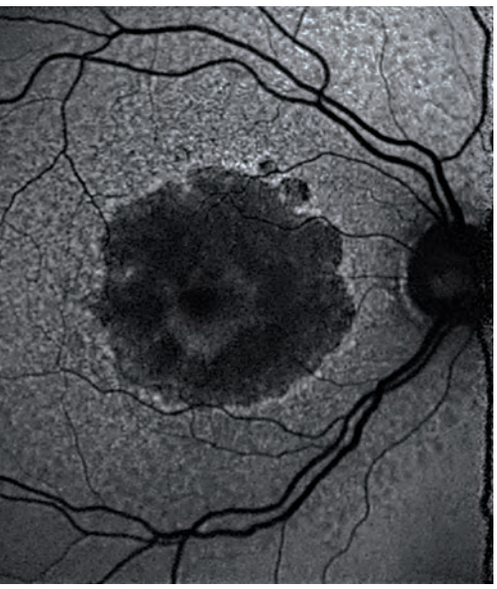
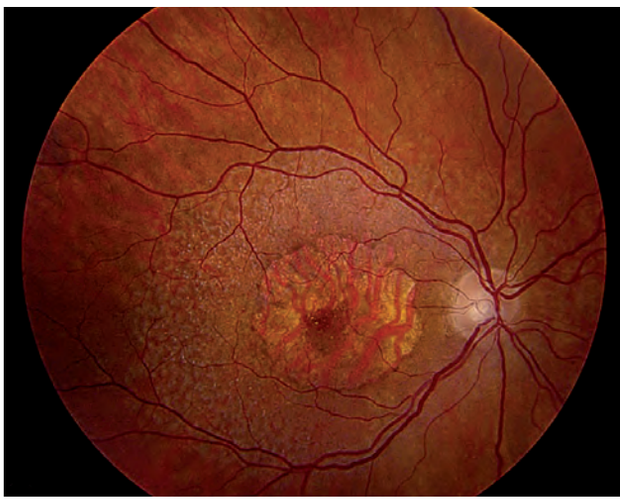
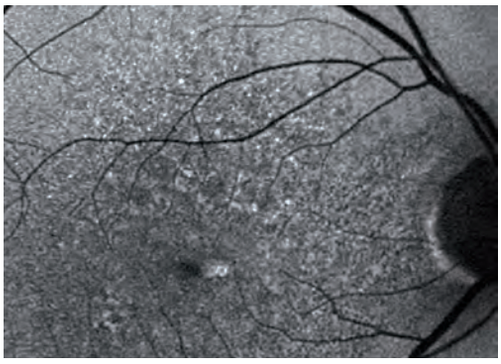
AMD YEAR BOOK 2012







Anita LEYS

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Théa

Driving innovation

Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in patients over the age of 60 in industrialized countries. It is divided in two different groups: non exudative or atrophic AMD and exudative or neovascular AMD. The first form is more common, but the second is responsible of 90% of the visual impairment related to AMD.

If untreated, the end stage of non-exudative AMD is geographic atrophy (GA), a circumscribed area of macular atrophy. On the other hand, Exudative AMD progresses to form an organized fibrous scar (disciform scar) which results in irreversible central visual loss.

Therefore the impact of AMD on quality of life and patients' autonomy can be devastating. During the last decade, the approach to these forms of AMD has changed.

Epidemiological studies have revealed the risk factors associated with AMD such as age, sex, diet, nutritional status, smoking, hypertension and genetic markers. On the other hand many mechanisms are known now about the pathogenesis of the disease and new imaging instrumentation can help ophthalmologist to obtain a early diagnosis and to carefully manage the patient. Finally new therapeutic approaches to AMD are available and new emerging and experimental treatments are on the horizon.

The aim of this AMD Year Book 2012 (Part I) is to summarize the new information about AMD that were presented during the last 8 months in international meetings.

Contents

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Chapter 1

Epidemiology and risk factors. Pathogenic mechanisms and genetics. AAO 2011 and EVER 2011

Doctor Anita LEYS (Belgium)

1 Progression of Geographic Atrophy in the AREDS1 study

Chew E. Retina Subspeciality Days AAO 2011

Although geographic atrophy (GA) is considered to be an endstage of AMD, the whole process from age-related precursor lesions leading to GA is progressive and the atrophic lesion itself is growing with time.

The progression of GA in the AREDS1 study has been analysed in 95 eyes of 77 participants who developed GA at least 4 years following enrollment in the study. The average time from baseline to initial identification of GA was 6.6 years (Figure 1). The early precursor lesions of GA included large and confluent drusen, then hyperpigmentation, drusen regression, and in 25% of cases highly refractile deposits, then hypopigmentation leading to GA. The mean time for progression from large drusen to GA was 5.9 years and for progression from hyperpigmentation and refractile deposits to GA 2.5 years. The growth rate of the geographic atrophy lesion was 2 mm^2 at 1 year, nearly 4 at 2 years, nearly 6 at 3 years or 1.78 mm^2/year overall. VA loss was 3.7 letters at first documentation of GA, and by 22 letters at year 5.

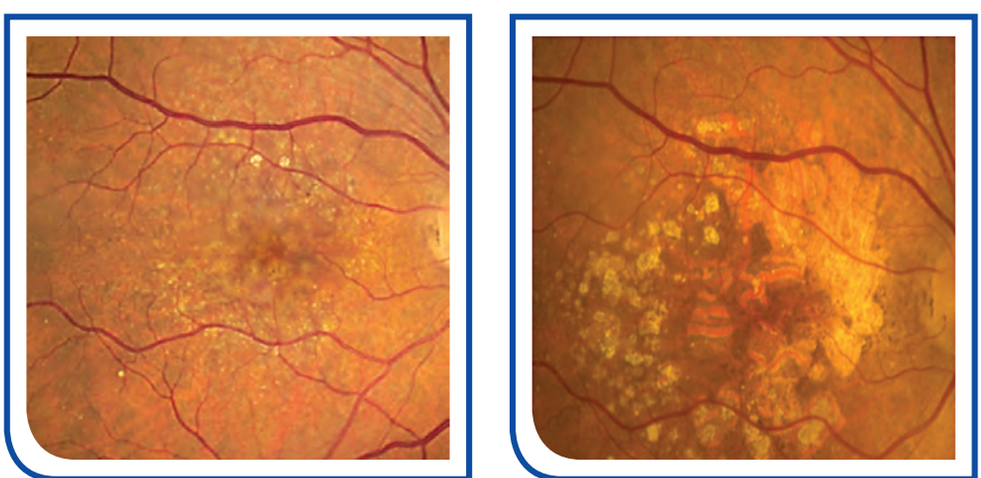

Figure 1:
Large and confluent macular drusen, and foci of hyperpigmentation in a 74 years-old patient (left), and progression to drusen regression, refractile deposits and GA at age 79 (right).

2 Reticular Pseudo-drusen as a risk factor for advanced AMD in AREDS2

Domalpally A. Late breaking developments. Retina Subspeciality Days AAO 2011.

An intermediate analysis of the AREDS2 study has identified reticular pseudo-drusen in 25% of participants. Further analysis showed that reticular pseudo-drusen are a risk factor for GA (Figure 2), but not for neovascular AMD.

