AMD

AGE-RELATED MACULAR DEGENERATION

Revised by Professor Francesco Bandello

Coordination:
Professor Rufino Silva
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</table>
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Age-related macular degeneration (AMD) is now one of the major causes of central vision loss. It involves the macular area and when it progresses and destroys the central fovea, quality of life is seriously compromised. The ability to read, drive, recognize faces or watch television is impaired or lost. It is a disease associated with aging and progressive tissue degeneration. Most of these senior citizens had anticipated the opportunity to enjoy life at leisure doing their preferred activities and find profound limitations and are deeply disappointed in their expectations. This was the dismal state of affairs until a few years ago when the introduction of new therapeutic agents changed dramatically the expected outcome. The most important development has been the clinical demonstration that agents inhibiting vascular endothelial growth factor and, therefore, the formation and development of new vessels not only preserve visual acuity but also improve visual function. This find was a true revolution in ophthalmology. The retina like the central nervous system, particularly in old age, was not considered capable of regeneration and, therefore, new therapies were expected to offer only stabilization of the disease. When laser photocoagulation was shown to be effective for the treatment of diabetic retinopathy this was a breakthrough but it offered only stabilization of the disease and maintenance of visual acuity present at time of treatment. Now, new available treatments offer real improvement in visual acuity, implicating improved visual function and some degree of recovery of the retinal neuronal network.

AMD is, therefore, an active scientific area with new information becoming available almost everyday on the pathophysiology, clinical phenotypes, markers of disease progression, new treatments and novel treatment regimens. It is a complex multifactorial disease where aging associates with genetic factors and inflammatory responses to local cell injury. This means that the treatment of AMD must also address the various factors involved in disease progression and, most likely, will involve a combination of therapies after clear identification of the different AMD phenotypes. This book reviews the new concepts on AMD and is, therefore, timely. It is a concerted effort of Portuguese ophthalmologists which together with a few other European experts in the field cover the subject paying particular attention to daily practice management of AMD.

I am particularly happy to see this collaborative effort coming from my country, Portugal. It shows that Portuguese ophthalmology is at the forefront of European and International ophthalmology. It shows also that there is in Portugal a real spirit of collaboration between colleagues working together to improve the vision of their patients.
Epidemiology of AMD

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1. Introduction

Although age-related macular degeneration (AMD) is the third cause of blindness worldwide, and the first in industrialized countries\(^1\), epidemiological data on this disease remain scarce and partial. The very first studies were published in the 1980’s. Since then, a number of population-based studies have been conducted, first in Caucasian populations of the United States and other industrialized countries (Australia, Europe). Studies have more recently been extended to other ethnical subgroups of industrialized countries (African Americans, Latinos) and to other parts of the world (India, China, and Japan).

2. Prevalence of late AMD in Caucasians from industrialized countries

The prevalence of late AMD in the main epidemiological studies performed in Caucasians of industrialized countries is presented in Table 1. The included studies were restricted to those having classified AMD from retinal photographs and used the international classification\(^2\), in order to make comparisons between studies easier. In these studies, late AMD is defined by the presence of neovascular AMD and/or geographic atrophy. In the United States, the prevalence of late AMD ranged from 0.2% to 1.6% according to studies. Since the prevalence of late AMD increases sharply with age, most of the differences between studies were due to differences in the age distribution. For instance, the lowest prevalence rate (0.2%) was observed for the Atherosclerosis Risk in Communities (ARIC) Study. In this study, the age range of participants was 48-72 years, thus excluding the oldest subjects, in which the prevalence of late AMD is the highest. This study also performed photographs in only one eye of each participant, which may have led to undetected unilateral cases and thus underestimation of the prevalence of late AMD. This is also the case for the Cardiovascular Health Study, which also found relatively low prevalence (1.3% in subjects aged 69 to 97 years).

Prevalence rates observed in Caucasians from Australia and Europe appear relatively similar to those observed in the United States (Table 1), when taking into account differing age distributions in the different studies. In European studies, the prevalence of late AMD ranged from 1.65% to 3.5%.

In order to better compare the prevalence rates among studies, age-specific prevalence rates need to be estimated. This has been done in a meta-analysis performed in 2004 by Friedman et al\(^3\). The authors concluded that prevalence rates were not different among Caucasian populations of industrialized countries, including the United States, Australia and the Netherlands. Similarly, in the EUREYE Study, which included 7 European countries (Norway, Estonia, Ireland, France, Italy, Greece, Spain), no significant differences were found among the participating countries\(^4\).

Therefore, the prevalence of late AMD appears to be similar in the Caucasian populations from the United States, Australia and European countries, despite major geographical and lifestyle differences. Thus, the meta-analysis from Friedman et al, probably constitutes the most reliable available estimate of prevalence rates for these countries, since it bears on studies gathering altogether more than 25000 subjects\(^5\). In this meta-analysis, the prevalence rates increase sharply with age (Fig. 1), from less than 0.5% in subjects 50 to 60 years, to 12% and 16% in men and women aged 80 years or more, respectively. While men tend to have higher prevalence rates than women in the younger age groups (less than 80 years), late AMD is more frequent in women than in men in the oldest age group (80 years or more). This may be due to the higher mortality in ageing men, which leads to a higher selection of the oldest subjects.
Finally, in the late AMD groups, two clinical entities exist: neovascular AMD and geographic atrophy. In most studies, neovascular cases represent about 55% of all late AMD cases\(^{(3)}\). Nordic European countries (in particular Iceland and Norway) may be an exception, since the few studies performed in these countries suggest a higher rate of geographic atrophy\(^{(5,6)}\).

### 3. Prevalence of late AMD in other ethnic groups

As shown in Table 2, the prevalence of late AMD is much lower in African-Americans than in Caucasians. Most of the cited studies were multi-ethnic and performed

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**Table 2. Prevalence of late AMD in Caucasians from industrialized countries**

<table>
<thead>
<tr>
<th>Author, Study</th>
<th>Years study conducted, Country</th>
<th>Number of subjects, age</th>
<th>Prevalence of late AMD (%)</th>
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<tr>
<td><strong>United States</strong></td>
<td></td>
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</tr>
<tr>
<td>Friedman, Baltimore Eye Study(^{(28)})</td>
<td>1985-1988, USA</td>
<td>N=2518, age ≥ 40 years</td>
<td>1.23</td>
</tr>
<tr>
<td>Klein, Beaver Dam Eye Study(^{(29)})</td>
<td>1988-1990, USA</td>
<td>N=4752, age ≥ 40 years</td>
<td>1.64</td>
</tr>
</tbody>
</table>
| Bressler, Salisbury Eye Evaluation Project\(^{(30)}\) | 1993-1995, USA | N=1773, age ≥ 65 years | Exudative AMD: 1.7
Geographic atrophy: 1.8 |
| Klein, Atherosclerosis Risk in Communities Study\(^{(31)}\) | 1993-1995, USA | N=8984, age 48-72 years (one eye only) | 0.2 |
| Klein, Cardiovascular Health Study\(^{(32)}\) | 1997-1998, USA | N=1998, age 69-72 years (one eye only) | 1.3 |
| Klein, Multi-Ethnic Study of Atherosclerosis\(^{(10)}\) | 2000-2002, USA | N=2315, age 45-84 years | 0.6 |
| **Australia** |                               |                         |                           |
| Mitchell, Blue Mountains Eye Study\(^{(33)}\) | 1992-1994, Australia | N=3632, age ≥ 50 | 2.06 |
| Van Newkirk, Melbourne VIP\(^{(34)}\) | 1992-1996, Australia | N=4345, age ≥ 40 years | 0.68 |
| **Europe** |                               |                         |                           |
| Vingerling, Rotterdam Study\(^{(35)}\) | 1990-1993, Netherlands | N=6774, age ≥ 55 years | 1.65 |
| Delcourt, POLA Study\(^{(36)}\) | 1995-1997, France | N=2196, age ≥ 60 years | 1.9 |
| Jonasson, Reykjavik Eye Study\(^{(5)}\) | 1996, Iceland | N=1022, age ≥ 50 years | 3.5 |
| Topouzis, Thessaloniki Eye Study\(^{(37)}\) | 2000, Greece | N=2554, age ≥ 60 years | 2.5 |
| Bjornsson, Oslo Macular Study\(^{(6)}\) | 2002, Norway | N=459, age ≥ 50 years | 2.8 |
| Augood, Eureye\(^{(4)}\) | 2001-2002, | N=4753, age ≥ 65 years | 3.3 |

7 European countries
direct comparisons between African-Americans and Caucasians. Statistical power was generally low for these comparisons, but these studies globally suggest that the prevalence of late AMD is at least 2-fold lower in African-Americans. In the Barbados Eye Study, bearing on subjects of African origin from the Barbados Island, the prevalence rate of late AMD (0.57%) was similar to those observed in African-Americans (Table 3). Therefore, people of African origin appear to be at much lower risk of late AMD than Caucasians, although no data are available from African countries. American Hispanics also appear to be at lower risk for late AMD than Caucasian, with prevalence rates ranging from 0.09% to 0.5% among studies (Table 2). The reasons for the lower prevalence rates in African-American and Hispanics are unclear. No data are available on minorities of other industrialized countries, in particular in Europe. This constitutes a limitation to the estimation of the prevalence of AMD in European countries, which have different types of ethnic groups than in the United States (populations originating from North Africa, Middle-east countries, India…). Given the major differences observed in ethnic groups in the United States, such epidemiological data in European minorities are warranted.

Other epidemiological studies have mainly been conducted in Asia (Japan, India and China). In contrast to what was originally thought, late AMD is not rare in these Asian populations, as shown in Table 3. In two Japanese studies, the prevalence rates in Japanese men were similar to those observed in Caucasian men from industrialized countries, while late AMD appeared rare in Japanese women. This gender-effect, which is not observed in industrialized countries, may be due to gender-related smoking habits in Japanese. Indeed, smoking is a major risk factor for AMD, in all studied populations. In these two studies, smoking was very frequent in Japanese men, while rare in Japanese women.
In three epidemiological studies performed in India, the prevalence rates of late AMD ranged from 0.6% to 1.9% (Table 3). Below the age of 80 years, the prevalence rates were rather similar to those observed in industrialized countries. Above 80 years, the figures are mostly unreliable because of the very high prevalence of unoperated cataracts in this population, leading to a very high percentage of ungradable retinal photographs. The global prevalence rates were therefore probably underestimated.

Three studies have been performed in subjects of Chinese origin: one in the United States (Table 2)\(^\text{(10)}\), one in mainland Beijing\(^\text{(11)}\) and one in Taiwan\(^\text{(12)}\). While prevalence rates in Chinese from the United States and Taiwan seemed similar to those in Caucasians, the prevalence rates in Chinese from Mainland China were much lower. The reasons for these differences are unclear, and may be related to gene-environment interactions, since Chinese from the United States and Taiwan tend to live a more industrialized lifestyle. More epidemiological data are needed to confirm and understand the reasons for these differences.

Finally, in a study from Singapore, the prevalence of late AMD was similar to that in Caucasians\(^\text{(13)}\). Globally, the prevalence of late AMD appears high in Asians, with the only exception of Chinese from Mainland China, which remains to be confirmed.

### Table 2. Prevalence of late AMD in other ethnic groups from the United States

<table>
<thead>
<tr>
<th>Author, Study</th>
<th>Years study conducted, Country</th>
<th>Number of subjects, age</th>
<th>Prevalence of late AMD(%)</th>
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</thead>
<tbody>
<tr>
<td><strong>African-Americans</strong></td>
<td></td>
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</tr>
<tr>
<td>Friedman, Baltimore Eye Study(^\text{(28)})</td>
<td>1985-1988, USA</td>
<td>N=1843, age &gt;= 40 years</td>
<td>0.22</td>
</tr>
<tr>
<td>Bressler, Salisbury Eye Evaluation Project(^\text{(30)})</td>
<td>1993-1995, USA</td>
<td>N=666, age &gt;= 65 years</td>
<td>Exudative AMD : 1.1 Geographic atrophy : 0.3</td>
</tr>
<tr>
<td>Klein, Atherosclerosis Risk in Communities Study(^\text{(31)})</td>
<td>1993-1995, USA</td>
<td>N=2548, age 48-72 years (one eye only)</td>
<td>0.04</td>
</tr>
<tr>
<td>Klein, Cardiovascular Health Study(^\text{(32)})</td>
<td>1997-1998, USA</td>
<td>N=363, age 69-97 years (one eye only)</td>
<td>0.3</td>
</tr>
<tr>
<td>Klein, Multi-Ethnic Study of Atherosclerosis(^\text{(10)})</td>
<td>2000-2002, USA</td>
<td>N=1590, age 45-84 years</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Hispanics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Luis Valley(^\text{(17)})</td>
<td>1983, USA</td>
<td>N=3995, age 43-74 years</td>
<td>0.09</td>
</tr>
<tr>
<td>Proyecto VER(^\text{(31)})</td>
<td>1997-1999, USA</td>
<td>N=2776, age &gt;= 50 years</td>
<td>0.5</td>
</tr>
<tr>
<td>Los Angeles Latino Eye Study(^\text{(30)})</td>
<td>2000-2003, USA</td>
<td>N=5875, age &gt;= 40 years</td>
<td>0.43</td>
</tr>
<tr>
<td>Klein, Multi-Ethnic Study of Atherosclerosis(^\text{(10)})</td>
<td>2000-2002, USA</td>
<td>N=1280, age 45-84 years</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Chinese</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klein, Multi-Ethnic Study of Atherosclerosis(^\text{(10)})</td>
<td>2000-2002, USA</td>
<td>N=699, age 45-84 years</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Table 3. Prevalence of late AMD in other countries

<table>
<thead>
<tr>
<th>Author, Study</th>
<th>Years study conducted, Country</th>
<th>Number of subjects, age</th>
<th>Prevalence of late AMD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbados</td>
<td>Schachat, Barbados Eye Study(7) 1987-1992, Barbados</td>
<td>N=3444, age 40-84 years</td>
<td>0.57</td>
</tr>
<tr>
<td>Japan</td>
<td>Oshima, Hisayama Study(8) 1998, Japan</td>
<td>N=889, age &gt;= 50 years</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Kawasaki, Funagata Study(9) 2000-2002, Japan</td>
<td>N=1625, age &gt;= 35 years</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N=1037, age &gt;= 55 years</td>
<td>0.5</td>
</tr>
<tr>
<td>India</td>
<td>Krishnaiah, Andrah Pradesh Study(10) 1996-2000, India</td>
<td>N=3723, age &gt;= 40 years</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Nirmalan, Aravind Eye Study(11) 1995-1997, India</td>
<td>N=4197, age &gt;= 40 years</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Krishnan, INDEYE(12) 2005-2007, India</td>
<td>N=4266, age &gt;= 60 years</td>
<td>1.2</td>
</tr>
<tr>
<td>China</td>
<td>Li, Beijing Eye Study(13) 2001, China</td>
<td>N=4376, age &gt;= 40 years</td>
<td>0.2</td>
</tr>
<tr>
<td>Taiwan</td>
<td>Chen, Shihpay Study(14) 1999-2000, Taiwan</td>
<td>N=1105, age &gt;= 65 years</td>
<td>1.9</td>
</tr>
<tr>
<td>Singapore</td>
<td>Kawasaki, Singapore Malay Eye Study(15) 2004-2006, Singapore</td>
<td>N=3265, age 40-80 years</td>
<td>0.7</td>
</tr>
<tr>
<td>Greenland</td>
<td>Andersen, Greenland Inuit Eye Study(16) 2002-2003, Greenland</td>
<td>N=642, age &gt;= 60 years</td>
<td>9.1</td>
</tr>
</tbody>
</table>

4. Prevalence of early AMD

Late AMD is preceded by early, usually asymptomatic, retinal abnormalities. The long-term cohort studies have helped to characterize the lesions that are the most predictive of incident late AMD. While large soft drusen (>125 microns) and pigmentary abnormalities clearly are the hallmarks of early AMD, there is no consensus on the precise definition of early AMD, and several classification systems coexist. This makes it difficult to assess and compare the prevalence of early AMD among geographical areas and ethnic groups. Despite these difficulties, it appears that early AMD is frequent in the elderly. For instance, in the meta-analysis by Friedman et al, the prevalence of large drusen increased from about 1.5% in Caucasians aged 40-49 years, to more than 25% in those aged 80 years or more(8). Similar rates were observed in the EUREYE Study, performed in Europe(9).

Consistently with what was observed for late AMD, early AMD appears less frequent in African-Americans than in Caucasian(3,10). By contrast, while late AMD is also less frequent in Hispanic-Americans, the prevalence of early abnormalities appears similar, or even higher than in Caucasians(10,15-17). The reasons for these differences are unclear, and suggest that different factors may be implicated in the etiology of early and late AMD.
In most studies performed in Asians, the prevalence of early AMD appears similar to that observed in Caucasians, consistently to what was found for late AMD\(^8\)\(^-\)\(^{10}\),\(^{12}\),\(^{13}\),\(^{18}\),\(^{19}\).

### 5. Prevalence of late AMD in Portugal

To our knowledge, there are no published data on the prevalence of AMD in Portugal. What can therefore be extrapolated from available data in other countries? As explained above, the prevalence of late AMD appears to be similar in Caucasian populations from industrialized countries. Its best estimate therefore derives from the meta-analysis by Friedman et al\(^9\). We, thus, applied the age and gender-specific prevalence rates of the meta-analysis to the demographic data from Portugal, leading to an estimation of about 84 000 cases of late AMD in Portugal (Table 4). A major limitation to this approach is that it does not take into account the prevalence rates of AMD in non Caucasians living in Portugal. As shown above, the prevalence of late AMD appear lower in subjects of African origin. However, no data are available on the prevalence of AMD in subjects of African, or other ethnic origins (for instance North Africa and Asia) in European populations. It appears difficult to assess the prevalence of AMD while taking into account the multi-ethnic structure of the Portuguese population. However, these ethnic subgroups represent small minorities in Portugal, so that this may only marginally affect the global prevalence rates.

According to these estimations, about two thirds of cases are women, and two thirds of cases are aged 80 years or more. Since it represents about 55% of late cases, neovascular AMD probably affects about 46 000 subjects.

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**Table 4. Prevalence of late AMD in Portugal according to age and gender**

<table>
<thead>
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<th>Population of Portugal</th>
<th>AMD prevalence (%) *</th>
<th>Number with AMD</th>
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<td><strong>Total women</strong></td>
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<tr>
<td><strong>Total</strong></td>
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6. Incidence of AMD

Data on the incidence of AMD were mainly provided by a few population-based studies from industrialized countries. They confirm the observations from prevalence studies, with an exponential increase of the incidence of late AMD with age, and similar incidence rates in Caucasian populations from the United States (20), Australia (21-22) and Europe (23,24,25). These studies have largely contributed to the recognition of large soft drusen and pigmentary abnormalities as the precursors of late AMD, thereby giving a more precise outline of the definition of early AMD, although differences in classifications do persist.

Very few data are available on the incidence of late AMD in other ethnic groups and geographical areas. The Barbados study confirmed a low incidence of early and late AMD in subjects of African origin (20). In the Hisayama Study, the incidence rates of early and late AMD were similar to those observed in Caucasians, confirming the prevalence observations (22).

7. Conclusion

In conclusion, the worldwide epidemiology of AMD is slowly emerging. There is still a total lack of data in large areas of the world (South America, Africa) and in many ethnic groups (in particular ethnic minorities from Europe). The initial feeling that AMD is more frequent in Caucasians and less frequent in “pigmented” populations is challenged by observations. Indeed, while African-Americans and Hispanic-Americans appear at lower risk of late AMD than Caucasians, the frequency of AMD seems as high in Asians as in Caucasians. While the picture of the epidemiology of AMD gets clearer, it becomes more complicated. Understanding the reasons of these ethnic and geographical variations will contribute to a better knowledge of the determinants of AMD.

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References:


The epidemiological studies conducted in the past 25 years have helped identifying major modifiable risk factors for AMD. In particular, smoking and nutrition appear ever more important in determining the occurrence of AMD, and may, in the future, lead to prevention strategies.

2. Smoking

Smoking is the best characterized risk factor for AMD(1). The initial observations performed in Caucasian populations from Western countries(2), are now being confirmed in other ethnic groups, such as African-Americans(3), Latino-American(4), or Asian populations(5-7). In most studies, the risk for late AMD was multiplied by 2.5 to 4.5 in current smokers. In addition, the dose-response relationship was explored in some studies(6,8-12). Most of these studies found that the risk for AMD increased with increasing number of cigarettes smoked per day, and, even more, with number of pack-years smoked, which is an indicator of cumulative smoking over the lifetime (mean number of packs smoked/day x duration of smoking (years)). Moreover, the risk for AMD appeared to decrease with time from cessation of smoking. Former smokers generally demonstrated a lower risk for AMD than current smokers. Several studies have shown that the risk for AMD in subjects having ceased smoking for more than 20 years was similar to the risk in never smokers(8-13,15). One study suggested that passive smoking is also associated with an increased risk for AMD(15), while this association did not reach statistical significance in another study(16). Overall, the strength of the association (about 3-fold increased risk in current smokers), its consistency across different populations, the observation of a clear dose-response relationship in most studies, and the decrease of the risk with stopping smoking are all strong arguments in favour of a causal role of tobacco smoking in the aetiology of late AMD.

The exact mechanisms by which smoking increases the risk for AMD are unclear, and probably multiple, including oxidative stress, inflammation and decreased macular pigment. Finally, recent studies gave important insights on the joint effects of smoking and genetic polymorphisms, showing that the risk for AMD is particularly high in smokers bearing at-risk polymorphisms in the CFH or LOC387715 genes(19-21).

Other vascular risk factors, such as systemic hypertension, obesity, diabetes, plasma lipids or alcohol drinking may be associated with an increased risk of AMD, but epidemiological studies have been inconsistent in this field(22). At the time being, they remain putative, but not clearly identified risk factors for AMD.

3. Nutritional factors

More recently, epidemiological studies have focused on the potential association of AMD with nutritional factors. Mainly three types of nutritional factors have been investigated for their potential protection against eye ageing: antioxidants (mainly vitamins C and E, zinc), the carotenoids lutein and zeaxanthin and omega 3 polyunsaturated fatty acids (PUFA).

The retina is particularly susceptible to oxidative stress because of the high level of in-site reactive oxygen species production, due in particular to light exposure and high metabolic activity(23). Epidemiological studies are
mostly in favour of a protective role of antioxidants for AMD\textsuperscript{24}. Moreover, the Age-Related Eye Diseases Study (AREDS), a randomized clinical trial performed in the United States and including on almost 5000 subjects supplemented for five years, showed a significant 25\% reduction of the incidence of late AMD with supplementation in antioxidants and zinc, by comparison with placebo\textsuperscript{25}. In this field, data from the United States should be extrapolated to European populations with caution. Indeed, vitamin supplements are widely used in the American population, while this is rarely the case in Europe. For instance, two thirds of the AREDS participants used vitamin supplements, in addition to the supplementation tested in the study\textsuperscript{25}. Plasma vitamin C concentration at baseline in the AREDS (before the initiation of the study supplementation) was 62 micromol/l\textsuperscript{25}, whereas it was 31.6 micromol/l in men and 40.5 micromol/l in women of the Pathologies Oculaires Liées à l’Age (POLA) Study, performed in the South of France\textsuperscript{26}. Similarly, in the EUREYE Study, plasma vitamin C concentrations ranged from 35.5 micromol/l to 48.4 micromol/l in seven European countries\textsuperscript{27}. Therefore, antioxidant intake is much lower in European populations than in the United States, with part of European populations being at risk of clinical deficiency in these vitamins. Two European studies suggested that the benefit to be expected from increased antioxidant intake may be more important in our populations with low antioxidant intake. Indeed, in the French POLA Study, we observed an 80\% decreased risk for late AMD in the subjects with higher plasma vitamin E, by comparison to those with lower concentrations\textsuperscript{28}, a much stronger effect than the 25\% reduction in risk observed in the AREDS Study. Moreover, we observed a 25\% reduction in risk for early ARM, whereas the AREDS Study showed no benefit of antioxidant supplements for early ARM. Similarly, results from the Rotterdam Study

Figure 1. Association of the risk of AMD with plasma levels of lutein and zeaxanthin in cross-sectional and case-control studies (odds-ratios with 95\% confidence interval)

OR below 1 suggest a protective role and OR greater than 1 suggest a deleterious role. References of the cited studies: Beaver Dam\textsuperscript{31}; EDCC\textsuperscript{32}; NHANES III\textsuperscript{33}; Gale et al\textsuperscript{34}; POLA\textsuperscript{35}
showed a decreased risk for early ARM in subjects with high dietary intake of vitamin E or zinc, by comparison with those with low intake. A European supplementation study would be needed to better assess the benefit of antioxidant supplementation in European populations.

A more recent research domain evaluated the role of two carotenoids, lutein and zeaxanthin, in the protection of the retina and the lens. These carotenoids accumulate in the macula, where they are known as the macular pigment. Besides their antioxidant properties, they probably act as a filter against the phototoxic effects of blue light. To date, five epidemiological studies have assessed the associations of the risk of AMD with plasma concentration of lutein and zeaxanthin. As shown in Fig. 1, all five studies showed a decreased risk for AMD in subjects with high plasma concentrations of lutein and zeaxanthin, although the association was statistically significant only in 2 studies. With regard to dietary intake, four prospective population-based studies were published. These studies assessed the risk for developing AMD (in subjects initially free of AMD), according to their dietary intake of lutein and zeaxanthin. As shown in Fig. 2, the results for these dietary studies are less clear than for those on plasma measurements. Only one study found a significantly reduced risk for AMD in subjects with high dietary intake of lutein and zeaxanthin. However, dietary assessment methods rely on the subjects’ memory and perceptions and face the difficulties of the extreme day-to-day variability of human diet, the bias in reporting due to social standards and nutritional recommendations and the estimations of nutritional contents of food items. Biomarkers have the advantages of being objective, and of taking into account individual variations in bioavailability and metabolism. For instance, smoking and obesity are known to decrease the bioavailability of carotenoids. Despite normal dietary...

Figure 2. Associations of the risk for AMD with dietary lutein and zeaxanthin, in published epidemiological prospective studies. References of the cited studies: Beaver Dam; Health Professionals; Rotterdam; Blue Mountains.
intake in lutein and zeaxanthin, subjects may be at higher risk for AMD because of decreased bioavailability, associated with lower plasma concentrations of these components. However, currently available studies including plasma measurements are cross-sectional or case-control studies, where the plasma measurements were performed in subjects already affected by the disease. The stronger associations found in these studies may therefore be explained by reverse causality (i.e. plasma carotenoids were lower because of change of dietary habits in subjects with AMD, for instance).

**Globally, the few available epidemiological studies suggest a protective role of lutein and zeaxanthin in AMD, but other studies are needed in this field, in particular larger, prospective studies including dietary and plasma measurements.**

Finally, omega 3 PUFA include a precursor (alpha-linolenic acid (ALA)), and three long-chain derivatives (eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA)). ALA is an essential nutrient, since humans cannot synthesize it de novo and therefore rely on diet as its sole source. Synthesis of the long-chain derivatives is very limited in humans,$^{41}$ who must therefore rely on their dietary supply, mainly by fish and seafood. Long-chain omega-3 PUFA have important structural and protective functions in the retina. DHA is a major component of the photoreceptors.$^{42}$ They also have protective functions, including the systemic anti-inflammatory, anti-angiogenic and anti-apoptotic functions and specific actions in the retina such as increase in lysosomal acid lipase, leading to increased lipid degradation in the retinal pigment epithelium.$^{43}$

As shown in Fig. 3 and Fig. 4, seven case-control$^{43-44}$ or cross-sectional studies$^{45-49}$ and five prospective studies$^{50-54}$ assessed the associations of dietary intake of long-chain omega 3 PUFA or fish with AMD. In spite of differences in populations, methods and types of studies, all studies showed a reduced risk for AMD in subjects with high dietary intake of long chain omega 3 PUFA or fish, although some of these associations did not reach statistical significance. Most of these studies were grouped in a meta-analysis performed in 2008, which concluded at

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**Figure 3. Association of the risk for AMD with dietary long chain omega 3 or fish (cross-sectional and case-control studies). References of the cited studies: EDCC$^{43}$; Twin Study$^{45}$; AREDS$^{44}$; Beaver Dam$^{46}$; NHANES$^{47}$; POLA$^{48}$; EUREYE$^{49}$**
Modifiable risk factors for AMD

a 38% reduction in risk for AMD in subjects with high dietary long-chain omega 3 PUFA\(^{(55)}\). By contrast, two recent studies found an increased risk for ARM in subjects with high omega 3 PUFA\(^{(56-57)}\). However, in these studies, only total omega 3 PUFA intake was studied, including ALA and long-chain omega 3 PUFA, while most studies found a reduced risk for ARM only with high long chain omega 3 PUFA, in accordance with the scientific rationale. As stated in one of these studies\(^{(57)}\), the main source for ALA is vegetable oil, which is also the main source of omega 6 PUFA, and was found to increase the risk for ARM in some studies\(^{(43,53)}\). Future studies need to separate the precursor from the long chain derivatives.

Globally, available epidemiological studies strongly suggest a reduced risk for AMD in subjects with high consumption of long chain omega 3 fatty acids and fish.

In addition, some studies have suggested that the risk for AMD may be decreased in subjects with high intake of vitamins B\(^{(56-63)}\). Consistently, supplementation with vitamins B reduced the incidence of AMD in a randomized interventional study\(^{(64)}\). A role for vitamin D in the aetiology of AMD has also been suggested\(^{(65)}\).

4. Light exposure and cataract extraction

Sunlight exposure has been investigated as a potential risk factor for AMD. Light exposure may have deleterious effect on the eye, in particular through the production of reactive oxygen species\(^{(66)}\). Only blue light reaches the retina, since ultraviolet radiations are absorbed by the cornea and the lens. Intense blue light exposure has been shown to induce retinal damage\(^{(66)}\), and the macular pigment, which absorbs blue light, is thought to protect the macula against photo-toxic damage\(^{(60)}\). However, results have been inconsistent in the few studies that have investigated the associations of the light exposure with AMD in humans\(^{(67,68,74)}\). Globally, the available studies suggest that the effect of sunlight exposure in the aetiology of
AMD is at most modest. Interestingly, a recent study evidenced an association of the risk for AMD with blue light exposure, only in those subjects with low plasma antioxidants and zeaxanthin(27). This suggests that light exposure may increase the risk for AMD only when defences against the produced reactive oxygen species are not appropriate. Sunlight exposure therefore does not appear to be a major determinant of AMD, but may be a risk factor in susceptible individuals. Appropriate nutritional intake in antioxidants and macular pigment may be particularly important in subjects highly exposed to light. These data will need to be confirmed in future studies.

Besides, cataract surgery was associated with a major increase in AMD incidence in a few studies(7,75-81), although not all(82-83). For instance, in a pooled analysis of two major population-based studies (Beaver Dam and Blue Mountains), eyes which had undergone lens extraction had a 5.7-fold increased risk of developing late AMD(79). The reasons for increased risk of AMD in aphakic and pseudophakic eyes are unknown, but may include increased light exposure. Indeed, the lens naturally absorbs ultraviolet light, and, with the lens yellowing observed with ageing and cataract, also part of blue light. In this context, use of blue light filters in the implanted artificial lenses have been proposed(84), and are currently widely used, although their potential effect on the reduction of incidence of AMD has not been evaluated.

**Because cataract surgery is the most frequent surgical procedure in most industrialized countries, the potential increased risk of AMD in operated eye needs further study, in order to better characterize it, to determine its causes and to identify strategies to limit this potentially deleterious effect.**

In conclusion, AMD is emerging as a disease resulting from major genetic susceptibility, the effect of which is modulated by lifestyle. Among lifestyle factors, smoking is the best characterized risk factor, now considered as causal, while the role of nutrition is increasingly identified. The respective roles of antioxidants, macular pigment and omega 3 fatty acids, together with other potential nutritional factors such as vitamins B and D, will be better understood in the future. In this field, several new epidemiological studies are being conducted, among which, in France, the Alienor Study (85). This population-based cohort study aims at assessing the association of AMD with nutritional, vascular and genetic risk factors. It bears on almost 1000 subjects, recruited from an existing cohort study on brain ageing (the 3C Study). The main nutritional factors studied are lutein and zeaxanthin, antioxidants (vitamins C, E, zinc) and omega 3 fatty acids, and are measured both in the diet, in plasma and, for lutein and zeaxanthin, on the retina. This study will add to the existing literature in this field, which is still relatively scarce and partially inconsistent. Moreover, several large controlled interventional trials, including the AREDS2 Study, will help demonstrating their causal role in the aetiology of AMD. Finally, the role of light exposure (in particular blue light) does not seem to be major determinant in this disease, but may be important in subgroups of the population (subjects with low antioxidant and macular pigment intake, subjects undergoing cataract surgery).
References:


Pathogenic Mechanisms

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1. The outer retina

Age-related changes that predispose to age-related macular degeneration (AMD) occur in the outer retina, more specifically the region that includes the photoreceptors (PR), the retinal pigment epithelium (RPE), Bruch’s membrane and the choriocapillaris.

