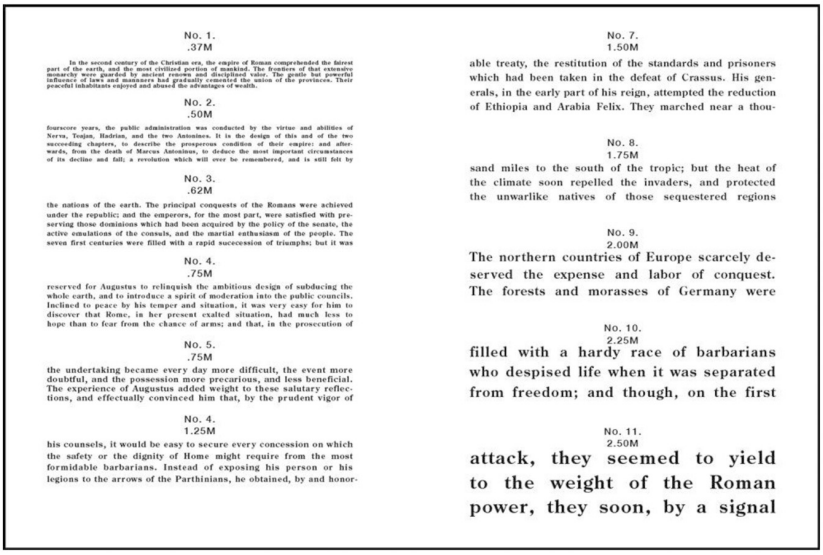

Fig. 10. - The Jaeger chart. (See Methods for details).

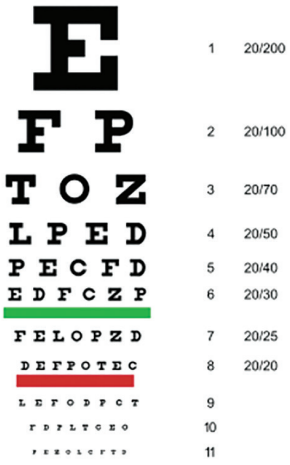

Fig. 11. - Snellen chart. (See Methods for details).

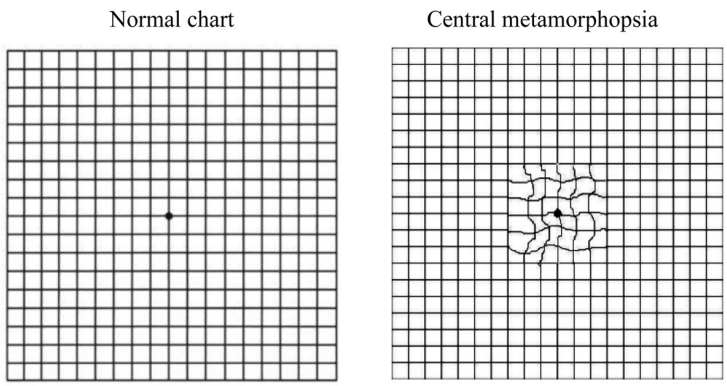

Fig. 12. - Amsler Grid. (See Methods for details).

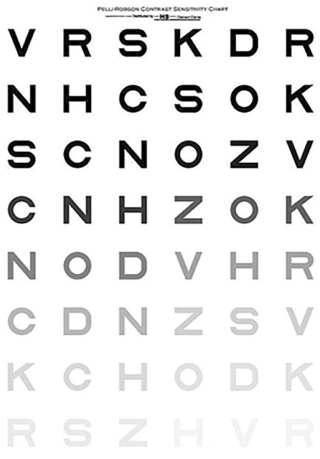

Fig. 13. - Pelli-Robson chart. (See Methods for details).

Visual tests

Each patient (n = 141) underwent four subjective tests that measure visual function. The tests were repeated 3 times in each patient in order to express the mean value. The tests here used were the following: the Jaeger Chart test, the Snellen Chart test, the Amsler grid test and the Pelli-Robson Chart test (Contrast sensitivity test).

Jaeger Chart test

The Jaeger Chart test was used to determine the near best corrected visual acuity (BCVA). The apparatus consists in a chart reporting short blocks of text in different sizes. The chart was held at a specified reading distance (35 cm) and the patient is asked to read the smallest block of lines he can focus from the biggest block (J10 or 1/10) to the smallest block (J1 or 10/10) (Figure 10). If the patient read a specific block of letters without squeezing, that block was considered solved. Different blocks were shown during each detection in order to avoid learning words by heart. In this study the score J10 to J1 was converted in percentage (10% to 100%) where 100% is the maximum visual acuity for near (J1).

Snellen Chart test

The Snellen Chart test was used to determine the far best corrected visual acuity (BCVA). A retro-illuminated wall-mounted Snellen chart was used with the patient standing at 6 m from the chart (Johnson et al., 1998; Chen et al., 2014). The chart includes red and green color bars for an easy and helpful place to start administering the test (Figure 11). The patient was asked to read 5 letters per row (from row 1/10 to row 10/10) There are 10 rows of decreasing size at a pre-determined distance. If the patient read at least 3/5 of letters in a specific row and 1 or 2 letters of the subsequent row, that row was considered solved. Different letters were shown during each detection in order to avoid learning letters by heart. In this study the score 1/10 to 10/10 is converted in percentage (10% to 100%) where 100% is the maximum visual acuity for far (10/10).

Amsler Grid test

It is a grid of horizontal and vertical lines used to monitor a person's central visual field. It is a diagnostic tool that aids in the detection of visual disturbances caused by changes in the retina, particularly the macula, as well as the optic nerve and the visual pathway to the brain. Amsler Grid test usually helps detecting defects in central 20 degrees of the visual field.

The apparatus consists in a white square-shaped grid divided by horizontal and vertical black lines in approximately 20 small squares in each side of the grid. A central black dot was present for fixation (Figure 12). The illumination of the chart was kept steady and optimal to allow the best resolution. The grid was kept at least 33 cm far from the eye. The patient was asked to close one eye and each eye was tested separately. In patients with altered vision the lines of the square appear distorted, otherwise they look parallel (Su et al., 2016).

Patients with macular disease may see wavy, interrupted or disturbed lines or some lines may be missing. In this study, the score ranges from 10 to 1, and it is indicated as follows: **10** (0 wavy lines). **9** (1 wavy, interrupted, disturbed horizontal and vertical line). **8** (2 wavy, interrupted, disturbed horizontal and vertical line). **7** (3 wavy, interrupted, disturbed horizontal and vertical line). **6** (4 wavy, interrupted, disturbed horizontal and vertical line). **5** (5 wavy, interrupted, disturbed horizontal and vertical line). **4** (6 wavy, interrupted, disturbed horizontal and vertical line). **3** (7 wavy, interrupted, disturbed horizontal and vertical line). **2** (8 wavy, interrupted, disturbed horizontal and vertical line). **1** (9 wavy, interrupted, disturbed horizontal and vertical line).