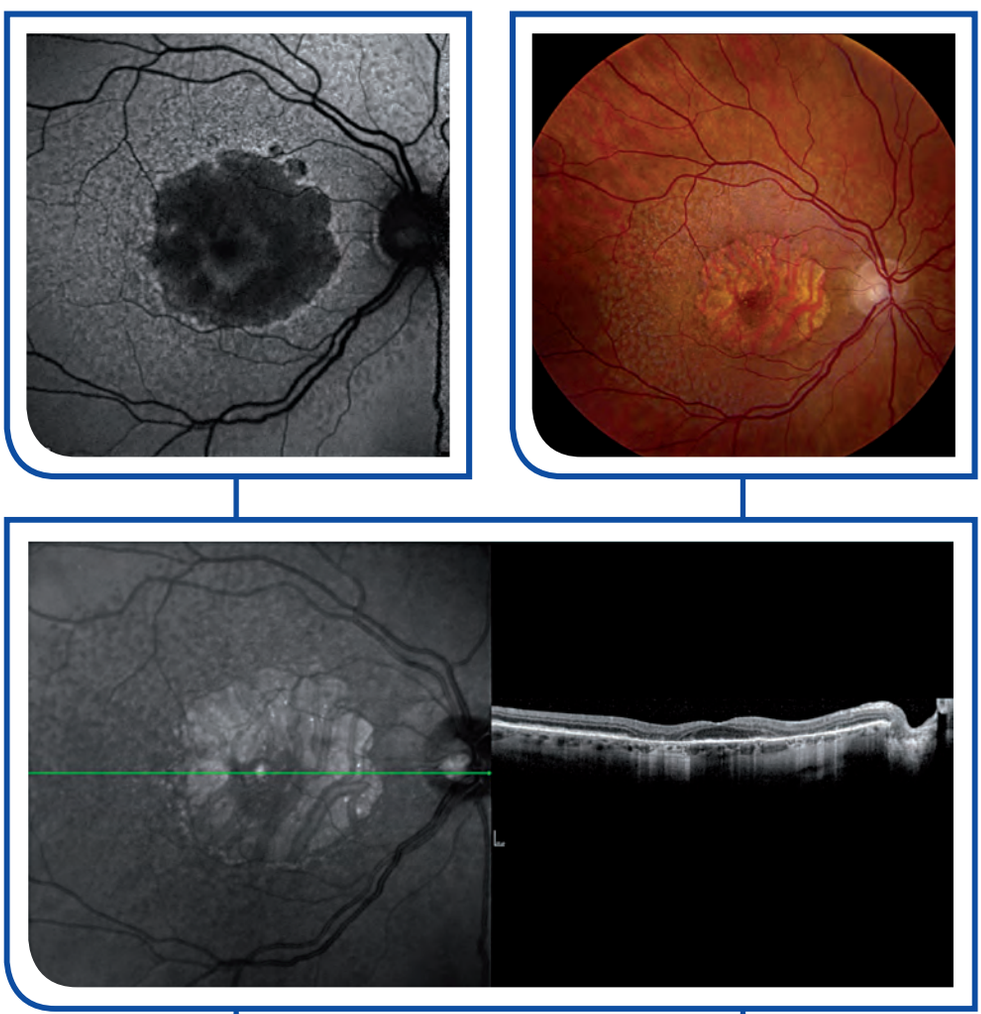

Figure 2:

Reticular pseudo-drusen and geographic atrophy in a 82 year-old pseudophakic patient. VA is 20/80. The reticular pseudo-drusen are imaged with blue light (upper left), are also evident on the colour images (upper right), and are identified on the Spectralis OCT as a strongly hyperreflective band with an irregular surface at the inner side of the RPE (bottom). The area of geographic atrophy is parafoveal and deeply autohypofluorescent (upper left). The fovea is relatively spared of atrophic changes. The outer border of the geographic atrophic lesion has banded autohyperfluorescence.

3 Hypovitaminosis D as a risk factor in AMD

Mauget-Faÿsse EVER 2011 (3215)

Hypovitaminose D is frequent in the older age group. In a case-control study conducted in Lyon and Angers, France, 37/65 participants had hypovitaminose D (serum 25-hydroxyvitamin D < 50 nmol/ml). The mean age of the participants was 76 years. Of the total of 65 participants 31 were AMD patients treated in Lyon. From a geriatric center in Angers, 34 patients without AMD were prospectively recruited as controls. Subjects with hypovitaminose D had more often AMD than those without hypovitaminose, and the association was significant (p = 0.029). Odds-ratios (OD) were 3.10 for increased risk of AMD and 3.50 for risk of advanced AMD.

These data and the reports of Parech *et al.* [1], and Millen *et al.* [2], suggest a role for vitamin D in AMD and show a correlation between reduced serum vitamin D levels and risk for AMD. In the third National Health and Nutrition Examination Survey1 7752 adults were evaluated to access a possible association of serum vitamin D and early and advanced AMD. Levels of serum vitamin D were inversely associated with early but not advanced AMD. OR was 0.64 for early AMD in the highest versus the lowest quintile of serum 25-hydroxyvitamin D. Millen *et al.* [2] confirmed the association of increased serum vitamin D and decreased OR of early AMD. Seddon *et al.* [3] studied twin pairs with discordant AMD phenotypes and detected higher dietary vitamin D intake in the twin with less severe AMD compared with the co-twin. On the other hand, Golan *et al.* [4] found no association of vitamin D plasma levels and AMD in a large cross section study of AMD and non AMD participants.

Recent reports have shed light in mechanisms of interaction of vitamin D with AMD. Lee *et al.* [5] demonstrated that vitamin D administration for 6 weeks in aged mice significantly impacts on the aging process. Treated mice showed significant reductions in retinal inflammation, in levels of amyloid β accumulation, and in retinal macrophage numbers and showed improvement in visual function. The protection of vitamin D against age-related macular degeneration, suggested in epidemiologic studies, could be due to rejuvenating action of vitamin D in the aging eyes by reducing inflammation and clearing amyloid β.

Moreover, a genetic association between vitamin D metabolism and AMD risk has been demonstrated [6]. In an initial study extremely phenotypically discordant sibling pairs were used to evaluate the association of neovascular AMD and vitamin/sunlight-related epidemiological factors. After controlling for established AMD risk factors (polymorphisms CFH and ARMS2/HTRA1, smoking) the study team found that ultraviolet irradiance was protective for neovascular AMD (p = 0.001). Serum vitamin D levels were higher in unaffected individuals than in their affected siblings (statistically not significant). Genetic studies were performed in the initial cohort and replicated in the extended family cohort, and in unrelated case-control cohorts with a total of 2.528 individuals.

Hypovitaminosis D as a risk factor in AMD

These genetic studies resulted in the identification of single point variants in CYP24A1 (the gene encoding the catabolizing enzyme of the vitamin D pathway) that influence the AMD risk after controlling for smoking, sex and age in all populations, both separately and in a meta-analysis.

The problem of vitamin D deficiency is prevalent among elderly, because of diminished ability to produce vitamin D with advanced age. Moreover, hypovitaminosis is not restricted to the elderly. As of 2005, approximately 40% of men and 50% of women aged over 18 from the USA were estimated to have inadequate levels of serum 25-hydroxy vitamin D [7]. Recommendations for vitamin D intake (sun exposure, milk, supplements) are important to maintain adequate serum 25-hydroxyvitamin D levels, taking into account the requirement of vitamin D for bones, CNS, and other organs and the protective role in many diseases, including protection for AMD.

4 Gene testing and AMD: are we ready to start?

Kim I. Retina Subspeciality Days AAO 2011

AMD is a complex genetic disorder. Alleles and haplotypes (combinations of alleles at a given locus that are inherited together) on chromosome 1 in complement Factor H (CFH) and on chromosome 10 in Age-Related Maculopathy Susceptibility 2 (ARMS2) have large influences on risk for all AMD subtypes in populations of various ethnicities [8]. However, the combination of these genes alone is insufficient to correctly predict the development and progression of AMD. Genome wide scans and genome wide association studies have revealed a number of other genes associated with AMD or candidate genes, including lipid metabolism genes. AMD genes intervene in the alternative complement pathway (CFH, C2, CFB, C3 and CFI), in the high-density lipoprotein (HDL) cholesterol pathway (LIPC,A ABCA1 and CETP),in the extracellular matrix pathway (TIMP3, COL10A1, and COL8A1) and angiogenesis pathway (VEGFA). The function of ARMS2/HTRA1 is still not confirmed. HDL genes seem to play important roles in drusen initiation in the early stages of AMD. As drusen accumulate between RPE and Bruch's membrane, genes in the complement pathway are activated [9]. Complete characterization of alleles that influence AMD, also including those with a weaker association, will be necessary for optimal and accurate determination of an individual's overall genetic risk of developing advanced disease as well as further understanding of the pathogenesis of AMD and identification of new targets for therapeutic intervention.

Currently recognized genes and environmental factors may help to identify patients most susceptible to AMD. Risk assessment models for development of advanced AMD are available [10-11] and are useful for designing clinical trials and for AMD surveillance.

Genetic testing for individuals is becoming widely available (Macular Risk, RetinaGene, deCODE genetics, 23 and me or ARUP Laboratories), but are we ready and willing to start? A first concern is the value of the test. Gene testing should be evaluated by ACCE. ACCE Model Project is the first public-available analytical process for evaluating scientific data on emerging genetic tests. The ACCE framework has been adopted by various entities worldwide for evaluating genetic tests. ACCE takes its name from the four main criteria for evaluating a genetic test: Analytic validity, Clinical validity, Clinical utility and associated Ethical, legal and social implications. In clinical validity, AMD current models achieve area under the receiver-operating curve (AUC) near 0.80. In clinical utility, correlations between genotype and response to anti-VEGF treatment have been inconsistent. On the other hand, there are some suggestions of decreased benefits from AREDS supplements in patients with CFH Y402H risk allele.