Retinal anatomy is highly organized and vascular and avascular compartments are strictly segregated in the retina(1). The blood-retinal barriers, inner and outer, are fundamental for the integrity of structure and optimization of function in neuro-sensorial retina(2).

The outer blood-retinal barrier is formed, among its various components, by the RPE tight junctions. The intercellular cohesiveness of the RPE is not easily disrupted. Tight junctions appear as a necklace of strands that encircle each cell, binding each cell to its neighbours in a monolayer that separates the outer layer of the neural retina from the choriocapillaris(3). Choriocapillaris is a great vascular network of fenestrated capillaries with high blood flow, fundamental for the metabolism of outer retina. This outer blood-retinal barrier retards transepithelial diffusion through the paracellular spaces(3).

The RPE is a polarized epithelium that consists of a continuous pavement-like monolayer of cuboidal shaped cells that in macular area are tall, narrow and highly uniform in size and shape(4). Interdigitation of the apical processes of the RPE with the cone and rod outer segments provides only a tenuous adhesion of the RPE to the sensory retina(5).

RPE cells have at least ten known functions, but regeneration of visual pigments, transport of fluids and ions between photoreceptors and choriocapillaris, formation and maintenance of the interphotocapillaris and Bruch’s membrane and phagocytosis of outer segments of PR should be emphasized(6).

Bruch’s membrane is a thin, acellular and well-delineated membrane with five layers. From internal to external, these layers are: the basement membrane of the RPE, the inner collagenous zone, the elastic tissue layer, the outer collagenous zone and the basement membrane of the choriocapillaris. It is composed of elements from both, the retina and choroid, but is an integral part of the choroid(7). Its inner surface is smooth, whereas its outer surface is composed of a series of collagenous protrusions that extend externally to form the pillars separating and supporting the choriocapillaris(5). Due to its specific location and properties, this tissue is thought to be a vital limiting layer for metabolic transport between the RPE cells and the choriocapillaris(8).

The choriocapillaris consists of a continuous layer of fenestrated endothelial cells surrounded by a basement membrane. In the macula the choriocapillaris is arranged in a lobular pattern of highly concentrated interconnecting capillaries supplied by a central arteriole and drained by circumferential venules(5,9). The fenestrations, 60-80 nm in diameter, are abundant and seem to play an important role in permitting the passage of glucose and vitamin A to the RPE and retina. The choriocapillaris supplies oxygen and nutrients to Bruch’s membrane and the outer third of the retina, except in the macula, where it supplies the entire retina(5). The peculiar structure of the choroidal vascular tree in the macula provides this area with the highest rate of blood flow of any tissue in the body(5).

2. Aging changes in outer retina and early AMD

With aging the lumina of the choriocapillaris and the choroidal thickness become reduced by half(10).
With the advancement of age, both the thickness and complexity of Bruch’s membrane increase primarily due to extracellular matrix remodelling and accumulation of inclusions in this region\(^1\). Bruch’s membrane calcifies and doubles in thickness between the ages of 10 and 90 years\(^2\). There is a linear thickening due to deposits of collagen, lipids and debris. After the 30’s its lipid concentration increases during life and consequently the fluid permeability and nutrient transport across the membrane decreases\(^3\). In normal conditions Bruch’s membrane acts as an intercellular matrix regulating survival of adjacent RPE and choriocapillaris cells. Its diminished function results in apoptosis of these cells from incorrect cell adhesion\(^4\). On the other hand extracellular deposits around Bruch’s membrane instigate chronic inflammation, invasion by dendritic cells and release of inflammatory cytokines, angiogenic factors and immune complexes\(^5\). The RPE is a monolayer of regularly arranged hexagonal cells that spans the retina from the margins of the optic disc anteriorly to the ora serrata. The number of RPE cells diminishes with age. Macular RPE cells become wider, flatter and increase in height with advancing age\(^6\). In each RPE cell there is a progressive accumulation of lipofuscin during life and in people over 80 years of age, the debris can occupy more than one fifth of the total volume of an RPE cell\(^7\). RPE cells have a brown color in young eyes but with age, they become increasingly more golden colored, owing to the accumulation of lipofuscin pigment granules\(^8\). Lipofuscin in the RPE is the source of fundus autofluorescence. The major component of lipofuscin is N-retinylidene-N-retinaldehyde (A2E) a retinoid product of the visual cycle\(^9\). The A2E produced interferes with the function of RPE cells, leading to its apoptosis and subsequent geographic atrophy\(^10\). Age-related changes also include a decrease in the number of melanin granules, loss of basal digitations and irregularity in shape. The RPE cells, become separated from their basal membrane by membranous debris and abnormal secretory products and, subsequently occurs deposition of collagen and fibronectin and latter formation of basal laminar deposits\(^11\). Basal laminar deposits are composed of basement membrane protein and long-spacing collagen located between the RPE plasma and basement membranes\(^12\). Basal laminar deposits are considered the precursors of age-related macular degeneration and can appear around the age of 40 years\(^13\). Basal linear deposits consist of granular, vesicular or membranous lipid-rich material located external to the basement membrane of the RPE, in the inner collagenous layer of Bruch’s membrane, and represent a specific marker of AMD\(^14\). These two types of deposits can only be shown on pathological specimens and not by clinical evaluation\(^15\). The combination of the deposits with secondary changes in the RPE results in the formation of drusen. Drusen are localized deposits of extracellular material lying between the basement membrane of the RPE and the inner collagen layer of Bruch’s membrane\(^16,17\). Drusen often have a core of glycoproteins but they also contain fragments of RPE cells, crystallins, apolipoproteins B and E, and proteins related to inflammation such as amyloid P and ß, C5 and C5b-9 complement complex\(^18-21\). Drusen change in size, shape, color, distribution and consistency with the passing years\(^22\). Small drusen are defined as being less than 63μm in diameter\(^23\). The presence of small, hard drusen alone is not sufficient to diagnose early AMD. These deposits are ubiquitous and the new development of small drusen in an adult eye without prior evidence of hard drusen is not age dependent\(^24\). Hard drusen are discrete nodules or deposits composed of hyaline-like material. During fluorescein angiography hard drusen behave as pin-point window defects\(^25\). Soft drusen are larger and associated with pigment epithelium detachment and diffuse abnormal Bruch’s membrane alterations\(^26,27\). Soft drusen have a tendency to cluster and merge with one another demonstrating confluence\(^28\). During fluorescein angiography soft drusen hyperfluoresce early and either fade or stain in the late phase\(^29\). Drusen can be visible in opthalmoscopy when their diameter exceeds 25μm as dots ranging in color from white to yellow\(^30\). However soft drusen are clinically identified whenever there is sufficient RPE hypopigmentation or atrophy overlying diffuse Bruch’s membrane thickening, or, when there are focal detachments within this material. These findings suggest that the clinical identification of soft drusen identifies an eye with diffuse changes at the RPE-Bruch’s membrane complex\(^31\). When they become larger (>125μm), and greater the area that they cover, the risk of late AMD becomes higher\(^32\). The RPE degeneration and non-geographic atrophy of the RPE are characterized by pigment mottling and stippled hypopigmentation with thinning of the neurosensory retina\(^33\). Histopathology shows mottled areas of RPE hypopigmentation or atrophy overlying diffuse basal linear and basal laminar deposits\(^34\). Incidence and prevalence rates of RPE depigmentation are age dependent\(^35\). Focal hyperpigmentation of the RPE, clinically evident as pig-
ment clumping at the level of the outer retina or sub-retinal space, increases the risk of progression to the late phases of the disease[31,33,37].

3. Late AMD

The primary clinical characteristic of late dry AMD is the appearance of geographic atrophy (GA) of RPE. On microscopy, GA is seen as abnormal RPE cells with hypotrophy, atrophy, hypertrophy, hyperpigmentation, hypopigmentation, migration, loss of photoreceptors, attenuation of Bruch’s membrane and choriocapillaris degeneration[38,39]. Geographic atrophy is clinically characterized by roughly oval areas of hypopigmentation that allows the increase visualization of the underlying choroidal vessels and is the consequence of RPE cell loss. Loss of RPE cells leads to gradual degeneration of photoreceptors and thinning of the retina that may extend to the outer plexiform and inner nuclear layers[6,24]. Compensatory RPE cell proliferation leads to hyperpigmentary changes frequently observed at the periphery of the hypopigmented areas[60]. The atrophy of RPE is usually more severe than the loss of choriocapillaris but the choriocapillaris seem to be highly constricted in areas of complete RPE cell loss[38].

In neovascular AMD early choroidal neovascularization occurs under the RPE[40] and eventually breaks through[41], leading to accumulation of lipid-rich fluid under the retinal pigment epithelium or neuroretina. In haemorrhagic forms blood breaks through the RPE into the subretinal space and sometimes through the retina and into the vitreous[32].

The pattern of growth of CNV often simulates that of a sea fan with radial arterioles and venules supplying and draining a circumferential dilated capillary sinus[5]. As neovascularization of the sub-RPE space occurs, initially the blood flow through the neovascular network is sluggish and there is little or no exudation. This is a period of occult neovascularization and the overlying RPE and neuroretina may be minimally affected[5]. With an increase of blood flow through the network, the endothelium decompensates and exudation extends into the subpigment epithelial space creating in some cases RPE detachments. The exudation may also extend through the RPE and detach the overlying retina.

In type II CNV the new vessels extend from the choroid through defects in Bruch’s membrane enters the space between the photoreceptors and RPE cells and grow laterally in the subretinal space[5]. This is usually accompanied by varying amounts of subretinal exudates and/or blood.

Macrophages have been documented both morphologically and functionally in neovascular AMD[42,43]. Activated macrophages and microglia may secret cytokines and chemokines that promote cellular damage and angiogenesis[44].

Involution of CNV eventually occurs and is associated with varying degrees of subretinal scar tissue, reactive hyperplasia of the RPE and/or atrophy, and can partially or totally replace the neuroretina[19]. The outer nuclear layer can be severely attenuated with a reduction of photoreceptor length of almost 70%[45]. Often anastomosis between the retinal circulation and the underlying choroidal circulation develops within these old disciforme scars[29,46].

Other factors like complement factor H, that down-regulates the alternative complement pathway[47], HtrA1 – a secretory protein and an inhibitor of transforming growth factor ß (TGF-ß)[48] – and ARMS2[49] play a role in development of AMD. However its specific role and relevance in development and progression to neovascular and atrophic forms of age-related macular degeneration are discussed in others chapters of this book.

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References:


Age-related macular degeneration (AMD) is a complex disease with demographic and environmental risk factors (age, diet, and smoking) but also genetic risk factors. In fact, instead of having a single contributory gene, there are multiple genes of variable effects that seem to be involved turning the issue of genetics of AMD a complex one: AMD involves environmental factors and varying susceptibilities to these external factors based upon different genetic backgrounds. The genetic component of the disease has been suspected from family, twin and sibling studies. According to several family studies, patients with a family history of AMD have an increased risk for developing AMD. The concordance for the presence of the disease is greater among hoizogous twins than among heterozygous twins. In 2008, Luo et al. estimated the magnitude of familial risks in a population-based cross-sectional and case-control study. Recurrence risk in relatives indicate increased relative risks in siblings (2.95), first cousins (1.29), second cousins (1.13) and parents (5.66) of affected elements.

Many linkage and association studies have showed that chromosomes 1q (1q25-31) and 10q (10q26) had genes involved in this pathology. It was the completion of the Human Genoma Project, 5 years ago, resulting in the knowledge of the sequencing of the human genome that allowed improved DNA sequencing and mapping technologies and consequently, identification of Single Nucleotide Polymorphism (SNPs).

There are several types of genetic sequences variations (polymorphisms) in the human genome: repeated polymorphisms, insertions and deletions. However the majority of the DNA sequence variations in the human genome is in the form of SNPs which are persistent single changes, substitutions or variants of a single base in at least a population and with a frequency of more than 1% referred as alleles and representing altered forms of a gene: different alleles may produce variations in inherited characteristics. These variants are also important because they serve as genetic markers and in this way they can help in determining those which confer increased or decreased risk of several diseases including AMD.

Dissection of the genetic background of AMD has undergone tremendous progress in the last 2 years. We know, now, some polymorphisms which modulate AMD risk.

### 2. Complement system factors and AMD

#### 2.1 Complement factor H (CFH)

CFH is a negative regulator of alternative pathway of the complement system which means that in normal conditions, it inhibits the alternative pathway complement system. It is encoded by a gene localized in 1q23-32 and its dysfunction may lead to excessive inflammation and tissue damage. Complement activity is very important for the immune responses against pathogens and dying cells but, over-activation can result in complement-mediated damage to nearby healthy tissue cells. It is now accepted that CFH gene is an important susceptibility gene, harbouring variants and haplotypes (short DNA sequences containing alleles) associated with increased and reduced risk of AMD. Six CFH gene variants have been reported in AMD association studies as major genetic factors for developing AMD in Caucasians: rs1061170, (CFH Y402H); rs3753394; rs800292; rs1061147; rs380390; rs1329428. However in the Chinese and Japanese populations only three of these CFH SNPs (rs1329498, rs800292 and rs3753394) were associated with risk of AMD. So it is possible that CFH could play a central role in AMD pathogenesis and that multiple SNPs
that alter CFH function might contribute to the development of AMD. Their importance varies among the race of the population.

In the variant (polymorphism) CFH Y402H of the CFH gene, there is a substitution on the nucleotide in exon 9 (1277) where thymine (T) is changed for cytosine (C) (rs1061170) which is the allele risk. This change leads to the substitution of the aminoacid in the position 402 in the protein, from tyrosine (Y) to histidine (H).

Homozygote CC or heterozygote TC can account for 50% of AMD cases. The risk attributable for a disease is the rate of disease among individuals with a given characteristic minus the rate of the disease among individuals without that characteristic. The population attributable risk (PAR) in individuals with this polymorphism for developing AMD is 43% to 50%[11,12,18].

When compared with those with no risk allele TT, one copy of the Tyr402His polymorphism (heterozygous for the risk allele TC), increases the risk of AMD by a factor of 2.2 to 4.6 (these individuals are at least twice and half more likely to develop AMD) and two copies of the risk allele (homozygous for the risk allele CC) increases the risk by a factor of 3.3 to 7.4 in Whites[19].

In addition to the common risk haplotype carrying the C allele of CFH Y402H, haplotype analysis of CFH has revealed two common protective haplotypes: homozygous deletions CFHR1 or CFHR3.

The gene cluster of CFH includes other “CFH-related genes”: CFHR1, CFHR2, CFHR3, CFHR4 and CFHR5. This means that the CFH gene resides within the region of complement activation (RCA), which includes also five “CFH–related” genes. While the function of the CFH related genes is largely unknown, the high degree of sequence similarity between these genes and the suggestion that they arose out of duplication events with CFH, suggest an overlapping function of the CFH-related genes in immune system function and/or regulation.

There is a common and widespread (commonly found in many different populations in the world) deletion within the RCA locus that encompasses the CFHR1 and CFHR3 genes. However the frequency of homozygous CFHR1 or CFHR3 deletion shows considerable variation between ethnic groups and occurs in 17.3% of African populations, 15.9% of African-American, 6.8% of Hispanic, 4.7% of Caucasian and 2.2% of Chinese cohorts[20]. This is in agreement with AMD less frequency among African-Americans compared with Caucasians and Chinese populations. CFH1 and CFH3 protein may compete with CFH for C3 binding and therefore interfere with normal regulation of the complement system.

Those individuals, who are homozygous for the CFHR1/CFHR3 deletions and, therefore do not express the respective proteins, are highly protected from developing AMD[20].

2.2 Complement factor B (BF), complement component 2 (C2)

Complement factor B (BF) is involved in the activation of the complement alternative pathway and complement component 2 (C2) is involved in the activation of the classical pathway of the complement and both have adjacent genes located 500 base pair apart on chromosome 6p21.3 within the major histocompatibility complex class III region[21]. Haplotypes in BF and C2 have been linked to AMD. In particular, the L9H in BF and the E318D in C2 and also the R32Q in BF and a variant in intron 10 of C2 have been showed to be protective for AMD by Gold et al.[21]. They hypothesized that the significance of the haplotypes is due largely to the BF variants, which are in strong linkage disequilibrium with C2.

BF is a complement activating factor and studies have demonstrated that at least one of the two variants associated with AMD (R32Q BF) leads to an impairment in the complement activation function of BF. This means that the absence of these variants C2/BF can predispose patients to AMD[21].

Thus, much like impaired CFH-mediated complement inhibition confers AMD risk, decreased complement activation by BF might serve to protect against AMD risk.

2.3 Complement component 3 (C3)

C3 is the central element of the complement cascade and a candidate gene to be involved in AMD, since its cleavage product, C3a, not only was found in drusen but also was proved to induce vascular endothelial growth factor expression and promote choroidal neovascularization in both in vitro and in vivo[22-24]. The variants R102G and P314L of the C3 gene significantly increase the risk of early and all subtypes of AMD and this risk seems to be independent of CFH Y402H, LOC387715 A69S and smoking[25].

3. LOC387715/ARMS2 and HTRA1

In 2003 Majewski et al. suggested that chromosome
10q26 might contain an AMD gene(7). Later this finding has been replicated by other genome-wide linkage studies(26) and supported by a genome–scan meta-analysis(5). This locus contains three tightly linked genes: PLEKHA1, LOC387715/ARMS2 (age related maculopathy susceptibility gene 2) and HTRA1, a secreted heat shock serine protease. In 2005 Jakobsdottir et al.(27) found that the strongest association was over LOC387715/ARMS2 and HTRA1, which share an extensive linkage disequilibrium (LD) block harbouring the high risk haplotype.

There has been more dispute than agreement between the studies in what concerns this locus. All initial genetic studies, about ten years ago, lacked statistical power (because small samples were used), used cumbersome genotyping technologies and poorly defined cohort. In recent years, there are some publications of preliminary and unconfirmed genetic associations of the genes in this locus to AMD(28).

### 3.1 ARMS2 (LOC387715) SNP and AMD

The association of ARMS2 gene and AMD has been now replicated in various independent studies especially the advanced form of the disease, that means to say, the “wet” or with choroidal neovascularization and the “dry” or geographic atrophy form of the disease(29-32).

The risk conferring polymorphism consists in a change in the 69 position aminoacid alanine (A) to serine (S).

According to Ross(31), heterozygosis at the ARMS2/LOC387715 (A69A/A69S) is associated with odds ratio (OR) of 1.69-3.0 for advanced AMD while homozygozity for the risk conferring allele (A69S/A69S) results in a OR of 2.20-12.1. The frequency of the risk allele is higher in patients with advanced AMD than in those with early or intermediate AMD(27,33).

Later two studies, based on semiquantitavive expression data of allele associated differences in HTRA1 mRNA or protein levels, suggested a diferent variant (rs11200638) in the promoter of HTRA1 gene as the functional variant(34,35) .

### 3.2 HTRA1 (high temperature required factor A-1) SNP and AMD

HTRA1 gene is located on chromosome 10q26.3, extremely close to the locus of the ARMS2 gene (10q26.13) and because of its role in extracellular matrix homeostasis (its extracellular protease activity may favour neovascularization) and in cellular growth or survival (it is an inhibitor of TGF-β family member(36) and it could play a critical role in controlling TGF-β dependent neuronal survival(37) it seems a possible functional candidate gene.

Four significant SNPs have been reported in the promoter and the first exon of HTRA1: G625A (rs11200638); T487C (rs2672598); C102T, A34A (rs1049331); G108T, G36G (rs2293870). However the most well documented, statistically significant AMD associated SNP is rs11200638 (G625A) in the promoter region.

Caucasians, Chinese and Japanese heterozygous for the risk allele (G/A) have a high OR of 1.60-2.61 and Caucasians, Chinese and Japanese homzygous for the risk allele (A/A), 6.56-10.0(34,35,38-40).

According to Tam et al.(40), there is an increase in population attributable risk (about 5.5 fold increase) by the joint effect of smoking and HTRA1 allele. This means that smokers homozygous for the risk allele had a substantially higher risk of developing wet AMD than non smokers with the risk allele. However Deangelis et al(41), in 2008 reported no interaction between this SNP and smoking.

In what concerns the studies which relate HRTA1 promoter polymorphisms to risk factors for developing AMD, three problems arise according to Allikmets and Dean(28).

The variant encoding the A69S (rs10490924) in ARMS2 and the rs11200638 variant in HTRA1 are almost in complete LD, so it is impossible to assign causality on the basis of allele frequency alone.

10q26 locus doesn’t harbour a wet AMD gene as the authors claimed but a late AMD gene as showned by Weber and colleagues in 2005(30).

All subsequent studies have failed to replicate the functional data(32,42).

This basically means that, as there is strong linkage disequilibrium (LD) across ARMS2-HTRA1 region, genetic association studies alone are insufficient to distinguish between the two candidates. It is also necessary not only the characterization of the extent of the variants associated to the disease but also the analysis of their possible functional relevance in the disease process(42).

Doing this, Fritsche et al.(42) claimed that the functional variant in this locus is the deletion-insertion polymorphism variant 372-815delins54 in the ARMS2 gene. The deletion removes the polyadenilation signal sequence at position 395-400 exclusively used for the addition of a poly A tract 19 bp downstream. The insertion introduces a 64 bp AU-rich element, known for its properties to control mRNA decay in many transcripts that encode a wide variety of proteins(43,45). They demonstrated that it is a major risk factor for AMD: individuals carrying a single copy of the risk allele deletion-insertion in ARMS2 gene have a 2.8-fold increased risk compared with an 8.1-fold increase.
increased risk in homozigous individuals. Their work, also revealed that in homozogous for the deletion-insertion variant, expression of ARMS2 is absent. They localized the ARMS2 protein within the photoreceptor layer namely, to the mitochondria–enriched ellipsoid region of the inner segments and in accordance; they proposed a functional role of ARMS2 in mitochondrial homeostasis. According to Fritsche et al., this suggests, that this polymorphism is the sought-after functional variant with relevance in AMD etiology in 10q26 locus.

However, as Fritsche et al. recognize, it is ultimately required formal exclusion of functional consequences for the remaining polymorphisms on the risk haplotype namely, A69S in ARMS2 gene and HTRA1 promoter variant. The A69S and the InDel are in 100% LD and on the same haplotype and so the effects are not independent to each other(46). The work of Fritsche and colleagues does not eliminate all other possibilities(28), nobody disputes the role of complement genes in AMD in spite of the functional consequences of the disease associated variants being not known for CFH, CFB/C2 and/or C3.

4. Apolipoprotein E gene (ApoE)

The ApoE gene, located on chromosome 19q13.2, is polymorphic and has three isoforms which are common, E2, E3 and E4, coded by different alleles: the ancestral E3 and the SNPs, E2 and E4(47).

Most studies favour a protective role for the ApoE4 SNP and a slight risk-conferring role for ApoE2(47-51). However other studies do not(52-55).

5. SNP genotype and therapeutic responses

5.1 Genotype and response to antioxidative/zinc therapeutics

One of the first works in this area was that of Michael Klein et al.(56). These authors correlated the CFH and LOC387715 A69S genotypes with the therapeutic responses to supplementation with antioxidants and zinc. They concluded that, in homozygous individuals for the non-risk phenotype (Y402Y/Y402Y), 34% of those treated with placebo progressed to advanced AMD, compared with 39% of those treated with antioxidants plus zinc: a reduction of only 11%. A similar interaction was observed in the groups taking zinc versus those not taking zinc: intake had a more protective effect in patients with non-risk alleles compared to patients with risk alleles. These results suggest that the zinc plus antioxidative treatment seems to have less impact on those with the high-risk CFH variant. These authors found no association between LOC387715/ARMS2 A69S and the response to AREDS treatment.

5.2 Genotype and response to intravitreous bevacizumab

Brantley et al(57) investigated 86 wet AMD patients for the association between CFH and LOC387715/ARMS2 genotypes and the response to treatment with bevacizumab. For the CFH genotypes results show that only 10.5% of patients homozygous for the risk - conferring allele Y402H/Y402H genotype demonstrated improved vision with treatment compared with 53.7% of patients homozygous for the non-risk allele Y402Y/Y402Y and the heterozygous for the non-risk allele Y402Y/Y402H variants. They found that the CFH variants are associated to the responses to bevacizumab but that LOC387715/ARMS2 are not.

5.3 Genotype and response to photodynamic therapy

Goverdhan et al(58) and Brantley et al(59) studied the association of the CFH and LOC387715 genotypes with the response to PDT. They found no statistical association with the LOC387715 genotype but a statistical association with CFH genotype: risk allele genotypes have better results than non-risk allele genotypes. However more studies are warranted before any definitive conclusions.

6. Conclusions

1. Genetics variants at two chromosomal loci, 1q31 and 10q26, confer major disease risks, together accounting for more than 50% of AMD pathology(11-14, 27,30).

At present SNPs are the best available markers of AMD risk: SNPs in complement factor H and ARMS2/ HTRA1 capture a substantial fraction of AMD risk and permit the identification of individuals at high risk of developing AMD. Genetic markers can successfully identify individuals whose lifetime risk of age-related macular
2. Understanding the genetic basis of AMD has important implications for the ophthalmologists as it allows the identification of the biochemical pathway for a large proportion of AMD patients, raises the possibility to perform pre-symptomatic diagnostic testing of risk genotypes and to stratify the response to therapy based on genetic risks and supports the development of new therapies being the inhibition of complement a potential one among others that are already being tested.
References


Angiogenesis

1. Definition

Blood vessels develop and grow by three different basic mechanisms: vasculogenesis in which vessels form by concatenation of vascular precursor cells into solid cords that then lumenize; angiogenesis that is the growth of new blood vessels from pre-existing ones; and intussusception in which new blood vessels form by the proliferation of endothelial cells (EC) that form a pre-existing vessel into the vessel lumen, originating two blood vessels that split into two opposite sides(1).

Angiogenesis, the growth of new vessels from pre-existing ones by sprouting of EC into a previously avascular tissue, is an essential process both in embryonic development and in adulthood(1,2). It is a complex multistep process involving extracellular matrix degradation and proliferation, survival, migration and anastomosis of EC(2). The release of extracellular matrix proteases leads to the degradation of blood vessels basal membrane, EC change shape, proliferate, invade stroma and form tubular structures that coalesce. This requires the coordinated action of a variety of anti and pro-angiogenic factors and cell-adhesion molecules in endothelial cells. However, if on one side it promotes tissue repair, on the other hand if imbalanced it promotes tissue damage. If not tightly regulated, the angiogenic process is frequently imbalanced, and associated with several pathological situations(1,3).

2. Angiogenic mediators and modulation of their expression

Angiogenic process requires the activation of series of receptors by numerous ligands including Placental Growth Factor (PIGF), Fibroblast Growth Factors (FGFs), Angiopoietin-1 and -2 (Ang-1 and -2), Platelet-derived Growth Factor (PDGF), Hepatocyte Growth Factor (HGF), Connective Tissue Growth Factor (CTGF) and Transforming Growth Factors (TGF-α e TGF-β), among many others(1,3-9).

However, there is a consensus that the Vascular Endothelial Growth Factor (VEGF) is the most important angiogenic factor and represents the crucial rate-limiting step during angiogenesis(3,10,11). VEGF-A is the prototype member of a gene family that also includes placenta growth factor (PIGF), VEGF-B, VEGF-C, VEGF-D, and the orf-virus-encoded VEGF-E(11). Alternative exon splicing results in the generation of four main VEGF isoforms, which have respectively 121, 165, 189, and 206 amino acids after the signal sequence is cleaved (VEGF121, VEGF165, VEGF189, and VEGF206). Less frequent splice variants have also been reported, including VEGF145, VEGF183, VEGF162, and VEGF165b(8,11).

VEGF mediates its biological functions in endothelium through binding two highly related receptor tyrosine kinases (RTKs), VEGFR-1 and VEGFR-2. It is generally agreed that VEGFR-2 is the major mediator of the mitogenic, angiogenic and permeability-enhancing effects of VEGF-A(3). VEGFR-1 binds not only VEGF-A, but also Placenta Growth Factor (PGF) and fails to mediate a strong mitogenic signal in endothelial cells. It is now generally agreed that VEGFR-1 plays a role in modulation of activity of VEGF(10).

The mediators of angiogenic process can be modulated by some molecules and microenvironmental conditions. VEGF is upregulated by cyclo-oxygenase (COX-2)(12); inflammation with the hypoxic environment and the cells involved in inflammatory process, release huge amounts of factors that exert effects on EC and degrade the extracellular matrix(2).

Angiogenesis can also be suppressed by inhibitory molecules, such as interferon-α, thrombospondin-1, angiostatin, endostatin or pigment epithelial-derived factor (PEDF)(13-17).
It is the balance of stimulators and inhibitors that tightly controls the normally quiescent capillary vasculature. When this balance is upset pathological angiogenesis develops\(^\text{(18)}\).

### 3. Angiogenesis during development of retinal vasculature

During embryogenesis retinal vascularization begins in the most superficial (or inner) retinal layers at the optic nerve head, and radiates outwards from this central point. It reaches the retinal periphery just before birth\(^\text{(19)}\). The migration of large numbers of vascular precursor cells (VPCs) from the optic disc is the first event in human retinal vascularization, apparent before 12 weeks gestation\(^\text{(20)}\). They proliferate and differentiate to form a primordial vascular bed centered on the optic disc. Thus, vasculogenesis is responsible for the formation of the primordial vessels of the inner (superficial) plexus in the central human retina\(^\text{(21)}\). Formation of retinal vessels via vasculogenesis appears independent of metabolic demand and hypoxia-induced VEGF expression\(^\text{(22)}\).

Angiogenesis is responsible for the formation of the remaining retinal vessels, including increasing vascular density in the central retina, vessel formation in the peripheral retina of the inner plexus, and formation of the outer plexus and the radial peri-papillary capillaries\(^\text{(22)}\). Formation of the outer plexus begins around the incipient fovea between 25 and 26 weeks of gestation, coincident with the signals indicative of a functional visual pathway and photoreceptor activity\(^\text{(21)}\). The timing and topography of angiogenesis in the human retina supports the “physiological hypoxia” model of retinal vascular formation, in which angiogenesis is induced by a transient but physiological level of hypoxia as a result of the increased metabolic activity of retinal neurons as they differentiate and become functional\(^\text{(23)}\).

### 4. Angiogenesis in retina and choroidal pathologies

Retinal anatomy is highly organized and vascular and avascular compartments are strictly segregated in the retina\(^\text{(1)}\). The blood-retinal barriers, inner and outer, are fundamental for the integrity of structure and optimization of function in neuro-sensorial retina\(^\text{(24)}\). Pathological retinal and choroidal angiogenesis generates chaotically orientated and physiologically deficient vessels that do not conform to neuronal histology, which can lead to vision-threatening exudation and haemorrhage\(^\text{(2)}\).

Angiogenesis is a key aspect in many ocular pathologies that are leading causes of blindness in the world, such as neovascular age-related macular degeneration (AMD), diabetic retinopathy, retinopathy of prematurity (ROP), central retinal vein occlusion and other diseases associated with ischemia and neovascularization\(^\text{(25)}\).

Although angiogenesis is a highly complex and coordinated process requiring multiple receptors and ligands in endothelial cells, VEGF is a hypoxia-inducible cytokine that appears to be a pivotal element required for the process in a variety of normal and pathological circumstances\(^\text{(3,10)}\). Vascular endothelial growth factor is a surrogate angiogenic marker, since it acts not only as a mitogen, but also as a survival factor for endothelial cells (EC)\(^\text{(2)}\). Furthermore, it is also involved in the stimulation of the invasive and migration capacity of EC and in the enhancement of vascular permeability\(^\text{(10)}\).

### 5. Angiogenesis and Age-related Macular Degeneration

The diagnosis of age-related macular degeneration rests on signs in the macula, irrespective of visual acuity\(^\text{(4)}\). The stages of age-related macular degeneration are categorized as early, in which visual symptoms are inconspicuous, or late usually associated with severe loss of vision\(^\text{(27)}\). Early AMD is characterized by the presence of drusen and/or hyperpigmentations or small hypopigmentations\(^\text{(26)}\). Late AMD has “dry” and “wet” forms. However, in the same patient we can find either the dry form in one eye and the wet in the other eye, or the two forms in the same eye. Moreover with time we can see the conversion of wet in dry or dry becoming wet\(^\text{(28)}\).

Age-related changes that predispose to AMD occur in the outer retina, more specifically in the region that includes the photoreceptors, the retinal pigment epithelium (RPE), Bruch’s membrane and the choriocapillaris. The aging-dependent alterations in the outer retina have been already discussed in another chapter. AMD-related visual loss is a complex process starting by the deposition of debris in the outer retina\(^\text{(29)}\). The deposition of insoluble material, the calcification and increase in thickness of Bruch’s membrane, and a less fenestrated and thinner choriocapillaris leads to photoreceptors/retinal pigment epithelium hypoxia resulting in a stimulus for VEGF release\(^\text{(28,30-32)}\).

All the aging changes in outer retina compromise the
nutrition of photoreceptors and RPE and create a favourable ambiance for the development of choroidal neovascularization. However other factors – genetic and environmental – are also important, but their roles in the development of CNV is discussed in other chapters of this book.