Pelli-Robson Chart test

Pelli-Robson Chart test measures the contrast sensitivity defined as the ability to perceive slight change in luminance between regions, which are not separated by sharp borders.

The chart is composed of letters (6 per horizontal line) arranged in groups whose contrast varies from high to low (Figure 13). Patients read the letters, starting from the highest contrast, until they are unable to read two or three letters in a single group. Each group has three letters of the same contrast level, so there are three trials per each contrast level. The score is based on the contrast of the last group in which two or three letters were correctly read. A Pelli-Robson score of **2.0** indicates normal contrast sensitivity, a score of less than **1.5** is consistent with visual impairment and a score of less than **1.0** represents visual disability.

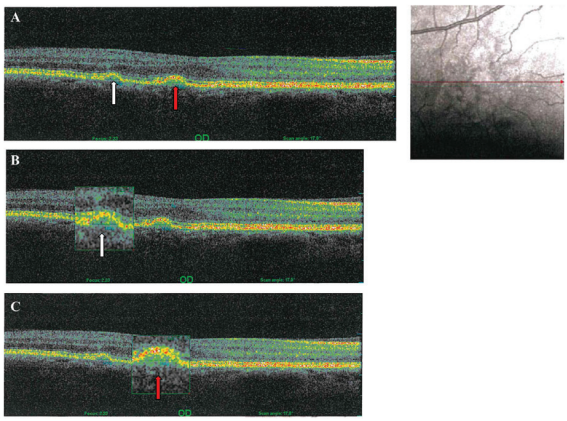

Fig. 14. - Representative OCT scan from a dry AMD patient. (A) The OCT scan shows two drusen (arrows). (B) The OCT shows the magnification of the peripheral drusen (white arrow). (C) The OCT shows the magnification of the central drusen (red arrow).

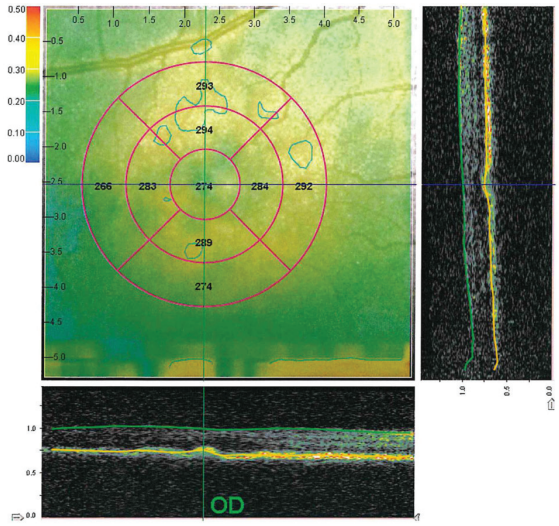

Fig. 15. - Representative retinal topography with map diagram of a patient affected by dry AMD. The disruption of the retinal planar arrangement induced by the drusen is evident in the retinal topography according to two different OCT detected in the horizontal and vertical axis.

Statistical analysis

In order to produce useful descriptive statistics to document drusen and visual impairment in dry AMD we started data analysis by expressing the drusen number and size to assess whether this was significant to predict visual impairment. We used the classic parameters for drusen number, drusen size and a combined size/number scoring system. According to the classic **drusen number** score is classified as following: absent (0 drusen) mild (1-3 drusen) moderate (4-5 drusen) severe (> 6 drusen). Again, according to the classic score for **drusen size**, the mean diameter of drusen in each retina was calculated. Based on the mean diameter the following scores are given: **small** (0-65 μm), **moderate** (65-120 μm), or **large** (> 120 μm).

The classic combined score to assess **drusen severity** considers three groups: **moderate** (1 to 3 small drusen up to 65 μm diameter), **mild** (3 to 6 small drusen or 1 to 3 medium drusen from 65 μm to 120 μm diameter), **severe** (more than 6 small drusen or more than 3 medium drusen or ≥ 1 large drusen more than 120 μm).

However, all these three systems, even when associated, are not appropriate to infer on the severity of drusen in impacting visual function.

In fact, several drusen which would score for a high drusen severity could be so small that they do not fill most of the macular area. Again, the mean drusen diameter may be heterogeneous as well the drusen severity since it remains unclear the real number of drusen possessing that specific diameter.

All these measurements and scoring do not allow to provide a quantitative evaluation of the macular area filled with drusen. In the hypothesis that visual impairment is related to drusen occurrence, we need to measure the amount of macular area, which is really filled by drusen. We referred to this measurement as “drusenoid area” which is expected to provide a reliable quantitative index of drusen severity in AMD. Based on the drusenoid area it is possible to infer the causal effect of drusen on visual impairment by drawing regression curves to establish whether a strong correlation exists between drusenoid area and visual impairment.

From a statistical point of view such a measurement, i.e. the drusenoid area, still remains imperfect since it assumes a monoplanar distribution for a structure which is indeed solid and owns a specific volume. Thus, the drusenoid area is indeed the surrogate of the drusenoid volume as much as the macular area is a surrogate of what indeed is the macular volume, the so called “macular cube”.

Assuming a spherical shape for the macular region as well as for the drusen, the measurement of the drusen volume would be expressed as a ratio of one cubic millimeter. In fact, for each axis the macular region could be assumed to extend roughly for one millimeter leading to a cubic

millimeter when expressing the macular volume. Such a measurement however would practically extend the macular volume anteriorly to surpass the posterior edge of the vitreous. Therefore, in order to simplify the measurements and to avoid the bias of including a non-retinal region, we expressed both the macular shape and the drusen shape as an area rather than a volume, in which the drusenoid area can be expressed as a percentage (or a ratio) of the macular area.

The drusenoid area is useful both to get a reliable measurement of drusen severity in AMD and to infer whether drusen are likely to cause visual impairment. In fact, through the measurement of the drusenoid area the impact of drusen severity can be directly related to each visual symptoms occurring in AMD. Therefore data analysis was carried out for each specific visual measurement both considering traditional scoring (mean drusen diameter, drusen number, combined calculation i.e. drusen severity), and finally it was validated by applying the drusenoid area. The occurrence of a significant loss of vision for high values of classic drusen scoring (number, diameter, combined) does not tell the real impact of drusen in causing visual alterations. It is rather the strength of the correlation between the quantitative measurement of the absolute drusenoid area with the loss of specific visual functions, which is expected to provide the impact of drusen in causing AMD-induced visual impairment.