Another concern is the added value of genetic testing of an individual. In a recently published Seminar in ophthalmology [8], the authors explain in detail the current concepts in the field of genetics of AMD and genetic testing. Lack of specific efficacious preventative treatments severely limits the utility of genetic testing for individuals without any signs of disease. For those already affected by some degree of AMD, retinal findings remain the strongest predictor of advanced AMD, with genotype adding minimally to risk prediction. For those who develop advanced AMD, no consistent correlations have been made with respect to treatment response and genotype.

The authors conclude that while genetic studies should be an integral aspect of clinical studies of AMD, routine genotyping of AMD patients may not yet be indicated.

5 Emerging Role of Biomarkers for AMD

Sternberg P. Jr. Retina Subspeciality Days AAO 2011

A biomarker is a naturally occurring molecule, gene, clinical feature or characteristic that is objectively measured and evaluated as an indicator of normal biologic and pathogenic processes, or pharmacologic responses to a therapeutic intervention.

AMD biomarkers are important in the prediction of the disease. They can be classified in:

1. Morphologic Predictors of AMD and clinical/phenotypic predictors of treatment response. Drusen size and number can predict the development of AMD. AREDS severity study has a 9-step scale, which associates drusen and pigmentary abnormality with risk. The AREDS simplified scale is designed for clinical practice. Size of AMD lesion is a predictor, with poor prognosis of large lesions. Angiographic characteristics of CNV are indicative for prognosis and treatment response.

2. Genetic predictors of AMD include mitochondria DNA polymorphisms and somatic mutations with increased risk for carriers of at risk alleles. Complement factor H (CFH) at 1q31 and ARMS2/HTRA1/LOC387715 at 10q26, and other complement cascade genes - C2/BF, C3 and CFI - are genetic predictors of disease. Besides, CFH Y402H and LOC387715 are genetic predictors of progression of disease. CFH is a genetic predictor of treatment response for antioxidants and zinc therapy. CFH Y402H CC carriers proved to have poorer outcome with bevacizumab and were more likely to need reinjection with ranibizumab. However, correlations between genotype and response to anti-VEGF treatment have been inconsistent. In CATT, no correlation was demonstrated between genotype and response to anti-VEGF treatment.

3. Systemic quantitative AMD predictors include inflammatory markers, oxidative stress markers and lipid profiles.

   - A significant elevation of Apo B levels has been demonstrated in serum of AMD patients [15a]. In AMD decreased HDL and increased LDL have been observed in one study [15], but other studies showed conflicting results.
   - Inflammatory markers are CRP, IL-6, Fas Ligand, and complement components and fragments.
   - Oxidative stress markers include carboxyethylpyrroles (CEPs), malondialdehyde (MDA), homocysteine, and thiol redox status in plasma, and 8-OHdG in aqueous [12-14].

Salomon *et al.* [13], in a review article in Chemical Research in Toxicology, demonstrated the importance of CEP-oxidative protein modification of DHA in AMD. In the outer retina oxidative cleavage occurs of docosahexaenate (DHA)-containing phospholipids derived from old photoreceptor disc membranes. This process is associated with production of CEPs. CEPs modify proteins and CEP-modified proteins accumulate in DHA-rich photoreceptor outer segments of retina, endocytosis occurs in RPE, and accumulate in drusen. CEP-modified protein levels are elevated in AMD retina, drusen, RPE, and blood. CEP-modified proteins induce phagocytosis of rod outer segments, induce angiogenesis (CNV) and induce immune response. The autoimmune tissue destruction results in atrophy (GA).

Emerging Role of Biomarkers for AMD

Weismann *et al.*, in a report in Nature [14], has focused attention on malondialdehyde. Malondialdehyde (MDA) and its condensation products are reliable markers for oxidative stress and have been associated with many disorders including atherosclerosis and AMD. Drusen have been shown to contain MDA. Complement factor H binds MDA and protects from oxidative stress, can block uptake of MDA-modified proteins by macrophages, and can block MDA-induced proinflammatory effects. The CFH polymorphism H402Y, which is strongly associated with AMD markedly reduces the ability of CFH to bind MDA, indicating a causal link to disease etiology.

6 Lipids and AMD

SIS EVER 2011: Cholesterol and Retina

6.1 - Lecerf JM: Plasma cholesterol and lipid metabolism

Plasma cholesterol is carried in blood by lipoproteins. Lipoproteins contain free and esterified cholesterol, triglycerids, phospholipids and apolipoproteins. Very low-density lipoproteins are liver derived and contain triglycerides and cholesterol. Low-density lipoproteins contain cholesterol and apolipoprotein B. High-density lipoproteins are involved in the reverse transport of cholesterol. Lipoproteins deliver cholesterol to gonads, adrenal glands, liver and other tissues. Triglycerides are transported and stored in adipose tissue and are a source of energy for muscles.

The plasma lipid metabolism is complex. Genetic factors control apolipoprotein synthesis, control proteins for transfer and exchanges, and control receptors. Diet and abdominal adiposity modulate the lipid metabolism.

Statins do not reduce risk of AMD [16].

In the recently published PIMAVOSA Study, macular pigment density was associated not only with plasma lutein and zeaxantin but also with omega-3 long-chain PUFAs, particularly with EPA and DPA [17].

6.2 - Bretillon L: Cholesterol in retina and RPE

Cholesterol in the neuroretina derives from biosynthesis and from the circulation. RPE cells express lipoprotein and scavenger receptors that allow identification of cholesterol-rich lipoprotein, and enhance uptake of cholesterol in neuroretina. The neuroretina and RPE cells express proteins that can remove cholesterol.

Deposits of free cholesterol and cholesterol esters at the basement of Bruchs' membrane are hallmarks of aging and of AMD.

Lipids and AMD

6.3 - Rudolf M: Cholesterol, drusen and AMD

The authors propose a biochemical model of AMD [18].

- RPE secretes apolipoprotein B-lipoprotein particles of unusual composition, resulting in drusen formation and accumulation into Bruchs' membrane eventually forming a lipid wall, a precursor of basal laminar deposit.
- In addition, apolipoprotein B-lipoprotein particles are perhaps partially delivered from nutrients and lipophylic recycling system.
- Constituents of these eye lesions interact with reactive oxygen species to form pro-inflammatory peroxidised lipids that elicit neovascularisation.

Directions for research and therapeutic strategies should be based on the oil-spill in Bruchs' membrane and taking into account associations between AMD and previously identified CFH, C2, C3, CFB, CFI, APOε and ARMS/HTRA1 genes/regions and the novel genes LIPC, CETP, ABCA1 in the HDL cholesterol pathway [19-21].

7 Recent data on Apolipoprotein ε (APOε)

Apolipoprotein ε is a lipid transport protein involved in low-density cholesterol modulation.

A pooled analysis [22] of 15 studies showed an association of late AMD and APOε. Following adjustment for age, sex, and smoking, the APOε 4 haplotype was protective for AMD with an OR of 0.72 per haplotype and the APOε 2 haplotype was a risk factor for AMD with an OR of 1.83 for homozygote carriers.

Ever smokers had a significant increased risk relative to never smokers for both CNV and GA but not early AMD implicating smoking as a major contributing factor to disease progression from early signs to late AMD.

8 Amyloidβ accumulation in Alzheimer's disease and AMD. How to reduce amyloidβ accumulation?

T helper 2 inducing dendritic cell vaccine against A-β (EVER 2011 p. 3432, Possemiers et al.)

In addition to age as a risk factor, Alzheimer's disease and AMD have many characteristics in common, including amyloid in senile plaques of the Alzheimer's disease brain and in drusen of AMD patients [23]. To reduce amyloid β accumulation several research projects are ongoing.

- Based on previous studies that have shown that T helper 2 responses are effective in degrading amyloidβ Possemiers *et al.* want to develop a T helper 2 inducing dendritic cell vaccine against amyloidβ.
- The monoclonal antibody against A-β Ponezumab (PF-04360365) had a significant therapeutic effect in mouse model of AMD [24], and is used in a phase 2 trial for Alzheimer's disease.
- Synthetic apolipoprotein mimetics are used in animal models and early human clinical trials, based on the fact that apolipoproteins naturally regulate lipid transport within the bloodstream. These synthetic apolipoprotein mimetics have anti-inflammatory properties and are highly effective for binding lipids, for clearance of plasma cholesterol, and to remove lipid accumulation in vessel walls. 4F (oral apolipoprotein A-I mimetic peptide D-4F), used in a mouse model of Alzheimer's disease, inhibited amyloid β deposition and improved cognitive function. 4F intravitreal injection, used in an animal model of AMD, reduced the lipid deposition and the thickness of Bruchs' membrane and structural remodeling was obtained without adverse events [25]. Apparently, 4F accepts lipids from accumulated lipoproteins in Bruchs 'membrane and facilitates removal.