In general terms there are two basic CNV growth patterns, based on the anatomical position of the abnormal vessels with respect to the RPE monolayer, which are related to the Gass classification of choroidal neovascularization. Type 1 signifies CNV located in the plane between the RPE and Bruch’s membrane and type 2 neovascularization means that the vessels have penetrated the RPE layer to proliferate in the subneurosensory space.

In type 1 growth pattern after breaking through Bruch’s membrane, tufts of CNV extend laterally under the RPE in a horizontal fashion facilitated by the natural cleavage plane between basal laminar deposits and a lipid rich Bruch’s membrane. This growth pattern recapitulates the choriocapillaris and can provide some nutrients and oxygen to an ischemic RPE/outer retina.

The type 2 growth pattern occurs usually with one or few ingrowth sites with vascular leakage under the RPE/outer retina, leading to acute visual symptoms.

Recently, Yannuzzi proposed a type 3 neovascularization, for retinal angiomatous proliferation (RAP), indicating proliferating vessels within or below the retina itself. This mixed neovascularization, with a presumed dual origin, may have intraretinal neovascularization driven by angiogenic cytokines from Müller cells, endothelial cells, pericytes, and retinal glial cells, and CNV driven by cytokines from the RPE. There is hypothetically neovascularization extending anteriorly from the choroid in conjunction with retinal neovascularization progressing posteriorly, with both circulations destined to anastomose. The reason that growth patterns vary according to disease and individual may be related to genetic predispositions, environmental mechanisms, variations in composition and anatomy of Bruch’s membrane, cytokine distribution, or other causes.

During the dynamic process of development of CNV there is a balance of angiogenesis promoters and inhibitors. In the initiation stage, the RPE and photoreceptors produce VEGF. There is also production by RPE of Interleukin-8 (IL-8) and Monocyte Chemoattractant Protein-1 (MCP), which attract monocytes from the choriocapillaris along the outer surface of Bruch’s membrane. The macrophages tend to concentrate around sites of vascular ingrowth through the Bruch’s membrane and express Tumor Necrosis Factor-α (TNF-α) and Interleukin-1 (IL-1), which up-regulate complement factor-B, activate the complement alternative pathway in the subretinal space, and stimulates RPE cells to produce more VEGF.

After initiation, CNV grows to a certain size and progresses through the tissue planes by the action of Matrix Metalloproteinases (MMP) produced by EC and macrophages. During this stage of active growth, Angiopoietins (Ang-1 and 2) are expressed, FGFs are produced by RPE and EC, and TGF-β is produced by the RPE.

CNV stabilizes during the active stage due to a steady state established between MMP and tissue inhibitors of metalloproteinases, Ang-1 and 2, PEDF and VEGF, PDGF and VEGF, plasminogen and fibrin, and others. At some point the balance shifts toward antiangiogenic, antiproteolytic and antimigratory activity resulting in the involutional stage of CNV. When this occurs the angiogenic/proteolytic/migratory cytokine production decreases with a shift toward TGF-β and tissue inhibitors of metalloproteinases production by the RPE. In this involution stage the CNV may become collagenized and form a disciforme scar.
References:


Age-related macular degeneration (AMD) remains the leading cause of irreversible vision loss in the developed world among individuals older than 50 years \((1,2,3)\). Patients with intermediate to large soft/confluent drusen with or without hyper or hypopigmentation areas in the macula and no neovascular membrane or geographic atrophy are considered to have early age related maculopathy (ARM). Geographic atrophy and wet age related degeneration (AMD) are more advanced forms of AMD that are more often associated with vision disability.

Although the treatment of AMD has evolved to include laser photocoagulation, photodynamic therapy, surgical macular translocation and antiangiogenic agents, treatment options for advanced AMD are limited. Furthermore, the early form of ARM, albeit less devastating than the wet form, has even fewer viable treatment options.

AMD is characterized by ageing changes at photoreceptors, retinal pigment epithelium (RPE), Bruch’s membrane and choroid \((4,5,6)\). Such ageing changes are considered to play a major role in development and progression of AMD. AMD is a bilateral disease and approximately 10 to 20 % of patients with early ARM will develop the wet form of AMD. Whereas patients with early ARM in both eyes are at increased risk of developing either geographic atrophy or wet AMD, once wet AMD develops in one eye there is a higher risk of subsequent development of choroidal neovascularization (CNV) in the second eye \((7,8)\). Several funduscopic findings have been associated with increased risk of development of CNV in fellow eyes of patients with unilateral neovascular AMD. Various reports have emphasized the devastating effects of CNV in the visual function of these patients \((7,9,10)\).

It is crucial to understand the natural history of the development of CNV or the process underlying the conversion from early ARM to late AMD. A better understanding of the involved pathophysiological process or the identification of biomarkers for the conversion would enhance our ability to diagnose and treat the wet AMD, to develop better therapies and eventually to prevent vision loss associated with the disease \((13)\).

This review summarizes the various biomarkers of AMD and analyzes whether or not they may, one day, be exploited to determine risks of disease onset, measure progression of disease or even assess the effects of treatment of AMD. Potential biomarkers are important to identify since some might be utilized to reflect the disease state of a particular patient and to individualize therapy. Although studies have yielded promising results for nutrient and inflammatory biomarkers, these results have been inconsistent. At present, the best available marker of AMD risk is single nucleotide polymorphisms (SNPs). SNPs in complement factor H (CFH) and PLEKHA1/ARMS2/HtrA1 capture a substantial fraction of AMD risk and permit the identification of individuals at high risk of developing AMD.

Patients with AMD in the first eye are known to have high risk of bilateral involvement. In prospective studies in white populations, the annual rate of fellow eye involvement was reported to be around 6% to 9% \((9,12,13)\). The characterization of early ARM phenotypes is challenging. By combining different imaging modalities of the macula and correlating this information, we are better able to determine the presence of functional macular alterations in the fellow eye of patients with this disease.
To identify morphological and/or functional early markers of CNV development in fellow eyes of patients with exudative AMD in the other eye, our group performed a single-center, prospective, observational, longitudinal two-year study, enrolling patients with neovascular AMD in one eye (the non-study eye) and early ARM in the fellow eye (study eye) at risk for the development of CNV. It was possible to identify a sequence of alterations at the chorioretinal interface during the development of CNV and progression of early ARM to neovascular AMD using different imaging methods simultaneously and at regular intervals to characterize markers or predictors of conversion to CNV. It was also possible to correlate the evolution of the identified alterations with the development of CNV and to demonstrate the reliability and relative value of different clinical methodologies used to identify AMD disease progression.

2. Genetic biomarkers

AMD is a complex disease caused by the combination of genetic predisposition and environmental factors. The prevalence of AMD increases with age. The adverse effect of smoking is well established. Genetic predisposition has been demonstrated by familial aggregation studies and twin studies. Using genome linkage scan and association studies, multiple potentially causative genes have been identified. The chromosomes most commonly implicated are 1q25-31 and 10q26. In particular, variants in the gene for the complement factor H (CFH) and the genes PLEKHA1/LOC387715/HTRA1, Factor B (BF) and complement component 2 (C2) have been implicated as major risk or protective factors for the development of AMD. There have been some advances in the treatment of this condition; however, a complete cure remains remote but hopeful. Understanding the causative environmental and genetic interactions will facilitate the development of future preventive methods and treatments.

AMD-associated SNPs may eventually serve to identify at-risk individuals and separate AMD patients into homogeneous groups for preventive and therapeutic studies. These genetic biomarkers also serve as powerful tools in the elucidation of the underlying etiology of AMD. We limit our discussion predominantly to those SNPs that are consistently associated with AMD in multiple case-control studies and, thus, have the strongest potential to serve as genetic biomarkers. In early 2005, four groups reported independently that common variants in the gene encoding CFH confer major susceptibility to AMD.14,15,16,17. A year later, at-risk and protective haplotypes were identified in two other genes encoding complement proteins, BF and C2.[18]

These markers include:

- Complement factor H (Chromosome 1q32, Entrez Gene ID 3075) Polymorphisms in the complement factor H gene (CFH) are associated with a significantly increased risk for the development of age-related macular degeneration (AMD). The most documented risk-conferring single-nucleotide polymorphism results in a tyrosine-to-histidine substitution at position 402 (Y402H) of the CFH protein.
- Complement factor B (chromosome 6p21.3, Entrez Gene ID 629)
- Complement Component 2 (chromosome 6p21.3, Entrez Gene ID 717)
- PLEKHA1/ARMS2/HtrA1 (chromosome 10q26, Entrez Gene ID 387715/5654/59338)
- Excision repair cross-complementing rodent repair deficiency, complementation group 6 (chromosome 10q11.23, Entrez Gene ID 2074)
- VEGF (chromosome 6p12, Entrez Gene ID 7422)

3. Inflammatory biomarkers

Various immunological molecules and inflammatory mediators have been identified at the site of AMD lesions.[19] Pro-inflammatory cytokines are released from immune cells during an inflammatory response. These cytokines mediate inflammatory effects. Elevated levels of AMD biomarkers, e.g., markers of systemic inflammation: C-Reactive Protein (CRP), Interleukin 6 (IL-6), Tumor Necrosis Factor alpha-Receptor II (TNF α-RII), Intercellular Adhesion Molecule (ICAM), Vascular Cell Adhesion Molecule (VCAM ); lipid biomarkers: Apolipoprotein B (ApoLP B) or Lipoprotein (a) (Lp-a); homocysteine (Hc); and fibrinogen (Fbg), are predictive of development and progression of AMD.

4. C-reactive protein

CRP may be involved in the pathogenesis of AMD through chronic inflammation leading to oxidative damage, endothelial dysfunction, drusen development or the degeneration of Bruch’s membrane.[20] CRP may also have a direct role in AMD development through its
ability to induce complement activation. Several studies performed by Seddon and colleagues\(^\text{21,22}\) showed an association between CRP levels and AMD and elevated CRP levels may serve as a marker for AMD progression. However, Hogg and colleagues found no significant association between CRP plasma levels and AMD or AMD progression in both case–control and prospective studies\(^\text{23}\).

5. Interleukin-6

IL-6 is a marker for systemic inflammation, such as acute pancreatitis, chronic arthritis and geriatric syndromes. Seddon and colleagues found a correlation between the level of IL-6 and chances of AMD progression\(^\text{22}\). This study shows that elevated IL-6 levels may serve as a marker for progression of AMD. However, Klein and colleagues found no significant association between IL-6 plasma levels and AMD or AMD progression\(^\text{24}\).

6. Fibrinogen

Fibrinogen is an established biomarker of acute and chronic inflammation\(^\text{25,26}\). Lip and colleagues found elevated levels of plasma fibrinogen in AMD cases compared with controls\(^\text{25}\). A case–control analysis from the large Blue Mountains Eye Study in Australia detected significantly elevated plasma fibrinogen levels in AMD patients compared with controls \(p < 0.05\)\(^\text{27}\). In another study using patients recruited from the Muenster Aging and Retina Study population in Münster (Germany), no association between plasma fibrinogen levels and AMD was showed\(^\text{28}\).

7. Vascular endothelial growth factor

There is strong evidence suggesting that VEGF is a good candidate AMD biomarker. VEGF is also elevated in RPE cells of AMD patients and in the AMD patient post-mortem eyes\(^\text{29}\). This evidence points to a role for elevated VEGF levels in AMD. A study by Lip found increased plasma VEGF levels in 78 AMD patients compared with 25 age-matched controls \(p = 0.0196\)\(^\text{25}\) with no significant difference found between dry and wet AMD cases in a comparison of plasma VEGF values\(^\text{25}\). Tsai and colleagues found increased plasma VEGF levels in 77 AMD patients compared with 42 controls \(p < 0.001\)\(^\text{30}\) and significantly higher plasma VEGF levels in wet AMD patients compared with dry AMD patients \(p < 0.05\)\(^\text{30}\). These results suggest that high VEGF levels may play a role in predisposing individuals to neovascular AMD. Additional studies must be undertaken to establish its role as a biomarker for this disease.

8. Functional multimodal imaging of the macula

Multimodal imaging of the macula provides improved visualization of the macular alterations seen in early ARM.

- Color Fundus Imaging
- Fluorescein Angiography
- Indocianine Green Angiography
- Fundus Autofluorescence
- Optical Coherence Tomography
- Retinal Leakage Analyzer

8.1 Color fundus imaging

Grading of stereoscopic color photographs collected during 5 years of follow-up in more than 3000 AREDS participants were used to develop a detailed grading scale\(^\text{29}\). A simplified scale was developed based on the presence or absence of 2 features characteristic of AMD that were easily identified clinically (drusen size and pigment abnormalities) and highly associated with the development of advanced AMD, especially when the status of both eyes was considered.

Color fundus imaging and AREDS photographic grading helps us to make an AMD severity scale that would provide clinically useful risk categories for the development of advanced AMD in persons with earlier stages of AMD. Defining risk factors and counting them provides a convenient way to define risk categories. These categories may be useful in discussing with patients their risk of progression to vision-threatening AMD and in developing inclusion criteria for clinical studies of AMD. The simplest scheme counts the presence of at least 1 large drusen (diameter greater than or equal to that of a large vein at the disc margin) and the presence of any pigment abnormality as 1 risk factor each and sums their presence across both eyes when both are free of advanced AMD. One risk factor is assigned to patients who have no large drusen in either eye but intermediate-sized drusen (diameter ≥ one half that of a large drusen) in both eyes (Table 1).

The 5-year risk of advanced AMD using this scale increases
in the approximate sequence of: 0 factors, 0.5%; 1 factor, 3%; 2 factors, 12%; 3 factors, 25%; and 4 factors, 50%. This scale may be useful clinically, either with ophthalmoscopy or slit lamp biomicroscopy, or in less optimal photographs using less complex grading procedures than those used in AREDS.

For clinical purposes, as the number of risk factors increases from 0 to 4, the 5-year risk of advanced AMD in at least one eye increases in the easily remembered approximate sequence of 0.5%, 3%, 12%, 25%, and 50%. Extensive drusen area, as seen on fundus photograph, is the greatest risk factor for the progression of AMD(31). When examiners are asked to mentally aggregate the amount of drusen occupying a given macular subfield(32), as in the International System, where drusen areas were estimated to within 10% to 25% or 25% to 50%, and so on(33), these semi-quantitative estimates prove difficult for human observers. Clearly, there is a need to implement more precise techniques to improve the quality of data being gathered in clinical trials and epidemiological studies.

8.2 Fluorescein angiography

Fluorescein angiography is the standard examination for diagnosis and classification of conversion from early ARM to exudative AMD.

8.3 Indocyanine green angiography

In AMD, digital indocyanine green (ICG) angiography is a technique that may enable improved imaging of occult CNV(34). Hot spots are observed frequently in retinal angiomatous proliferation (RAP), polyoidal choroidal vasculopathy and focal occult CNV(35). ICG

<table>
<thead>
<tr>
<th>Table 1- Risk factors for AMD (from AREDS).</th>
<th>Large Drusen and Pigment Changes</th>
<th>Patient Severity Score = 4 Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Eye</td>
<td>Large Drusen</td>
<td>No = 0</td>
</tr>
<tr>
<td>Pigment Changes</td>
<td>No = 0</td>
<td>Yes = 1</td>
</tr>
<tr>
<td>Left Eye</td>
<td>Large Drusen</td>
<td>No = 0</td>
</tr>
<tr>
<td>Pigment Changes</td>
<td>No = 0</td>
<td>Yes = 1</td>
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</tbody>
</table>
Development and Progression of AMD angiography may demonstrate neovascular connections between the choroid and retina, in the form of anastomoses. The presence of late ICG hot-spots may be an important biomarker in the development of neovascular membrane. They appeared not only when a RAP developed but in other forms of CNV as well. The hot spots may appear not only when conversion occurs but even before conversion suggesting that this finding may be associated to future conversion in some subtypes of neovascular AMD.

8.4 Fundus autofluorescence

Fundus autofluorescence (FAF) imaging is a non-invasive method for examination and follow-up patients with macular disease that supplies additional information to that obtained using fundus photography and fluorescence angiography[36]. It is based on the detection of fundus autofluorescence, which is associated primarily with the lipofuscin content of retinal pigment epithelium[36]. Areas of increased FAF may correlate to areas of hyperpigmentation, yellowish soft drusen, or normal fundus appearance. However some drusen are related to areas of decreased FAF. Areas covered with reticular drusen usually show a reticular FAF pattern with small areas of decreased FAF surrounded by normal FAF. The areas of hypopigmentation on fundus photographs may be associated with decreased FAF suggestive of absence of retinal pigment epithelium or degeneration. There are also FAF images with no or minimal changes in patients with funduscopically visible drusen, and vice versa[36]. Smith et al[37] have retrospectively studied the relationship between reticular patterns of autofluorescence and choroidal neovascularization. They showed that reticular hyperfluorescence appears to be a marker for reticular pseudodrusen, which are known to be frequently associated with CNV development. Other studies have suggested a higher risk of CNV development in eyes showing a patchy pattern of autofluorescence[38-39]. In our series, among the eyes which developed CNV in study eye during follow-up no clear pattern of association could be established to their predominant FAF pattern at baseline. In this study the presence of a pattern of minimal change in fundus autofluorescence at baseline in eyes with neovascular AMD in the fellow eye appears to indicate a lower risk of developing CNV at two years. Eyes with fewer abnormalities in FAF pattern at baseline appear to have a slower progression to neovascular AMD in patients with unilateral neovascular AMD. The relatively small population studied makes necessary further studies with a more prolonged follow-up in order to clarify the relationship between fundus autofluorescence and the risk for progression to neovascular AMD, which was not apparent in this two-year study. Fundus autofluorescence imaging can be even more effective in evaluating ARM progression when it is combined with Optical Coherence Tomography, as with Spectralis HRA+OCT (Heidelberg Engineering). With the simultaneous recordings of high-resolution OCT, it is possible to evaluate corresponding morphological substrates like underlying microstructural changes in the retina and the retinal pigment epithelium[40,41].

8.5 Stratus OCT

Stratus OCT showed increased retinal thickness from intraretinal fluid accumulation in every eye that developed CNV. This increase in fluid accumulation, however, could not be identified before conversion, in any of the eyes. OCT findings confirm that this method is a good indicator of the presence of active CNV and therefore can be used reliably to monitor CNV treatments[42].

8.6 Retinal leakage analyser

The retinal leakage analyzer showed leakage of fluorescein into the vitreous associated with the area of CNV, confirming the observations of Merin et al[43], using vitreous fluorometry. Leakage location correlated well with the CNV site. Furthermore, it showed, before conversion, sites of abnormal breakdown of the blood-retinal barrier in 13 of the 17 eyes (76%) that converted to exudative AMD. An alteration of the blood-retinal barrier appears, in this study, to be a particularly promising predictor of conversion from dry to wet AMD. There were also sites of alteration of the blood-retinal barrier in 23% of the study eyes that did not convert during the two-year period of the study. It is now of particular interest to follow closely the future evolution of these eyes to see if these eyes with early ARM and localized alterations of the blood-retinal barrier demonstrated by the retinal leakage analyzer are the first to develop CNV.

We believe that by examining the natural history of eyes at high risk of converting from early ARM to exudative AMD designing a different imaging methodologies of these will help us to obtain a better understanding of what
happens on the retina and in the chorio-retinal interface. Our current research is focusing on understanding the alteration in the blood-retinal barrier at the RPE level. Other studies are looking at photoreceptor function to determine how much of it is altered and damaged before, after, or simultaneously with disease. We believe that studies such as these can help us to understand the natural history of age-related maculopathy.
References:


25. Lip PL, Blann AD, Hope-Ross M, Gibson JM, Lip GY. Age-relat-


Fluorescein Angiography

1. Introduction

Fluorescein angiography (FA) was introduced in ophthalmology by Novotny and Alvis in the sixties of the last century. They took serial fundus photographs after intravenous injection of sodium fluorescein to study the retinal and choroidal circulation\(^\text{1}\). Initially, they used this technique in diabetic and hypertensive patients and after, the technique was used in age-related macular degeneration (AMD). Although the clinical diagnosis of AMD can be established based on patient’s history and fundus examination, FA is the most important ancillary test for classifying the disease in its different subtypes, especially in its wet form. Nowadays, optical coherence tomography (OCT) is being more used than FA for monitoring the response to treatment, although FA is still very useful in some cases.

Sodium fluorescein is a small molecule, with a molecular weight of 376.27 daltons, and it is highly soluble in water. It is stimulated by light in the range of 465-490 nm and then it enters into a higher energy state. The molecule emits longer wavelength fluorescence, between 520 and 530 nm, as it decays to a lower energy state. In clinical application, two filters are used. An excitation filter allows the passage of blue light, which stimulates the fluorescein in the eye, which emits yellow-green light. In addition, a barrier filter is used to block some reflected blue light, allowing only the yellow-green light to pass through. This resultant fluorescence is recorded by a camera as an image\(^\text{2}\).

Sodium fluorescein diffuses through the fenestrated vessels of the choriocapillaris, but does not cross the internal and the external blood-retinal barriers. Thus, any condition that compromises these barriers, obstructs blood flow, or changes the normal pigmentation of the retinal pigment epithelium (RPE) can cause abnormalities on FA.

The dye is removed from the vascular compartment by the kidney. It is relatively inert, making intravenous injection safe and severe adverse reactions rare. Nevertheless, the patient should be properly informed of the potential risks of FA injection\(^\text{3}\).

2. Interpretation

Several FA patterns can be observed in AMD patients. They can be classified at those leading to decreased fluorescence (hypofluorescence) or increased fluorescence (hyperfluorescence). Hypofluorescence either represents blocked fluorescence or a vascular filling defect (Table 1). Hyperfluorescence can be the result of loss of the normal barrier to background choroidal fluorescence known as transmitted fluorescence. A second reason for hyperfluorescence can arise from extravascular accumulation of the dye or from leakage from abnormal vessels (Table 2)\(^\text{4}\).

By convention, leakage of fluorescein into a space is referred to as pooling, while leakage into a tissue is called staining\(^\text{5}\).
## Hypofluorescence

### Blocked

- Intraretinal or subretinal hemorrhage/exudate
- Sub-RPE hemorrhage
- Pigment proliferation
- Pigment epithelial clumping (RPE rip)

### Vascular filling defect

- Choroidal vascular atrophy
- Retinal capillary occlusion

## Hyperfluorescence

### Transmitted fluorescence

- RPE atrophy
- RPE rip
- Hard, basal laminar drusen

### Extravascular fluorescence

- Serous pigment epithelial detachment
- Soft drusen
- Disciform scar
- Loculated fluid
- Cystoid macular edema

### Abnormal vessels

- Choroidal neovascularization
- Retinal angiomatous proliferation

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Table 1 - Causes of hypofluorescence.

Table 2 - Causes of hyperfluorescence.
3. Angiographic patterns in AMD

3.1 Drusen and RPE abnormalities

The majority of patients with AMD have drusen and RPE abnormalities with no significant visual loss. FA is not usually indicated in these cases unless we suspect the presence of choroidal neovascularization (CNV). Several types of drusen can be identified. Hard drusen are small (<63 μm), round, well-defined, yellowish deposits that correspond to accumulation of hyaline material in the inner and outer collagenous zones of Bruch's membrane. On FA, they appear hyperfluorescent as transmission defects due to overlying RPE thinning(6). On occasion there may be a myriad of small drusen, termed cuticular or basal laminar drusen, which appear as a “starry sky” on FA (Fig. 1). Soft drusen are larger (>63 μm) with poorly defined borders and they tend to coalesce and become confluent. Their angiographic appearance depends on the thinning of the overlying RPE, the histochemical composition and the age of the patient. They are hyperfluorescent with phospholipid accumulation and in younger patients(7). Soft drusen represent localized detachments of the RPE. It is very usual to find both hard and soft drusen in the same eye of a patient (Fig. 2). The confluence of soft drusen can produce a drusenoid pigment epithelial detachment (PED), which shows hyperfluorescence and dye pooling without leakage beyond its margin with typical areas of focal hyperpigmentation (Fig. 3).

In addition to drusen we can find RPE abnormalities, namely hyperpigmentation. Focal hyperpigmentation is a risk factor for the development of choroidal neovascularization (CNV) and angiographically appears as a
Figure 3 - Drusenoid pigment epithelial detachment.

Figure 4 - RPE abnormalities with focal hyperpigmentation.
3.2 Atrophic AMD

Atrophy can occur in sharply defined areas of severe atrophy, known as geographic atrophy (GA), or in less well-defined, more granular regions of less severe atrophy, known as non-GA. Both forms share the feature of RPE loss, more extensive and with associated atrophy of the overlying retina and underlying choriocapillaris in GA. The angiographic appearance depends on the remaining pigment within the RPE and choriocapillaris vessels. Non-GA shows mottled early hyperfluorescence, which fades late consistent with window defect (Fig. 5). GA typically shows late well-defined hyperfluorescence from staining of the exposed deep choroid and sclera\(^6\). In these cases, visual acuity depends on the foveal involvement (Fig. 6). In advanced cases, larger choroidal vessels show a sclerotic appearance (Fig. 7).

3.3 Classic CNV

Classic CNV is characterized by well-demarcated hyperfluorescence in early phases on FA and late leakage that obscures the boundaries of the lesion (Fig. 8). As defined by Donald Gass, classic CNV lies between the neurosensory retina and the RPE (type II CNV)\(^10\). Angiographic classic CNV appears as a lacy or bicycle-wheel pattern. Depending on its location, it can be classified as extrafoveal (>200 microns from the foveal center) (Fig. 9), juxtafoveal (1-199 microns from the foveal center) (Fig. 10) or subfoveal (involving the foveal center) (Fig. 11). Sometimes, a feeder vessel can be localized (Fig. 12). Another typical feature is the presence of a hyperpigmented rim, hypofluorescent on FA, surrounding the CNV (Fig. 12). On occasion, classic CNV can be associated to loculated fluid (Fig. 13). In loculated fluid, dye pooling is well-demarcated in a confined space of a localized sensory retinal detachment or within intraretinal cystic spaces. It was a common finding in patients with new subfoveal CNV in the Macular Photocoagulation Study (MPS) and may confuse the treating physician as to the boundary of the lesion\(^11\). Depending on their sizes, classic CNV can be classified as small (Fig. 9-11) or medium (Fig. 14) or large (Fig. 15).
Figure 8 - Classic CNV.

Figure 9 - Extrafoveal classic CNV.
Figure 10 - Juxtafoveal classic CNV.

Figure 11 - Subfoveal classic CNV.
Figure 12 - Classic CNV with feeder vessel.

Figure 13 - Classic CNV with loculated fluid.
Figure 14 - Medium size classic CNV.

Figure 15 - Large classic CNV.
Figure 16 - Old classic CNV with fibrosis.

Figure 17 - Predominantly classic CNV.
Importantly, larger classic CNV are associated with a poorer visual prognosis since they represent long-term duration of the pathological disorder. Classic CNV is an emergency and it requires early treatment to halt the progression of the disease. Without treatment, CNV tend to enlarge and irreversible fibrosis appears (Fig. 16).

In the last decade of the last century, the advent of photodynamic therapy (PDT) with verteporfin promoted a classification of the lesions depending on the percentage of classic CNV. Thus, predominantly classic lesions were defined as having 50% or more of the total lesion size comprised of classic CNV (Fig. 17). On the other hand, minimally classic lesions were characterized by classic CNV occupying less than 50% of the total lesion size (Fig. 18)(12). The best results with PDT in wet AMD patients were obtained in the treatment of predominantly classic lesions. Nowadays, in the antiangiogenic therapy era, this classification has lost popularity among ophthalmologists since lesion composition does not seem to be as relevant as it was with PDT.

Lesion components associated with neovascular AMD that can obscure the boundaries of CNV include changes that block fluorescence, such as blood, fibrous tissue, RPE hyperplasia, or RPE redundancy (from an RPE tear). Likewise, CNV can be obscured by greater fluorescence from staining or pooling.

3.4 Occult CNV

Occult CNV has been categorized as fibrovascular PED or late leakage of undetermined source(13). Fibrovascular PED (type I occult CNV) is defined as an irregular elevation of the RPE associated with stippled hyperfluorescence apparent 1 to 2 minutes after fluorescein injection and ill-defined staining or leakage in the late frames (Fig. 19-20). It differs from classic CNV in that the early hyperfluorescence is not as bright and the boundaries usually are indeterminate. Late leakage of undetermined source (type II occult CNV) lacks a discernible, well-demarcated area of leakage in the early angiographic frames. Speckled hyperfluorescence with no visible source becomes apparent 2 to 5 minutes after dye injection (Fig. 21).

3.5 Serous PED

Although serous PEDs can occur in the context of non-neovascular AMD, most of them are related to CNV. On fundus biomicroscopy, a serous PED appears as a round or oval translucent elevation of the RPE. On FA, it is
Figure 19 - Fibrovascular PED (type I occult CNV).

Figure 20 - Fibrovascular plaque
Figure 21 - Late leakage of undetermined source (type II occult CNV).

Figure 22 - Serous PED.
Figure 23 - Serous PED associated to occult CNV.

Figure 24 - Serous PED associated to classic CNV.
Figure 25 - RAP (stage I).

Figure 26 - RAP (stage II).
characterized by progressive and uniform hyperfluorescence from early frames with intense pooling of fluorescein in late phases (Fig. 22). PEDs with a notch usually have occult CNV in the notch (Fig. 23). The association of occult CNV and a serous PED is frequently termed "vascularized PED"\(^{(14, 15)}\). On occasion, a serous PED is associated to classic CNV (Fig. 24).

3.6 Retinal angiomatous proliferation

Retinal angiomatous proliferation (RAP) has been described and classified by Yannuzzi et al.\(^{(16)}\). In RAP, the vasogenic process originates in the retina and begins as intraretinal neovascularization (stage I), which progresses to subretinal neovascularization (stage II) and finally to CNV (stage III). In some cases it is possible to find a retinal-retinal anastomosis. Angiographically, early lesions (stage I) show a focal area of intraretinal hyperfluorescence with indistinct borders corresponding to the intraretinal neovascularization and surrounding intraretinal edema (Fig. 25). Sometimes, these early lesions can mimic the appearance of a classic CNV. Later stages of RAP are often classified as minimally classic or occult CNV.

In stage II, it is very characteristic to find a serous PED with occult CNV associated to overlying cystoid macular edema (Fig. 26). Indocyanine green (ICG) angiography is often more useful than FA for the diagnosis and evaluation of RAP lesions.

Figure 27 - Polypoidal choroidal vasculopathy.
3.7 Polypoidal Choroidal Vasculopathy

In polypoidal choroidal vasculopathy (PCV), the primary abnormality involves the choroidal circulation, and the characteristic lesion is an inner choroidal vascular network of vessels ending in an aneurismal bulge. Clinically, PCV is associated with multiple, recurrent, serosanguineous detachments of the RPE and neurosensory retina secondary to leakage and bleeding from the choroidal vascular lesion (Fig. 27). Although FA can sometimes confirm the diagnosis of PCV, ICG angiography is the choice for imaging this entity.

3.8 RPE tears

Although RPE tears can occur spontaneously, it is not uncommon for them to occur after treatment with thermal laser, PDT or antiangiogenic therapy. RPE tears are commonly related to PEDs, although they have been described in classic lesions too (18). The detached monolayer of RPE scrolls toward the CNV, leaving a denuded area of choroid exposed. On FA, the denuded area becomes hyperfluorescent and the scrolled RPE is dark and blocks the underlying fluorescence (Fig. 28).

3.9 Hemorrhagic AMD

FA is not very useful in hemorrhagic forms of macular degeneration since blood blocks the underlying fluorescence (Fig. 29). ICG angiography can detect the presence of occult CNV.

3.10 Disciform scar

A disciform scar is the end-stage manifestation of untreated CNV, namely formed by fibroblasts and inflammatory cells. Angiographically, it typically shows late staining (Fig. 30).

4. FA for monitoring AMD treatment

In the era of PDT with verteporfin, FA was the gold standard for monitoring the response to treatment (19). Nowadays, with antiangiogenic therapy, OCT scanning has replaced FA for this purpose since it is highly effective to detect lesion activity and it is a non-invasive procedure (20). However, in some cases FA is still very useful in the evaluation of treated patients.
Figure 29 - Hemorrhagic AMD.

Figure 30 - Disciform scar.
References:


1. Introduction

1.1 Definitions

Fluorescence is the capability of absorbing light at a specific wavelength and releasing it at a longer, less energetic wavelength. This phenomenon raises an especial interest when the released radiation is found within the spectrum of visible light permitting its visualization, recording and measurement.

**Autofluorescence** is the spontaneous fluorescence that some substances present naturally.

**Fluorophore** is the part of a molecule that makes it fluoresce.

The human eye contains autofluorescent substances in the retina, especially within the retinal pigmentary epithelium (RPE). The main autofluorescent component of the RPE is lipofuscin (LF), containing at least ten different fluorophores presenting discrete emission spectra within the green, golden-yellow, yellow-green, and orange-red emitting range\(^{[1]}\). When LF granules are stimulated with light within the blue range, a characteristic yellow fluorescence is emitted\(^{[3]}\).

1.2 Basic considerations on fundus autofluorescence

The RPE plays an important role in the physiopathology of age-related macular degeneration (AMD)\(^{[3, 4]}\). The study of RPE can help to achieve a better understanding of AMD and to find new ways of early diagnosis as well as new prognostic and progression markers for this condition.