An additional group of 20 patients was reported for statistical purposes to demonstrate whether a so-called “paradox effect” (such as the lack of visual deficit in the presence of drusen) may occur. In these patients with optimal visual function, the number of drusen ranges from 1 to 5 and the size may reach 65 μm diameter. The opposite paradox was tested by measuring the absence of drusen in a single patient with severe AMD-related loss of visual impairment (Figures 3 and 4).

In the wet AMD drusen are difficult to detect and the measurement of visual impairment is the retinal thickness. In the case of wet AMD indirect measures of exudates provides a scoring which was useful to correct for potential non-drusen related visual impairment. Exudates were scored in each groups by measuring the thickness of the retina considering both the foveal and the extra-foveal region. In detail, the exudate was: **mild** when foveal thickness ranges from 150 ± 20 μm to 250 ± 20 μm and macular thickness ranges from 250 ± 50 to 350 ± 50 μm; **moderate** when foveal thickness ranges from 250 ± 20 μm to 350 ± 20 μm and macular thickness ranges from 350 ± 50 μm to 450 ± 50 μm; **severe** when foveal thickness is ≥ 350 ± 20 μm and macular thickness is ≥ 450 ± 50 μm. This scoring system was adopted based on the assumption that the severity of wet AMD eventually leads to an involvement of the extra-foveal region.

However even in this case, just like drusen for dry AMD, we found the significant visual loss for those cases where exudate was scored, but the amount of exudates did not indicate any powerful correlation with visual impairment.

Data concerning drusen morphometry in dry AMD and partial data about exudates in wet AMD were reported as well as the scoring of visual symptoms which occurred in AMD namely visual acuity, far and near, metamorphopsia and contrast sensitivity. Each test was repeated three times for each patients and it was expressed as follow:

- Visual acuity near and far: for the AMD patients group and the additional patients group, we reported the mean percentage ± SEM of the score where 100% is the maximum visual acuity for near and far BCVA.
- Metamorphopsia: for the AMD patients group and the additional patients group we reported the mean score ± SEM. The score value ranges from 10, no wavy lines detected, to 1, wavy, interrupted and disturbed horizontal and vertical lines.
- Contrast sensitivity: for the AMD patients group and the additional patients group we reported the mean score ± SEM. The score ranges from 2, i.e. normal contrast sensitivity, to 0, visual disability.

For each eye, we specify the number of drusen, the average diameter of drusen, the combined classic score and the drusenoid area, which is obtained by the sum of the areas of each drusen in the macular region. Once calculated the sum of the areas of drusen, this was related it to the total macular area in order to obtain drusen density. This corresponds to the ratio between drusenoid area and the macular square. For a simple conversion, the reader may just consider the number reported for the drusenoid area which is expressed in μm^2 and

compare it with 1,000,000 μm^2 which corresponds to the macular square. Thus, the patient with the largest drusenoid area owns a density which surpasses at large a quarter of the total macular square (276,300 μm^2 equals to 27.63% of the macular square).

We drew bar graphs each reporting one items related to different drusen features expressed as four parameters, the number of drusen, the average diameter of the drusen, the classic score combining the number with the volume of the drusen, and the drusenoid area. Each range of values was plotted as histograms where each bar represented a subgroups of range values.

The comparison between symptoms and classes of drusen severity was carried out by using the Kruskal-Wallis test for non-parametric data. Null hypothesis H_0 was rejected for $p < 0.05$.

In addition, to analyze the power of the correlation between drusen (or macular irregularities in wet AMD) and visual impairment we plotted for each patients the score of the visual symptoms against drusen severity. This was carried out as regression curves specific for metamorphopsia, loss of visual acuity and loss of contrast sensitivity. The scattered points in each plot refer to single patients affected by AMD or to additional patients carrying drusen in the absence of visual disability. The association of the points was measured with the linear regression and the power of the correlation was analyzed by using the correlation coefficient (R^2).

When using the classic scoring, for dry AMD in the x axis the term "drusen +" refers to mild presence of drusen, "drusen ++" refers to moderate presence of drusen and "drusen +++"refers to severe presence of drusen.

For wet AMD in the x axis the term "exudates +" refers to mild presence of exudates, "exudates ++" refers to moderate presence of exudates and "exudates +++"refers to severe presence of exudates.

Results

Data collected from the four tests indicate a significant decrease in visual abilities at most severe stages in both dry AMD and, mostly, wet AMD.

Classic drusen measurement and visual function for dry AMD

When focusing on the association of drusen and visual function in dry AMD (see representative Figures 1, 2, 9, 14 and 15), the number of drusen (Figure 16) does not significantly associate with any visual impairment even considering the group of patients owing a drusen number scored as high (> 6 foveal drusen) (Figure 16). Thus, low, moderate and even high foveal drusen number does not associate with any decline in the four visual measurements. In detail, no significant effect was induced on visual acuity score related to the near BCVA (A) and to the far BCVA (B), the occurrence of visual distortion by using the Amsler test (C) and the worsen of contrast sensitivity (D) for a low, moderate and high amount of foveal drusen. Instead, an effect was obtained when the visual tests were associated with drusen size (Figure 17). In this case, no significant visual impairment was produced in those patients owing small or intermediate mean drusen diameter, whereas a significant visual deterioration for all tests, including Amsler grid was present in patients bearing large drusen. Thus, the mean drusen diameter when exceeding 120 mm (large average diameter) associated with a loss of visual acuity (Figure 17A and 17B) and contrast sensitivity (Figure 17D) along with occurrence of any metamorphopsia (Figure 17C). These data were confirmed in Figure 18 by using the classic combined scoring system, which balances both average drusen diameter and drusen number. In this case, for the highest score, a significant deterioration was measured in all test (Figure 18C), while visual acuity was impaired at near distance (Figure 18A) and it was optimal at far distance (Figure 18B). A significant loss of contrast sensitivity was also detected for the highest combined score (Figure 18D). No significant AMD-related visual deficit was associated with mild and moderate drusen score. Remarkably, when a correlation analysis was carried out considering drusen number (Figure 19) the curves did not evidence any powerful correlation between the number of drusen and all four visual test carried out by the patients (Figure 19A-D). The symptoms mostly, though non-significantly, correlated was the perception of line distortion at the Amsler test (Figure 19C). When the regression curve was drawn for drusen diameter (Figure 20)