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Chapter 2

The evolving imaging of AMD for diagnosis and monitoring the disease progression

Doctor Salomon-Yves COHEN (France)

1 Disease progression

Progression of Geographic Atrophy in the Age-Related Eye Disease Study (AREDS) was reported by EY Chew (Subspeciality day, AAO).

Geographic atrophy (GA) associated with AMD remains a major cause of vision loss with no proven effective therapy. Regulatory agencies have accepted structural changes, such as growth of atrophic lesions, as reasonable outcome measurements in clinical trials. The growth of lesions associated with GA was measured in the AREDS study. AREDS is a randomized, controlled trial of high-dose antioxidants and zinc to reduce progression of AMD.

AREDS participants who had developed GA at least 4 years following enrolment in the study were included in the analysis and followed for a median of 10 years. Retrospectively, annual fundus photographs were evaluated prior to the development of GA to identify specific fundus lesions.

Ninety-five eyes of 77 participants developed GA at least 4 years following enrolment in the study, where average time from baseline to initial GA was 6.6 years. Large drusen formation was initially detected, followed by hyperpigmentation, drusen regression, and then hypopigmentation, which lead to GA.

The developing GA was found in drusen (100%), drusen > 125 µm (96%), confluent drusen (94%) and hyperpigmentation (82%). Time from lesion appearance to GA onset varied from large drusen (5.9 years) to hyperpigmentation and refractile deposits (2.5 years). The study also measured the growth rate of the lesions: 2.03 mm^2 at 1 year, 3.78 mm^2 at 2 years, 5.93 mm^2 at 3 years and 1.78 mm^2 per year overall. Median time to developing central GA after any GA diagnosis was 2.5 years. Average visual acuity decreased by 3.7 letters at first documentation of central GA, and by 22 letters at year 5.

Thus, AREDS data confirmed that eyes with AMD usually develop GA following the regression of pre-existing large and confluent drusen. Regression of these large drusen is associated first with hyperpigmentation, followed by hypopigmentation. It is also often accompanied by deposition of refractile deposits and terminates in some cases with GA. These AREDS data will contribute to our knowledge of the natural history of GA development associated with AMD.

2 Imaging AMD

It is a challenge for the imaging devices to achieve a resolution, which is able to detect the very early changes in the macula, predicting the occurrence of ARM/AMD. Commonly used or new devices imaging techniques for diagnosis of ARM/AMD are:

1. Fundus color photography and fundus autofluorescence (FAF) imaging

2. Optical coherence tomography (OCT)

3. Fluorescein angiography (FA)

4. Indocyanine green angiography (ICGA)

5. Adaptative optics

2.1 - FAF imaging

Fundus autofluorescence gives information about changes especially important for early GA detection. Widefield autofluorescence (e.g. Optos 200Tx widefield instrument) is a further development (SR Sadda, subspeciality day, AAO). It contains a simultaneous phase SLO technology, which permits additional autofluorescence imaging. It is able to image up to a 200-degree field of view without medical mydriasis of the pupils, but one has to accept peripheral distortion. Indeed, it was discovered that central retinal fundus autofluorescence (FAF) changes are accompanied with FAF changes in the periphery in retinal degeneration, inflammatory diseases and AMD. In AMD patients, over 60% of patients showed such peripheral FAF changes.

The OPERA substudy and AREDS2 trial will study these changes and their importance more detailed, especially for neovascular AMD. They may aid in the diagnosis and pathogenesis of disease. Meanwhile, the presence of FAF changes were reported in a second study, recently released (Reznicek L *et al.* Peripheral Fundus Autofluorescence is Increased in Age-related Macular Degeneration. Invest Ophthalmol Vis Sci. 2012 Mar 12). In this study, consecutive series of 71 normal eyes, 71 eyes with neovascular AMD having received anti-VEGF treatment and 43 eyes with untreated AMD were investigated. In all subjects, wide-field FAF imaging was performed applying a wide-field scanning laser ophthalmoscope (Optomap® Panoramic 200Tx, Optos). Fundus autofluorescence increased with age not only in the perifoveal retinal area, but also in the retinal periphery. For age corrected measurements peripheral FAF was significantly increased for both, treated and untreated AMD groups compared to normal subjects.

Imaging AMD

2.2 - OCT

Huang *et al.* first introduced the optical coherence tomography (OCT) in 1991 "as a high resolution, noninvasive, *in vivo* ophthalmic imaging technique with low coherence interferometry to detect echo time delays of light, as opposed to sound". Originally, time-domain (TD) OCT was used. The reference arm moves mechanically. About 400 A-Scans/second were achieved. The spectral-domain OCT (SD-OCT; fourier domain technique) has a stationary reference arm, a high-speed spectrometer and a charge coupled device (CCD) camera to detect light echoes simultaneously. With this technique, the acquisition speed was increased to 25.000 to 27.000 A-Scans/second and the axial resolution was enhanced to 3-7 µm.

Two main technical improvements allow to visualizing the choroid (R Spaide, subspeciality day, AAO); this is of importance because imaging the choroid has become more important to analyze pathogenic pathways of AMD, but also to evaluate the treatment success of anti-VEGF therapy.

Enhanced depth imaging (EDI; available mode in SD-OCTs), at least enables to measure choroidal thickness. The peak sensitivity is shifted to the choroidal scleral junction and therefore images of deeper layers can be taken. This works through zero delay inversion and A-Scan averaging with a wavelength of 840 nm.

The swept-source OCT (SS-OCT; Carl Zeiss Meditec) is equipped with a light source that only produces a single wavelength (1050 nm) of light at any instance and the output of light is swept across a range of frequencies. A photodiode serves as detector, which detects signals faster than a commonly used CCD (charge coupled device) in the SD-OCT. Scattering is reduced and deeper tissue penetration is possible. The scan rates are higher (up to 300.000 A-Scans/second) in the SS-OCT. The scan depth is about 5 mm due to less sensitivity loss. The peak sensitivity is similar to that in the SD-OCT. Simultaneous imaging of the vitreous, the retina and the choroid becomes possible. Choroidal thickness can also be measured.

With these OCT improvements, choroidal thinning was frequently observed in AMD patients. In examinations of healthy eyes, regional variations of choroidal thickness with inter-individual differences were observed and have to be considered when measuring choroidal thickness in diseased patients. In normal eyes, the choroid is thickest subfoveally and decreases rapidly nasally and less temporally.

A recent paper also showed the improvements in OCT technology to monitor the course of drusen and their evolution towards RPE atrophy (Yehoshua *et al.* Natural history of drusen morphology in age-related macular degeneration using spectral domain optical coherence tomography. Ophthalmology 2011; 118: 2434-41). The authors report the results of a prospective, and longitudinal study of 143 eyes of 100 patients. They used a custom software to quantify volumetric changes in drusen over a period of ≥ 6 months. On average, drusen volume and drusen area increased over time with the magnitude of the increase dependent on the length of follow-up ($p = 0.001$, 3 mm circle). However, drusen volume decreased in 12% of eyes, with occurrence of RPE atrophy.

Thus, OCT is now used as a routine tool to quantify lesions which were previously only qualitative. For example, an eye with small areas of RPE atrophy could be imaged with autofluorescence pictures, that give information about the location of RPE atrophy, and its future development; and with OCT which allows to observe thinning of the choroids, and to quantify the course of progression of the geographic atrophy (Figure 1).