LF originates from the constant phagocytosis of the shed outer segment disks of the photoreceptors and is accumulated in the cytoplasm of RPE cells\(^{[5, 6]}\). This accumulation is considered to be a hallmark of RPE aging\(^{[7]}\). Recent experimental studies have addressed the molecular mechanisms of the interaction of excessive LF with the normal cellular functions of RPE\(^{[8, 9]}\). According to these studies, A2-E (N-retinylidene-N-retinylethanolamine) has been identified as the main fluorophore of LF. A2-E may play a toxic effect including phototoxic and detergent actions, as well as an inhibitory effect on lysosomal function\(^{[10, 11]}\). It has been suggested that the photooxidation by products related to LF may trigger the complement cascade, thus contributing to the pathological chronic inflammation of the macular area\(^{[12]}\). New studies have been designed to improve our understanding of potential underlying molecular mechanisms. The autofluorescence of LF, its distribution in postmitotic human RPE cells and its accumulation with age have been extensively studied in post-mortem eyes with fluorescence microscopy\(^{[14, 15]}\). In the past few years we have started to study the autofluorescence in vivo. Ultraviolet light is frequently used to visualize LF by fluorescence microscopy ex vivo, since the absorption properties of the eye limit the transmission of ultraviolet light within the retina in human living eyes. However, due to the wide range of excitation of LF (from 300 nm to 600 nm), visible light can be used to visualize its fluorescence in vivo. The emitted spectrum ranges from 480 to 800 nm and peaks within the range of 600 to 640 nm\(^{[15]}\). Fundus autofluorescence (FAF) imaging of the human living eye is a relatively new imaging method that provides a topographic map of the distribution of LF in the RPE.
The detection of FAF is limited by its low intensity (approximately two orders of magnitude lower than the peak background fluorescence of an ordinary fluorescein angiography), and by the autofluorescence characteristics of the anatomic structures of the eye, including those of the optical media, especially of the lens\(^{16}\).

2. Imaging methods

2.1 Fundus spectrophotometry

Fundus spectrophotometry was developed by Delori et al. and was designed to determine the spectrum of excitation and fluorescence emission from small areas of the retina (2° diameter)\(^{17,18}\). The authors were able to determine the amount of autofluorescence and compare it with in vitro fluorescence microscopy. They found out that the spectrum of in vivo excitation was slightly broader, and peaked at a longer wavelength than that of A2E and native LF. The authors concluded that considering the spatial distribution, spectral characteristics and age relationship, LF is the main source of fluorescence in the FAF in vivo\(^{18}\).

Presently, two systems are available to examine the autofluorescence of the human eye in vivo in the clinical practice: confocal scanning laser ophthalmoscope and fundus camera.

2.2 Scanning laser ophthalmoscope

Confocal scanning laser ophthalmoscope (cSLO) was originally developed by Webb et al. using a low-energy laser source to scan the retina in two directions: termed as x and y\(^{19}\). The confocal nature of the optics ensures that the reflectance and fluorescence correspond to the same focal plane. cSLO overcomes the limitations of the low-intensity signal of FAF and the lens interferences. The defocused light is almost completely suppressed, thus reducing the autofluorescence from the optical media anterior to the retina, such as the lens or the cornea.

In order to reduce the background noise and to increase the contrast of the image, a series of FAF images are usually recorded\(^{20,21}\). Following the aligning of the images in order to correct the movement of the eye during the acquisition, the final image is calculated (usually from 4-32 frames) and the values of the pixels are normalised. The FAF image can be obtained with low excitation energies within the limits of maximum retinal irradiance established by the American National Standard Institute and other international standards\(^{22}\).

cSLO enables the acquisition of FAF images from wide areas of the retina (55° with one frame and even larger areas using the composite mode)\(^{20,22}\). Although limited by the optical properties of the human eye, SLO succeeds in imaging the posterior pole with a high contrast. Currently there are three different cSLO systems for FAF imaging: the Heidelberg Retinal Angiograph (HRA) (based on the HRA classic, HRA 2 and the Spectralis HRA) (Heidelberg Engineering, Dossenheim, Germany); the Rodenstock cSLO (RcSLO; Rodenstock, Weco, Düsseldorf, Germany); and the Zeiss prototype SM 30 4024 (ZcSLO; Zeiss, Obercochen, Germany). HRA is the only currently commercially available system with the cSLO system to capture FAF images. HRA uses an excitation wavelength of 488 nm from an Argon laser or a solid-state laser. A barrier filter with a short-wavelenght cutoff at 500 nm is inserted just opposite the detector, blocking the laser light and letting the autofluorescent light through. Recently, it has been made possible to acquire real time images, a technique known as real-time averaging.

The Rodenstock cSLO and the Zeiss prototype SM 30 4024 have also been used to acquire clinical FAF images. Both systems use an excitation wavelength of 488 nm (the same as the HRA), and barrier filters at 515 nm and 521 nm, respectively\(^{20,21,23}\).

Bellmann et al. have noticed marked contrast and brightness differences as well as in the grey range (an important marker of the image quality between the different systems of cSLO). These limitations must be taken into consideration when comparing images from different cSLO systems\(^{24}\).

The default software of the HRA system normalizes the pixel distribution of the final image in order to improve the distribution of the FAF intensity. Even though this final step facilitates the evaluation of the localized topographic differences, it allows a relative estimation of the intensities of the FAF. Thus, it should not be used for quantitative calculation and absolute comparison between different FAF images. The normalization of the average images can be easily turned off, and brightness and contrast can be manually adjusted to permit an adequate visualization of the distribution of autofluorescence in areas with a very high or very low signal in order to improve the visualization of small details.
2.3 Fundus camera

Fundus cameras are widely used in clinical routine for imaging the retina as fundus photographs, reflectance photographs and fluorescein angiography. Fundus cameras use a single flash to capture images from large retinal areas. When confocal optics are not available, the autofluorescent signal from all the ocular structures with fluorescent properties reaches the camera, and scattered light, anterior and posterior to the plane of interest can influence the detected signal [25-27]. The lens contributes significantly to the autofluorescent signal when similar wavelengths are used in the blue-range, as for the cSLO ($\lambda = 488$ nm), particularly in older patients with lens yellowing and nuclear opacities. Flashlight intensities and detector gain have to be set at relatively high levels in order to obtain reasonable FAF images. However, the signal-to-noise ratio decreases simultaneously which may result in reduced image quality. To reduce interference from lens fluorophores which mainly emit in the range between 510 to 670 nm, Spaide modified the excitation filter (peak 580 nm, bandwidth 500-610 nm) and the barrier filter (peak 695 nm, bandwidth 675-715 nm). A further modification was introduced in 2007 using a slightly different filter set (excitation bandwidth 535-580 nm, emission bandwidth 615-715 nm) [28], thus improving signal-to-noise ratio and image quality. Furthermore, as this setup of the fundus camera uses different excitation and emission filters compared with the cSLO, it may even visualize other retinal fluorophores. However, a systematic comparison of different pathologies with clinico pathological correlations between cSLO and fundus camera, particularly in patients with AMD, has not yet been performed. Originally, a fundus camera that enabled imaging with a field of 13º was used. Recently, Spaide has obtained images of the spatial distribution of FAF intensities over larger retinal areas up to 50º with his new modified fundus camera [25-28].

In the near future we may improve FAF imaging with the aid of scientists and investigators developing filters and some other innovations, and increased experience. Furthermore, it is already possible to visualise different fluorophores from the retina with the configuration of the fundus camera using excitation and emission filters for the cSLO. A systematic comparison of clinical images with different pathologies obtained by the cSLO and the fundus camera, (especially in AMD patients) has not been performed yet.

3. Autofluorescence imaging in the human eye in vivo

FAF images show the spatial distribution of the intensity of autofluorescence of each pixel in grey values (arbitrary values from 0 to 225); low intensities are commonly known as low pixel values (dark) and high intensities as high pixel values (light).

3.1 Normal fundus

FAF imaging shows a consistent pattern of autofluorescence distribution in normal eyes [22]. Such common findings have been reported in children as young as four years old [29]. The macular FAF signal is reduced at the fovea because it is limited by the presence of lutein and zeaxanthin in the neurosensory retina. The signal is higher in the parafoveal area and tends to increase as we move away from it, peaking at the most peripheral retinal areas. It has been suggested that this FAF pattern is caused by the melanin deposition and density of LF granules at the different areas of the retina [18,30]. The optic nerve head typically appears dark mainly due to the absence of RPE. The retinal vessels are associated with a markedly reduced FAF signal because of the blocked fluorescence (Fig. 1).

The common ratios of grey intensity between the fovea and the perifoveal area have been established [31,32]. Considering these findings, any deviation from the normal pattern in a specific location can be easily identified; hence the qualitative description of the local changes in the FAF is widely used. The changes in signal intensity are qualitatively described as decreased, normal, or increased as compared to the background signal of the same eye.

3.2 FAF imaging in AMD

When examined with autofluorescence, the fundus of patients with AMD may show a range of signal changes [20,33-37]. Assuming that RPE has an important role in the pathophysiology of AMD and that the major fluorophores in the retina are located within RPE cells, FAF imaging can show changes in the concentration and distribution of RPE LF and hence establish the condition of RPE in patients with AMD. Therefore, atrophic RPE typically appears as dark patches in FAF and can be clearly delineated, even better than in normal fundus photograph [21,38], (Fig. 2). All this information can
Figure 1: Colour fundus and fundus autofluorescence from a normal subject. (A) Right eye colour fundus photograph and (B) fundus autofluorescence. (C) Left eye colour fundus photograph and (D) fundus autofluorescence.

Figure 2: Atrophic area of RPE. These areas typically appear as dark patches in FAF images and can be clearly delineated. (A) Colour fundus and (B) fundus autofluorescence photographs.
Figure 3 - Fresh haemorrhage in the left eye from a patient with choroidal neovascularization secondary to AMD. Fresh haemorrhages typically appear dark due to blocked fluorescence. (A) Colour fundus and (B) fundus autofluorescence photographs.

be obtained from a quick and minimally invasive exploration with FAF. The decreased FAF intensity may also be associated with hyperpigmented areas due to the melamin absorption of light\(^\text{(35,39)}\). However, it should be considered that other fluorophores than LF can be found in RPE and become more prominent in AMD patients, and hyperpigmented areas may also cause an increase in the signal, which is supposed to result from the accumulation of melanolipofuscin.

Other changes in FAF which are not related to RPE defects may appear in AMD. Fresh haemorrhages typically appear dark due to blocked fluorescence (Fig. 3). However, these haemorrhagic areas eventually synthesize substances and fluorophores, which are observed in the fundus as yellowish areas and in FAF images as increased signals\(^\text{(40)}\) (Fig. 4). Pigment epithelial and neurosensory detachment and areas with extracellular fluid accumulation associated with exudative lesions can be observed in FAF as increased or decreased signal intensity.

Fluid accumulation under pigment epithelium detachment, extracellular deposition of material under the RPE (drusen), and fluid originated from CNV can occur with increased, normal or decreased FAF intensity. This phenomenon is a consequence of the presence of unknown autofluorescent molecules other than LF in the same spectral range than LF. FAF imaging alone may not distinguish between melanolipofuscin from RPE cells migrated into the neurosensory retina and LF within the normal RPE layer. It is always necessary to compare the FAF findings with those from other techniques such as fundus photograph, reflectance image, fluorescein angiography or optical coherence tomography (OCT)\(^\text{(35,39)}\).

3.2.1 FAF in early AMD

Early AMD is characterized by the appearance of localized RPE hypo or hyper pigmentation and drusen. Drusen are formed by the accumulation of extracellular deposits in the inner aspects of Bruch’s membrane\(^\text{(3)}\). Depending on their size and morphology, they can be classified as hard or soft drusen. The molecular composition of drusen is quite complex and has not been completely elucidated (Fig. 5).

FAF changes in early AMD have already been reported by several authors\(^\text{(9,21,25,27,33-36,41)}\); all of them concluding that the changes in ophthalmoscopy and fluorescein angiography are not necessarily related with FAF, suggesting that FAF may provide new information regarding the stages and activity of the disease. Differentiation between RPE LF and sub-RPE deposits with FAF images in vivo can be a hard work.

An analysis of the variability of FAF in patients with early AMD was recently reported by an international workshop on FAF phenotype in early AMD. Among their conclusions, a new classification system with eight different FAF patterns was given\(^\text{(39)}\).

**Normal pattern** characterized by a homogeneous background autofluorescence with a gradual fluorescence decrease in the inner macula towards the foveola (blocked fluorescence caused by yellow macular pigments). FAF may be normal even in the presence of soft or hard drusen.

**Minimal change pattern** characterized by a limited and irregular decrease or increase of background AF, not associated to any obvious or important topographic pattern.
Figure 4 - Haemorrhage in the left eye of a patient with choroidal neovascularization secondary to AMD. Fluorophores eventually appear in haemorrhagic areas which are observed as yellowish areas in the fundus and as an increased PAF signal. (A) Colour fundus photograph of the fresh haemorrhage. (B) Colour fundus photograph from the same eye one month later. (C) Fundus autofluorescence of the haemorrhage.

Figure 5 - Small drusen from a patient with early AMD in the left eye. (A) Colour fundus and (B) fundus autofluorescence photographs.
Focal increased pattern is defined by the presence of at least one well defined spot (<200 micron diameter) of markedly increased FAF much brighter than the surrounding background fluorescence. These areas may or may not correspond to large, soft drusen and to areas of hyperpigmentation.

**Patchy pattern** characterized by the presence of at least one large area with well defined borders (>200 micron diameter) of markedly increased FAF. Again, these areas may or may not correspond to large, soft drusen and areas of hyperpigmentation.

**Linear pattern** defined by the presence of at least one linear area of markedly increased FAF with well defined borders. Linear structures of increased FAF usually correspond to hyperpigmented lines.

**Lacelike pattern** This pattern shows multiple branching lines of increased FAF forming a lacelike pattern. The borders may be hard to define, and FAF may gradually decrease from the centre of the linear area towards the surrounding background. This pattern may correspond to hyperpigmentation or to non visible abnormalities.

**Reticular pattern** is defined by the presence of multiple small, ill defined areas (<200 micron diameter) of decreased FAF. This pattern has been found to occur not only in the macular area, and may be associated with multiple small soft drusen, hard drusen or areas with pigment changes or non visible abnormalities.

**Speckled pattern** is characterized by the simultaneous presence of different types of abnormalities in a large area. The changes reach beyond the macular area and may cover the entire posterior fundus. These abnormalities include multiple small areas of irregular increased or decreased FAF corresponding to hyper and hypopigmented areas and multiple subconfluent and confluent

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**Figure 6 - Drusen in a patient with early bilateral AMD.** (A) Right eye colour fundus photograph and (B) fundus autofluorescence. (C) Left eye colour fundus photograph and (D) fundus autofluorescence.
drusen.
The speckled pattern has been reported to be the most frequent (26%) followed by the patchy pattern (23%). The most infrequent patterns are the normal pattern (2%) and the lacelike pattern (2%). The study confirmed that visible drusen on fundus photography are not always correlated with noticeable FAF changes and that areas of increased FAF may or may not correspond to areas of hyperpigmentation or soft or hard drusen.
Several authors have also mentioned the different FAF patterns in eyes with drusen. Delori et al. described a pattern consisting of decreased FAF in the centre of the drusen surrounded in most of the cases by a ring of increased FAF(27). They also observed that the decreased drusen signal was not as intense as in the areas with RPE atrophy. The authors hypothesized that it might be caused by a displacement of the cytoplasm and LF granules in RPE cells instead of an actual RPE atrophy(35).
Von Rückmann et al. further reported that crystalline drusen are characterised by a decrease in FAF signal, signalling the onset of atrophy. Lois et al. confirmed that areas of confluent drusen are usually associated with focal, mildly increased FAF and that only large subfoveal soft drusen (drusenoid RPE detachments) topographically correspond with focal changes of FAF(33) (Fig. 6). Smith et al. recently reported their results after using image analysis software to study drusen and pigmented areas on fundus photographs from AMD patients(41). The authors initially used image analysis algorithms, including automated background levelling and thresholding. Areas of focally increased FAF intensities were compared to the normal background signal. By overlapping fundus photographs and FAF, the topographic correlation of drusen and pigmented areas with focally increased FAF signals was established. Smith and co-workers reported that eyes with isolated drusen or pigment abnormalities were better correlated with FAF abnormalities than eyes with geographic atrophy(41).
Regarding areas with changes in RPE, hypopigmented areas are usually associated with a corresponding decreased FAF signal, suggesting an absence or degeneration of RPE cells, with reduced content of LF granules. (Fig. 7 and 8). However, hyperpigmented areas frequently show a higher FAF signal, which may be caused by a higher concentration of autofluorescent melanolipofuscin(35) (Fig. 9).

3.2.2 Advanced AMD

Advanced AMD is characterized by geographic atrophy (GA), choroidal neovascularization (CNV), pigment epithelial detachment (PED), RPE tears and disciform scars.

3.2.2.1 Geographic atrophy

Geographic atrophy is thought to be the natural end stage of the atrophic AMD process when CNV does not appear. GA occurs in areas where the RPE is dead and the outer neurosensory retina and choriocapillaris disappeared(42, 43).
Due to the loss of RPE and LF, the atrophic area appears dark in FAF imaging(35). High contrast between the atrophic and the non atrophic retina defines the area of GA

Figure 7 - RPE hypopigmentation in the macular area secondary to AMD. Hypopigmented areas are usually associated with correspondingly decreased FAF signals, suggestive of RPE cells loss or degeneration with reduced content of LF granules. (A) Colour fundus and (B) fundus autofluorescence photographs.
more precisely than colour fundus photographs, permitting a clearer and more specific study of GA, as well as its natural development and evolution\(^{(38,44)}\) (Fig. 10 and 11). The GA patches usually become larger and coalesce as AMD progresses\(^{(45,46)}\). An excessive accumulation of LF, and therefore an increased FAF in the junction are highly suggestive of the appearance or progression of pre-existing GA (Fig. 12). Preliminary observations suggest that different phenotypes may appear associated with junction FAF changes\(^{(47)}\). Recently, a new classification for junction FAF patterns has been proposed in GA patients\(^{(48)}\) (Fig. 13).

- **Focal increased autofluorescence** is defined by single or multiple spots of focal markedly increased FAF localized at the border of the atrophic patch.
- **Band pattern of increased autofluorescence** is characterized by a continuous stippled band of increased FAF surrounding the entire atrophic area.
- **Patchy increased autofluorescence** are large patches of increased FAF outside the GA area. FAF tends to be less intense than that in the focal pattern described above.
- **Diffuse increase autofluorescence** is the most frequent pattern of increased FAF in eyes with GA. FAF changes are not limited to the border of the atrophic area and may show interindividual differences that have been further classified into four subtypes.
- **Reticular pattern**, characterised by several lines of increased FAF usually following a radial pattern.
- **Branching pattern** shows a diffusely increased FAF.
Figure 10 - Geographic atrophy secondary to AMD. The loss of RPE and LF causes a characteristic absence of FAF signal (a dark area in FAF images). (A) Right eye colour fundus photograph and (B) fundus autofluorescence. (C) Left eye colour fundus photograph and (D) fundus autofluorescence.

Figure 11 - Geographic atrophy secondary to AMD. The high contrast between atrophic and non-atrophic retina enables the delineation of the atrophic area more precisely than can be performed from conventional fundus photographs. (A) Colour fundus and (B) fundus autofluorescence photographs.
Fundus autofluorescence in age-related macular degeneration

Figure 12 - Geographic atrophy (GA) secondary to AMD with increased autofluorescence in the junction. New areas of GA and the extension of pre-existing areas are characterised by an excessive accumulation of LF, and therefore an increased FAF signal. (A) Colour fundus and (B) fundus autofluorescence photographs.

Figure 13 - Geographic atrophy secondary to AMD with a banded pattern of increased autofluorescence in the junction. (A) Colour fundus and (B) fundus autofluorescence photographs.

with a fine branching pattern of increased FAF.

- **Fine granular pattern**, is defined by a large area of increased FAF with a granular appearance surrounding the GA area and a clear border between the granular increased FAF and the surrounding normal background FAF.

- **Fine granular with peripheral** punctate spots pattern is characterised by diffuse FAF changes surrounding the atrophic area with elongated small lesions and increased FAF.

Refined phenotypes help to identify the prognosis and seem to be a prerequisite to determine specific genetic factors in a complex, multifactorial disease such as AMD.

A recent analysis of the follow-up of junction FAF patterns in GA and the rate of progression of atrophic lesions revealed that variation in GA growth rates are dependent on the specific phenotype of FAF at baseline. Atrophy enlargement was slowest in eyes with normal FAF pattern (median, 0.38 mm²/year), followed by focal FAF pattern (median, 0.81 mm²/year), diffuse FAF pattern (median, 1.77 mm²/year), and banded FAF pattern (median, 1.81 mm²/year). The rate of progression of GA in eyes with patchy FAF pattern were not included in this analysis because of their low frequency, insufficient for statistical analysis. The rate of progression of “banded” and “diffuse” FAF patterns were significantly
higher compared to eyes without FAF abnormalities and “focal” FAF pattern. Another interesting finding of this study was the identification of eyes with extremely rapid progression of the atrophy, showing distinct FAF features of atrophy that had not been previously reported. The authors introduced the term “diffuse trickling” for a pattern associated with a significantly faster enlargement of atrophy. Areas with increased FAF and consequently higher concentrations of RPE LF precede the development of new areas of GA or the enlargement of the pre-existing atrophic areas.

The phenotypic features of FAF abnormalities may play a stronger influence on the progression of atrophy than any other previously reported risk factors such as smoking, arterial hypertension or diabetes. The different rates of enlargement of atrophy may be related to heterogeneity at a cellular and molecular level in the disease. The high degree of symmetry in GA suggests that genetic determinants may be involved, rather than nonspecific aging changes.

### 3.2.2.2 Choroidal neovascularization

Choroidal neovascularization is considered to cause almost 90% of the cases of severe visual loss related to AMD\(^{50}\). CNV is usually studied by fluorescein angiography and OCT to assess the extent, location and nature of the lesion\(^{50}\). Fluorescein angiography shows changes in retinal vascularity, but does not reveal how deeply RPE is affected. FAF imaging shows RPE damage, with the advantage that it is a non-invasive test, less time-consuming than angiography.

Several studies have reported that CNV may show irregular FAF alternating areas of increased, normal and decreased fluorescence intensity\(^{20,34–37}\). (Fig. 14). Areas of abnormal FAF extend beyond the edge of the angiographically defined lesion. As in other exudative retinal diseases such as central serous chorioretinopathy, areas of increased FAF next to CNV are frequently found inferior to the leaking areas in fluorescein angiography.

The hypothesis was that they might represent areas with subretinal fluid and that their location was influenced by gravity. Other fluids may typically decrease FAF, as occurs with haemorrhages and exudates. Decreased FAF is caused by blocked fluorescence. It is usually necessary to compare the results of FAF with colour photographs. Recent research has examined early CNV in FAF\(^{30,52–55}\), reporting that early CNV lesions tend to show normal FAF in areas that were hyperfluorescent in fluorescein angiography, whereas eyes with a history of one month or more since CNV was diagnosed, showed decreased FAF in areas of previous fluorescein leakage\(^{52}\). These data suggest that RPE affected by the CNV may still be viable in the early stages of the disease (Fig. 15). These studies have also reported that areas with previously high levels of FAF may show decreased FAF six months later\(^{30}\). These changes may be secondary to photoreceptor loss, RPE atrophy, replacement of normal phenotypes of RPE cells with scar, and increased melanin deposition. These findings may have therapeutic implications and clarify long-term visual prognosis.

For example, a person with an active CNV on fluorescein angiography, and normal FAF, may show a much better outcome than another with an abnormal basal FAF.

Data comparing FAF findings in occult and classic CNV are limited. Spital et al. reported that classic CNV usually shows more focal areas of decreased FAF than occult CNV\(^{34}\). These findings have been confirmed by McBain et al.\(^{54}\) who guessed that low FAF at the site of the CNV are related to blocked fluorescence induced by the presence of CNV in the subretinal space, rather than to severe damage to the RPE.

A more recent study did not find significant differences in FAF patterns in early classic and occult CNV secondary to AMD\(^{53}\) (Fig. 16). A continuous preserved autofluorescence pattern was observed in the central macula in most of the cases. These findings suggest that neovascular complexes, regardless if classic or occult, would be external to the RPE in most cases.

Additional studies with a higher number of patients and longer monitoring are required to verify with these changes in patients with CNV (Fig. 17).

### 3.2.2.3 Pigment epithelial detachment (PED)

PED can show different FAF patterns\(^{11,36,31,59}\). FAF can provide complementary information to that of fundus colour photograph and fluorescein angiography. In most of the cases, a moderately and diffusely increased FAF can be found, surrounded by a clearly defined ring with decreased fluorescence\(^{34,55}\). Occasionally, intermediate or even decreased FAF can be found that may not correspond to the atrophic RPE or to fibrovascular scars. These changes in the FAF could correspond to different stages in the PED evolution\(^{16}\). The findings in FAF should be compared with those in fluorescein angiography. We should bear in mind that areas with increased FAF do not always correspond to increased or decreased LF. Besides, the presence of
Figure 14 - Occult choroidal neovascularization. These lesions show irregular FAF intensities with alternating areas of increased, normal and decreased signal intensity. (A) Colour fundus and (B) fundus autofluorescence photographs. (C) & (D) Early frames fluorescein angiography. (E) Late frames fluorescein angiography.
Figure 15 - Early choroidal neovascularization. Early CNV lesions are frequently characterised by few alterations of FAF signal. (A) Colour fundus and (B) fundus autofluorescence photographs.

Figure 16 - Classic choroidal neovascularization. (A) Colour fundus and (B) fundus autofluorescence photographs.
Figure 17 - Choroidal neovascularization with chorioretinal anastomoses. (A) Colour fundus and (B) fundus autofluorescence photographs. (C) Early frame fluorescein angiography. (D) Late frame fluorescein angiography.

Figure 18 - Retinal pigment epithelial detachment. (A) Colour fundus and (B) fundus autofluorescence photographs.
other fluorophores, extracellular fluid or degraded photoreceptor remnants should be considered (16) (Fig. 18).

### 3.2.2.4 RPE tears

RPE tears usually occur in association with pigment epithelial detachments (PED) in patients with neovascular AMD, either spontaneously or following therapy (51). FAF imaging reveals absence of autofluorescence in the area denuded from RPE. These areas are clearly identifiable by their very low signal, whereas a heterogeneous FAF signal is seen in the area where the RPE is rolled. Therefore, the exact location of the tear can be delineated in most cases. FAF imaging is a very good tool to diagnose RPE tears (34).

### 3.2.2.5 Disciform scars

The appearance of disciform scars in FAF imaging depends on their duration and evolution (34,36). Disciform scars may show different variations and alterations of FAF signal. A decreased signal is typically observed in scarred and fibrotic areas. It has been reported that approximately 50% of the disciform scars may be surrounded by a rim of increased FAF (34,36). These areas of increased autofluorescence correspond to irregularly pigmented areas and may have been caused by a multilayered RPE, a well illustrated finding in histopathology (Fig. 19 and 20) (35).

Figure 19 - Choroidal neovascularization with fibrosis. FAF outlines the marked atrophic lesions in the RPE surrounding the CNV/fibrosis. These changes are inconspicuous in colour photographs. (A) Colour fundus and (B) fundus autofluorescence photographs.

Figure 20 - Fibrous scar secondary to CNV after treatment with anti-VEGF. The damaged RPE appears hyperpigmented in fundus photograph, whereas FAF imaging shows an increased signal. (A) Colour fundus and (B) fundus autofluorescence photographs.
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Geographic atrophy (GA) is considered the late stage of the dry form of Age-related Macular Degeneration (AMD)\(^1\). GA is less common than neovascular AMD and it is responsible for 10-20% of cases of legal blindness in this condition\(^2,6,7\).

### 2. Definition

Usually defined as any sharply delineated round or oval area of hypopigmentation, or apparent absence of the retinal pigment epithelium (RPE), in which choroidal vessels are more visible than in surrounding areas, that must be at least 175 μm in diameter\(^1\). Other dimensions as 200 μm, 500 μm and 700 μm have been proposed as minimum limit for GA because the regression of a single soft drusen can be followed by a small atrophic area (Fig. 1)\(^3,28\).

In Age-related Eye Disease Study (AREDS), GA is included in category 3 or intermediate form (if not involving the center of the macula), and 4 or advanced form (involving the center of the macula)\(^9\). One eye with GA and choroidal new vessels (CNV) is considered as having exudative form\(^9\).

### 3. Epidemiology and risk factors

The global prevalence of GA is 0.66% in all ages but it occurs in 0.34% between 65-74 years old, 1.3% between 75-84 and 4.4% over 85 years old. The prevalence increases to 22% after 90 years of age\(^2\). It corresponds to half of exudative form prevalence\(^7,8,10,11\).

The most consistent risk factors are age, familiar history and tobacco smoking like in other forms of AMD\(^2,7,10\). In AREDS, GA was also associated with: high BMI (body mass index); use of calcium channel blockers and β-blockers; not using anti-acids; not using hormone replacement (woman); light iris color and less education level\(^11,12\).

The prevalence of GA is lower in blacks than in whites.
ments as lipofuscin that accumulate in RPE cells, contribute to a decline of the cell function and degeneration conducting to GA\(^{(27)}\).

Although RPE cells are the most involved, the outer nuclear layer is also severely affected with dysfunction and death of photoreceptors. Probably rods are the first affected photoreceptors\(^{(31,32)}\).

The mechanisms of RPE death are best studied at junctional zone. Here, lipofuscin may occupy 30% of RPE cell and may interfere with its metabolism, conducting to death. These mechanisms include oxidative stress and inflammation\(^{(33,34,35,36)}\).

Macrophages are often seen in areas of GA, apparently phagocytosing pigment and debris resulting of normal cells deletion\(^{(37)}\).

5. Diagnosis

5.1 Fundus

Fundoscopy in GA typically shows a well-circumscribed oval or round area of pigment epithelium atrophy, usually sparing the fovea until late stages (Fig. 2). All precursor lesions of this final appearance can also be present: large drusen (>125 microns), focal pigmentation changes and refractile deposits\(^{(3,23)}\).

A prolonged choroid filling phase has been described (2.1% vs 4.8%) like in exudative forms\(^{(14,15)}\).

In Rotterdam and Beaver Dam Eye Study, serum HDL cholesterol was directly associated with GA, however this association was not found in Blue Mountains Eye Study\(^{(16)}\). In this study, diabetes and the ratio total/HDL cholesterol were linked to increased risk of GA\(^{(18)}\).

Genetic risk factors have been described in association with AMD. As complement system seems to play an essential role in this disease, the complement factor H (CFH) gene located at chromosome 1q32, and others as CFB, LOC HTC1, C2 and C3, have been implicated in the development of both forms of AMD\(^{(18-20)}\). Some studies have linked specifically 5p region and 4q 32 region with GA\(^{(21,22)}\).

4. Pathology

Accordingly to AREDS the most common sequence of events leading to GA is the progression of a large drusen to hyperpigmentation, followed by regression of the drusen, hypopigmentation and ultimately RPE cell death, with development of an atrophic area of retina and underlying choriocapillaris, sometimes preceded by the appearance of refractile deposits. This evolution can be longer than 6 years\(^{(3,23,24)}\).

Less frequently, GA can follow a drusenoid RPE detachment, regression of a CNV membrane or a RPE rupture\(^{(5,25,26)}\). In some eyes, atrophy was related to a micro reticular pigment pattern distributed around the perimeter of the fovea\(^{(26)}\).

Most histopathologic studies suggest that RPE cells are the primary target in GA and its death results in choriocapillaris atrophy\(^{(29,30)}\). Autofluorescent pig-ments as lipofuscin that accumulate in RPE cells, contribute to a decline of the cell function and degeneration conducting to GA\(^{(27)}\).

Although RPE cells are the most involved, the outer nuclear layer is also severely affected with dysfunc-

![Figure 2. Fundus photography showing a well-circumscribed round area of pigment epithelium atrophy, sparing the fovea in right and left eyes.](image-url)
Geographic Atrophy

as a clinical marker for changes in Bruch’s membrane, and as a risk factor for development of geographic atrophy\(^{(24)}\). Despite these aspects in GA, fluorescein angiography may be indicated only in atypical cases, in order to allow the correct diagnosis\(^{(38)}\).

5.3 Optical coherence tomography (OCT)

OCT scan shows thinning of hyperreflective external band, corresponding to attenuation of RPE/Bruch’s complex, and deeper hyperreflectivity because of loss of outer layers including photoreceptors (Fig. 4)\(^{(39,40)}\). In high resolution OCT the atrophic area shows hyperreflective clumps at different levels, segmented plaques of the outer band and elevations with variable reflectivity\(^{(41)}\).

In the perilesional area there are elevations of the outer retinal layers, as well as thickening of outer hyperreflective band. At the junction area the outer band shows different degrees of loss\(^{(41)}\).