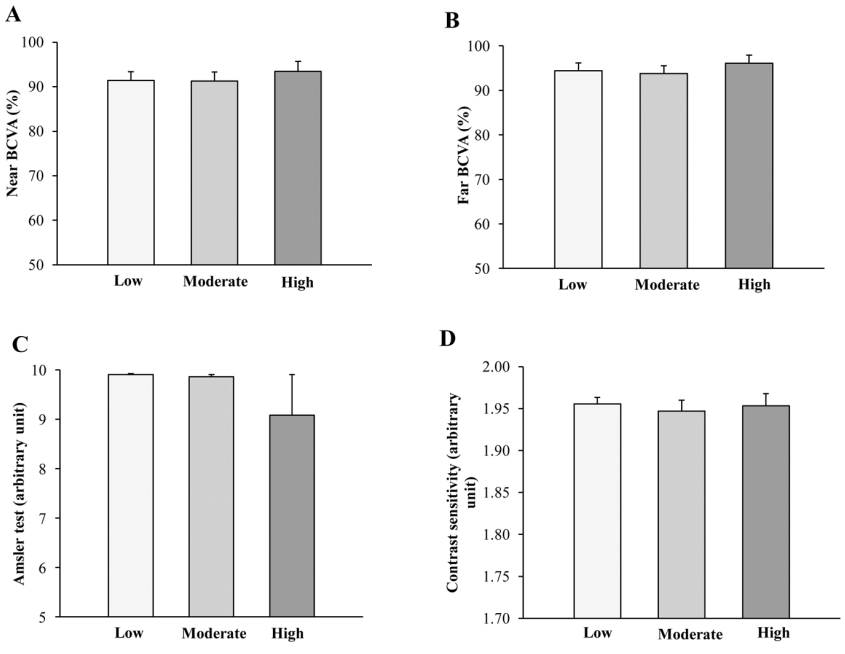

Fig. 16. -Association of drusen number and visual function in patients suffering from dry AMD. Graphs show the visual acuity, the near BCVA (A) and the far BCVA (B), the Amsler test score (C) and the contrast sensitivity score (D).

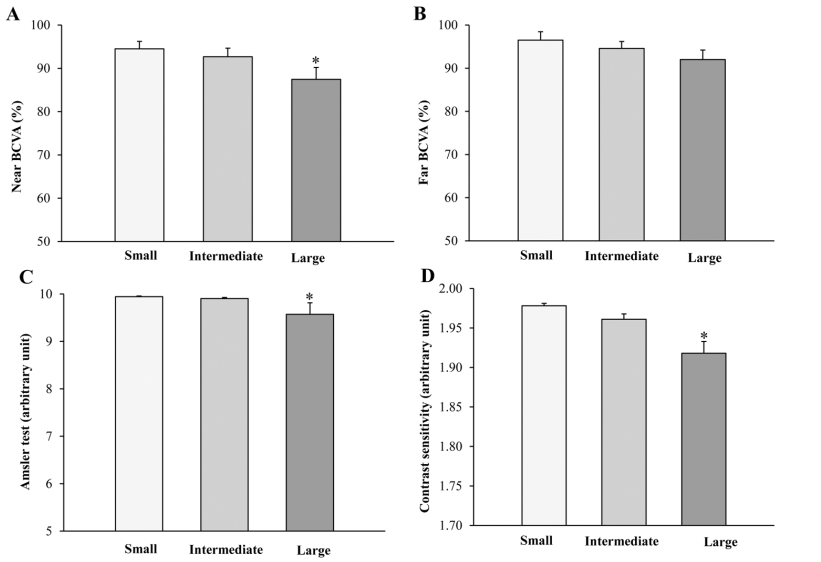

Fig. 17. - Association of mean drusen diameter and visual function in dry AMD patients. Graphs show the visual acuity percentage related to the near BCVA (A) and to the far BCVA (B), the Amsler test score (C) and the contrast sensitivity score (D). $^{*}p < 0.05$ vs other groups.

the correlation was neither powerful nor significant for any visual test with the highest value for near and far visual acuity (Figures 20A and 20B). Any significant or powerful correlation was measured for the correlation of visual function loss with the three scores for classic scoring of dry AMD (Figure 21). The drusenoid area (fully correlated with the drusenoid density) calculated in Figure 22 provides the direct quantitative measurement of drusen severity. This reproduced the significant effect for the largest drusenoid area, while no significant phenomenon was detected for intermediate and small areas expressed as three ranges of drusenoid area (small < 20,000 mm^2 intermediate from 20,000 to 50,000 mm^2; large > 50,000 mm^2). The large drusenoid area provided a similar association with all four visual test. As shown in Figure 23 the regression analysis of the curves expressing the distribution of values for the drusenoid area never significantly correlated with any test carried out for visual impairment. The drusenoid area provides the best correlation, which indicates a more reliable

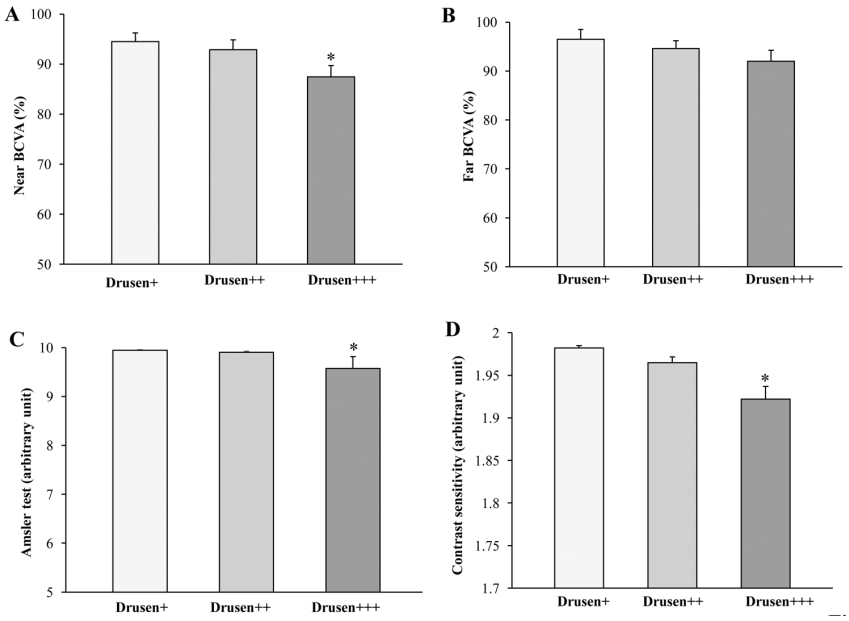

Fig. 18. - Association of classic combined scoring system for drusen with visual function in patients suffering from dry AMD. Graphs show visual acuity, near BCVA (A) and far BCVA (B), the Amsler test score (C) and the contrast sensitivity score (D). $^{*}p < 0.05$ vs other groups.

index measurement in AMD. Such a correlation was neither significant nor powerful for any tests.

In fact, despite owing the highest R^2, which corresponds to 0.439 for contrast sensitivity, the drusenoid area does not impact visual function (Figure 23D). In fact a patient which owns a drusenoid area of 276,300 μm^2 suffer a loss of visual acuity for near and far BCVA (visual acuity 76.67% and 81.66%, respectively), which is comparable to patients owing a 1,000 times smaller drusenoid area.