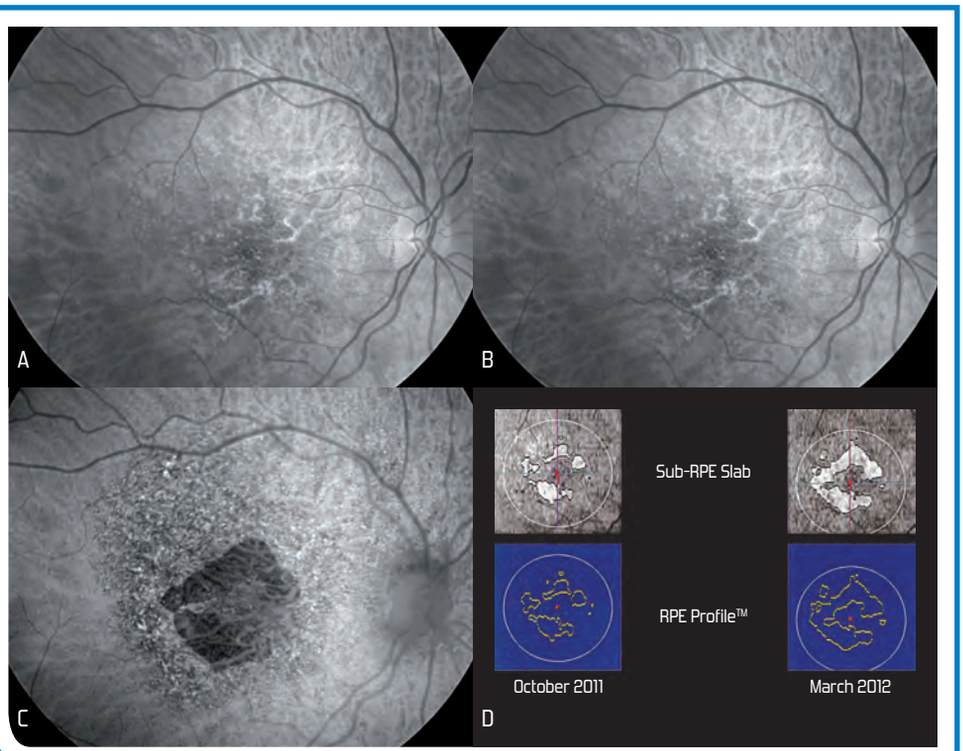

Figure 1:

Geographic atrophy imaged with red-free pictures in October 2011 (a), and in March 2012 (b). Improvements of imaging allowed to better visualize geographic atrophy in March 2012, thanks to the auto-fluorescence fundus photograph (c), showing the limits of the atrophy and the surrounding hyperfluorecencent areas, in which geographic atrophy is likely to extend. Furthermore, it was possible to automatically measure the area involved by atrophy in March and, retrospectively, 5 months before, showing, in this case, a growth rate of 96%, and a diminution of the closest distance to fovea of 50%.

(Image Dr S.Y. Cohen)

Recently, it has been shown that eyes with reticular pseudodrusen also have a thin choroid, as measured by EDI-OCT (Querques G. Choroidal changes associated with reticular pseudodrusen. Invest Ophthalmol Vis Sci 2012; 53: 1258-1263). The authors compared 22 consecutive patients (22 eyes) with reticular pseudodrusen, and without medium/large drusen, with 21 age and sex-matched subjects (21 eyes) with early age-related macular degeneration (AMD), and without pseudodrusen. The mean subfoveal choroidal thickness was significantly reduced in the group with reticular pseudodrusen compared with that in the control group (174.6 ± 10.1 and + 241.4 ± 16.5, respectively; $p < 0.001$).

With the Fourier-domain mode locking (FDML), the tuning speed of the laser light source is improved and up to 370.000 A-Scans/second can be achieved. Further software modifications improve the resolution (e.g. with eye tracking). Internal choroidal structures can be visualized and provide information for a better understanding of pathophysiological processes in ocular diseases.

2.3 - Indocyanine green angiography

The role of indocyanine green angiography (ICGA) in management of AMD seems to decrease significantly in the last years. This is pointed out in a recent review (SY Cohen *et al.* Is indocyanine green still relevant ? Retina 2011; 31: 209-221. In 1992, Yannuzzi *et al.* showed that late frames of ICGA allowed visualization of the entire choroidal neovascular membrane, and ICGA-guided photocoagulation of occult CNV became part of standard care in exudative AMD without classic CNV, i.e. in occult CNV, especially when the latter was located inside or at the margin of a pigment epithelial detachment (PED). Anti-VEGF therapy was available in France in 2007, and inaugurated a new era in the management of exudative AMD. This method of treatment differs radically from laser photocoagulation or verteporfin PDT, because there is no longer any point in limits of the CNV, since it is sufficient simply to inject the anti-VEGF drug into the vitreous. Thus, precise delineation of the CNV membrane is obviously less important than before, and this may explain the marked reduction in the use of ICGA in AMD. However, there are useful indications of ICGA in AMD, especially when diagnosis associated polypoidal choroidal vasculopathy is considered.

Recently, a retrospective study analysed the charts of 44 consecutive patients (55 eyes) with newly diagnosed occult CNV secondary to AMD treated by intravitreal ranibizumab (Querques G. *et al.* Anatomic response of occult choroidal neovascularization to intravitreal ranibizumab: a study by indocyanine green angiography. Graefes Arch Clin Exp Ophthalmol 2012; 250: 479-484). In all patients, optical coherence tomography (OCT) and ICGA were performed at baseline, after 3 months and 12 months. The mean follow-up was 20.3 ± 6.2 months. Central macular thickness (CMT) significantly improved during follow-up (229.0 ± 54.7 µm vs 281.0 ± 61.3 µm at baseline, $p = 0.003$). An overall stabilization was observed on ICGA in both the lesion area (5.27 ± 3.9 mm^2 at baseline vs 4.60 ± 3.5 mm^2 at month 12, $p = 0.4$), and greatest linear dimension (GLD 2.66 ± 1.2 mm at baseline vs 2.55 ± 1.0 mm at month 12, $p = 0.3$). Thus, the study suggests that functional outcomes after intravitreal ranibizumab were related to CMT reduction rather than CNV regression. ICGA does not seem to be useful in the follow-up of patients treated with anti-VEGF therapy.

2.4 - Adaptative optics

Adaptive optics (AO) enables the approach of retinal imaging on a single cell level (M Ching, subspeciality day, AAO). It can be distinguished between rods and cones due to a high resolution of the retina (transverse resolution of about 2 microns). Optical aberrations are measured by a wavefront sensor and corrected by a deformable mirror. It is even possible to differentiate between the three different subtypes of cones. Thereby, a different cone mosaic was detected in patients with color vision deficiency.

Several combinations were attained with adaptive optics and already existing techniques. Fluorescence AO (FAO) combines adaptive optics with scanning laser ophthalmoscopy (SLO). Intracellular lipofuscin is used to detect single retinal pigment epithelial cells (RPE cells) simultaneously to the overlaying cones. A quantitative analysis is possible. Every single cell can be labeled by software features and counted. The measurement of cone density (foveal cones), rod imaging, RNFL imaging or the illustration of retinal eccentricity is feasible. The application for pharmaceutical testing, stem cell implants etc. is also feasible. This device can be meaningful for monitoring in diseases of photoreceptor degenerations, macular telangiectasia, geographic atrophy, and for the evaluation of therapeutic efficiency.

The combination of SD-OCT with a confocal microscopy developed an AO-OCT with an resolution of 3.5 x 3.5 x 3.5 µm (Imagine Eyes rtx1 TM). The area of interest can be marked and scanned. Quantitative analysis of cells/area and retinal vessel wall analysis is possible. The limitations of the device are achieved through eye movements and visual pathway opacities. Besides, it produces large datasets and operator skills are more important again than in other modern imaging devices.

Imaging AMD

Adaptative optics (AO) is currently used at the Institut de la Vision, Paris, to analyze Geographic Atrophy (GA) (Paques M. *et al.* ARVO 2012). In this study, the authors examined eight eyes of eight patients with GA, which underwent 850 nm AO imaging (rtx1, Imagine Eyes, France) of the macula. Results were compared to reflectance and autofluorescence scanning laser ophthalmoscope imaging and to optical coherence tomography. Compared to the other imaging modalities, AO allowed a better delineation of GA limits (Figure 2) and showed more details about melanin redistribution within and around GA areas. The authors conclude that AO imaging imaging may provide novel biomarkers for detecting the earliest stages, documenting the retinal pathology and monitoring progression of GA.

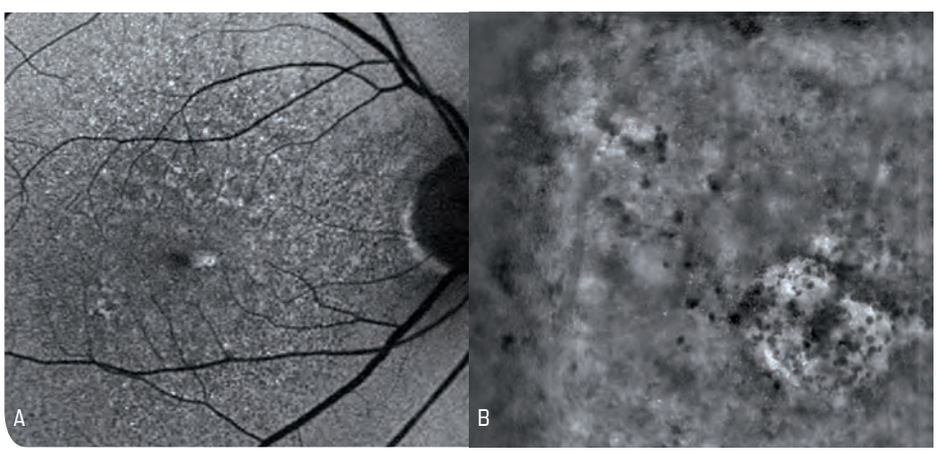

Figure 2:

Geographic atrophy imaged with autofluorescence fundus photography (a), and adaptative optics, performed on the paracentral atrophic area (b). Note the disappearance of photoreceptors and black dots corresponding to melanine clumps.