5.4 Fundus autofluorescence (FAF)

Fundus spectrophotometric studies \textit{in vivo} by Delori and co-workers, have shown that FAF represents an accumulation of lipofuscin in the lysosomes of RPE cells, mainly derived from photoreceptors outer segments degradation. The compound is found as micrometer-sized spherical particles and is characterized by yellow autofluorescence when exposed to blue light\(^{(42,43,44)}\).

It has been shown with confocal scanning laser ophthalmoscopy (cSLO) that FAF response is very low or extinguished in areas of atrophy. The lack of RPE cells or its low number and therefore of lipofuscin, (the dominant fluorophore) explain this reduction\(^{(45)}\). Increased FAF precedes development of GA\(^{(46,47)}\).

FAF is increased in junctional zone around areas of atrophy, and intensity seems to correlate with extension of the atrophic area, and also with reduction of retinal sensitivity detected by fundus perimetry\(^{(48,49)}\).
Despite the works of Holz and Valkenberg, the prognostic value of FAF remains controversial. In a recent study, FAF was not a strong risk factor for development or extension of GA\(^{(50)}\).

**6. Clinical evolution**

Eyes with GA may also develop CNV. In one study, 7% of eyes with GA developed CNV in 2 years. The strongest risk factor for developing CNV in one eye with GA is the presence of CNV in the fellow eye\(^{(51)}\).

The 4-year rate of developing CNV is 11% if the other eye has pure GA but increases to 34% if there is CNV. As other forms of late AMD, GA tends to be bilateral (over 50% of cases) and there is high symmetry between eyes for total atrophic area, presence of peripapillary atrophy and enlargement rate\(^{(51,53,58)}\). On the other hand there is a high interindividual variability\(^{(54)}\).

The mean overall enlargement rate of atrophic area is 2.6 mm\(^2\)/year and eyes with larger areas of atrophy at baseline tend to have larger enlargement rates\(^{(53)}\).

GA often first develops surrounding the fovea, sparing the central area\(^{(55,56,57)}\). Because of that, the correlation between visual acuity (VA) and area of atrophy can be complex. The loss of three lines (ETDRS scale) was observed by Suness et al. in 31% of studied eyes by 2 years and 53% by 4 years. The risk of acuity loss was higher in eyes with better visual acuity at baseline and with lightest iris color\(^{(57)}\).

Usually vision loss is bilateral because of lesion symmetry, and evolution since first signs to legal blindness is quite variable\(^{(53,58)}\).

Because of rods paucity, reduction of foveal cone function and also because of switching from central to eccentric fixation, other visual functions as the contrast sensitivity, reading rate and dark adaptation are decreased in GA\(^{(52,55,57)}\).

The low luminance visual dysfunction and the maximum reading rate seem to be significant risk factors for subsequent VA loss\(^{(55)}\).

**7. Management**

**7.1 Prevention**

In AREDS, dietary supplements as zinc and anti-oxidants vitamins C, E and beta-carotene, have been shown to reduce the risk of progression in participants in

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Figure 5. FAF showing increased fluorescence in the junctional zone around areas of atrophy.
categories 3 and 4 to advanced AMD (25% in 5 years), however, in the group with GA away from the center (category 3), this reduction was not statistically significant. Despite of that the AREDS Report n° 8 concluded, that those with noncentral GA also should consider taking a supplement of antioxidants plus zinc(4,12).

Macular xanthophylls and polyunsaturated fatty acids seem to be associated with a lower risk of advanced age-related macular degeneration(59,60). Because of that antioxidant effect of macular pigments as lutein, zeaxanthin and omega-3 fatty acids has been tested in the Age-related Eye Disease Study 2 (AREDS 2)(59,61). Low dietary glycemic index also seems to reduce the risk of evolution to advanced AMD(61). Other behavioral factors such as stop smoking and control of BIM may play an important role on prevention(13).

7.2 New treatments

So far there is no proven drug treatment for dry AMD. However, several trials are investigating many strategies regarding three major targets: Neuroprotection, oxidative stress protection and suppression of inflammation(62). Ciliary neurotrophic factor (CNTF) is a potential new treatment protecting photoreceptors of degeneration. CNTF is released from an encapsulated cell device implanted in vitreous cavity(63,64).

ACU-4429 is a modulator of visual cycle, inhibiting the generation of lipofuscin precursors and is selective to rod system(62).

OT-551 is a new topical anti-inflammatory, antiangiogenic and antioxidant agent, that protects photoreceptor cells by inhibiting lipid peroxidation(64,65).

POT-4 is a C3 inhibitor administered as an intravitreal gel and suppresses local inflammation(66). Other inflammation suppressor is fluocinolone acetonide, a glucocorticoid used as an intravitreal implant.

Although currently there is no drug proven effective, in the next decade some of the research lines will probably be able to find a more effective treatment for the atrophic form of AMD.
References:


Geographic atrophy (GA) and choroidal neovascularization (CNV) represent the advanced forms of age-related macular degeneration (AMD). GA is defined as a well-circumscribed area of atrophy of the retinal pigment epithelium (RPE) where the large choroidal vessels can be seen by ophthalmoscopy and show thinning or absence of the RPE, closure of the choriocapillaris and degeneration of the overlying photoreceptors \(^{(1,2)}\).

Visual loss in GA is due to areas of atrophy of the RPE larger than 175 μm and subsequent loss of tissue in the outer retina (photoreceptors) and choriocapillaris; these areas tend to coalesce progressively and may not affect the fovea until late in the course of the disease (the so-called “foveal sparing”), when visual acuity (VA) finally ensues. Due to the large paracentral areas of atrophy but preservation of the foveal visual function is often very poor in spite of an apparently good VA. Patients with a VA of 20/20 may be functionally blind (Fig. 1). Relatives of the patients, and even ophthalmologist, have often confused VA for visual function, thus, frequently these patients have felt poorly understood \(^{(1)}\). GA is responsible for one-third of the cases of end stage disease \(^{(3)}\) and accounts for 20% of cases of severe visual loss due to the disorder \(^{(4)}\).

At the age of 85 years or older incidence of GA is four times the one of CNV. GA is not a benign disease; the atrophy of the external layers may progress at a speed of 1.5-2.6 mm\(^2\) per year (Fig. 2) \(^{(5)}\).

Figure 1: Foveal sparing may allow a 20/20 visual acuity in spite of very severe visual function impairment
It remains a significant challenge because of its potential to cause blindness, its relentless progression and the lack of current effective treatment. With the progressive increase of the longevity in developed countries GA secondary to AMD represents a true epidemic.

Recent research has pointed towards lipofuscin, a fluorophore that accumulates in the RPE, as a triggering agent in the development of atrophy. Lipofuscin derives mainly from phagocytosed photoreceptor outer segments and accumulates in RPE lysosomes, where one of its many compounds, A2E (N-retinylidene-N-retinylethanol-amine), increases the pH by inhibition of ATPase proton pump function, dificulting its phagocitation and inducing cellular apoptosis\(^\text{[6-7]}\). Using a fundus spectrophotometer, Delori et al.\(^\text{[8,9]}\) were able to visualize lipofuscin due to its autofluorescent properties (when stimulated with blue light in the range of 488 nm, lipofuscin emits a yellow fluorescence). Current developments allow the clinical in vivo visualization of the distribution of lipofuscin by means of confocal scanning laser ophthalmoscope (cSLO) or specific filters in the fundus cameras.

2. Fundus autofluorescence

Fundus autofluorescence (FAF) is a novel, non-invasive method for imaging the fluorescence properties of lipofuscin (and possibly other molecules with a range of absorption and emission spectra close to that of this fluorophore) at the level of the RPE. Using a commercially available cSLO, the distribution of FAF in the normal eye can be seen in Fig. 3. It is characterized by a uniform grayish signal in the fundus and a marked dark appearance in the optic nerve (absence of RPE) and retinal vessels (absorption of fluorescence by hemoglobin and other blood contents). The macular area shows progressive diminished signal intensity towards the fovea because of absorption phenomena of short wavelengths by macular pigment, lutein and zeaxanthin. A relatively high degree of inter-individual variability and technical difficulties limit the use of the absolute quantification of pixel gray values for longitudinal or transversal studies, and therefore the interpretation of the images is based on qualitative observations, ie decreased (dark), normal or increased (white) FAF, in a similar way to that of conventional fluorescein angiography.

A decreased signal is commonly due to RPE atrophy (absence of lipofuscin), an increase in RPE melanin content and absorption from extracellular material anterior to the RPE (intraretinal fluid, fibrosis and media opacities, being cataract a common cause of decreased FAF intensity and poor image quality, specially with cSLO). On the other hand, an increased
signal may be due to lipofuscin accumulation in the RPE (which is the main fluorophore in FAF imaging), presence of other fluorophores not in the RPE (drusen -including those in the optic nerve head-, older hemorrhages), lack of absorbing material and artifacts(10).

FAF imaging in patients with GA is characterized by a decreased signal with sharp borders corresponding to the area of atrophy on conventional retinography (Fig. 4). However, many patients show increased FAF at the borders of atrophy (Fig. 5), which has been histopathologically confirmed as areas of increased lipofuscin-filled RPE cells between atrophic and normal retina. This finding has not been identified by any other imaging modality. Holz et al. showed in a longitudinal study that atrophy developed selectively in the junction areas of increased FAF(11) but not elsewhere, a finding that could not be confirmed on a small sample by Hwang et al.(12). Based on this information, the FAM (Fundus autofluorescence in age-related macular degeneration) study initially described 8 patterns of FAF in the junction zone of GA(13), which were later modified to incorporate a ninth pattern (Fig. 6)(14). According to FAF in the junction zone of atrophy, eyes are classified as none (when there is no increased FAF at the borders of the GA), localized (focal, banded, patchy) and diffuse (fine granular, branching, trickling, reticular and fine granular with punctuated spots)(14).

The relevance of these patterns relays in the fact that they may represent different phenotypic manifestations of the disease. It has been shown in natural history studies that rates of growth differ between subtypes of FAF and that a strong correlation exists between FAF pattern and progression of atrophy in GA(14,15). In the FAM study(14) 195 eyes of 129 patients with GA were followed a median of 1.80 years and classified according to FAF pattern at baseline. Those without abnormal FAF at the borders of the lesion experienced the slowest progression over time (0.38 mm2 /year, n = 17) compared to those with the focal (0.81 mm2 / year, n = 14) and diffuse (1.77 mm2 / year, n = 112) subtype (p<0.0001). FAF was more strongly associated with GA growth than other classic risk factors, such as size of baseline atrophy, smoking, age or family history. Nowadays current devices of autofluorescence equipped with semiautomated software allow quantification of total area of GA and measurement of its progression in time (Fig. 7).

Furthermore, studies using fine-matrix mapping(16) and SLO microperimetry(17) have found impaired rod photoreceptor function and photopic sensitivity respectively in areas of increased FAF in the junction zone, which underscores abnormalities associated with increased fundus autofluorescence. Taken together, these results suggest that presence of increased FAF at the borders of GA is associated with a greater rate of progression of atrophy and that different patterns of FAF may reflect differences at the cellular and molecular level that may explain the different evolution of the disease process. This information is relevant for understanding its physiopathology, natural history and to evaluate future therapeutic strategies.
Recent development of high resolution, high speed spectral domain optical coherence tomography (SD OCT) improves the visualization of the RPE, outer and inner segment of the photoreceptors and external limiting membrane over previous time-domain based technology. Given the aforementioned reasons, simultaneous imaging techniques that combine SD-OCT and FAF are highly desirable for the evaluation of the atrophic and junction areas of patients with GA (Fig. 8). Currently there are instruments that fulfill these criteria and allow the study of the correlation between areas of increased or decreased autofluorescence with the morphologic changes detected in the external retina by SD OCT. SD OCT in atrophic areas of patients with GA show retinal thinning due to atrophy of the RPE and disappearance of the external retina, that includes inner and outer segment of the photoreceptors and, very frequently, the external limiting membrane\(^\text{18}\); in the severe forms, the outer nuclear layer may be no longer identifiable and therefore the outer plexiform layer may be in direct contact with Bruch’s membrane. The thinned retina permits the deeper penetration of light and a corresponding increased signal from the choroid within the atrophic area (Fig. 9). SD OCT is also useful to identify absence of exudative signs (intra-retinal or sub-retinal pockets of fluid, RPE detachments, maintenance of the continuity of Bruch’s membrane)\(^\text{19}\).

Several abnormalities have been found with SD OCT in the junction zone\(^\text{18,20,21}\), such as disruption of external retinal layers with different shape of band endings, disappearance of the external limiting membrane and/or of the retina encompassing inner segments of the photoreceptor layer to Bruch’s membrane at the same or at a different transverse planes, small elevations of RPE thought to represent sub-RPE deposits or increased distance between inner and outer photoreceptors segment and RPE, presumably due to debris between these layers. Smooth margins with no structural changes from normal to abnormal retina exist in the junction zone when there is no FAF abnormality, which underscores the significance of abnormal FAF\(^\text{21}\) (Fig. 9).

In summary, precise quantification of the GA and its progression by fundus autofluorescence imaging and the detailed morphologic study of the external retina by the high resolution SD OCT allow to show the relative slow progressing abnormalities of the outer retinal layers in dry AMD. This may prove essential for prognostic and interventional strategies, in order to detect potential benefit by slowing down the degenerative process or perhaps detect signs of RPE and/or photoreceptors rescue.
Figure 6. FAM-Study classification of fundus autofluorescence patterns of geographic atrophy in age-related macular degeneration (14). "Reproduced with permission from Rightslink (Elsevier)."
Figure 7. Semi-automated software allows quantification of total area of GA and measurement of its progression in time (Spectralis Heidelberg Retinal Angiograph/OCT; Heidelberg Engineering, Heidelberg, Germany).

Figure 8 - High resolution optical coherence tomography correlated with the autofluorescence image.
Figure 9 - SD OCT in normal retina (up left). In the lower images, SD OCT of the junction area between preserved retina and the geographic atrophy. Atrophic areas show retinal thinning due to atrophy of the RPE and disappearance of the external retina that includes inner and outer segment of the photoreceptors and, very frequently, the external limiting membrane. The outer nuclear layer may be no longer identifiable and therefore the outer plexiform layer may be in direct contact with Bruch’s membrane. The thinned retina permits the deeper penetration of light and a corresponding increased signal from the choroid within the atrophic area.

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References


Over 100 years ago, Oller described for the first time the presence of anastomoses between the retinal and choroidal circulations in eyes with disciform scars. Chorioretinal anastomoses were later described, associated with laser photocoagulation, radiotherapy, chorioretinal inflammatory diseases, and parafoveal telangiectasias. Anatomopathological studies of these anastomoses were also undertaken in disciform scars resulting from late age-related macular degeneration. In 1992, Hartnett et al. described nine cases of retinal neovascularization, to which they referred as “deep retinal vascular anomalous complex”. In 2000, Slakter et al. described chorioretinal anastomoses in eyes with pigment epithelial detachment and indocyanine green (ICG) hot spots. In 2001, Yannuzzi et al. described chorioretinal anastomosis as neovascular proliferation with origin in the retina, and proposed the designation of RAP – retinal angiomatous proliferation. Several authors maintained the designation of chorioretinal anastomosis, proposing a choroidal origin for this clinical entity. In 2008, Yannuzzi et al. described 5 cases of RAP with the neovascular complex originating in the choroid instead of the retina, and proposed that RAP should be called type 3 neovascularization. However, RAP is still the most common designation.

2. Classification

According to Yannuzzi et al., the initial neovascularization process in RAP consists of 3 stages:

i) Stage I – Intraretinal neovascularization. This stage is mainly diagnosed when the second eye is already affected. The presence of associated small retinal haemorrhages constitutes a very useful sign in early RAP diagnosis. A small elevation of the inner/intermediate retina caused by angiomatous tissue may be observed under a slit lamp; this elevation may extend tangentially, assuming telangiectatic appearance. In FA, the angiomatous complex is observed as a hyperfluorescent area in front of the RPE, identical to that occurring in classic choroidal and, more frequently occult neovascularization. Dilated retinal vessels may perfuse and drain the intraretinal neovascularization (IRN) and form retino-retinal anastomoses. ICG may reveal a hot spot with staining and leakage.

ii) Stage II – Involvement of the subretinal space with localized neurosensory detachment of the retina, oedema and retinal haemorrhages at the edges may already be observed in fundus colour photography. The angiomatous formation draining vein becomes visible, originating in the deep retina. PED is observed in 94% of eyes; FA may reveal well-delimited hyperfluorescence in the diffuse leakage area associated with PED, identical to that occurring for a minimally classic choroidal neovascular membrane. ICG clearly establishes stage II diagnosis as the presence of a hot spot with leakage, as well as a hyperfluorescent area associated with serous pigment epithelium detachment (SPED). Leakage might not be revealed by FA, probably because fibrin from retinal exudates cannot be impregnated with fluorescein, contrary to what occurs with ICG. FA is rarely useful in differential diagnosis between classic, occult or minimally classic membranes and stages I and II, contrary to ICG, where a slow-growing extra, juxta or subfoveal hot spot, sometimes asymptomatic, is observed in stages I and II. Retino-retinal anastomoses may also be observed in stage II.

iii) Stage III – Choroidal neovascularization is clearly visible, sometimes with the appearance of vascularized PED. According to Gass, RAP would progress in 5 stages instead of 3, easily identifiable by FA and ICG:

i) Pre-clinical stage – Atrophy of the outer retina, with retinal capillaries moving closer to a choroidal neovascular complex
and more frequently in stage I), with early focal hyperfluorescence (corresponding to the angiomatous formation), leakage and staining, in the intermediate and late stages. Angiographic appearance in stages II and III is often that of minimally classic or occult membranes. However, it is very difficult to distinguish between intraretinal neovascularization and choroidal neovascularization or vascularized retinal pigment epithelial (RPE) detachment by FA (Fig. 1 and 3).

Hot spots normally appear in areas with no window effect, being observed before filling of the RPE detachment with fluorescein. Stereo FA allows viewing of leakage in the deep retina or the subretinal space(8,19). The initial stages in the filling of deep vascular lesions and afferent arterioles are often only visible in videoangiography, since filling occurs simultaneously. Angiomatous lesions may continue to fill even after venous drainage has started(17,19).

Indocyanine green angiography (ICG)

In ICG, a hyperfluorescent focus is visible, which may be observed in all 3 stages, fading only in later stages (Fig. 2). Late staining or intraretinal oedema, which are typical occurrences in RAP, may also be observed. Intraretinal exudates contain fibrin(18), which may be stained with indocyanine but not with fluorescein. A hypofluorescent area corresponding to serous RPE detachment may also be observed. With confocal high-speed ICG, early frames allow the establishment of a relationship between RAP and retinal circulation, although without denying the existence of chorioretinal anastomosis(7,10). High-speed videoangiography also allows identification of retino-retinal anastomoses, chorioretinal anastomoses, feeding arterioles and draining venules (Fig. 2).

Stereo ICG with scanning laser ophthalmoscopy (SLO) shows that RAP consists of a neovascular complex with two components: one is the hot spot (which fills one to three seconds after filling of choroidal arteries, with moderate late leakage); the other consisting of one or more retinal vessels (arteries and particularly veins (70% vs. 30%) (plunging into or emerging from the hot spot)(19). In stage III, vascularized pigment epithelial detachment (PED) may be observed in ICG. The serous component of PED is hypofluorescent; the vascular component remains hyperfluorescent.

3. Diagnosis of RAP

3.1 Clinical observation

The presence of small central macular haemorrhages, sometimes punctiform, associated with oedema in an eye with soft drusen, is highly suggestive of RAP in its initial stages (Fig 1).

The following lesions suggest RAP in AMD(8-17):

i) Small multiple haemorrhages, pre, intra or subretinal, normally not observed in macular neurosensory detachments with choroidal neovascularization.

ii) Tortuous, dilated retinal vessels, sometimes showing retino-retinal anastomoses (Fig. 2).

iii) Telangiectasias.

iv) Microaneurysms.

v) Sudden disappearance of a retinal vessel that appears to have moved deeper.

vi) Hard exudates around the retinal lesion (Fig. 1,3,4).

3.2 Angiography

Fluorescein angiography (FA)

Angiographic appearance varies with the stage of the disease. It may be identical to classic neovascularization (rarely located below the RPE – type 1 choroidal neovascularization – and no clinical signs of chorioretinal anastomosis. ICG would be necessary to identify type 1 neovascularization.

ii) Early clinical signs – Anastomosis between dilated capillaries of the deep retina and the choroidal neovascular complex is associated with small intraretinal haemorrhages, which would constitute the first clinical sign of chorioretinal anastomosis. This stage may occur weeks or months before stage 3, where subretinal choroidal neovascularization is already observed.

iii) Proliferation of choroidal neovascularization over the RPE – subretinal neovascularization – type 2 CNV.

iiii) Appearance of serous PED caused by activation of newly formed subepithelial vessels.

iiii) Mixed neovascularization – piggyback-type neovascularization, with two levels – type 1 and type 2 with cicatricial disciform lesion, making chorioretinal anastomosis visible.

Stage 3 in the Gass classification corresponds to stage I in the Yannuzzi classification, with stages 4 and 5 in the Gass classification corresponding to stages II and III, respectively. Currently, the Yannuzzi classification is the most widely used.

3.3 Optical coherence tomography (OCT)

OCT imaging is an important tool in the diagnosis of RAP. Stage I and II lesions are often associated with a
Neovascular Phenotypes: RAP (Retinal angiomatous proliferation)

Figure 1 - RAP lesion. A: Fundus colour photography (A) with intra-retinal haemorrhages, hard exudates and neurosensory detachment. Fluorescein angiography shows a retinal hiperfluorescent spot apparently at the end of one retinal vessel, an intra-retinal haemorrhage, neurosensory detachment and pigment epithelium detachment. Early phase of ICG (C) with an intra-retinal hot spot (angiomatous proliferation) over diffuse choroidal hyperfluorescence. Late phase ICG (D) reveals a subfoveal hiperfluorescent plaque (subretinal neovascularization).

Figure 2 - ICG. A RAP lesion with retinal-retinal anastomosis, an apparently intraretinal angiomatous mass and a serous PED.
Figure 3 - RAP lesion. Fundus colour photography (A) with intra-retinal haemorrhages, hard exudates and neurosensory detachment. Red-free (B) with two juxtafoveal and extrafoveal small haemorrhages. Fluorescein angiography (C) shows two juxtafoveal hyperfluorescent spots, neurosensory detachment and pigment epithelium detachment. Late ICG reveals two juxtafoveal hyperfluorescent hot spots. E and F: Fluorescein angiography and ICG after laser photocoagulation with resolution of exudation.
Figure 4 - Bilateral RAP lesion: Fundus colour photography and red-free images (A,B,C,D) clearly show retinal oedema, small retinal haemorrhages and lipidic exudation. E and F: late ICG both eyes with a hot spot and subfoveal hypofluorescence (serous PED). Stratus OCT reveals, in both eyes (G and H), serous PED, neurosensory detachment and intra-retinal fluid.
focal hyperreflective area located in the deep retina (intraretinal neovascularization), as well as being often associated with intraretinal fluid with cystic spaces, subretinal fluid and PED (Fig. 4). In stage III lesions, neovascular proliferation associated with PED may be observed. With time-domain OCT (TD-OCT) a typical pattern of structural changes in RAP may be observed, characterized by increased foveal thickness, cystoid macular oedema (CME) mainly located in outer retinal layers, serous retinal detachment and a highly reflective intraretinal mass overlying a highly or moderately elevated retinal pigment epithelium. This mass corresponds to the hot spot observed in ICG angiography. With Fourier-domain OCT (TD-OCT) it is possible to obtain unprecedented in vivo detail of the anatomy of RAP lesions, with images nearly resembling histological specimens. OCT findings may vary with the stage of the disease and type of early neovascularization, including areas of intraretinal neovascularization (IRN) in the deep retina, adjacent to PED, anterior and posterior neovascular proliferation through a break in the RPE, intact and ruptured portions of Bruch’s membrane, subretinal fluid and subretinal and/or sub-RPE neovascular membranes (Fig. 5).

4. Differential diagnosis

Differential diagnosis is mandatory for parafoveal telangiectasias, other forms of choroidal neovascularization and polypoidal choroidal vasculopathy. Idiopathic parafoveal telangiectasia is a condition involving dilation of retinal capillaries located near the fovea, in one or both eyes. RPE hyperplasia may also occur, with refractive punctiform deposits and macular leakage being observed in FA. Migration of one or more venules to the deep retina may also be observed. Anastomoses between retinal vessels and the choroidal circulation have been described, as well as new choroidal vessels. The most significant differences are the fact that telangiectasias are not associated with serous PED, the RPE is healthier and choroidal neovascularization associated with parafoveal telangiectasias occurs less frequently.

Differential diagnosis should also be performed for other forms of choroidal neovascularization (CNV) with ICG hot spots (occult CNV) and polypoidal choroidal vasculopathy (PCV). Small intraretinal haemorrhages, sometimes punctiform, in patients with soft drusen, are very typical in RAP, as are telangiectasias and retino-retinal anastomoses. Retinal haemorrhages in PCV are normally larger, with round reddish-orange macular lesions being observed in the eye fundus. OCT is also a useful differential diagnosis tool in RAP, PCV and occult membranes. In RAP, intraretinal hyperreflectivity may be observed, corresponding to angiomatous proliferation associated with intraretinal fluid and/or RPE detachment. In PCV, polyps appear in OCT as abrupt protrusions from the RPE/Bruch’s membrane band, often associated with neurosensory detachment.

5. Natural progression

Natural history may be highly variable and probably similar to that of other CNV lesions. However, many reports ascribe a poor prognosis to RAP lesions. Kuhn et al. studied 22 eyes and observed structural evolution towards classic membranes, signs of RPE rupture and fibrous scars in 36.4%, 4.6% and 31.8% of cases, respectively. In functional terms, decrease in visual acuity occurred in 77% of cases. Final visual acuity (VA) values equal or inferior to 20/200 were observed in 14 eyes studied by Hartnett et al. In a retrospective and one-year study performed by Silva et al. in 17 consecutive patients with RAP, a
significant VA loss was observed in 69% of eyes and vision did not improve in any case\textsuperscript{14}. Significant loss of vision was observed in the first 3 months after diagnosis; a trend towards stabilization was observed subsequently, with no significant differences in average VA being observed at 3, 6 and 12 months and at the end of the study (p>0.05).

Viola et al.\textsuperscript{24} obtained also poor results: in 81% of cases, visual acuity had decreased by 2 ETDRS lines or worse by occasion of the 6-month examination. On final examination (6 - 44 months), 62% of study subjects showed subretinal fibrosis, with 56% showing chorioretinal anastomosis.

6. Epidemiology

RAP was observed in 5 to 28% of new cases of occult exudative neovascularization (Table 1).

Table 1: Percentage of RAP

<table>
<thead>
<tr>
<th>Country-Year</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>France (1995)\textsuperscript{13}</td>
<td>26.8%</td>
</tr>
<tr>
<td>Israel (2002)\textsuperscript{20}</td>
<td>28%</td>
</tr>
<tr>
<td>USA (2002)\textsuperscript{26}</td>
<td>5%</td>
</tr>
<tr>
<td>Portugal (2004)\textsuperscript{15}</td>
<td>9.4%</td>
</tr>
<tr>
<td>Italy (2008)\textsuperscript{27}</td>
<td>25%</td>
</tr>
</tbody>
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Kuhn et al.\textsuperscript{12} and Axer-Siegel et al.\textsuperscript{19} used SLO angiography, while Fernandes et al.\textsuperscript{25} used videoangiography, which may explain the differences observed. Using the ImageNet 1024 videoangiography system, our team observed a prevalence of 9.4% in a consecutive series of 563 patients\textsuperscript{14}. Women appear to be more affected, corresponding from 64.7% to 71% of patients\textsuperscript{8,12}.

Patients with RAP are normally older than those showing occult membranes or membranes with classic components – average age of 79 vs. 76 years\textsuperscript{6,8,9}. The average and median ages of a series of 108 patients studied by Yannuzzi et al.\textsuperscript{9} were 80 and 81 years respectively. Both eyes were similarly affected in 51.9% of patients, with RAP occurring in the second eye an average 15 months after appearing in the first eye. At 3 years, both eyes were affected in 100% of patients\textsuperscript{26,27}. Prevalence appears to be greater in hyperpigmented eyes\textsuperscript{8,9,28}.

7. Anatomopathology

Lafaut et al.\textsuperscript{18} studied six “deep retinal vascular anomalous complex” lesion specimens from retinal translocation surgery in eyes studied with FA and ICG. The deep vascular anomalous complex was located in front of the RPE, thus not representing choroidal neovascularization. Nodular fibrovascular complexes surrounded by a ring of diffuse drusen – basal linear deposits under electron microscopy –, pigment epithelium and an amorphous fibrous material containing photoreceptor outer segment debris were observed. The lesion was not covered by pigment epithelium; however, the latter was preserved around the lesion. The amorphous material did not cover the retinal surface of the membrane, which adhered directly to the outer nuclear layer in 50% of cases. Anastomoses between fibrovascular nodules and the choroidal circulation were only observed in cases showing isolated disciform scars. Avascular fibrocellular membranes were observed on the inside of Bruch’s membrane and, in three cases, on the choroidal side of diffuse drusen. Fibrin was present in affected retinas; choroidal anastomosis was not observed. However, the latter possibility was not excluded, since the specimens studied may not have included the entire lesion. Serous PED without CNV was observed in three cases.

Gass et al.\textsuperscript{13} described chorioretinal anastomosis and atrophy of the outer nuclear layer in a pre-clinical case, with outer retinal capillaries moving close to a choroidal neovascularization focus, having proposed a choroidal origin for RAP, instead of the retinal origin proposed by Yannuzzi.

In a histopathological study performed in nine neovascular lesions classed as RAP, Shimada et al.\textsuperscript{29} found only intraretinal neovascularization in stage 2 cases. In stage 3 cases, these authors found choroidal and intraretinal neovascularization; they concluded that their findings were in agreement with the classification proposed by Yannuzzi. These authors observed expression of VEGF (in intraretinal neovascularization and in the RPE), CD68-positive macrophages (in the neovascularization area) and expression of hypoxia-inducing factors alpha 1 and alpha 2 (HIF 1 alpha and 2 alpha) in neovascular endothelial cells. According to these authors, intraretinal neovascularization would appear before the occurrence of choroidal neovascularization and be associated with ischemia and increased expression of VEGF and inflammatory factors.

Monson et al.\textsuperscript{30} described the histopathological characteristics of RAP in an 87-year-old woman. The images obtained by fundus examination and fluorescein angiography were histopathologically consistent with a neovascular intraretinal angiomatous complex without...
subretinal pigment epithelial neovascularization.

Therefore, the origin of the neovascularization process (choroidal or retinal) is a controversial issue. However, it is known that like in other forms of AMD-related choroidal neovascularization RAP lesions are associated with increased VEGF and macrophage expression, ischemia and age-related macular alterations.

8. Pathogenesis

The exact mechanism and origin of RAP are not yet known. Kuhn et al.\textsuperscript{(12)} studied the evolution of RAP in the second eye of two patients. These authors assumed the existence of two asymmetrical membranes, a smaller retinal membrane – the angiomatous anomaly – over a larger membrane – the choroidal membrane, secondary to failure of a diseased pigment epithelium, unable to modulate and inhibit neovascular factors. Pigment epithelial decompensation might also lead to deposition of materials in the subretinal space, under the basal lamina, and consequent thickening of Bruch’s membrane, which would decrease inner retinal oxygenation. The presence of serous PED might increase hypoxia by moving the retina even further from the choroid\textsuperscript{(17)}. Choriocapillaris atrophy and hypoxia may lead to intense neovascular activity, with reactive synthesis of growth factors, such as VEGF\textsuperscript{(31)}. Overexpression of VEGF is sufficient to produce IRN and subretinal neovascular membranes (SRN) in animal and human models\textsuperscript{(31,32)}. The relatively good response of these lesions to ranibizumab and bevacizumab confirms the important role of VEGF in the pathogenesis of RAP lesions.

According to Yannuzzi et al.\textsuperscript{(9)}, the initial neovascular process in RAP occurs in the deep retina and consists of 3 stages. Gass\textsuperscript{(13)} contested a retinal origin for anastomosis, with basis on the following facts:

i) no communication between retinal vessels and the choroid was found in surgical specimens;

ii) the location of vascular anomalous complexes on the outer nuclear layer of atrophic retinas is more compatible with a choroidal origin hypothesis;

iii) although the macular retina is not excised in surgery, the retinal neovascular complex disappears and no macular hole is left, which contradicts its retinal origin.

It is clear that no definitive sequential histopathological or imaging evidence exists to support intrinsic retinal versus choroidal origin of RAP lesions. Yannuzzi et al.\textsuperscript{(16)} re-evaluated the early stages of RAP lesions in five eyes, using FA, ICG, td-OCT and fd-OCT, having concluded that the initial lesion may have its origin not only in deep retinal capillaries but also in the choroid. They described RAP disease as “type 3 neovascularization”, a type of neovascularization with preference for the retina, displaying the following manifestations:\textsuperscript{(16)}:

i) Focal neovascular proliferation from the deep retinal layer (originally RAP)

ii) Intraretinal neovascular extension from underlying occult type I CNV (originally occult chorioretinal anastomoses)

iii) De novo breaks in Bruch’s membrane with neovascular infiltration into the retina.