Paradoxical associations between drusen and visual function

The lack of a tighten correlation between drusen and visual impairment was magnified in the group of 20 patients briefly mentioned in the introduction. Despite the occurrence of drusen, none of these patients experienced AMD-related visual alterations. In detail, the near BCVA was 99.05% ± 0.01, the far BCVA was 102.55% ± 0.02, the Amsler test score was 10 ± 0 and the contrast sensitivity score was 1.99 ± 0.004. In these patients owing a significant drusenoid area, no decline in visual function was detected and the R^2 was the lowest, approaching zero (Figure 24). This was backed by the onset of AMD-related visual impairment with slight metamorphopsia in the paradox patient reported in Figures 3 and 4.

Classic scoring of wet AMD and visual impairment

In Figure 25, the classic scoring based on retinal thickness and visual impairment was reported. Despite being more severe for visual function only the patients bearing the thickest values experienced a significant deterioration in all visual functions. No significant effect was detected when the scoring system was plotted against the thickness in the regression curve.

Admittedly, the results obtained from both dry AMD (Figures 19, 20, 22 and 23) and wet AMD (Figure 26) indicate no significant correlation between the severity of macula irregularities and decrease of visual acuity, or onset of metamorphopsia or decrease of contrast sensitivity. The regression curves for 60 random points for drusen in dry AMD patients did not reveal any relationship between the two variables as demonstrated by the value of the R^2.

Discussion

According to Pfeiffer (2020) occurrence of proteinaceous inclusions and widespread degeneration in retinal degenerative disorders place the retina as the ideal gateway to understand proteinopathies, and mechanisms of neurodegeneration that drive a wide spectrum of devastating central nervous system (CNS) degenerative disorders.

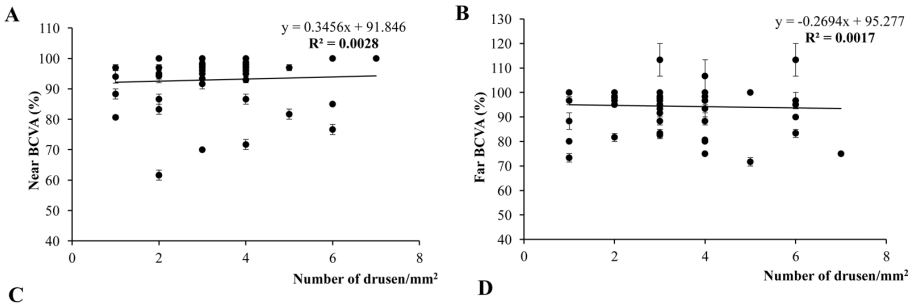

Fig. 19. - Correlation between visual ability and drusen number in patients suffering from dry AMD. Graphs report the regression curve between the visual acuity, near BCVA and far BCVA (A and B respectively), the Amsler test score (C), the contrast sensitivity test score (D) which were plotted vs the drusen number.

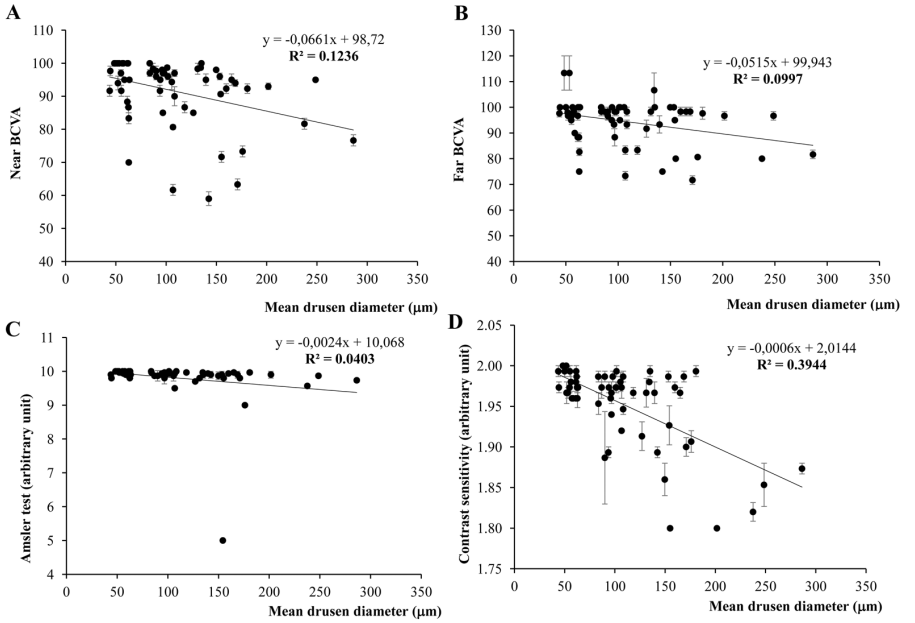

Fig. 20. - Correlation between visual ability and mean drusen diameter in patients suffering from dry AMD. Graphs report the regression curve between the visual acuity, near BCVA and far BCVA (A and B respectively), the Amsler test score (C), the contrast sensitivity score (D) which were plotted versus the mean drusen diameter.

In keeping with this, drusen are the protein aggregates, which characterize AMD. Despite being a hallmark of early disease, they are expected to alter vision since: (i) they represent a mechanical source of derangement in the finely tuned architecture at the choroid-pigment border; (ii) they alter the planar distribution of photoreceptors, which may specifically induce visual distortion. Nonetheless, moving beyond classic concepts, the occurrence of drusen may be independent. These may be regarded as the consequence of a primary alterations in the molecular and cell biology of the pigment epithelium. In fact, this external layer of the retina provides key functions to maintain the homeostasis of photoreceptors. In the classic view, drusen *per se* are conceived as detrimental agents fostering the loss of vision. In fact, drusen relate to the disease state since the overall amount, site specificity (behind the macular region of the retina), and their size all determines the severity of