Courtesy Pr Michel Paques, Institut de la Vision, Paris.

New imaging of geographic atrophy could thus include adaptative optics, fundus photography, and OCT (Figure 3).

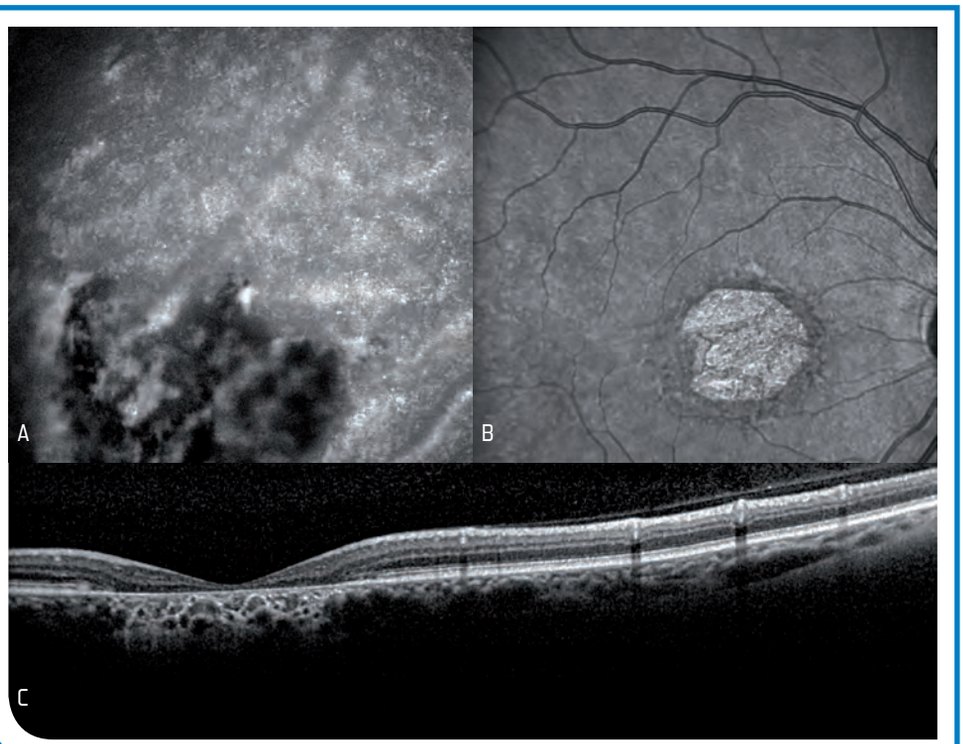

Figure 3:
Geographic atrophy imaged with adaptative optics (a), fundus photography and horizontal scan of the OCT. The area of photoreceptors loss correspond to the area of RPE atrophy.
Courtesy Dr Massamba, Department of Ophthalmology of Pr Souied, Creteil, France).

3 Future developments of imaging in AMD

The polarization sensitive OCT (PS-OCT) is based upon a depth-resolved tissue birefringence. Thereby, a better tissue-specific contrast between the retinal nerve fiber layer (RNFL) and retinal tissue is achieved due to their individual interactions with light. Retinal layers, e.g. the retinal pigment epithelium, can be displayed individually.

Meanwhile, retinal blood flow imaging can be performed with OCT. So far, angiography (qualitative), doppler ultrasound (inaccurate) and the laser doppler flow meter (time consuming) were the only possibilities to visualize retinal blood flow. The Doppler-OCT measures the retinal blood flow velocity due to light reflectivity changes over a period of time. A double circular scan of the optic nerve head scans all retinal branch vessels during the same time in different distances to their origin (6 times per second) over a short time period. For quantification, a special retinal circulation software is used. With the color Doppler-OCT (CD-OCT), real time blood flow visualization is possible and discrimination between arteries and veins can be made. In C-Mode imaging, microstructures and anatomic relationships can be visualized. At the present, usefulness of these developments for imaging chorioretinal diseases, and especially AMD, is not clear.

However, with these further developments, early disease detection, monitoring of disease progression and therapy experience a huge improvement. The diversity of applications is augmented. OCT is no longer a simple diagnostic tool, but can get access to intraoperative use. Moreover, an OCT biomicroscopy is conceivable. A binocular OCT, integrated in a "normal" slit lamp, could be used in the examination routine. The applications of the optical coherence tomography pass beyond the identification of macular edema, subretinal fluid, RNFL thickness, macular thickness and longitudinal measurements in glaucoma, AMD and diabetes. Quantification of eye motility measurements could be possible and used for the detection of strabismus and ocular alignment. Also, visual field testing is feasible with binocular OCT.

The rising knowledge of disease development and progression and the continuing development of imaging techniques to higher scanning speed and higher resolution on single-cell level, lead to a stage of diagnosis standard and treatment control, on which we have never been before. The variety of treatment options can be compared and optimal treatment intervals evaluated. This in turn leads to a good effectiveness and reduction of costs. Additionally, treatment can interfere earlier in the pathologic pathway and thus result in a better outcome of visual acuity. It is obvious that we have reached a new area of diagnosis and imaging in ophthalmology.

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Dr BANDELLO was the scientific coordinator of the Ophthalmology Monographs of the Italian Society of Ophthalmology for 12 years.

Dr Bandello was Vice-President of the European Board of Ophthalmology and chairman of the Recidency Review Committee of the same association.

Dr BANDELLO was the General Secretary and Treasurer of the Italian Society of the Retina.

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Dr BANDELLO is member of the Panel Discussion (tavolo oftalmologico) of AIFA (Italian Agency for Drug).

Dr BANDELLO is member of the Steering Committee of the Osservatorio Malattie Rare (O.Ma.R).

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Dr BANDELLO is member of the Scientific Committee of Vision+ Onlus.

Dr BANDELLO is Novartis Ophthalmology Vision Award (NOVA) Committee Member.

Dr BANDELLO is Member of the Grants Review and Awards Committee (GRAC) of the Bayer Ophthalmology Awards Program (BOAP).

Dr BANDELLO is candidate for Membership of American Ophthalmological Society (AOS).

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Dr BANDELLO is Advisory Board Member of "Ophthalmology and Therapy".

Dr BANDELLO served as trained principal investigator in several clinical trials performed following ICH/GCP and mainly concerning age-related macular degeneration and diabetic retinopathy.

Dr BANDELLO is co-author of five books. He has published 165 Pub-Med papers. He presented well over hundreds of presentations at different meetings. These primarily relate to retinal diseases; diabetic retinopathy; age-related macular degenerations; fluorescein and indocyanine green angiographies of different retinal vascular disorders.

Chapter 3

Disease management and treatment

Professor Francesco BANDELLO (Italy)

1 Non exudative AMD

The aim of non exudative AMD treatment is to restore loss of visual function. However the realistic goal of available treatments is its preservation. New therapies that modulate risk factors such as incorrect diet and nutritional status are currently available. However, these are able to prevent the development or progression of the pathology but do not completely cure patients affected by AMD.

The Age Related Eye Disease Study (AREDS), a multi-center, randomized, controlled clinical trial demonstrated that oral supplementation of a combination of vitamin C (500 mg), vitamin E (400 UI), beta-carotene (15 mg), zinc oxide (80 mg) and cupric oxide (2 mg) in patients with intermediate or advanced AMD in one eye had a 25% relative risk reduction over 5 years of developing advanced AMD in the other eye. The risk of vision loss of three or more lines was reduced by 19% with this treatment.

Deficiencies of Essential fatty acids (docosahexaenoic acid, DHA) have been implicated in AMD onset, and long chain omega-3 fatty acids may also help to prevent the oxidative, inflammatory and age-related retinal damage that occurs during AMD development.

Hyperhomocysteinemia is responsible for vascular damage and is also implicated in neovascular AMD. Vitamin B12 and folate are involved in reducing blood homocysteine concentration. The WAFACS Study showed that the patients treated with vitamin B12 and folate had a statistically significant 35% to 40% decreased risk of developing AMD.