This new category, type 3 neovascularization, helps to resolve the various conflicting theories and descriptions of RAP lesion origin: neovascularization in RAP may originate not only from deep retinal capillaries but also from the choroid. However, the main issue regarding RAP lesions is not the intraretinal or choroidal origin of neovascularization but its unique characteristic of two neovascular foci: one located in the deep retina and the other at the choroidal level.

9. Treatment

RAP lesions were never included in randomized clinical trials as a separate AMD subtype. All available reports concerning treatment of RAP lesions using different treatment modalities refer to non-randomized studies.

Thermal laser photocoagulation, surgical ablation, PDT, intravitreal triamcinolone, intravitreal antiangiogenic drugs and combined treatments have been used for treating RAP lesions.

Thermal laser – Kuhn et al.\textsuperscript{(12)} photoagulated hot spots in 28 eyes with “occlusion” in 25% of cases, including one eye with recurrence. No occlusion occurred in the remaining 75% of cases, despite multiple treatment sessions. Treatment caused tearing of the pigment epithelium in 45% of cases and visual acuity decreased in 86% of eyes. The low success rate observed might have been related to several factors, such as inadequate laser penetration – given serous PED height or absorption of radiation by the subretinal fluid –, incorrect location or presence of multiple afferent vessels. Slakter et al.\textsuperscript{(8)} confirmed this poor prognosis, particularly in serous PED cases. Occlusion
was not possible in 86% of eyes and progressive disciform evolution was observed. In a series of 108 eyes with RAP, Bottini et al.\(^{(33)}\) achieved full obliteration of chorioretinal anastomosis in 57.1% of cases classed as stage 1, according to Yannuzzi. Clinical experience shows that some extrafoveal stage 1 lesions (Yannuzzi classification) are amenable to laser photocoagulation\(^{(34)}\). However, the risk of complications needs to be evaluated\(^{(11)}\) and careful follow-up is mandatory, given the high rates of persistence and recurrence.

**Surgical ablation** – Borrillo et al.\(^{(35)}\) performed vitrectomy with detachment of the posterior hyaloid face, surgical section of afferent arterioles and draining veins and membrane excision in 4 eyes with RAP and PED – stage II – with resolution of intraretinal oedema, collapse of the PED after 7-10 days and increased average visual acuity (20/200 pre-operative vs. 20/70).

Shimada et al.\(^{(36)}\) excised the neovascular complex in 9 eyes from 8 patients – stages II and III. VA remained stable in the post-operative period; however, significant destruction of the RPE and the choriocapillaris occurred in patients with serous PED. The authors concluded that surgery may stabilise VA in stage III but is not indicated in stage II. Surgical section of the afferent vessel associated with intravitreal injection of triamcinolone was performed in one eye, with resolution of exudation and visual acuity of 20/320 at 6 months\(^{(37)}\). Nakata M et al.\(^{(38)}\) concluded that surgery may stabilise VA in stage III but is not indicated in stage II.

Surgical section of the afferent vessel associated with intravitreal injection of triamcinolone was performed in one eye, with resolution of exudation and visual acuity of 20/320 at 6 months\(^{(37)}\). Nakata M et al.\(^{(38)}\) observed recurrence of RAP lesions in 7 of 13 treated eyes after 24 months. Shiragami C et al.\(^{(39)}\) excised the neovascular complex in 9 eyes from 8 patients – stages II and III. VA remained stable in the post-operative period; however, significant destruction of the RPE and the choriocapillaris occurred in patients with serous PED. The authors concluded that surgery may stabilise VA in stage III but is not indicated in stage II. Surgical section of the afferent vessel associated with intravitreal injection of triamcinolone was performed in one eye, with resolution of exudation and visual acuity of 20/320 at 6 months\(^{(37)}\). Nakata M et al.\(^{(38)}\) observed recurrence of RAP lesions in 7 of 13 treated eyes after 24 months. Shiragami C et al.\(^{(39)}\) excised the neovascular complex in 9 eyes from 8 patients – stages II and III. VA remained stable in the post-operative period; however, significant destruction of the RPE and the choriocapillaris occurred in patients with serous PED. The authors concluded that surgery may stabilise VA in stage III but is not indicated in stage II.

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**Photodynamic therapy alone or associated with triamcinolone acetate.** In studies of photodynamic therapy in monkeys with neovascular complexes and chorioretinal anastomosis, occlusion of the complex was observed, but not of anastomoses. Anastomoses would be responsible for neovascular complex repermeabilization\(^{(40)}\). Kusserow et al.\(^{(41)}\) treated 6 eyes with predominantly classic membranes and chorioretinal anastomosis without success. No improvements in VA were observed for any of the treated eyes. Membranes continued to grow; no post-treatment hyperfluorescence was observed in FA. Stabilization or improvement in visual acuity was observed after PDT in 73.3% of eyes (<3 lines loss) at 12 months, which represent better outcomes compared to natural evolution\(^{(14)}\). However, significant VA decline was observed in the second year, mainly due to recurrence\(^{(15)}\).

Boscia et al.\(^{(42)}\) treated 13 eyes with PDT, having concluded that PDT would only be useful in cases where serous PED represents less than 50% of the lesion. In 2006, these authors referred that early treatment of eyes with smaller lesions using PDT with verteporfin potentially led to a beneficial effect on vision, whereas it might worsen the natural progression of larger lesions, with most eyes undergoing enlargement, disciform transformation or RPE tear\(^{(43)}\).

Reported short-term results of non-randomized studies on RAP lesions treated with PDT and IVTA\(^{(44,45,46)}\) revealed apparently better VA outcomes and/or a reduced number of treatment sessions compared to PDT alone. However, recurrences were also frequent\(^{(47,48)}\). Krebs I. et al.\(^{(49)}\) found no significant differences between the PDT monotherapy group and the combined PDT and Intravitreal Triamcinolone (IVTA) group regarding evolution of distance VA, retinal thickness and lesion size, having concluded that new therapeutic strategies might be required in RAP lesions, probably including therapy with antiangiogenic agents.

**Antiangiogenic agents:** Similarly to what occurs in classic and occult lesions, intravitreal antiangiogenic drugs appear to lead to better outcomes in the treatment of RAP lesions than PDT alone. Treatment with ranibizumab\(^{(50,51)}\) and bevacizumab\(^{(52,53)}\) has shown very promising results up to 12 months, with treated eyes displaying significant functional and anatomical improvements and no apparent short-term safety concerns. Improved VA and short-term oedema reduction or elimination, were also observed in combined treatments using PDT and ranibizumab or bevacizumab\(^{(54)}\). Information from clinical trials suggests that the response of RAP lesions to CNV treatments may be similar to that of other variants of neovascular AMD\(^{(55)}\). The superiority of combined treatment with PDT and ranibizumab or bevacizumab has not yet been demonstrated. Longer follow-up will be necessary to evaluate whether the well-known tendency for recurrence observed with PDT and entailing VA decline is also observed with antiangiogenic drug monotherapies or combined treatment with PDT.
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Neovascular Phenotypes:
Polypoidal Choroidal Vasculopathy

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1. Introduction

Polypoidal choroidal vasculopathy (PCV) was described for the first time in 1982(1). Different names were proposed like posterior uveal bleeding syndrome(2) or multiple recurrent retinal pigment epithelium detachments in black women(3). It has a characteristic imaging expression on indocyanine green angiography (ICG), peculiar characteristics in optical coherent tomography (OCT) and apparently different responses to treatments when compared to occult or classic choroidal neovascularization. Diagnosis is based on ICG and confirmed with fundus characteristics and OCT findings. The primary abnormality involves the choroidal circulation and the characteristic lesion is an inner choroidal vascular network of vessels ending, in the great majority of cases, in an aneurismal dilatation. Clinically a reddish orange, spheroid, polyp-like structure may be observed. The natural course of the disease often follows a remitting-relapsing course, and clinically, it is associated with chronic, multiple, recurrent serosanguineous detachments of the retinal pigment epithelium and neurosensory retina with long-term preservation of good vision(4). A more recent knowledge has expanded the spectrum of PCV allowing us a clear characterization of PCV as a distinct subtype of exudative age-related macular degeneration (AMD) easily differentiated from other diseases and other subtypes of choroidal neovascularization associated with AMD.

2. Epidemiology

PCV is usually diagnosed in patients between 50 and 65 years old but the age of diagnosis may range between 20 and more than 80 years. Most patients with PCV are likely to have AMD signs. Prevalence of PCV in patients with AMD signs, ranged between 4.8% and 23% in different series and different countries(3-9). It is considered to be more prevalent in Asian population(5) and African American than in Caucasian(3,4,6,7) as it seems to preferentially affect pigmented individuals. Preference for female gender is referred in Caucasian(4,6,7) whilst in Asian population male are more affected(5,8). Bilateral involvement is common and may be as high as 86%(6).

3. Pathogenesis

The pathogenesis of PCV is not completely understood. It is widely accepted to be originated at the inner choroidal level. Polyps may develop from a choroidal vascular network or from a plaque of occult new vessels(4,7,8). Yuzawa et al.(8) described the filling of PCV lesion simultaneously with the surrounding choroidal arteries suggesting that PCV lesions grow from inner choroidal vessels. Few clinicopathological studies have been reported. MacCumber et al.(9) examined an enucleated high myopic eye with rubeosis and vitreous haemorrhage from a diabetic patient without diabetic retinopathy, with high blood pressure and history of multiple, bilateral, recurrent neurosensory and pigment epithelium detachments. Bruch's membrane was crossed by choroidal vessels and an extensive fibrovascular proliferation was disclosed within Bruch's membrane and the inner retinal space. They did not observe choroidal saccular dilatations except that some choroidal veins were quite large. Inflammation was expressed by the presence of B and T lymphocytes at the level of choroid and fibrovascular tissue and the expression of intercellular adhesion cytokines like ICAM-1 was shown. Lafaut et al.(10) reported the histopathologic features of surgically removed submacular tissue from an elderly patient with a pattern of polypoidal choroidal vasculopathy on indocyanine green angiography. A thick fibrovascular membrane located on the choroidal side of the retinal pigment epithelium (RPE) was described. The RPE layer was discontinuous.
whereas on its choroidal side an almost intact layer of diffuse drusen was observed. A group of dilated thin-walled vessels were found and located directly under diffuse drusen within a sub-RPE, intra-Bruch’s fibrovascular membrane. Dilatations appeared to be of venular rather than arteriolar origin and some lesions were associated with lymphocytic infiltration. The presence of choroidal infiltration by inflammatory cells was also referred by Rosa et al. Okubo et al. described unusually dilated venules adjacent to an arteriole with marked sclerotic changes and newly formed capillaries within the wall of the degenerate arteriole and near the dilated venule. Therasaki et al. described clusters of dilated thin-walled vessels surrounded by macrophages and fibrinous material in neovascular membranes obtained from submacular surgery for PCV. Hyalinization of choroidal vessels and massive exudation of fibrin and blood plasma were observed in all the five specimens of PCV lesions studied by Nakashizuka H et al. They also found some blood vessels located above the RPE in two of the five eyes. Immunohistochemically, CD68-positive cells were described by them around the hyalinized vessels. There were no alpha-SMA-positive cells in the vessels of PCV. CD34 staining showed endothelial discontinuity. Vascular endothelial cells within the PCV specimens were negative for VEGF. HIF-1alpha positive inflammatory cells were located in the stroma of specimens. Hyalinization of choroidal vessels, like arteriosclerosis, seems to be characteristic of PCV.

All these previous histopathological studies identifying a large spectrum features (like dilated choroidal vessels, intra-Bruch’s neovascularization, inflammatory cells, drusen material, thick membranes, single saccular dilatations or clusters of dilated thin walled vessels) may partially being expression the influence of disease stage. For many authors and following the results of clinicopathological studies, PCV may represent a subtype of exudative AMD. Yannuzzi et al. found a prevalence of 7.8% of PCV in a population with signs of exudative AMD and Laffaut et al. described the presence of late ICG hyperfluorescent plaques in 58% of 45 cases with PCV, proposing that PCV should be considered a subtype of exudative AMD. Many other authors describe PCV cases with subretinal neovascularization. Ahuja et al. described a prevalence of PCV in 47% of a consecutive series with 16 eyes diagnosed as exudative AMD and showing a PED greater than 2 mm of diameter, haemorrhage or retinal neurosensory exudation. According to Yannuzzi, PCV and exudative AMD may differ in mean age of onset (PCV affected patients are younger), presence of exudative peripapillary lesions (more frequent in PCV), prevalence of soft drusen (greater in AMD patients) and ethnicity (PCV more prevalent in non-white population). PCV also has less tendency to develop fibrous proliferation and a higher incidence of neurosensory and PE detachments.

PCV and AMD share common genetic factors, which suggests that PCV and wet AMD are similar in some pathophysiologic aspects. A common genetic background may exist between typical exudative AMD and PCV patients. Complement pathway plays a substantial role in the pathogenesis of PCV, like in AMD. The nonsynonymous variant I62V in the complement factor H gene is strongly associated with polypoidal choroidal vasculopathy and may be a plausible candidate for a causal polymorphism leading to the development of PCV, given its potential for functional consequences on the CFH protein. The LOC387715/HTRA1 variants are associated with PCV and wet AMD in the Japanese population. The associations are stronger in AMD than in PCV. The de1443ins54 polymorphism is a common variant between White and Japanese populations and is strongly associated not only with AMD but also with PCV. Among the patients with AMD and PCV, those with a homozygous HTRA1 rs11200638 risk allele seem to have larger CNV lesions. However, some genetic differences seem to exist. A variation in the elastin gene may be associated with PCV but not with neovascular AMD suggesting that a different pathogenic process may be involved in the phenotypic expression of neovascular AMD and PCV.

PCV has been referred to be associated with other ocular disorders like macroaneurysms or inflammatory diseases. However, this association is still inconclusive and deserves further investigation. A relation between the retinal vascular changes in hypertensive retinopathy, like vascular remodelling, aneurismal dilatations and focal vascular constrictions, and choroidal alterations in PCV was proposed by Ross et al.

4. Natural evolution

The disease has a remitting-relapsing course and is associated with chronic, multiple recurrent serosanguineous detachment of the neurosensory retina and RPE, with long-term preservation of vision. Visual acuity (VA) loss is associated with central macular involvement and may range from mild to severe VA loss or blindness. Treatment of central macular lesions with PDT and more
recently with antiangiogenic drugs has precluded a better knowledge of natural history of PCV with macular involvement. Approximately half the patients with PCV lesions in the posterior pole may have a favourable course without treatment\(^\text{26}\). In the remaining half the disorder may persist for a long time with occasional repeated bleeding and leakage, resulting in severe macular damage and VA loss. Eyes with a cluster of grape-like polypoidal dilations of the vessels may have a higher risk for severe visual loss\(^\text{27,28}\). Choroidal vascular lesions may be located in the peripapillary area, in central macula or in midperiphery. Most of the PCV natural history series describe lesions in the posterior pole, differentiating macular from extramacular and/or peripapillary polyps\(^\text{5,7,28,29}\). Macular involvement ranged from 25% to 94%. The analysis of VA outcome must consider location of polyps and/or abnormal vascular network. Kwok et al.\(^\text{28}\) followed the natural history of nine eyes with macular involvement after a follow-up ranging from 5 to 60 months and found VA improvement of two lines in only one eye (11.1%), VA change of one line in one eye, and VA decrease of two lines in seven eyes (77.7%). Uhyama et al.\(^\text{26}\) followed 14 eyes with PCV (13 with macular involvement) for a mean period of 39.9 months and described VA improvement of two lines in five eyes (35.7%) and VA decrease of two lines in four eyes (28.5%). A favorable course was demonstrated in 50% of the cases with the remaining half of the cases showing recurrent leakage and hemorrhages and progressive VA loss.

Lesions may grow by enlargement with proliferation and hypertrophy of the vascular component but, apparently not by confluence. Polyps may bleed, grow, regress or leak and a choroidal neovascularization may appear. A massive spontaneous choroidal hemorrhage is rare but may constitute a severe complication associated with blindness\(^\text{27}\). Progression to RPE atrophy is common and may be related with resolution of PED, chronic or recurrent leakage with PE or neurosensory detachment, auto-infarction, regression or flattening of the lesion. Chronic atrophy and foveal cystic degeneration is associated with severe VA loss\(^\text{2,4,10,17,18,19,29}\).

5. Diagnosis

The diagnosis of PCV is based on ICG imaging (Fig. 1) and may be complemented with OCT, fluorescein angiography and fundus findings (Fig. 2, 3, 4).

Clinical examination may show one or more redish-orange, spheroid, subretinal mass located at the macular or juxtapapillary area (Fig. 2). This mass may correspond to the anterior projection of multiple polyps and is very suggestive of PCV. Also very suggestive of PCV is the Figure 1. Polypoidal choroidal vasculopathy. Red-free (A) image with circinate lipidic exudation. Intermediate phase ICG shows an abnormal choroidal vascular network and multiple polyps in the centre of the circinate exudation.
Figure 2: Fundus colour photography with lipidic exudation and a redish-orange, spheroid, subretinal mass located at the macular area associated with macular edema. Late ICG (top, right) reveals the presence of two polypoidal lesions in the papilomacular bundle. On OCT (bottom) the polyp is well delineated and a sub-foveal neurosensory detachment is observed.
Figure 3- Same eye of Figure 2 after one photodynamic therapy session. The two polyps show fluorescein staining (A; FA late phase). A complete polyps resolution is observed on late ICG (B). OCT (C): An intermediate reflectivity is now registered inside the polyp limits (no fluid) associated with a complete resolution of the neurosensory detachment.
serous or serous-hemorrhagic PED and/or neurosensory detachment associated with extensive subretinal haemorrhages and circinate hard exudates (Fig. 1, 2, 4).

Fluorescein angiography alone is useful for PCV diagnosis. Neurosensory detachment and serous or sero-hemorrhagic PED may suggest the diagnosis but polypoidal lesions are only visualized if the overlying pigment epithelium is atrophic.

Intermediate or late leakage on fluorescein angiography is very often diagnosed as occult choroidal neovascularization with late leakage from undetermined source or may be confused with chronic central serous chorioretinopathy.

The characteristic PCV lesion in ICG is an inner abnormal choroidal vascular network of vessels ending, in the great majority of cases, in aneurysmatic dilatations.

The lesion may be juxtapapillary, macular or may be rarely located in the midperiphery. Juxtapapillary lesions often show, in early ICG images, a radial arching pattern, and the vascular channels may be interconnected by smaller spanning branches more numerous at the edge of the lesions. When the polypoidal lesion is located in the macular area the vascular network often arise in the macula and follows an oval distribution. The area surrounding the vascular network is hypofluorescent during early phases of ICG and in late phase ICG angiography often shows a reversal of the pattern: the area surrounding the polypoidal lesion becomes hyperfluorescent and the centre shows hypofluorescence. In very late phases ICG shows disappearance of the fluorescence (washout) in non-leaking lesions (Fig. 4-d).

OCT and particularly 3-D OCT is very useful for...
diagnosis confirmation. Documentation of polyps (number, location, size) and associated features (neurosensory detachment, serous or haemorrhagic PED, haemorrhage) may be assessed by OCT. Polypoidal lesion and branching vascular network identified in ICG may be visualized on spectral domain OCT in near 95% of the cases as areas of moderate reflectivity between the clearly delineated abnormal section of retinal pigment epithelium and Bruch’s membrane[30]. Polypoidal lesions are visualized as anterior protrusions of a highly reflective RPE line. With “en face OCT”[31] branching vascular networks were detected as elevations of the RPE. Serous pigment epithelial detachments may be seen as round protrusions of the RPE and are often accompanied by adjacent round protrusions of the RPE, consistent with polypoidal lesions. These protrusions of the RPE are often fused and typically appeared as a ‘snowman’. Subsequent longitudinal examination reveals the polypoidal lesions to be sharp protrusions of the RPE with moderate inner reflectivity. Consistent with the location of the branching vascular network, a highly reflective line may be seen often just beneath the slightly elevated reflective line of RPE[30,31].

6. Differential diagnosis

Differential diagnosis of PCV with central serous chorioretinopathy, AMD with CNV, inflammatory conditions and tumors is not always easy and needs ICG for a clear differentiation.

Polypoidal choroidal vasculopathy is a primary cause of macular serous retinal detachment without hemorrhage in patients over 50 years of age in Asian population[32]. Since clinical and fluorescein angiographic findings are often indistinguishable among central serous chorioretinopathy, PCV, and occult choroidal neovascularization, indocyanine green angiography might help to establish a more definitive diagnosis[16,33]. Central serous chorioretinopathy shows staining or late leakage but not an abnormal choroidal vascular network neither polyps. The differential diagnosis becomes more challenged when lipid exudation and small PED are associated. ICG may be helpful differentiating PED from polypoidal lesions. Small PED from central serous chorioretinopathy become hypofluorescent in late phases ICG and hyperfluorescent in late phases fluorescein angiography. In contrast, polypoidal lesions are usually hyperfluorescent in late phases ICG because of its vascular nature[16]. Polypoidal choroidal neovascularization is a subtype of CNV in AMD[16,10,17,18,19]. However some features distinguish PCV from other subtypes of CNV: eyes with PCV are characterized by a higher incidence of neurosensory detachments, greater neurosensory detachment height, and less intraretinal edema than eyes with occult or predominantly classic CNV[34]. Non polypoidal lesions in exudative AMD patients tend to produce small calibre vessels that are associated with grayish membranes not easily observed clinically, in contrast with the redish-orange lesions clinically observed in PCV and corresponding to vascular spherical polypoidal lesions[16,34,35,36]. Stromal choroidal fibrosis is common in predominantly classic and occult lesions but is quite rare in PCV. PED associated with CNV in AMD has a poor prognosis whilst PED in PCV lesions virtually never forms fibrotic scars[16,18,29].

Tumoral lesions like choroidal circumscribed hemangioma, renal cell carcinoma or metastasis from carcinoid syndrome may also be confused with PCV[29]. Again ICG is essential for differentiation. Choroidal hemangiomas show, in general, a rapid filling of dye in very early phases and a washout in late phases. ICG characteristic lesions of PCV are not observed in choroidal or metastic tumors and ultrasound is also effective for characterization of the tumoral mass.

Inflammatory lesions like, posterior scleritis, multifocal choroiditis, panuveitis, acute posterior multifocal placoid pigment epitheliopathy, Harada disease, sympathetic uveitis, birdshot chorioretinopathy may also be confused with PCV. PCV does not course with anterior or posterior uveitis neither with pain or staining of the optic disc in fluorescein angiography[16,18,29]. Lipid deposition, often observed in PCV is not commonly seen in inflammatory conditions. Scleral or choroidal thickening and liquid in the subtenon space have never been described in PCV eyes[18,29].

7. Treatment

Treatment of PCV lesions is only recommended when central vision is being threatened by persistent and progressive exudative changes. Otherwise, a conservative approach is recommended. Different treatment modalities like laser photocoagulation, transpupillary thermotherapy, photodynamic therapy with verteporfin (PDT), and surgery or intravitreal antiangiogenic drugs have been reported. However no randomized, controlled studies have been performed to
prove the efficacy or safety of anyone.

Direct laser photocoagulation of leaking polyps has proven short-term safety and efficacy for extrafoveal lesions\(^{35,36,37,38}\). Other authors however described poor results\(^ {39}\) and persistence or even increasing exudation in up to 44% of the cases\(^ {10}\), or VA decrease of 2 or more lines in near half of the eyes and legal blindness in up to two thirds of the eyes\(^ {28}\). Yuzawa et al.\(^ {38}\) reported more lines in near half of the eyes and legal blindness in and 0.5 in the second and third years respectively\(^ {47}\). Yuzawa et al.\(^ {38}\) reported good efficacy of laser photocoagulation in near 90% of the eyes if all the polyps and abnormal vascular network were treated. If the treatment involved only the polyps more than half of the eyes suffered VA decrease related with exudation, recurrences, or foveal scars. Direct laser photocoagulation of feeder vessels, identified in ICG, was also reported as showing VA improvement of 2 or more lines in 8 out of 15 eyes\(^ {40}\). Considering the possibility of using other treatment modalities, laser photocoagulation should be reserved for well defined extrafoveal active polyps.

Transpupillary thermotherapy has shown to be useful in PCV\(^ {41}\). A large number of reports on photodynamic therapy with verteporfin in subfoveal PCV has been published\(^ {17,20,42-46}\). Results at one year and even at two years are apparently superior to those of PDT in predominantly classic or occult CNV. A longer follow-up shows a trend to a progressive but not significant VA decline at two\(^ {46}\) and 3 years follow-up\(^ {47}\): the rate of eyes gaining a significant amount of vision dropped from 26% in the first year to 15% in the third year, and the rate of eyes with significant VA loss (3 or more ETDRS lines) increased from 17% to 26%. A high rate of recurrence (44%) occurred during the 3-years follow-up but it was not associated with significant VA decline\(^ {47}\). This high rate of recurrences may be associated with a poor response of the vascular network to PDT\(^ {48}\). VA decline at 3 years may partially be explained by photoreceptors death due to chronic or recurrent neurosensory detachment, massive hemorrhages and progressive retinal PE atrophy. The number of treatments decreases markedly after the first year from an average of 2.0 to 0.4 and 0.5 in the second and third years respectively\(^ {47}\).

Complications like subretinal haemorrhages, haemorrhagic PED or even massive hemorrhages have been associated to PDT in PCV eyes\(^ {49}\). However, all of these complications may also occur without any treatment. Surgical removal of polypoidal lesions and associated hemorrhages has been reported\(^ {10-14,50}\) with and without macular translocation. Considering the potential alternative treatments and the high rate of serious complication macular translocation is no longer being considered for PCV\(^ {51}\). The relatively large vascular lesions of PCV patients needs to be considered if a vitrectomy is planned in cases without associated massive hemorrhage.

Intravitreal antiangiogenic drugs, like bevacizumab\(^ {52}\) and ranibizumab\(^ {53}\) have been used for treating PCV eyes. Ranibizumab short-term results\(^ {53}\) seem to be promising in terms of maintenance of VA or VA improvement, resolution of subretinal fluid and PED. Polyps were reported to disappear in 69% of the cases, at 3 months when using ranibizumab\(^ {53}\) but not with bevacizumab\(^ {50}\). Intravitreal bevacizumab appeared also to result in stabilisation of vision and reduction of exudative retinal detachment in PCV patients in short-term evaluation. However, it had limited effectiveness in causing regression of the polypoidal lesions\(^ {52}\). Some exudative AMD eyes refractory to ranibizumab or bevacizumab may be, in fact, PCV cases. Combined treatments associating PDT and intravitreal ranibizumab or bevacizumab have been reported to show good efficacy in these refractory cases of AMD\(^ {50}\).

The EVEREST study is the first multi-center, double-masked, indocyanine green angiography (ICG-A)-guided randomized controlled trial with an angiographic treatment outcome designed to assess the effect of Visudyne\(^ {a}\) (verteporfin photodynamic therapy) alone or in combination with Lucentis\(^ {a}\) (ranibizumab) compared with Lucentis\(^ {a}\) alone in patients with symptomatic macular polypoidal choroidal vasculopathy (PCV).

A total of 61 PCV patients of Asian ethnicity from 5 countries (Hong Kong, Taiwan, Korea, Thailand, and Singapore) participated in the study. The six months EVEREST study results\(^ {55}\) suggests that in a majority of patients, Visudyne\(^ {a}\) therapy, with or without Lucentis\(^ {a}\), may lead to complete regression of the polyps that can cause vision loss in patients with PCV. A complete polyp regression (primary endpoint) was achieved in 77.8% of patients who received the Visudyne\(^ {a}\) – Lucentis\(^ {a}\) combination, in 71.4% of Visudyne\(^ {a}\) monotherapy patients and in 28.6% of patients in the Lucentis\(^ {a}\) monotherapy group (p=0.0018 for combination, p=0.0037 for Visudyne\(^ {a}\) vs. Lucentis\(^ {a}\)). Best corrected visual acuity from baseline to month six improved in average in all groups with patients in the combination group achieving the highest gain (+10.9 letters from baseline). Lucentis\(^ {a}\) monotherapy patients gained +9.2 letters, and Visudyne\(^ {a}\) monotherapy patients +7.5 letters. Differences between the groups are not statistically significant. All therapies were well tolerated and the safety findings were consistent with the established safety profiles of Visudyne\(^ {a}\) and Lucentis\(^ {a}\).
PCV may be considered a subtype of exudative AMD. ICG is always mandatory for the diagnosis of PCV, showing a unique lesion – an abnormal inner choroidal vascular network with polypoidal structures at the borders. OCT and fundus findings may complement the diagnosis. PCV needs to be differentiated from other forms of exudative AMD, central serous chorioretinopathy, inflammatory conditions and some choroidal tumors. Photodynamic therapy with Visudyne, alone or in combination with antiangiogenic drugs, seems to be necessary for a complete resolution of the polypoidal lesions.

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Retinal pigment epithelial detachment (PED) is part of age-related macular degeneration (AMD) clinical spectrum. Different types of PED have been reported in the literature, related or not with AMD. Serous PED is defined as an area of sharply demarcated, dome-shaped serous elevation of the retinal pigment epithelium. In AMD, serous PED is frequently associated with chorioidal neovascularisation (CNV). The presence of this lesion worsens the prognosis of the disease, precluding to the formation of a large disciform scar that ends with a poor visual outcome. Even though no clear therapeutic indications are so far set for its treatment, the early detection of a serous PED is therefore important for the prognosis and the management of these patients.

The histopathology of a serous PED is consistent with the detachment of the RPE basement membrane along with the overlying retinal pigment epithelium from the remaining Bruch’s membrane by the accumulation of fluid(1).

Retinal pigment epithelium (RPE) monolayer is anatomically located between the external retinal layer, the outer segments apex, and the Bruch’s membrane. RPE functions, fundamental for the photoreceptors metabolism, include the outer blood-retinal barrier formation and the fluid exchange balance, that physiologically flows from the vitreous into the choroid. In normal conditions, RPE basement membrane is attached to the inner collagenuous layer of Bruch’s membrane. Although the correct pathology of the serous PED formation is not completely known, there are some evidences concerning AMD aspects that can at least partially explain it. In AMD, there are degenerative changes in Bruch’s membrane that are likely implicated in the adhesion loss between these two layers and can explain the pathogenesis of the serous PED. Aging thickening of Bruch’s membrane, more evident in AMD, has been shown to be secondary to accumulation of debris and lipids, most of them phospholipids that greatly decrease its hydraulic conductivity(2,3,4). Moreover, the localized accumulation of basal linear deposits between the RPE basement membrane and the outer collagen layer of Bruch’s membrane, increases the pathologic damage through the drusen formation(5). These two facts significantly combine to bring about an hydrophobic barrier that prevent the fluid outflow towards the choroid, that causes liquid accumulation in the subretinal pigment epithelium space(6-9).

In AMD, serous PED can be either associated or not with CNV, although the vascularised sort is by far the most observed. The formation of soft drusen predispose the progression to advanced AMD, with the development of CNV. Various theories concerning the relationship between serous PED and CNV have been proposed. To explain its pathogenesis, firstly Gass theorized the growth of newvessels from the choroid inside the Bruch’s membrane thickness, that actively leak increasing the hydrostatic pressure causing RPE detachment among the less adherent layers(10). This concept has been later sustained by the evidence that the development of CNV comes with inflammatory mechanisms that add more damage to Bruch’s membrane, supporting RPE separation from the inner collagenuous layer(11,12,13). When the growth of newvessels arises from the inner retina, more recently described as retino-choroidal anastomoses (RCA) or retinal angiomatous proliferation (RAP), it has been hypothesized that the serous PED formation, very frequently associated, can be reconducted to a RPE invasion by the neovascular complex(14-16). On the contrary, other authors observed that the presence of a PED can represent a pre-existing condition that can promote the CNV’s growth through a further Bruch’s membrane damage, expression of the same disease ongoing(2,17).
Even though the pathogenesis of the PED is not completely understood, from these studies the CNVs formation seems to be a pivotal moment. At fundus examination, a serous PED appears as a round or oval, distinct dome-shaped area of regular detachment of the RPE and the overlying neurosensory retina, yellow to orange color and smooth surfaced. Margins are typically sharply demarcated; focal RPE atrophy and pigment figures are frequently observed. However, the concurrent presence of a CNV can generate a variety of associated ophthalmoscopic aspects, such as hemorrhagic and exudative components, areas of irregular elevation of the RPE and serous detachment of the surrounding neuroretina. A CNV located at the margin of the PED can vary its shape, resulting in a reniform or notched aspect, or a flat-sided RPE detachment.