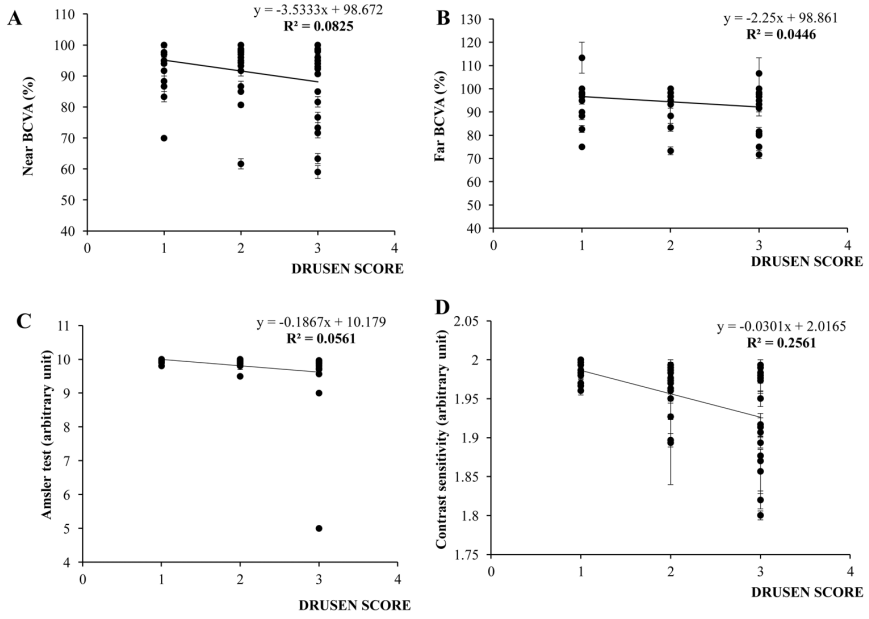

Fig. 21. - Correlation between visual ability and classic combined scoring system for drusen in patients suffering from dry AMD. Graphs report the regression curve between the visual acuity, near BCVA and far BCVA (A and B respectively), the Amsler test score (C), the contrast sensitivity score (D) which were plotted versus the classic combined scoring system for drusen.

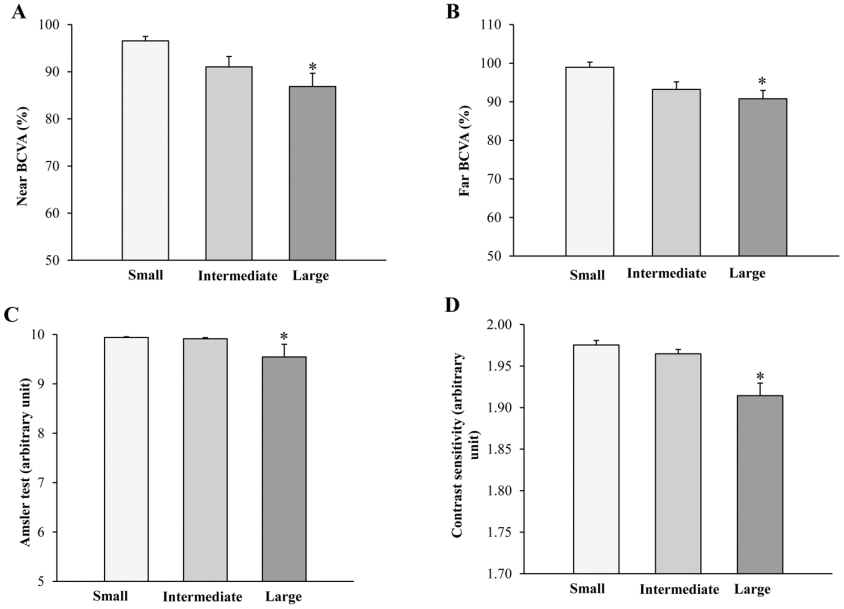

Fig. 22. - Association of drusenoid area with visual function in patients suffering from dry AMD. Graphs show visual acuity, near BCVA and far BCVA (A and B respectively), the Amsler test score (C) and the contrast sensitivity score (D). $^{*}p < 0.05$ vs other groups.

AMD. In the wet AMD, drusen are associated with neo-angiogenesis trespassing the choroid-retina border. These newly formed overwhelming vessels often lead to bleeding and fluid accumulation in the extracellular space, which is absent in the dry variant (Jager et al., 2008).

The anatomical site, which needs to be considered when studying AMD-related drusen is placed around the retinal pigment epithelium, the Bruch's membrane and the capillary vessels of the inner choroid as visible at light microscopy (Crabb, 2014; Zarbin et a., 2014; Roher, 2018). In the wet AMD phenotype, the specific alteration subsides within the semipermeable Bruch's membrane, which in baseline conditions occludes the spreading of blood vessels from capillary of the inner choroid towards the pigmented epithelium. In physiological conditions the Bruch's membrane modulates the exchanges between the blood and the retina but it also acts as a barrier, which separates the retinal pigment

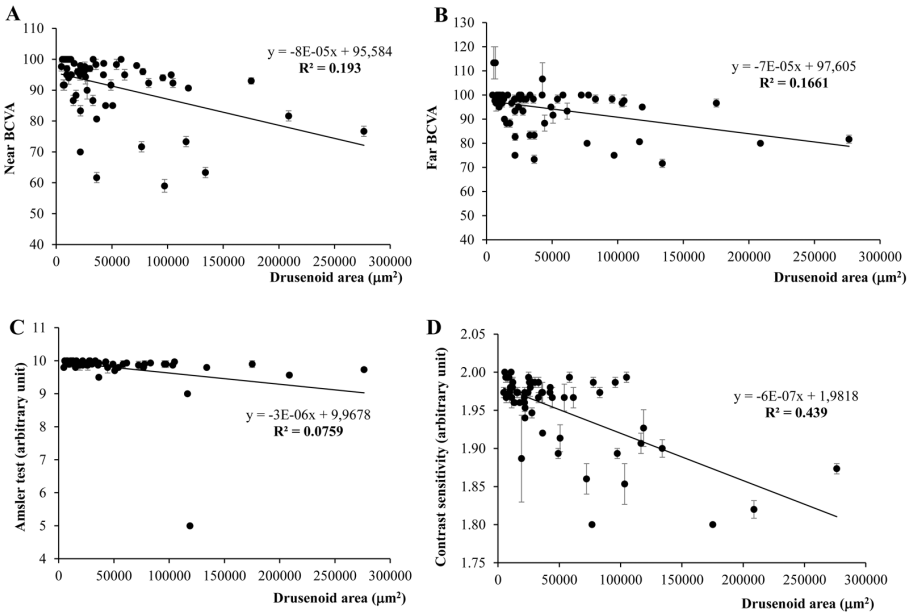

Fig. 23. - Correlation between visual ability and drusenoid area in patients suffering from dry AMD. Graphs report the regression curve between the visual acuity, near BCVA and far BCVA (A and B respectively), the Amsler test score (C), the contrast sensitivity test score (D) which were plotted vs the drusenoid area. In this way, data concerning the drusen provide a real information on the amount of drusen material filling the macular square.