Macular pigment is composed primarily of the xanthophylls lutein and zeaxanthin, also members of the carotenoid family. Their antioxidant properties, as well as their ability to filter short-wavelength light, may help to protect the outer retina and RPE from oxidative stress and aid in cell membrane stability.

Several epidemiologic studies demonstrated that carotenoid intake reduced the risk for advanced AMD and that lutein and zeaxanthin based diet may protect against intermediate AMD in female patients.

In conclusion, the published papers are not sufficient to recommend routine nutritional supplementation for primary prevention of AMD. However, patients with intermediate risk of AMD or advanced AMD in one eye are recommended to take AREDS type supplements, because this formulation is able to reduce the risk of progression to advanced AMD. Also the dietary intake of additional nutrients such as carotenoids and omega-3 fatty acids could be helpful to slow AMD progression.

Non exudative AMD

1.1 - New therapeutic perspectives

During the last years, the molecular mechanisms and pathophysiology of nonexudative AMD has been discovered, so new therapeutic strategies have been developed.

The aim of neuroprotective therapy is to tip the balance in favor of cell survival by blocking cell death signals and enhancing cell survival signaling. Because of the slow progression of cell apoptosis in AMD, ongoing studies are testing drug delivery implants characterized by progressive controlled release of neuroprotective agents.

Currently 3 different drugs are being tested in patients affected by geographic atrophy (GA):

• Neurotech encapsulated cell technology which releases ciliary neurotrophic factor (CNTF).

• Allergan biodegradable brimonidine drug delivery system.

• Alimera Iluvien fluocinolone acetonide drug delivery implant.

Neurotech managed to create a new intraocular implant (NT-501) that contains live human RPE cells encapsulated in a particular membrane that allows cellular CNTF and other metabolites to enter the retina, protecting the implant itself from rejection by the host immune system. The principal characteristic of this implant is that it does not release a primary stored drug, but produces the therapeutic drug directly *in situ*. The resulting vitreous CNTF levels are consistent over time and are effective in photoreceptor preservation and visual acuity stabilization.

It is well known that brimonidine protects the human retina from degeneration secondary to a variety of mechanic, ischemic or toxic insults, modulating different cellular pathways involved in cellular apoptosis. Brimo PS DDS® consists in a drug delivery system composed by a biodegradable polymer matrix containing brimonidine tartrate. It is intravitreally injected and the drug is released slowly, degrading itself completely. The results of a phase 2 clinical trial that is evaluating the effects of this delivery system in patients affected by GA are pending.

Inflammation has been demonstrated to have role in the ethiopathogenesis of AMD and *in vivo* studies have documented the positive effects of fluocinolone acetonide in rat's retina. Alimera has developed Iluvien (sustained release of fluocinolone acetonide) for diabetic macular edema and is starting a trial to evaluate the effects of steroids in the development of GA. This study is ongoing and enrollment is still opened.

Experimental findings suggest that certain molecular compounds of lipofuscin (LF), derived from the chemically modified residues of incompletely digested photoreceptor outer segment discs, may interfere with normal cell function leading to photoreceptor apoptosis. This is the basis of a new therapeutic approach to dry AMD that consists in modulating the visual cellular cycle.

4-Hydroxy (phenyl) retinamide (Fenretinide, Sirion Therapeutics, Tampa, FL) is a synthetic retinoid that strongly binds to excess vitamin A, thus decreasing the amount of retinol available in the visual cycle and inhibiting the production of toxic metabolites in RPE cells. The safety of this drug has already been documented. A reduction in lesion growth was documented, but the results were not statistically different from the placebo group. However, a difference was reported if the patient received fenretide with a smaller particle size. New studies, with a larger number of enrolled patients, are necessary to clarify the effects of this new therapeutic approach.

Inflammation, particularly via the complement system, plays an important role in the ethiopathogenesis of AMD. Since 2005, different genetic polymorphisms in the complement factor H (CFH) gene, complement component 3 (C3) gene and complement factor B/complement component 2 loci have been associated with the ethiopathogenesis of AMD. Later, a similar association was also reported for the complement factor 1 gene.

Therefore new approaches that interfere with different pathways of the complement cascade are being tested.

POT-4, a compstatin derivative, targets reversibly C3 (a point of convergence for all three pathways of complement activation). A phase 1 dose escalation trial is ongoing, and a phase 2 study is being organized to also investigate the anti-VEGF properties of POT-4.

Complement inhibition with Eculizumab for the treatment of Non exudative age related macular degeneration (COMPLETE) study is a phase 2 study that is testing the safety and the efficacy of Eculizumab (humanized monoclonal antibody that specifically binds C5, blocking the activation of complement) for the treatment of dry AMD.

The most innovative field regarding the treatment of Dry AMD is stem cell transplantation. It is very interesting because, when it will be a concrete therapeutic approach, it could completely replace the dead cells that characterize many retinal diseases such as GA or retinitis pigmentosa. Preclinical studies demonstrate the efficacy of using embryonic stem cells for treating retinal degenerative disease. However, the use of these cells in clinical practice is currently limited by many aspects such as the immunogenicity and stability of the cells and the propensity to form tumors *in situ*.

2 Neovascular AMD

In the last decade, the role of VEGF and neoangiogenesis in the pathogenesis of neovascular AMD was stressed. Consequently, the advent of intravitreous VEGF inhibitors has revolutionized the management of this disease.

The first anti-VEGF agent used in AMD treatment was pegaptanib sodium. Other anti-VEGF drugs are ranibizumab and bevacizumab.

Today, on the basis of results from the pivotal phase 3 clinical trials, ranibizumab dosed monthly represents the reference product to which all other therapeutic regimens are to be compared.

Recently new trials are testing the possibility to reduce the number of intravitreal injections administered to the patient (PRN or treat and extend approaches) without reducing the positive effects of anti-VEGF therapy.

PRN (Pro Re Nata "as needed") approach consists in regular monthly follow up visits and retreatment decided on the basis of the presence of retinal exudation. Unlike traditional PRN, a treat and extend approach initially involves regular and frequent treatment until the macula is dry, followed by a gradual extension of the treatment interval and corresponding follow-up visit. Treatment is rendered at every visit and this extension continues until there are signs of recurrence, at which point the treatment interval is then reduced.

About this topic, a formal head-to-head comparison of bevacizumab and ranibizumab is being conducted by the National Eye Institute of the National Institute of Health in the Comparisons of Age-Related Macular Degeneration Treatment Trials (CATTs). The CATT study design includes four treatment arms: either bevacizumab or ranibizumab on a variable schedule and either bevacizumab or ranibizumab on a fixed monthly schedule for 1 year followed by random assignment to either continued monthly injections or a variable schedule based on the treatment response. The primary outcome measure is mean change in BCVA; secondary outcome measures include number of treatments, anatomical changes in the retina, adverse events, and cost.

During the last AAO Meeting, Daniel Martin reported the results of this study*:

- Bevacizumab and ranibizumab were equivalent for visual acuity at all time points when administered at the same dose regimen.
- PRN dosing with monthly evaluation produced average gain that was 2 letters less than monthly dosing, but overall results still excellent.
- PRN dosing resulted in 4-5 fewer injections over 1 year than monthly dosing.
- Both drugs produced an immediate and substantial decrease of fluid.
- Neither drug eliminated fluid in the majority of eyes (although more eyes were completely dry with monthly ranibizumab).

* Martin DF, Comparison of AMD treatments trials (CATT): Bevacizumab-ranibizumab trial. AAO 2011.

Neovascular AMD

2.1 - Combined treatments

The establishment of new guidelines for the administrations of anti-VEGF drugs to minimize the side effects and the number of injections is still opened. It is well known that AMD is a multi-factorial disease, however all the available drugs are able to only interfere with a single pathogenetic mechanism. It is possible that the ongoing studies regarding the incorporation of different treatments will be capable of obtaining better results and potentially reach in treating also non responder patients.

Verteporfin PDT controls the progression of CNV in a particular way. It induces the occlusion of the microvasculature within the lesion, blocking its progression. The combination of this therapy with anti-VEGF injections may be positive compared with either modality alone, yielding longer treatment-free intervals and requiring fewer intravitreal injections.

Contrasting data regarding this combined therapy led to the initiation of two different large long term clinical trials: DENALI and MONT BLANC trials. These randomized, double-masked, controlled, multicenter studies were designed to assess the efficacy and safety outcomes of ranibizumab and verteporfin PDT compared with ranibizumab alone for any type of subfoveal CNV due to AMD. Both trials aim to show the non inferiority of combination therapy with respect to mean change in BCVA from baseline at month 12. Data released showed an average of 2.5 letters of VA improvement with combination therapy compared with 4.4 letters with monotherapy alone.