Serous PED imaging study includes fluorescein angiography (FA), indocyanine green angiography (ICGA) and optical coherence tomography (OCT). The diagnosis of a serous PED is made with fluorescein angiography. Examined by FA, a serous PED classically shows an early uniform hyperfluorescence of the entire lesion, slightly delayed compared to the background fluorescence, that progressively increases in brightness as the examination progresses (pooling). Serous PED’s hyperfluorescence typically does not change in size or shape during the angiographic phases. FA can also demonstrate the presence of CNV, usually associated to a serous PED as “occult CNV”, like areas of indistinct late subretinal staining, more evident when located at the margin of the RPE detachment or corresponding to the “notch”. The presence of a CNV can be also deducted by the presence of an hemorrhagic component of the PED, the dark meniscus described by Gass. However, a more precise localization of the neovascular component can be obtained with digital ICGA. Indocyanine green molecule has biophysical properties that, unlike fluorescein, make it useful to enhance vessel’s anatomy through RPE, blood and turbid exudation. In detail, ICGA enables to better delineate the presence and the type of newvessels associated with a serous PED and for this reason is considered a fundamental tool in the management of this disease.

On ICGA, serous PED appears as a hypofluorescent lesion, with sharply delineated margins, that remains constantly hypofluorescent during all the phases of the examination. When the newvessels are not present, no signs of localized hyperfluorescent areas are detectable; the outline of the PED is sharply round and it’s therefore considered a pure serous PED. In AMD patients, Yannuzzi found an incidence of 4% of non-vascularized PED among serous PED.

When the neovascular component is present, it has been suggested the term vascularized PED. Its frequency accounts for approximately 24% of newly diagnosed exudative AMD. Newvessels associated with serous PED are represented in different subtypes. High-speed videoangiography with scanning laser ophthalmoscope appears a precious tool that allows the ophthalmologist to identify the newvessel pattern and their angiographic behavior. To recognize the different types of neovascularization by distinguish angiographic findings is mandatory for the distinct natural course, visual prognosis and specially different response to the treatments of the three main kinds of newvessels associated to serous PED in AMD.

The more common type of newvessels associated with serous PED are those of choroidal origin, or CNV (Fig.1). In the early phases, ICGA shows the CNV’s feeder artery vessel that arises from the choroidal circulation, and subsequently the draining venule. At the same time the capillary network of the neovascular membrane can be detected. Unlike fluorescein, indocyanine green leaks slightly and the CNV’s hyperfluorescence is usually minimal, with the exception of some cases that show an intense leakage, considered as very active CNV. Frequently, in the late phases, a well-defined area of mild hyperfluorescence corresponding to the CNV network can be appreciable. The second type of newvessels that complicate serous PED are the so-called retinal angiomatosus proliferations (RAP) or more recently type 3 neovascularization. These vascular lesions, as reported by various authors, are referred to invade the outer retina and to involve the RPE, through a progression that, to the best of our knowledge, has been hypothesized to originate from either the retinal circulation or the choroid. ICGA shows the presence of a “hot-spot”, due to the early hyperfluorescence of the intraretinal neovascular complex, that increases during the angiography, with an intense leakage in the late phases. Its brightness is enhanced by the surrounding hypofluorescence of the underlying PED (Fig. 2).

Hallmark of the RAP, the neovascular complex is typically connected with one or more retinal vessels, tortuous and dilated, that suddenly deepen toward the vascular lesion. Single or multiple, the RAPs origin are classically extraroidal, and an intraretinal hemorrhage corresponding to the neovascular lesion is frequently observed.

The third type of newvessels associated with serous PED in AMD is consistent with polypoidal choroidal...
Figure 1 - Vascularized PED with choroidal neovascularization:
A - fluorescein angiography, B - indocyanine green angiography and C - OCT
Figure 2 - Vascularized PED with retinal angiomatous proliferation: fluorescein angiography, indocyanine green angiography early and late phases
Figure 3 - Vascularized PED with PCV: fluorescein angiography and early, mid and late phase ICGA
vasculopathy (PCV)\(^{(30)}\). PCV is a peculiar form of choroidal neovascularization, characterized by the presence of orange, aneurismatic, polyp-like round dilatations at the border of a branching vascular network from choroidal origin. Although PCV afflicts more frequently middle-age black and Asian populations, its clinical spectrum is expanded to whites, where it has been found to be present in 8-13% of patients with concomitant AMD lesions. In these cases, when the manifestations attributable to both PCV and AMD are present, some authors consider PCV as a subtype of CNV in AMD\(^{(30,31)}\). Hemorrhagic both PCV and AMD are present, some authors consider

In these cases, when the manifestations attributable to both PCV and AMD are present, some authors consider PCV as a subtype of CNV in AMD\(^{(30,31)}\). Hemorrhagic manifestation are common in patients with PCV. Serous PED associated with PCV show frequently a blood level in the lower portion of the detachment. ICGA is the state-of-the-art examination to distinguish the typical features of the two vascular components. The vascular network is characterized by the presence of one or more aneurismatic lesions that show a bright fluorescence since the early phases, followed in the late phases by a clearing of the dye, called “wash-out”, typical of this disease (Fig. 3).

Nevertheless, some polyp-like structure can actively leak showing late staining of their walls and surrounding exudation. The polypoidal lesions are usually located at the margin of the serous PED\(^{(32)}\). Recognition of these lesions appears fundamental because clinical course, prognosis and treatment response of PCV and RAP are different from that of CNV.

Optical coherence tomography (OCT) provides images that allows an exact correlation with both the angiographic exams findings. In OCT cross-sectional scans, serous PED appears as an optically empty dome-shaped elevation of the external high reflective band – the RPE –, that steeply detaches from Bruch’s membrane\(^{(33)}\). The overlying retina, usually adherent to the bullous PED, at the margins of the lesion can be slightly detached from the underlying RPE. More additional information can be provided by OCT in vascularized PED\(^{(34)}\). The tomographic sections, guided by FA and ICGA in the area corresponding to the CNV, show a smoother elevation of the RPE, continuous with the serous detachment, with a deeper backscattering, due to the presence of the fibrovascular tissue. Hyporeflective areas of homogeneous optically empty spaces referable to fluid accumulation are frequently present in the intraretinal and subretinal spaces\(^{(35)}\). Intraretinal optically empty spaces are more pronounced when the serous PED is associated with a RAP, specially with cystic shape. By positioning the scan line corresponding to the “hot-spot”, the neovascular abnormality is represented as a dense or hyporeflective pre-epithelial zone in the inner retinal layers, where the outer hyperreflectant layers are no longer detectable\(^{(36)}\). The RPE close to that lesion shows frequently effractions or interruptions in its hyperreflective layer\(^{(37)}\). The retinal topographic measurement sustains an increased retinal thickness. Even though it has been hypothesized that the intraretinal neovascularization should be connected to the choroid with an anastomotic vessel, no clear enough images that can bear its presence in the optical free area under the PED can be detected by OCT (Fig. 4-A).

In eyes with serous PED and PCV, the polypoidal ectasies show a sharp protrusion of RPE, similar to the PED but steeply sloped. The polyps cavity, usually optically empty, is contiguous to irregular RPE elevation, expression of the occult neovascular component of the lesion\(^{(38,39)}\). Subretinal and intraretinal fluid, observed as hypofluorescent optical empty areas, are related to the PCV’s activity (Fig. 4-B).

Serous PED natural course depends on the presence or not of the neovascular component\(^{(40)}\). In pure serous PED there is generally a slow enlargement of the lesion, with a minimal progression of visual loss over a long period (months or years). However, many of them can subsequently develop neovascularization, that worsens their prognosis\(^{(8)}\). Different natural course occurs indeed in vascularized PED, and it’s related to the type of neovessels associated. The most common acute complication is the tearing of the RPE\(^{(6,41-43)}\). It usually occurs at the edge of the PED, at the intersection of the detached and attached RPE. Clinically, a RPE tear or rip appears as a well-defined area of bare choroid, contiguous to a darker hyperpigmented rugate area, that corresponds to the mound of the RPE that has torn away\(^{(44,45)}\). The ripped RPE usually rolls towards the occult CNV, and its propensity to tear can be predicted by the observation of pre-tear characteristics, such as an increase in the size and a modification in the shape, the presence of small holes at the PED margins, the presence of hemorrhages or subretinal fluid, but the most noteworthy aspect is the irregular filling of the PED visible at the FA\(^{(46)}\). RPE tears occurs either spontaneously, either after a treatment, formally laser photocoagulation, photodynamic therapy and intravitreal injection of steroids or anti-VEGF agents\(^{(47-57)}\). The exact pathogenesis of RPE tears is poorly understood. Concerning PEDs’ natural course, it has been hypothesized that tangential shearing forces in the PED can cause the break of the RPE basement membrane at the edge of the detachment; however it is more likely the result of several variables, where the presence of a CNV plays a major role. When RPE tear occurs after a treatment, various other causal relationships have...
been reported, varying from the heat generated by photocoagulation, to an abrupt increase of intra-PED fluid, a contraction of the associated CNV or the concomitant sudden resolution of the sub RPE fluid. The combining presence of a vitreomacular traction and the deformation of the globe due to the mechanical trauma by the needle have also been reported as causative agents\(^{(58)}\).

After RPE rips, the majority of patients complain a sudden severe visual decrease. In a small percentage of eyes, where the tear spares the fovea, patients can even experience a temporary preservation of good visual function\(^{(59)}\). However, in the long term, the progression of a subretinal scar leads to a severe visual decrease. In the prognosis of serous PED, it must be also considered the high risk of bilaterality, with symmetrical behavior\(^{(60)}\).

Treatment of serous PED, associated or not with CNV, has always been a challenge and so far there are no recommended guidelines for their management. Pure serous PEDs have been treated in the past with laser grid or scattered photocoagulation, nevertheless with disappointing results\(^{(61)}\). No other approaches have been attempted to treat these lesions. On the other hand, when a neovascular net is present, treatment of serous PED has been focused on CNVs management. However, given that vascularized PEDs have never been included in the major RCTs, we must establish our treatment decision on published small series, often retrospective, that hint different therapeutic approaches. By and large, in the antiangiogenic therapy era, all the previous employed treatments appear unsatisfactory to the occasion. Laser photocoagulation, far-back widely employed, can still have a limited indication when an ICG-well defined CNV lies remote to the detached RPE\(^{(62)}\). Verteporfin photodynamic therapy alone has been proved to be harmful, increasing the risk of RPE tear, hemorrhages and sudden visual acuity decrease\(^{(43, 48, 49, 63)}\) (Fig. 5).

On the contrary, PDT combined with intravitreal triamcinolone acetonide injection has been demonstrated as potentially capable of visual acuity stabilization and recurrences reduction\(^{(64)}\). However, the high rate complications (cataract and glaucoma) has reduced the intravitreal triamcinolone use. The optimistic results obtained with the anti-VEGF intravitreal therapy in the treatment of occult CNV have extended its employment to the vascularized PED, however with disappointing results\(^{(65-68)}\). As a matter of fact, both acute complications

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**Figure 4 - Retinal Angiomatous Proliferation (right) and Polypoidal Choroidal Vasculopathy (left): ICGA and OCT patterns**
and scanty response to the treatment frequently invalidate our attempts to heal the lesion. RPE tears and subretinal hemorrhages are reported to complicate ranibizumab and bevacizumab treatments\(^{[51-57]}\). Moreover, the sub-RPE fluid hardly responds to the anti-VEGF therapy, probably due to the hydroconductivity changes of Bruch’s membrane\(^{[68]}\). In a recent retrospective case series of 328 patients treated with bevacizumab, ranibizumab, pegaptanib and PDT+IVTA respectively, after a mean follow-up of 42.4 weeks the authors reported a significant stabilization of visual acuity in each group, better in the bevacizumab and ranibizumab ones compared to the other two, and an overall RPE tears frequency of 12.5%. However, they conclude that with these treatments, only a partial regression of the lesions can be obtained, and the risk of RPE tears is not avoided\(^{[68]}\). In the future, new combination therapies and new therapeutic strategies, tested in multicentric clinical trials specifically designed, will help to improve the prognosis of the patients affected by vascularized PED.

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A better understanding of the pathophysiological processes occurring in “retinal aging” and age-related macular degeneration (AMD) has been achieved in recent years, leading to the emergence of new treatments and consequent long-term improvements in the quality of life of patients. AMD is one of the leading causes of severe, irreversible vision impairment in developed countries, in individuals over 50 years of age. Approximately 1.75 million people over 40 in the United States suffer from neovascular AMD or geographic atrophy; 7.3 million patients display large drusen (≥125 microns) in one or both eyes (1).

In the United States, AMD causes approximately 46% of severe visual loss cases (visual acuity of 20/200 or worse) in patients over 40 (1). Although, an estimated 80% of AMD patients display the non-neovascular form of this disease, the neovascular form is responsible, for almost 90%, of cases of severe visual loss (visual acuity of 20/200 or worse) caused by AMD (1). Data from three population-based studies – the Beaver Dam Eye Study, the Rotterdam Study and the Blue Mountains Eye Study – have led to an estimated prevalence of advanced AMD of 0-2% in patients aged 55–64, increasing to 13% in patients over 85 (1). Since there is no significant cure for AMD, prevention may be the first and logic approach to reduce vision loss, justifying an intensive search for some kinds of intervention able to prevent the onset of AMD or to delay its progression to more advanced and severe forms. Age is the main risk factor for AMD; all population-based studies confirm that the prevalence of AMD increases with age in white individuals (1). Belonging to the female gender may also constitute a risk factor in individuals aged over 75 years (1). Several studies also demonstrated that effective control of modifiable risk factors, such as smoking, hypertension and body-mass index, could reduce the risk of developing AMD by half (1). Since the early 90’s, when “large population studies” appeared, several hypotheses have been formulated around the idea that nutritional supplements such as antioxidants, vitamins and/or minerals may be able to reduce the risk of AMD development.

2. AREDS (Age-related Eye Disease Study)

2.1 AREDS 1

2.1.1 Design implications and study categories

AREDS (Age-related Eye Disease Study) was a prospective, multicentric, randomised clinical trial conducted between 1992 and 2006, mainly sponsored by the National Eye Institute (NEI) of the National Institutes of Health (NIH). This study was designed to evaluate the clinical aspects, natural course and risk factors associated with age-related cataract and AMD, as well as the effects...
of antioxidant vitamins and minerals on these two ocular conditions.

Eligible patients were aged 55-80 by occasion of enrolment and required to be free of any illness or condition that would make long-term follow-up or compliance with study medications unlikely or difficult. Participants were placed in one of several AMD categories according to fundus photographs graded by a central reading centre, best corrected visual acuity and ophthalmic examination:\footnote{10}

**AREDS category 1** – (No AMD) – this was the AREDS control group, consisting of patients with no or a few small drusen (\(<63\) microns in diameter).

**AREDS category 2** – (Early AMD) – characterised by a combination of multiple small drusen, a few intermediate drusen (63 to 124 microns in diameter) or RPE abnormalities.

**AREDS category 3** – (Intermediate AMD) – characterised by extensive intermediate drusen, at least one large drusen (125 microns in diameter) or geographic atrophy not involving the centre of the fovea.

**AREDS category 4** – (Advanced/Late AMD) – characterised by one or more of the following (in the absence of other causes), in one eye:
- Geographic atrophy of the RPE and choriocapillaris, including the centre of the fovea
- Neovascular maculopathies, such as the following:
  - Choroidal neovascularization (CNV)
  - Serous and/or haemorrhagic detachment of the sensory retina or the RPE
  - Hard exudates in the retina
  - Subretinal and sub-RPE fibrovascular proliferation
  - Disciform scar\footnote{10}.

### 2.1.2 Risk factors and categories

AREDS Report no. 18 described a simplified clinical scale that defines risk categories for the development of advanced AMD.

The grading system described assigns one risk factor to each eye for the presence of one or more large drusen (125 microns) and one risk factor for the presence of any pigment abnormality.

If no large drusen are present, the presence of intermediate drusen in both eyes is counted as one risk factor. Advanced AMD in one eye is counted as two risk factors; if this is observed together with large drusen and hypo/hyperpigmentary changes in the RPE, four risk factors are considered, which corresponds to the highest risk level for patients with AMD.

Risk factors are added for both eyes, leading to a 5-stage scale (0-4) according to which the approximate 5-year risk of developing advanced AMD in at least one eye increases as follows:\footnote{11}:

- Stage 0 (zero factors) – 0.5% in five years
- Stage 1 (one factor) – 3% in five years
- Stage 2 (two factors) – 12% in five years
- Stage 3 (three factors) – 25% in five years
- Stage 4 (four factors) – 50% in five years\footnote{11}

### 2.1.3 Results

AREDS results show an overall beneficial effect for high doses of antioxidant vitamin (vitamins C, E and beta-carotene) and zinc supplements in reducing the progression of intermediate or advanced AMD to advanced AMD in the fellow eye, corresponding to 25%.

Therefore, a formulation has been proposed:\footnote{12}:

**2.1.3.1 AREDS 1 formulation:**
- Antioxidant vitamins – 500 mg of vitamin C; 400 IU of vitamin E; 15 mg of beta-carotene
- 80 mg of zinc oxide and 2 mg of cupric oxide

This formulation has been shown to reduce the risk of developing advanced AMD and the associated visual loss by as much as 25%, over 5 years, in individuals with moderate to high risk of age-related macular degeneration (AREDS categories 3 and 4). These findings were accompanied by a 19% reduction in the risk of moderate vision loss (loss of three or more lines on the visual acuity chart), at 5 years\footnote{13}.

However, this formulation is not recommended for smokers, since beta-carotene has been shown to increase the risk of lung cancer\footnote{14,15}.

### 2.2 AREDS 2

The Age-related Eye Disease Study 2 (AREDS2), initiated in 2006 and still in course, enrolled 4000 patients with non-neovascular AMD consisting of large drusen in both eyes or advanced AMD in one eye and large drusen in the fellow eye (AREDS categories 3 and 4). The aim of this study is to evaluate the effect of dietary supplements – xanthophylls (10 mg of lutein and 2 mg of zeaxanthin) and/or long-chain omega-3 polyunsaturated fatty acids (1 g of docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]) – on the progression to advanced AMD. An additional goal of the study is to
assess whether forms of the AREDS nutritional supplement with reduced zinc and/or no beta-carotene works as well as the original supplement in reducing the risk of progression to advanced AMD.

2.2.1 AREDS 2 formulation:

Antioxidant vitamins – 500 mg of vitamin C; 400 IU of vitamin E
15 mg of beta-carotene
80 mg of zinc oxide and 2 mg of cupric oxide
Macular pigments – xanthophylls
10 mg of lutein
2 mg of zeaxanthin
1 g of omega-3 fatty acids (DHA + EPA)

This study will also investigate whether the current AREDS formulation might be modified by eliminating beta-carotene. As previously mentioned, beta-carotene, which is not present within the eye, constitutes a problem for smokers, due to the high incidence of lung cancer in this patient group. A secondary randomisation in AREDS 2 will evaluate the possibility of eliminating and/or lowering the amount of zinc in the AREDS formulation, since zinc levels in the current formulation are considered high and available evidence suggests that the body is only able to absorb 25 mg of zinc per day.

3. Ocular Micronutrition

3.1 Carotenoids

3.1.1 Macular pigments: lutein and zeaxanthin

Over 600 carotenoids have been identified to the present date; however, only about 50 are found in normal human diets. Vegetables are the only source of carotenoids. Macular pigments (MP) consist of two natural xanthophylls from the carotenoid family: lutein (L) and zeaxanthin (Z), as well as the products of their transformation in the body, notably meso-zeaxanthin. These pigments are not synthesised by the human body and have been investigated for their ability to promote visual health. Macular pigments are found in photoreceptor axons in the pigment epithelium and in the external segment. The latter contains very high levels of polyunsaturated fatty acids, at a high risk of oxidation. Macular concentrations of L and Z decrease with age, which exacerbates the harmful effects of blue light on photoreceptors. Their protective mechanism is unknown; however, two mechanisms have been proposed: macular pigments may act as an optical filter, due to their ability to absorb blue light, and they are strong antioxidants, neutralising free radicals generated by light. Several observational studies have demonstrated a correlation between plasma L and Z levels, macular pigment density and a lower risk of AMD. Increased intake of L and Z supplements resulted in increased plasma levels, which were positively and significantly associated with the optical density of macular pigment.

Furthermore, various well-conducted population-based longitudinal studies have suggested that high dietary antioxidant levels, specifically L and Z, may have protective and beneficial effects, delaying progression to advanced AMD. The following are some examples of observational studies investigating the relationship between dietary and/or serum antioxidant levels and the risk of AMD:

EDCCS (Eye Disease Case-Control Study): this study, published in 1992, demonstrated a high significant inverse relationship between the prevalence of AMD and serum L and Z concentrations: the risk of neovascular lesions was 70% lower in subjects with the highest serum L and Z concentrations, compared to those with the lowest levels (odds ratio: 0.4; 95% CI: 0.2-0.6; p= 0.0001). In 1994, the same group concluded that the risk of developing the most severe form of macular degeneration was 43% lower in individuals who consume large amounts of fruits and vegetables rich in L and Z (6mg/day) (odds ratio: 0.57; 95% CI: 0.35-0.92; p= 0.02).

Another observational study conducted in 2002 showed that average L and Z levels were 32% lower in AMD eyes than in control eyes, in elderly subjects, provided the latter were not consuming high doses of L supplements. This study demonstrated that average levels of macular pigments in patients who had begun to regularly consume supplements containing high doses of L (> 4 mg/day) after the initial diagnosis of AMD were within the normal range and significantly higher than in AMD patients not consuming this supplementation.

In 2006, the POLA study (Pathologies Oculaires Liées à l’Age) found that high plasma L and total L and Z concentrations are associated with a reduced risk of age-related maculopathy (ARM).

CAREDS (Carotenoids in Age-related Eye Disease Study): in this observational study, conducted in 2006, no statistically significant differences in intermediate AMD
prevalence were found between subjects with high and low dietary intakes of L plus Z. However, analysis of a sub-group of women under 75 with stable L plus Z intakes revealed a reduced risk of this subtype of AMD in association with a high dietary intake of those antioxidants. A diet rich in L plus Z may protect against intermediate AMD\(^\text{27}\).

In 2007, AREDS (Age-related Eye Disease Study report \text{22}), a case-control study with 4519 participants, concluded that a high dietary intake of L and Z was independently associated with a decreased likelihood of neovascular AMD, geographic atrophy, and large and extensive drusen\(^\text{28}\).

Up to the present date, seven important interventional studies have investigated the role of L supplementation in AMD patients\(^\text{29-35}\).

Of the aforementioned studies, two assume major importance:

**LAST** (Lutein Antioxidant Supplementation Trial) was the first study to show that L supplementation improved visual function in AMD patients. Furthermore, it reinforced the notion that AMD is a nutrition-responsive disease. The results of this trial were confirmed in 2004. In this trial, 90 AMD patients received either a daily supplement consisting of 10mg of L, a supplement consisting of 10 mg of L and a mixed antioxidant formula (containing vitamin A, beta-carotene, vitamin C, vitamin E, vitamin B complex, copper, zinc, manganese, magnesium, selenium and other minerals), or placebo, for 12 months. Results showed that patients receiving the L supplement displayed significant improvements in several objective visual function measures (contrast sensitivity or visual acuity) when compared to the placebo group. Slightly better results were observed in subjects consuming the combined supplement\(^\text{34}\).

**CARMA** (Carotenoids in Age-related Maculopathy Study) was an important European intervention study, published in 2008. This randomised, double-blind clinical trial of antioxidant supplementation versus placebo enrolled 433 patients from two centres in Ireland, with signs of early AMD of sufficient severity in at least one eye, or any level of AMD in one eye and late AMD (neovascular AMD or central geographic atrophy) in the fellow eye. The aim of the CARMA Study was to investigate whether administration of 12 mg of L and 2 mg of Z, in combination with antioxidants (120 mg of vitamin C, 15mg of vitamin E, 20mg of zinc and 0.4 mg of copper), had a beneficial effect on visual function and/or was able to delay progression of early to late disease stages.

The primary outcome was improved or distance visual acuity was preserved at 12 months. Although no beneficial effects were demonstrated in the primary outcome measure at the stated end point (12 months), secondary outcomes favoured the supplemented group\(^\text{35}\).

**3.1.2 Key points:**

The aforementioned findings are consistent with the hypothesis that low L and Z levels in the human macula may represent a pathogenic risk factor for AMD development. They also suggest that supplementation may contribute to maintaining eye health.

**3.2 Antioxidants**

**3.2.1 Vitamin and mineral supplements**

Antioxidants are recommended for AMD due to oxidative stress on photoreceptors in the retina and the fact that cumulative damage caused by blue light enhances free radical production. It has been proposed that antioxidants may prevent cellular damage in the retina by reacting with free radicals\(^\text{36}\).

The substances that possess antioxidant activity are vitamins C and E, beta-carotene and some minerals, such as zinc, copper, selenium and manganese.

Some studies indicate that diets rich in antioxidants may protect against the appearance of signs of early AMD; in common perception, a diet rich in antioxidants is capable of protecting against AMD.

Randomised control trials and observational studies have been conducted in well-nourished Western populations; however, the role of dietary antioxidants in the primary prevention of AMD remains unclear. In the 90’s, several studies reported a protective effect against AMD development for high intakes of antioxidant vitamins and minerals.

In 1993, the EDCCS (Eye Disease Case-Control Study), performed a comparison between 421 patients with neovascular age-related macular degeneration and 615 control subjects, the results revealed that high plasma levels of antioxidants (vitamins A, C, E, selenium and carotenoids) are associated with a lower risk of developing neovascular AMD. Additional carotenoid intakes, particularly of those present in the retina, are associated with a lower risk of developing AMD\(^\text{37}\).

In 1994, the authors of the Baltimore Longitudinal Study on Aging, a study involving 976 patients, reported a protective effect against AMD for high plasma concentrations of Vitamin E. The authors also found an antioxidant combination of Vitamin C, Vitamin E and beta-carotene to be protective\(^\text{38}\).

In 1998, the Beaver Dam Eye Study, in which a cohort
of 1,700 patients was subject to a 5-year follow-up eye examination, showed that a high intake of carotenoids and vitamin E is associated with a lower risk of developing large drusen. High dietary zinc intakes would be associated with a lower number of retinal pigment epithelium anomalies.

In 2001, AREDS report no. 8, a large multicentric, randomised clinical trial, revealed that the risk of progression to advanced AMD was reduced by 28% in patients with intermediate AMD treated with high doses of antioxidant supplements (vitamins C and E, zinc and β-carotene), when compared to the placebo group (odds ratio: 0.72; 99% confidence interval: 0.52-0.98). This study did not specifically investigate whether antioxidant supplements were effective in the primary prevention of early AMD in individuals without signs of this condition.

In 2004, AREDS report no. 13 evaluated mortality rates in patients with ocular disorders taking high doses of antioxidants or zinc. Results showed that mortality was lower in patients taking zinc (alone or with antioxidants) (12% reduction), when compared to those not taking this mineral (RR: 0.73; 95% CI: 0.61-0.89).

In 2005, the Rotterdam Study, a population-based study involving 4170 participants, showed that an above-average intake of the 4 AREDS trial nutrients protected against AMD development or early AMD, as indicated by large drusen, and was associated with a 35% reduction in the risk of AMD (HR: 0.65; 95% CI: 0.46-0.92).

In 2007, Chong and colleagues undertook a systematic review and meta-analysis of nine prospective cohort studies and three randomised clinical trials. The results from the first studies indicated that vitamin A, vitamin C, vitamin E, zinc, L, Z, alpha-carotene, beta-carotene, beta-cryptoxanthin and lycopene have little or no effect in the primary prevention of early AMD. The three randomised clinical trials failed to show that antioxidant supplements prevented early AMD.

In 2008, a systematic review and meta-analysis undertaken with the objective of examining available evidence as to whether antioxidant vitamin or mineral supplements are able to prevent AMD development or delay its progression was published online. No evidence was found that antioxidant (vitamin E or beta-carotene) supplements are able to prevent AMD (RR 1.03; 95% CI: 0.74-1.43).

Some evidence was found that antioxidant (beta-carotene, vitamin C and E) and zinc supplements were able to delay progression to advanced AMD and prevent loss of visual acuity in individuals displaying signs of the disease (adjusted odds = 0.68; 95% CI: 0.53-0.57, and 0.77; 95% CI: 0.62-0.96, respectively).

3.2.2 Key points

According to current evidence, antioxidant vitamin supplements are unable to prevent AMD. Patients with AMD or early AMD may benefit from taking AREDS trial supplements. High-dose antioxidant supplementation may increase the risk of lung cancer in smokers (beta-carotene), heart failure in individuals with vascular disease or Diabetes (vitamin E) and hospitalisation in patients with genitourinary conditions.

3.3 Dietary fatty acids

3.3.1 Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)

The role of fatty acids in AMD was initially investigated because of the hypothesis that AMD and cardiovascular disease may share a similar pathogenesis and fat intake has been associated with atherosclerosis and cardiovascular disease. Fatty acids may be divided into three types:

- Saturated fat from dairy products and meat.
- Monounsaturated fatty acids (MUFA) from olive oil.
- Polyunsaturated fatty acids (PUFA), especially from fish and seafood.

Omega-3 fatty acids, also known as Long-Chain Polyunsaturated Fatty Acids (LCPUFAs), are essential to human health. Omega-3 fatty acids include alpha-linolenic acid (a short-chain omega-3 fatty acid) and long-chain omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

Omega-3 fatty acids, especially DHA, have morphological, functional and protective roles in the retina:

1. Morphological role – DHA is the main PUFA found within the outer segments of rods and has anti-apoptotic, anti-inflammatory and antiangiogenic functions.
2. Functional role – DHA provides an adequate environment for conformational changes in rhodopsin.
3. Protective role - DHA protects against aging of the retina and may reduce lipofuscin accumulation in the retinal pigment epithelium and lipid deposits in Bruch's membrane.

Several epidemiological studies have evaluated the relationship between total and specific fat intake and the risk of advanced AMD. Results confirm that higher intakes...
of vegetable and animal fat are associated with a greater risk of advanced AMD.

In 2008, a systematic review and meta-analysis was undertaken with the objective of evidencing the role of dietary omega-3 fatty acid and fish intakes in the primary prevention of AMD.

This review included three randomised, controlled, prospective cohort trials\(^{(45,46,47)}\), three case-control studies\(^{(48,49,50)}\) and three cross-sectional studies\(^{(51,52,53)}\).

The results of these nine studies demonstrated that high dietary omega-3 fatty acid intakes were associated with a 38% reduction in the risk of late AMD (OR: 0.62; 95% CI: 0.48-0.82). Eating fish at least twice a week was associated with a reduced risk of both early AMD (OR: 0.76; 95% CI: 0.64-0.90) and late AMD (OR: 0.67; 95% CI: 0.53-0.85).

Several other relevant studies evidence this fact:

A prospective study conducted by Cho et al. in 2001 evidenced a positive association between total fat intake and incidence of AMD. A diet rich in fat increases the risk of advanced AMD. Nevertheless, eating fish 4 or more times a week (fish is a major source of DHA) decreases the relative risk of AMD by 35%\(^{(54)}\).

A case-control study conducted by SanGiovanni et al. concluded that higher omega-3 and fish intakes are associated with a decreased risk of neovascular AMD\(^{(55)}\).

The objective of AREDS report n. 20 was to evaluate the association between lipid intake and age-related macular degeneration severity at baseline. The results of this study showed that total dietary intake of total long-chain omega-3 polyunsaturated fatty acids (LCPUFA) was inversely associated with neovascular (NV) AMD (odds ratio (OR): 0.61; 95% confidence interval (CI): 0.41-0.90), the same occurring for docosahexaenoic acid, a retinal omega-3 LCPUFA (OR: 0.54; 95% CI: 0.36-0.80), when the highest and lowest intake quintiles were compared, after adjustment for total energy intake and covariates. Higher fish intakes, both total and broiled/baked, were also inversely associated with NV AMD (OR: 0.61; 95% CI: 0.37-1.00, and OR: 0.65; 95% CI: 0.45-0.93, respectively). Dietary intake of arachidonic acid was directly associated with the prevalence of NV AMD (OR: 1.54; 95% CI: 1.04-2.29). No statistically significant relationships were found for other lipids or groups\(^{(56)}\).

More recently, in AREDS report n. 23, reduced likelihood of progression from bilateral drusen to CGA was observed in individuals reporting the highest EPA intakes (odds ratio (OR): 0.44; 95% confidence interval (CI): 0.23-0.87) and EPA + DHA intakes (OR: 0.45; 95% CI: 0.23-0.9). DHA levels were associated with CGA in age, gender and calorie adjusted models (OR: 0.51; 95% CI: 0.36-1.00). However, this statistical relationship was not observed in multivariable models. This study suggested that dietary intake, of long-chain omega-3 polyunsaturated fatty acids, is associated with a decreased risk of progression from bilateral drusen to CGA\(^{(57)}\).

European study Nat-2, performed at the University of Créteil, a double-blind, randomised, parallel, comparative study, compared oral DHA supplementation with placebo in the prevention of exsudative age-related macular degeneration in 298 patients with any type of drusen in the study eye and wet AMD in the fellow eye. Nat-2 supplementation consisted of 10 mg of L, 2 mg of Z, 1 mg of omega-3 (DHA plus EPA), 500 mg of vitamin C, 400 IU of vitamin E, 25 mg of zinc and 2 mg of copper. Patients took no other supplements and were followed for three years (2004-2008). The first study results included in NAT-2 report no. 1, revealed high HDL and low PUFA levels in exsudative AMD patients. These findings confirmed the benefits of DHA supplementation in these AMD patients\(^{(58)}\).