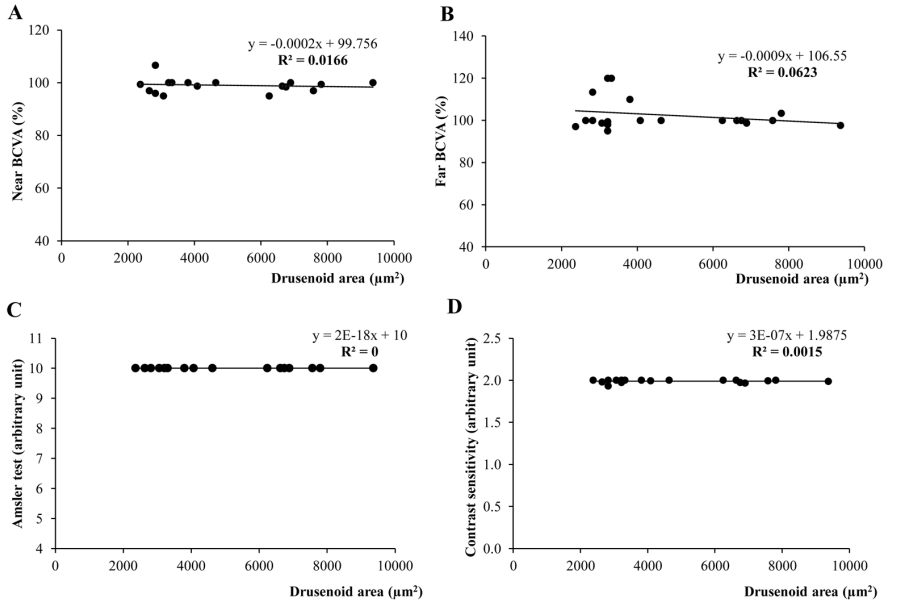

Fig. 24. - Paradoxical association between drusen area and visual function. The lack of a compulsory correlation between drusen onset and visual dysfunction was magnified in the group of 20 patients. Graphs report the regression curve between the visual acuity, near BCVA and far BCVA (A and B respectively), the Amsler test score (C), the contrast sensitivity test score (D) which were plotted vs the drusenoid area.

epithelium from the choroid. In contrast, in wet AMD an overproduction of blood vessels supplying the outer border of the retina occurs. This surpasses the border of choroid through ruptures of the Bruch's membrane, thus destroying the anatomical border between the choroid and the retina.

This represents the key distinguishing point between wet and dry AMD. In fact, in dry AMD, choroid vessels are not primarily recruited and pathology is solely grounded on drusen deposits externally to the pigment epithelium, which disrupt the Bruch's membrane without angiogenesis, bleeding or extracellular fluid accumulation. In the wet variant, drusen associate with bleeding of small vessels in the inner choroid layer, which in turn stimulate angiogenesis, which occurs following an abnormal pattern often leading to a detachment of the pigment epithelium. In the wet variant drusen become difficult to recognize by using OCT and 3D imaging due to a complex pathology. In dry AMD,

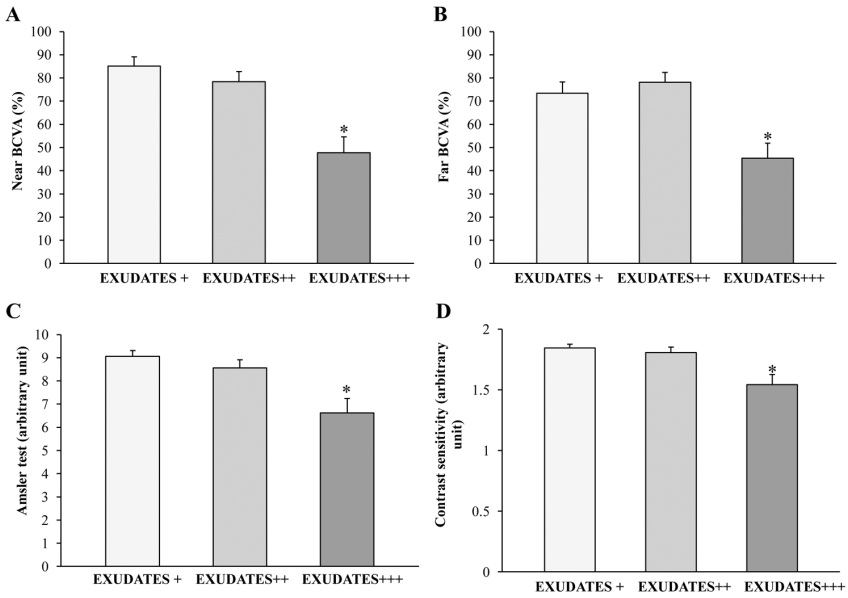

Fig. 25. - Association of the classic scoring system for the retinal thickness with visual function in wet AMD. Graphs show the patients' visual acuity, near BCVA (A) and far BCVA (B), the Amsler test score (C) and the contrast sensitivity score (D). $^{*}p < 0.05$ vs other groups.

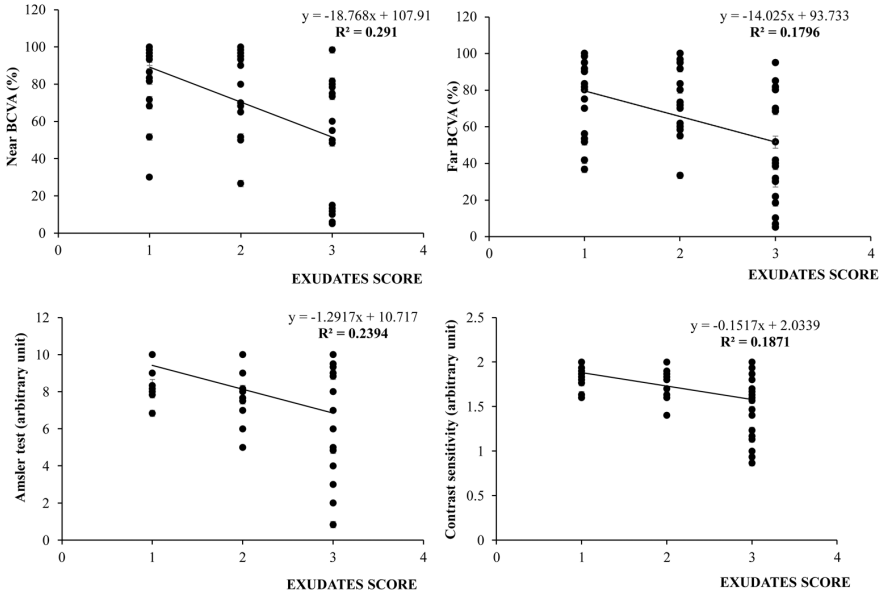

Fig. 26. - Correlation between visual ability and classic combined scoring system for the retinal thickness in wet AMD. Graphs report the regression curve between the visual acuity, near BCVA and far BCVA (A and B, respectively), the Amsler test score (C), the contrast sensitivity score (D), which were plotted versus the classic combined scoring system for retinal thickness.

drusen appear as small white or yellowish deposits, beneath the macula, the central area of the retina (as shown in Figure 9B and 9C).