2.2 - New therapeutic approach

2.2.1 - Radiation Therapy

Radiation has the ability to destroy different tissues with two principal mechanisms: producing DNA damage (directly or indirectly via H2O molecule's damage, producing OH- free-radicals) and releasing vasoactive substances resulting in additional tissue damage and vessel closure.

It is well known that endothelial cells in newly formed vessels are more sensitive than mature vessels or fibroblasts to radiation therapy. This is the basis of the employment of radiation in exudative AMD therapy.

It was demonstrated that this treatment has anti-angiogenic, anti-inflammatory and anti-fibrotic effects if addressed against CNV. The first two effects are secondary to the destruction of neovascular tissue, which is a characteristic of this promising therapy and that differentiates it from the effects of anti-VEGF therapy which is effective in limiting the increased vascular permeability, but doesn't lead to CNV regression. Finally the anti fibrotic effect is secondary to the direct inhibition of CNV metaplasia, leading targeted endothelial cells to apoptosis.

Two different types of radiotherapy have been employed in the treatment of neovascular AMD: external beam radiation in which the radiation source is outside the patient's body and is directed against a particular tissue; and brachytherapy, which consists in the placement of a radiation source inside the body, near or adjacent to the target tissue.

In brachytherapy the radioactive plaque is sewn directly into the sclera, posterior to the macula and this allows a reduced variability of the radiation dose. This type of radiation has a favorable physical characteristic: the rapid decline of the dose with increasing distance from the source (approximating 10% for every 0.1 mm away from the target). Unfortunately this type of therapy requires surgical removal of the plaque and is complicated in a major percentage of cases by cataract formation.

- The MERITAGE Study (Macular EpiRetinal Brachyteraphy in Treated Age-Related Macular Degeneration Patients) is a multicenter, non-randomized phase 2 study that is evaluating the effects of a single intraocular treatment with 24 Gy radiation (strontium-90) followed by intravitreal ranibizumab injections in patients with predominantly classic or occult (with no classic) CNV secondary to AMD.

  At 6 months, the mean number of injections was 1.8. At 6 months follow-up, about 50 % of patients lost fewer than 15 letters, and 5.7% gained at least 15 letters. One patient lost more than 30 letters. Twenty-eight patients (52.88%) gained 0 or more letters.

  The study results pointed to a favorable trend with respect to a reduced number of anti-VEGF injections at 12 months following delivery of Epimacular Brachytherapy (mean of 3.9) *versus* the period of time leading up to Epimacular Brachytherapy intervention (mean of 12.3). In addition, 25% of patients remained injection-free at 12 months following the Epimacular Brachytherapy procedure.

Study results at one year also suggest that a single procedure of Epimacular Brachytherapy can stabilize visual acuity in a majority of this patient population (79%) while decreasing the number of anti-VEGF injections required. Most importantly, 47% of patients enrolled in the study experienced some improvement in their visual acuity while 10% of patients gained 15 or more letters of visual acuity at 12 months.

Neovascular AMD

- CABERNET is a phase 3 trial comparing the effects of epiretinal strontium-90 radiation combined with ranibizumab, to that of ranibizumab alone. In this study, patients were randomized in two different arms: in arm A patients received a one-time treatment of strontium-90 and two ranibizumab injections (the first following surgery and the second during the 1 month follow up visit). Subjects enrolled in arm B received 3 monthly ranibizumab intravitreal injections followed by quarterly injections for 2 years.

This study demonstrated the non inferiority of radiation therapy compared to ranibizumab treatment in eyes losing 15 or more ETDRS letters and a superiority in eyes which gained 15 or more ETDRS letters.

- Oraya therapeutics (Newark, CA). More recent studies are examining the efficacy of radiotherapy for wet AMD utilizing a new divergent technique: the external, non-surgical, orthovoltage X-Ray IRayTM therapy (Oraya Therapeutics, Inc., Newark, CA).

The IRay™ system is a stereotactic radiosurgical device designed specifically to treat diseases of the eye. This instrumentation incorporates eye tracking, lesion targeting through coupling to OCT and A-scan ultrasound and gating.

The IRay is a robotically controlled, noninvasive, low-energy X-ray irradiation therapeutic platform that delivers highly collimated beams through the inferior pars plana that overlap at the macula to deliver precise doses of 16 to 24 Gy to a 4 mm spot size on the macula. The entire patient treatment process requires only a topical anesthetic, and patients are able to leave the hospital within 15 to 20 minutes of receiving treatment.

In a Phase 1 study, both treatment-naïve and previously treated patients were enrolled at sites in Mexico. In total, 3 radiation doses and 4 treatment strategies were employed:

Ranibizumab at Day 0, 16-Gy IRay treatment between days 1 and 14, ranibizumab at Day 30, and then monthly evaluation with OCT and quarterly fluoresecein angiography.

Ranibizumab at Day 0, 24 Gy IRay treatment between days 1 and 14, ranibizumab at Day 30, and then monthly evaluation with OCT and quarterly fluoresecein angiography.

Ranibizumab at Day 0, 11 Gy IRay treatment between days 1 and 14, ranibizumab at Day 30, and then monthly evaluation with OCT and quarterly fluoresecein angiography.

16 Gy treatment followed by monthly evaluation with OCT and quarterly fluorescein angiography.

2.2.2 - VEGF-Trap

Vascular endothelial growth factor Trap-Eye (VEGF Trap-Eye) is a potent, specific VEGF antagonist that binds and inactivates circulating VEGF in the extravascular space. It consists of extracellular portions of VEGF receptors 1 and 2 fused to the Fc portion of human immunoglobulin G. VEGF Trap-Eye may bind both VEGF-A or PlGF to form an inert 1:1 complex with one of the growth factors. Thus, VEGF Trap-Eye has broader anti-VEGF activity compared to pegaptanib, which binds only the VEGF-A165 isoform and ranibizumab, which neutralizes all active isoforms of VEGF-A, but not PlGF. Moreover, VEGF Trap-Eye has a longer half-life in the eye after intraocular injection and it binds other members of the VEGF family including placental growth factors 1 and 2, which have been shown to contribute to excessive vascular permeability.

Heier JS, at the last AAO meeting, presented the 1 year results of the CLEAR-IT 2 trial. It is a multicenter, randomized, double-masked trial designed to evaluate visual and anatomical outcomes, injection frequency, and safety during the PRN treatment phase of a study evaluating a 12-week fixed dosing period followed by PRN dosing to week 52 with VEGF Trap-Eye for neovascular AMD.

The study demonstrated that:

- All VEGF Trap-eye dosing groups were non inferior and clinically equivalent to ranibizumab dosing monthly for the primary end point on maintenance of vision.
- VEGF Trap-eye dosed 2 mg every two months demonstrated similar efficacy and safety to ranibizumab monthly dosed.

2.2.3 - Anti-VEGF and anti PDGF-B

PDGF-B regulates the recruitment of pericytes which are required for vessel maturation. E10030 (Ophthotech) is an antiplatelet derived growth factor aptamer. A phase 1 study demonstrated the safety of the combination Ranibizumab/E10030. This study also showed a main gain of 14 letters at week 12 in treated patients and that 59% of the enrolled patients gained 15 or more letters. All patients showed vascular regression. Currently, a phase 2 trial is recruiting patients with AMD complicated by classic CNVM to test a possible synergistic effect.

2.2.4 - Anti-VEGF and Anti-Endothelial Cell drug combinations

1. Integrins $\alpha\upsilon\beta3$ e $\alpha5\beta1$ are upregulated in angiogenesis and integrin $\alpha5\beta1$ is also upregulated in the RPE, macrophages and fibroblasts. Volociximab (Ophthotech) is a human/murine chimeric monoclonal antibody of $\alpha5\beta1$. A phase 1 study is evaluating the safety and efficacy of the combination Ranibizumab/volociximab in the treatment of CNV. At 8 weeks follow-up, treated patients gained 9.1 letters and mean retinal thickness reduction from 361 to 246 µm. Authors reported that 23% of patients gained 15 letters or more.

2. Sphingolisine-1 phosphate inhibition by monoclonal antibodies results in inhibition of retinal neovascularization and CNV in animals with a subsequent reduction of inflammation and fibrosis.

3. The inhibition of the Nicotin acetylcholine receptor has been demonstrated to successfully control laser induced CNV in animal models. A phase 1 trial developed to study the effects of ATG003 (mecamylamine) in combination with ranibizumab is ongoing.

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