Despite being present also in extra-macular regions, the macular placement and the size of drusen characterize advanced stages of AMD (Jager et al., 2008). This generates a progressive loss of central vision over time with deterioration in near and far visual acuity, loss of contrast and distortion of images.

In both phenotypes, the disease course is irreversible and, despite transient changes in the slope of the progression curve, no spontaneous recovery takes place. This led to recent efforts to rescue cell transplantation by using stem cell-related approaches for restoring or relenting macular degeneration in AMD (Zarbin et al., 2019). At present, there is no deep insights into the pathogenic mechanisms, which drive the disease, both concerning the wet and dry variants.

The present study provided a multifaceted evidence that despite dry AMD is characterized by the occurrence of proteinaceous drusen, there is no powerful correlation between the occurrence of drusen and the onset of visual impairment. Such a lack of correlation is obtained also when the count of the drusenoid area within the macular square is carried out using a quantitative measurement of the drusen filling the macula. Nonetheless, in this case the correlation is the highest suggesting that the use of the drusenoid area is an index, which provides a higher reliability of the macular involvement in this neurodegenerative disorder. Such a finding should be further considered when expressing the engagement of the macula region. Nonetheless, even when considering the largest cases of drusen filling the macula no powerful correlation could be drawn between visual symptoms and the ratio of the macula suffering a drusenoid engagement.

This observation rules out a causal effect of the drusenoid material to impair directly the visual processing. This is confirmed by the analysis of paradoxical conditions we brought up in the present study. In these cases no visual impairment is present in 20 patients bearing macular drusen, while metamorphopsia and visual alterations are detected in an anecdotal patient owing a magnificent macular architecture (Figures 3 and 4). The addition of data from patients suffering from wet AMD further confirmed the lack of a direct role of any kind of material accumulating beneath the macula in producing per se a visual deficit. In particular, the presence of drusen in dry AMD patients classified as mild, moderate or severe does not significantly correlate with the reduced visual acuity.

It is very likely that in dry and mostly wet macular degeneration an ongoing biochemical alteration underlies the defects in visual processing. Such a biochemical alteration is likely to involve cell-clearing pathways early in the retinal pigment and later on up- and down-stream. This metabolic defect would be responsible for the early accumulation of drusen and the progression towards inflammatory response which accompanies wet AMD. In fact, recently it was hypothesized that the main biochemical pathways which are activated within the pigment epithelium engage the autophagy flux and the proteasome system, which are critical to prevent neurodegeneration (Fornai et al., 2003; Pinelli et al., 2020a). Thus, it is likely that a dysfunction in the clearance of misfolded proteins including complement molecules (Rudolf et al., 2008) may produce extracellular accumulation of polymorphic debris in the form of drusen. If this is the case, one may expect that compounds which exert a stimulation of the autophagy machinery empower the pigment cells to metabolize properly such an excess of substrates. In fact, following administration of autophagy promoters it is likely to restore the paracrine homeostasis bridging photoreceptors with the pigment epithelium and the Bruch's lamina of the choroid. This is the case of prolonged administration of lutein or resveratrol which may occlude the formation and deposition of drusen, up to reabsorption of such a deleterious extracellular material (Pinelli et al., 2020b). This morphological regression of the dry AMD hallmark was associated with a recovery in visual acuity, loss of distortion and a gain of visual contrast. This confirms the involvement of protein clearing machinery in dry AMD (Bowes Rickman et al., 2013). In fact, as reported by these authors, autophagy dysfunction accompanied by lipofuscin accumulation and reactive oxygen species (ROS) activates inflammatory reactions, further promoting long-term and chronic inflammatory cascade thus accelerating cell senescence of the pigment epithelium (Kaarniranta et al., 2013, 2020). The effects of compounds administered to the patient involve all these pathogenic events, which are presently known to produce retinal damage in AMD (Munia et al., 2020; Neal et al., 2020). These compounds share anti-inflammatory effects (Bola et al., 2014; Wang et al., 2015; Mares, 2016; Buscemi et al., 2018; Schink et al., 2018; Bungau et al., 2019). Remarkably, the synergism between these compounds may extend to protein clearing pathways, which play a fundamental role in tuning the orchestration at the retinal-choroid border, where retinal pigment cells are the pivot. The occurrence of pathological regression joined with improvement of symptoms of visual acuity and contrast with the occlusion of distortion in this case of dry AMD may greatly rely on the multiple step synergism between these compounds to interfere with the biology of disease. Indeed, the secretion of debris in AMD occurs at both sides of the pigment epithelium, which suggests that physiological polarity (secretion towards the basal membrane) is lost and a bidirectional polarity of secretion occurs,

which concerns proteins mainly non-related with photoreceptors such as un-esterified cholesterol, apoE, complement factor H, and vitronectin (Rudolf et al., 2008). This calls to consider a rather generalized defect in protein handling by the retina-choroid junction in AMD, which is best targeted by a pharmacological synergism at multiple levels. Such a challenging hypothesis leads to tone down the role of drusen (Tolentino et al., 1994).

Conclusions

Our data provide evidence leading to interpret drusen mostly as innocent bystanders of a primary metabolic defect, which also produce visual alteration in AMD. This hypothesis is not bizarre when considering the general significance of protein aggregates in the pathogenesis of most degenerative disorders of the central nervous system. This is the case of Lewy bodies in Parkinson's disease and dementia with Lewy bodies, plaques and neurofibrillary tangles in Alzheimer disease, inclusion bodies in amyotrophic lateral sclerosis (Fornai et al., 2005). We posed this question two decades ago questioning whether neuronal inclusions in neurodegenerative disorders represent static pathogenic features or they rather represent a key to understand the previous dynamic steps generating neurodegeneration itself (Fornai et al., 2002, 2003a, 2003b, 2005). In fact, even in AMD, when considering the protein composition of drusen, these contain alpha-synuclein, which is also present in these central neurodegenerative disorders. As shown in the accompanying paper a deposit of alpha-synuclein may be demonstrated in baseline conditions around the pigment epithelium, where a release apparatus is also evident. Thus, it may be concluded that a metabolic alteration in the retina pigment epithelium is the core of AMD producing from one side the formation of drusen and, on the other side, altering the mechanisms of vision. Such a concept was partially inherent to the conclusion of Jones et al. (2016) when they stated that alterations of RPE in AMD may really predict the progression of the disease. In a way, we may conclude that nothing new is under the sun and the chance to observe the retina though the pupil represents a windows for observing ongoing mechanisms which govern neurodegeneration in the CNS.

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