Two important prospective observational studies clearly reveal that fish consumption and omega-3 fatty acid intake decrease the risk of AMD:

The Blue Mountains Eye Study and the Melbourne Collaborative Cohort Study.

Blue Mountains Eye Study: The objective of this longitudinal study was to investigate the association between baseline dietary fatty acids and 10-year incidence of AMD in an elderly Australian cohort. Nutrient intakes were estimated through a semi-quantitative food frequency questionnaire.

The risk of incidence of early AMD was lower in individuals consuming 1 to 2 servings of nuts per week (RR: 0.65; 95% CI: 0.47-0.91). These results were similar to those obtained for dietary consumption of long-chain omega 3 PUFAs, which also show a lower risk of incidence of early AMD in participants eating 1 serving of fish per week (RR: 0.69; 95% CI: 0.49-0.98). Participants consuming below-average amounts of linoleic acid contributed the most to this association (RR: 0.57; 95% CI: 0.36-0.89). Nut consumption was associated with a lower risk of pigmentary abnormalities in non-smokers, individuals with below-average total to high-density lipoprotein serum cholesterol ratios, and individuals with above-average beta-carotene intakes\(^{(59)}\).

Melbourne Collaborative Cohort Study: the aim of this study, carried out in 1990-1994, was to investigate the relationship between past dietary fat intake and the
prevalence of AMD in a cohort of 6734 Australian participants aged 58-69. The corresponding results showed that a higher dietary intake of trans unsaturated fats was associated with an increased prevalence of late AMD. Comparing the highest and lowest trans fat intake quartiles, the OR for late AMD was 1.76 (95% CI: 0.92-3.37; p = 0.02), whereas a higher intake of omega-3 fatty acids was inversely associated with early AMD (OR for highest quartile vs. lowest quartile: 0.85; 95% CI: 0.71-1.02; p = 0.03).

The prevalence of late AMD was lower for olive oil intakes equal to or higher than 100 mL/week vs. less than 1 mL/week (OR: 0.48; 95% CI: 0.22-1.04; P = 0.03). No significant associations were found between fish, total fat, butter and margarine intakes and AMD.

More recently, in 2009, the SanGiovanni AREDS Group investigated the relationship between dietary omega-3 LCPUFA intake and progression to advanced AMD in 1837 AREDS participants with a moderate risk for developing sight-threatening AMD (1211 participants in category 3a and 626 participants in category 4a).

It was observed that participants reporting the highest baseline omega-3 LCPUFA intakes were approximately 30% less likely to develop advanced AMD by the end of the 12-year follow-up period than those reporting the lowest omega-3 LC-PUFA intakes. Results for CGA and NV AMD were similar; the corresponding multivariate odds ratios were 0.65 (95% CI: 0.45-0.92; p ≤ 0.02) and 0.68 (95% CI: 0.49-0.94; p ≤ 0.02).

3.3.2 Key points:

Several studies support the benefits of regular dietary fish and omega-3 PUFA intakes on the risk of AMD, especially in individuals with lower omega-6 to omega-3 ratios. Other studies have also suggested that higher vegetable fat and trans unsaturated fatty acid intakes increase the prevalence of late AMD, while omega-3 fatty acids and olive oil were associated with a reduced prevalence of early and late AMD.

3.4 Preventive treatment pyramid

Indications for AMD prevention are based on the risk factors and disease stages described in AREDS report no. 18.

Therefore, a treatment “pyramid” may be defined for individuals over 50 years of age:

- **Stage 0** (zero factors): annual observation
- **Stage 1-2** (one-two factors, drusen < 125 + pigment abnormality): DHA +/- Lutein
- **Stage 3** (three factors): antioxidants + zinc (AREDS report no. 8)
- **Stage 4** (four or more factors): antioxidants + DHA

Based on AREDS data, individuals over 50-55 should be subject to dilated eye examinations to determine their risk of developing advanced AMD. Patients with extensive intermediate-size drusen, at least 1 large drusen, non-central GA in 1 or both eyes, advanced AMD or vision loss due to AMD in 1 eye, and without contraindications such as smoking, should consider taking an antioxidant and zinc supplement, such as those used in this study.

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Currently, three types of therapy are approved for the treatment of exudative age-related macular degeneration (AMD): laser photocoagulation, photodynamic therapy with Verteporfin and intravitreal injections of antiangiogenic agents (Ranibizumab and Pegaptanib). Other treatments revealed to be ineffective or even more aggressive than natural disease progression. Examples of these later are radiotherapy, surgical removal of the subfoveal membranes, alpha 2 Interferon, transpupillary thermal therapy and anecortave acetate injections.

2. Laser photocoagulation

2.1 Laser photocoagulation in exudative AMD

The Macular Photocoagulation Study Group (MPS, 1982-1997)\(^{1-16}\) has performed several randomized, double-blind, placebo-controlled studies in patients with exudative AMD. These studies showed that laser photocoagulation might be effective in reducing loss of vision in cases of well defined exudative AMD lesions. The importance of well-defined limits resulted from the absolute need to treat the entire lesion in order to maximally reduce recurrence and persistence rates, generally associated to greater loss of vision. For extrafoveal and juxtafoveal lesions, results were evaluated for well-defined lesions before any differentiation based on angiographic patterns (occult or classic) was made. In the subfoveal lesion study, results were evaluated for well-defined lesions with a classic component.

In addition to defining which cases to treat and the benefits expected from laser photocoagulation, the MPS defined the angiographic characteristics of neovascular lesions and guidelines for treating each type of membrane (extra, juxta or subfoveal), including preparation for treatment, treatment techniques, the wavelength to be selected, post-treatment care, special circumstances and expected complications.

Other studies of extra, juxta and subfoveal lesions with less impact on everyday clinical practice were performed by various authors using the same treatment technique studied by the MPS (for extra and juxtafoveal lesions) or macular grid photocoagulation (subfoveal lesions)\(^{17,18,9}\).

2.2 Macular Photocoagulation Study Group (MPSG): extrafoveal lesions.

The first study was performed by the MPSG in patients with well-defined extrafoveal neovascular lesions (located 200 to 2500 μm from the foveal centre), with drusen, age ≥ 50 years and VA ≥ 20/100. No differentiation was made between classic and occult membranes in this study. Choroidal neovascularization was angiographically defined as the presence of leakage in the external retina. Patients were enrolled in the study between 1979 and 1982 and treated with blue-green Argon laser. The first results were published in the latter year (MPS, 1982)\(^{1,2}\). The MPS concluded that laser photocoagulation with blue-green or green Argon laser of sufficient intensity to produce nearly white lesions in the retina and cover the entire neovascular lesion, as well as adjoining blood, reduces the risk of additional and severe loss of vision, when compared to natural progression of the disease. The benefits of laser were greater during the first year following treatment, having persisted after 5 years\(^{9}\). The probability of stabilizing or increasing VA doubled for treated eyes; a 58% reduction in the risk of severe loss of vision (6 lines in the ETDRS scale) was also observed. After 5 years, 48% of treated eyes and 62% of non-treated eyes had lost ≥ 6 lines. These results show reduced efficacy when evaluated in terms of the number needed to treat\(^{20}\). It was necessary to treat 7 patients
for one patient to benefit from the treatment. Average VA after 5 years was 20/125 in the treated group and 20/200 in the non-treated group (MPS, 1982, 1986). After 5 years, 54% of treated eyes had shown recurrence with severe loss of vision most of them occurring in the first 2 years after treatment, they have been responsible for the majority of cases of severe loss of vision in the treated group. Smokers had a greater risk of recurrence (recurrence was observed in 85% of patients smoking more than 10 cigarettes per day, compared to 51% of non-smokers).

Similar results were obtained in two other studies performed in the United Kingdom(17) and France(19).

2.3 Macular Photocoagulation Study Group (MPSG): juxtafoveal neovascular lesions

A second study was performed with juxtafoveal membranes and using a Krypton laser(11). Inclusion criteria allowed treatment of well-defined choroidal neovascular lesions located 1 to 199 μ from the foveal centre, or 200 to 2500 μm from the foveal centre but showing blood or pigmentation less than 200 microns from the foveal centre (resulting in a barrier effect in fluorescence). As opposed to the study with Argon laser in extrafoveal membranes, this trial did not require treatment of the entire area where bleeding occurred. Under no circumstances should treatment reach the foveal centre. After 3 years, severe loss of vision (≥6 lines) had occurred in 49% of treated eyes and 58% of non-treated eyes(14). The efficacy of this treatment in terms of the number needed to treat(20) was very low: 11.1. This treatment reduced the risk of severe loss of vision by 10%. However, this benefit was not observed in patients with hypertension or taking antihypertensive medication. Nevertheless, the MPS maintained the indication to treat for these non-normotensive patients due to the absence of similar findings in other MPS studies. After 5 years, the number of eyes with final VA ≥ 20/40 was double for treated eyes(14). Persistence (incomplete treatment) and recurrence (neovascularization later than six weeks after treatment) were responsible for the majority of loss of vision in the treated group.

The MPS reclassified membranes as 100% classic, classic with an occult component and 100% occult. Results were better in classic membranes: 54% of treated eyes and 72% of non-treated eyes lost 6 or more VA lines. No statistically significant differences were observed between the treated and non-treated groups in cases of mixed membranes (only the classic component was treated) and 100% occult membranes. Therefore, treatment of occult membranes and the classic component of mixed membranes was not effective in reducing loss of vision(14,16).

In conclusion, the MPS showed that laser photocoagulation of well-defined extrafoveal choroidal membranes and classic extra and juxtafoveal membranes secondary to AMD may prevent or delay loss of vision in patients fulfilling the inclusion criteria.

2.4 Macular Photocoagulation Study Group (MPSG): subfoveal neovascular lesions

In 1986, the MPS started two studies(12,13) to determine the efficacy of laser photocoagulation in subfoveal choroidal neovascularization. In the first study, the effect of laser photocoagulation (Argon or Krypton) was evaluated in eyes with subfoveal exudative AMD not previously treated; in the second study, the efficacy of laser treatment in subfoveal recurrence in eyes with extra or juxtafoveal membranes was evaluated. The results of this study and treatment recommendations generated a great deal of controversy. In fact, treated eyes displayed a very marked loss of vision immediately after treatment. After 4 years, 30% of treated eyes and 60% of non-treated eyes displayed VA ≤ 20/400, whereas 45% of non-treated eyes and 23% of treated eyes has suffered severe loss of vision. The efficacy of this treatment in terms of the number needed to treat was 4.5(20).

A large percentage of ophthalmologists did not agree with the MPS recommendations for treating subfoveal lesions. In fact, patients were losing 3 lines immediately after treatment. The MPS re-evaluated treatment efficacy in terms of lesion size and difference from baseline VA, having established treatment groups and criteria according to these two variables(13). Ophthalmologists could advise their patients and help them choose whether or not to undergo treatment according to lesion size and baseline VA.

With the emergence of photodynamic therapy with Verteporfin, laser photocoagulation for subfoveal lesion became obsolete. It remains indicated only for extrafoveal lesions and the angiographic control should be performed 15 days after treatment.

2.5 Treatment

2.5.1 Preparation for treatment

Regarding preparation for treatment, the MPS recommended that patients should be informed that
photocoagulation causes permanent paracentral scotoma in cases of juxta and extrafoveal choroidal membranes. Patients should also be informed that they may continue to lose vision, even under the best treatment conditions, and that treatment does not cure AMD but it is only a means of reducing the risk of marked loss of visual acuity. A fluorescein angiography (FA) should be performed 72 to 96 hours before photocoagulation in order to select treatable cases and to guide the ophthalmologist during treatment. Patients should undergo treatment as quickly as possible, since neovascular lesions may grow 10 to 18μ per day(21). Most neovascular lesions are extra or juxtafoveal at the onset, becoming subfoveal with rapid growth towards the fovea.

2.5.2 Treatment technique

The MPS recommends that treatment should be performed so that a white lesion in the retina is obtained. The neovascular lesion should be surrounded by laser marks with a diameter of 200μ and duration of 0.2 to 0.5 seconds. After surrounding the perimeter of the neovascular lesion, its central part is covered with 200μ burns; the remaining lesion is covered with 200 to 500μ burns, with duration of 0.5 to 1.0 seconds. In cases of juxtafoveal lesions, the foveal centre should be preserved, although it should be ensured that the entire lesion is treated. If bleeding extends to the area under the fovea, treatment should include the entire neovascularization area and stop at the limit of the fovea. Since the emergence of new treatments, namely intravitreal antiangiogenic treatments, laser photocoagulation of juxtafoveal lesions has become controversial. The MPS demonstrated that the wavelength selected does not affect laser results. Laser treatment should avoid retinal blood vessels and the optic nerve (treatment should start 10-200 μm from the optic nerve), as well as preserve at least 1.5 hours of the papillomacular bundle (no peripapillary treatment). Treatment of serous pigment epithelial detachment (PED) could be indicated when photocoagulation is used to treat subfoveal lesions including serous PED as a component(1,3,8,10,11).

2.5.3 Post-treatment follow-up

Follow-up of treated patients was also recommended and defined by the MPS. In addition to self-evaluation, it is necessary to perform medical examinations and control FA 2 to 3 weeks, 4 to 6 weeks, 3 to 4 months and 6, 8, 9 and 12 months after treatment. Recurrence is rare after 2 years. The greater risks exist 6 weeks to 12 months after treatment. Detection through biomicroscopy without FA is sometimes difficult. Angiography allows detection of approximately 12% of the cases that go unnoticed in medical examinations. Recurrence and persistence rates are much greater in cases of choroidal neovascular or disiform lesions caused by AMD in the non-treated eye. Other factors that appear to increase recurrence rates include smoking, hypertension and choroidal neovascularization with reduced pigmentation(1,2,8,11).

2.5.4 Treatment complications

Laser photocoagulation treatment may also lead to complications, including choroidal haemorrhage (rarer if spots ≥ 200 microns and time intervals ≥ 0.2 seconds are used), premacular fibrogliosis, accidental treatment of the fovea in extrafoveal or juxtafoveal lesions (minimised by retrobulbar anaesthesia, drawing of lesion limits and correct identification of the fovea), rupture of the pigment epithelium (more frequent in cases of PED) and atrophy of the RPE in the area adjoining the laser scar (immediately after treatment or years later)(2,3,7,8).

2.5.5 Treatment of occult membranes

The MPS also defined guidelines regarding occult membranes. When extrafoveal and juxtafoveal neovascular lesions caused by AMD started to be studied no distinction was made between classic and occult membranes. Subsequent analysis of all angiography results obtained during study of juxtafoveal lesions revealed that treatment was effective for classic neovascular lesions with no occult component. In cases where an occult component (not treated) coexisted with a classic component no benefits were gained from treatment(15). Photocoagulation may be reasonably considered in cases of well-defined, symptomatic, occult neovascular lesions with no classic component, in order to reduce the risk of membrane growth towards the fovea. However, little knowledge exists of the natural progression of these occult membranes and it would not be wrong to delay treatment while examining patients at regular intervals (of months, albeit varying according to the type of membrane), in order to wait for the appearance of a classic membrane that would benefit from laser photocoagulation treatment. Only 25% of occult choroidal membranes maintained baseline VA values after 3 years and approximately 50% suffer severe loss of vision within the same time period(13).
2.6 Other laser photocoagulation studies

In a retrospective study, Soubrane et al.\(^{18}\) demonstrated the absence of benefits for the treatment of extrafoveal and juxtafoveal occult neovascular lesions. Scatter or grid photocoagulation revealed to be ineffective in ill-defined neovascular lesions\(^{18}\).

In an attempt to preserve the foveal centre, Coscas et al\(^{19}\) described a form of ring treatment for subfoveal membranes. This randomized, placebo-controlled study included eyes with VA 20/200–20/1000. Treatment surrounded the 400 central μ of the central avascular area. After one year, baseline VA has been maintained or increased in 41% of treated eyes and only 20% of nontreated eyes. This technique was not well received.

Several groups determined the efficacy of laser photocoagulation in eyes with AMD and PED. In MPS studies, cases with PED were excluded. The Moorfields Macular Study Group\(^{17}\) showed that grid laser photocoagulation of “pure” PED (with no clinical or angiographic signs of choroidal membrane) had worsened prognosis in terms of VA.

With the advent of indocyanine green (ICG) angiography, enhanced imaging of occult CNV allowed a characterization of at least 2 forms of occult CNV: a plaque of late staining and a focal area of active vessel proliferation or a so-called “hot spot”\(^{22,23}\). ICG-laser photocoagulation was used in several centers\(^{23-28}\), in uncontrolled studies, to treat these hot spots with apparently relative success. Polypoidal choroidal vasculopathy (PCV) and retinal angiomatous proliferation (RAP), two AMD sub-types, represented the great majority of these treated cases. Laser photocoagulation in RAP lesions have shown very poor results with a high rate of persistence and recurrences\(^{29-30}\). Better results may be obtained in early lesions with extrafoveal hot spot resulting in stabilization of the pathology and visual acuity. However, an accurate follow-up is mandatory after the treatment due to the high rate of recurrences.

Direct laser photocoagulation of polypoidal lesions has shown controversial results\(^{31-34}\). Treatment of leaking polyps has proven short-term safety and efficacy for extrafoveal lesions\(^{31-32}\). Yuzawa et al\(^{35}\) reported good efficacy of laser photocoagulation in near 90% of the eyes if all the polyps and abnormal vascular network were treated. If the treatment involved only the polyps more than half of the eyes suffered VA decrease related with exudation, recurrences, or foveal scars. Considering the possibility of using other treatment modalities, laser photocoagulation should be reserved for well defined extrafoveal active polyps.

2.7 Conclusion:

Laser photocoagulation remains currently indicated for the treatment of well-defined extrafoveal choroidal membranes. For classic juxtafoveal membranes, laser photocoagulation could theoretically be considered as an option for cases in which the entire neovascular lesion can be treated without damaging the fovea. However, considering the great incidence of persistence and recurrences, intravitreal antiangiogenic agents are the first treatment option. Photodynamic therapy with verteporfin and antiangiogenic agents eliminated all indications for the treatment of subfoveal neovascular lesions, with laser photocoagulation.

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Bibliography


Photodynamic therapy was approved in 2000 as an alternative treatment for patients with AMD of the exudative form, having been the first effective pharmacological treatment for this form of the disease. Until then, laser photocoagulation was only successful in treating a small percentage of neovascular lesions (juxtafoveal and extrafoveal), excluding subfoveal lesions, which are more frequent.

With the emergence of antiangiogenic therapies, photodynamic therapy has been used less frequently. However, it remains current in three situations: in patients with systemic or ocular contraindications regarding intravitreal administration of antiangiogenic drugs, as an adjuvant, in combination with other drugs, and in the treatment of polypoidal choroidal vasculopathy.

2. Mechanism of action

Experimental studies\(^1\) suggest that photodynamic therapy (PTD) causes endothelial cell lesions, with formation of clots and selective vascular occlusion. Endothelial cell membrane lesions appear to be caused by free radicals released when verteporfin is activated by non-thermal laser light. These free radicals react with endothelial cell membranes and circulating blood cells, inducing platelet activation and local clot formation.

The mechanisms by which PTD induces tissue destruction are not exactly known. Three related mechanisms of action have been proposed: cellular, vascular and immune.\(^3\) The cellular mechanism, which is the most relevant, corresponds to the cytotoxic effects of free radicals on mitochondria, the endoplasmic reticulum and lysosomes. When exposed to these radicals, endothelial cell membranes rupture, exposing the basal membrane, which causes platelet adhesion and aggregation. Activated platelets release mediators such as histamine, thromboxane and TNF-\(\alpha\). These mediators trigger a sequence of events, namely vasoconstriction, thrombosis, increased vascular permeability, blood stasis and hypoxia. The proposed immune mechanism is based on the high concentrations of cytokines observed in patients subject to PDT, such as interleukin 2 and TNF–\(\alpha\). It is equally admitted that PDT may decrease immune response by reducing antigen-presenting cell activity.

Standard treatment consists of endovenous infusion of verteporfin at a dose of 6 mg/m\(^2\) body surface, for 10 minutes. Fifteen minutes after starting the infusion, the patient is treated with a diode laser with wavelength of 689 nm and light intensity of 600 mw/cm\(^2\), at a radiation dose of 50 J/cm\(^2\), with an exposure time of 83 seconds and a spot diameter corresponding to the diameter of the largest lesion plus 1mm.

These parameters have been studied and appear to be ideal, allowing maximum vascular effect with minimum photoreceptor and pigment epithelial cell damage. Verteporfin activation by the diode laser induces temporary closure of the choroidal neovascular complex, through the mechanisms already described, causing little damage to adjacent retinal structures. This characteristic doubtless represented a therapeutic advantage, since it allowed treatment of lesions whose location or size prevented use of other available therapies, namely conventional laser photocoagulation. However, photodynamic therapy does entail some damage, although induced retinal lesions are smaller than that occurring following thermal laser photocoagulation. Laser fluence reduction protocols have been proposed in the attempt to reduce the extent of this damage.

Therapy schemes with more intense treatment regimes, including treatment every 2 months in the first 6 months, were also tested. The efficacy and safety of the latter regime were compared with those of the standard
regime\(^{(40)}\). No statistically significant differences were found between the two regimes in terms of visual improvement, number of retreatments and safety. The intensive treatment regime in the first 6 months appears to be more effective in preventing severe loss of visual acuity; however, the difference observed after 24 months is not statistically significant, with loss of visual acuity greater than 6 lines being observed in 25\% of patients treated with the intensive regime and 38\% of patients treated with the standard regime.

### 3. Main clinical trials

The efficacy of PDT was evaluated in several multicentric, randomized clinical trials in patients with AMD with choroidal neovascularization, of which the following should be highlighted:
- Treatment of AMD with PDT (TAP studies)\(^{(5,6,7,8,9)}\)
- Verteporfin in PDT (VIP studies)\(^{(10,11)}\)
- Verteporfin in Minimally Classic Choroidal Neovascularization (VIM studies)\(^{(12)}\)
- Visudyne in Occult Classic Choroidal Neovascularization (VIO study)\(^{(13)}\)
- Meta-analysis of the TAP and VIP Studies\(^{(14)}\)
- Treatment of AMD with PDT – 5-year extension Study - TAP Extension\(^{(15)}\)

Many studies were subsequently performed (the results of some studies are still not available) in order to study and compare several therapeutic modalities, of which the following should be highlighted:
- Anti-Vascular endothelial growth factor (VEGF) Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization (CNV) in AMD (Anchor Study\(^{(16,17,18)}\))
- Ranibizumab Combined with Verteporfin Photodynamic Therapy in Neovascular AMD (Focus\(^{(19)}\))
- Summit Clinical Trial Program, which includes 3 studies: the Mont Blanc, Denali and Everest Studies\(^{(20)}\)

#### 3.1 TAP Study (Table 1)

This study provided the main evidence of PDT efficacy. It included two multicentric, double-blind, randomized, placebo-controlled studies, in Europe and the United States of America. Four hundred and two patients with classic subfoveal choroidal neovascularization were treated with PDT, while 207 patients were treated with placebo. The primary endpoint was the percentage of eyes for which losses of less than 15 ETDRS letters from baseline were observed at 12 and 24 months.

PDT was significantly more effective than the placebo, both at 12 months (61\% versus 46\%) and 24 months (55\% versus 38\%). These results were more significant in predominantly classic membranes.

<table>
<thead>
<tr>
<th>Study Number of patients</th>
<th>Pred. classic Verteporfin</th>
<th>Pred. classic Placebo</th>
<th>( P )</th>
<th>All membranes Verteporfin</th>
<th>All membranes Placebo</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAP: 12 months N=609</td>
<td>67.3%</td>
<td>39.8%</td>
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<td>61.2%</td>
<td>46.4%</td>
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<tr>
<td>TAP: 24 months N=609</td>
<td>59.1%</td>
<td>31.3%</td>
<td>&lt;0.001</td>
<td>53%</td>
<td>37.7%</td>
<td>&lt;0.001</td>
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<tr>
<td>TAP: 36 months N=476</td>
<td>58.1%</td>
<td>---</td>
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<td>---</td>
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<tr>
<td>TAP: 48 months</td>
<td>57%</td>
<td>---</td>
<td>---</td>
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</tr>
</tbody>
</table>

Table 1: TAP study: percentage of eyes with loss <3 lines in the ETDRS chart
3.2 VIP Study (Table 2)

In this study, the efficacy and safety of Photodynamic Therapy were evaluated in patients with occult lesions. Results after 12 months were somewhat disappointing; however, efficacy was demonstrated in the treated group at 24 months (46.2% versus 33.3%). Subgroup analysis led to the conclusion that greater benefits were achieved in patients with small lesions (less than 4 disc areas) and/or visual acuity worse than 20/50. In these patient subgroups, the differences between the PDT group and the placebo group had greater statistical significance (51% versus 25%).

3.3 VIM Study (Table 2)

The objective of this study was to determine the efficacy of photodynamic therapy in minimally classic membranes (where the classic component represents less than 50% of the neovascular lesion) sized below six disc areas. Additionally, the efficacy of reducing fluence to 50% (25J/cm²) relatively to standard parameters (50J/cm²) was also analysed. In the standard laser light activation protocol, a wavelength of 689 nm and an intensity of 600 mw/cm² are used for 83 seconds to achieve a fluence value of 50J/cm². In this study, no statistically significant efficacy was found at 12 and 24 months in the group of patients treated with the standard protocol. On the contrary, better results were observed for patients treated with the reduced fluence protocol, in terms of the primary endpoint (loss of visual acuity of less than 15 letters). Based on these results, the study authors advise treatment of small minimally classic lesions with PDT, concluding that the reduced fluence protocol may be beneficial. The percentage of conversion of minimally classic lesions into predominantly classic lesions was also studied and treatment efficacy was demonstrated, irrespective of the fluence used. The reduced fluence issue will also be referred in the Denali study. This study investigates the efficacy and safety of combined therapy involving PDT and antiangiogenic drugs, namely ranibizumab 0.5 mg, administered intravitreally. Patients were randomized to receive intravitreal injections of ranibizumab 0.5 mg, in monotherapy or combined with PDT, with standard or reduced fluence. This study, which started in May 2007, includes 321 patients and is currently in course in the United States and Canada. The results of the Denali study are not yet available.

3.4 VIO Study

The VIO study was designed to determine PDT indications in occult lesions with no classic component.

<table>
<thead>
<tr>
<th>Study</th>
<th>MTRI verteporfin</th>
<th>MTRI Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP 12 months</td>
<td>49%</td>
<td>45%</td>
<td>Ns</td>
</tr>
<tr>
<td>VIP 24 months</td>
<td>45%</td>
<td>32%</td>
<td>0.032</td>
</tr>
<tr>
<td>VIM 12 months</td>
<td>86%</td>
<td>53%</td>
<td>0.002</td>
</tr>
<tr>
<td>300 mw/cm²</td>
<td>72%</td>
<td>53%</td>
<td>0.08</td>
</tr>
<tr>
<td>VIM 24 months</td>
<td>74%</td>
<td>38%</td>
<td>0.003</td>
</tr>
<tr>
<td>300 mw cm²</td>
<td>47%</td>
<td>38%</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Table 2: VIP and VIM studies: percentage of eyes with loss <3 lines in the ETDRS chart.
Although the complete results report has not been published, the primary endpoint had not been reached at 12 and 24 months; therefore, no significant benefits were demonstrated for the treatment of occult membranes with PDT. These results led the EMEA to remove occult membranes from the list of photodynamic therapy indications (April 2007).

3.5 Meta-analysis of the TAP and VIP studies

The meta-analysis of the TAP and VIP studies was a retrospective analysis in which lesion size, composition and visual acuity at baseline were considered, as well as possible relations between these parameters and study results. The objective of this meta-analysis was to explain the apparent discrepancies found between the TAP and VIP study results, considering the following:

- in the TAP study, treatment was found to be beneficial in predominantly classic and occult lesions, whereas it was found not to be beneficial in minimally classic lesions;
- in the VIP study, treatment of occult lesions was found to be more beneficial in small lesions (≤4 disc areas) and/or visual acuity <20/50. This meta-analysis revealed that the most important factor in predicting final visual acuity in patients treated with PDT appears to be lesion size. Therefore, treatment of small lesions (≤4 disc areas) will be beneficial for all types of lesions, including occult lesions with no classic component, provided lesions are recent. Regarding classic membranes, treatment benefits extend to lesions > 4 DA and non-recent lesions.

3.6 TAP Extension

Some patients that completed the 2-year TAP were enrolled in a 3-year extension study, for a total duration of 5 years (60 months), under an open-label regime. The main objective of this study was to obtain long-term visual acuity and 5-year safety data in patients with subfoveal choroidal neovascularization treated with photodynamic therapy. Patients having completed month 24 of the TAP study were eligible to participate in the study extension, irrespectively of having been included in the treatment or the placebo group and of lesion characteristics at baseline. In the TAP study extension, visual outcomes remained stable between month 24 and month 60, even in patients with low retreatment rates. No safety problems were found leading to contraindications being associated to retreatment with photodynamic therapy in the 5 years of study duration. No safety problems were found in bilateral treatment.

4. PDT Safety

The most complete and extensive PDT safety data were published in the meta-analysis of the TAP and VIP studies, where a comparison with placebo was performed. PDT is considered a safe treatment, with rare side effects, of little significance (Table 3). Choroidal hypoperfusion associated to PDT has been documented in fluorescein and ICG angiography in the first days after treatment and, more rarely, in the following months. Controversy exists regarding the cumulative effect of treatment in permanent occlusion of the normal choriocapillaris and the association between this hypoperfusion and eventual functional consequences.

<table>
<thead>
<tr>
<th>Ocular effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient loss of visual acuity (18% vs. 0%)</td>
</tr>
<tr>
<td>Severe loss of visual acuity (≥ 20 letters up to 7 days after PDT) (0.7% vs. 0%)</td>
</tr>
<tr>
<td>Scotomatous alterations (6% vs. 3.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Systemic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection site reactions (13% vs. 5.6%)</td>
</tr>
<tr>
<td>Lower back pain (2.4% vs. 0%)</td>
</tr>
<tr>
<td>Hypersensitivity reactions (3% vs. 0%)</td>
</tr>
<tr>
<td>Sleep pattern alterations (1.6% vs. 0%)</td>
</tr>
</tbody>
</table>

Table 3 – PDT adverse effects

5. Combined treatments

Combined approaches for treating exudative AMD have been investigated as a mean of improving treatment efficacy and reducing treatment frequency. Many non-randomized studies reported successful treatment using combinations of PDT, corticosteroids and antiangiogenic agents. The Focus trial showed that combination therapy using PDT and Ranibizumab was superior to PDT alone in efficacy and also reduced the need for repeat PDT sessions. A merely illustrative comparison of
The Anchor and Focus trials showed more favourable results in terms of visual acuity gain in the Anchor patients, which included only treatment naïve patients, suggesting that adding PDT to Ranibizumab may not increase the visual acuity gain.

The SUMMIT program, which includes three randomized clinical trials - DENALI, EVEREST and MONT BLANC, was designed to compare a combination therapy with PDT and ranibizumab with ranibizumab monotherapy. The DENALI study is a two-year, randomized, double-blind multicentric study conducted at 45 centres in the United States and five centres in Canada. Enrolled patients with subfoveal CNV of all angiographic subtypes were randomized to receive either ranibizumab monotherapy, a combination of ranibizumab and standard fluence PDT or a combination of ranibizumab and reduced-fluence PDT. Results are being awaited. MONT BLANC, a similar study conducted at 50 centres throughout Europe, enrolled subjects with subfoveal CNV of all angiographic subtypes, who were randomized to receive either ranibizumab monotherapy or ranibizumab in combination with standard-fluence PDT. Preliminary visual acuity results at 12 months revealed the non-inferiority of the combined treatment (PDT+Ranibizumab), when compared with Ranibizumab alone; the number of treatments and safety evaluation were similar in both groups. These results and those from Focus trial suggest that PDT with standard fluence may be useful in combination with Ranibizumab for treating predominantly classic, minimally classic or occult AMD lesions. The awaited results from the Denali study will show whether reduced fluence entails any additional efficacy or safety.

Certain angiographic lesion subtypes, such as retinal angiomatous proliferation (RAP) and polypoidal choroidal vasculopathy appear to respond differently to PDT treatment when compared to predominantly classic, minimally classic or occult lesions. It is unclear whether they are more likely to benefit from a combination therapy.

Polypoidal choroidal vasculopathy (PCV) may be considered as a well-defined subtype of AMD with a distinct natural history characterized by multiple recurrences and specific response to treatment. PCV often follows a remission-relapsing course and usually has a good visual prognosis. However, up to half of patients may have persistent bleeding and leakage, leading to vision loss. Although no data are available from randomized controlled trials of verteporfin PDT in PCV, numerous cases demonstrated that total polyp regression or complete disappearance of PCV lesions occurred in 56–95% of ≥200 eyes treated with verteporfin PDT. These studies indicated that many verteporfin-treated patients had stable or improved vision (Table 4), with outcomes that compared favourably with the natural history of PCV. Few reports have been published on the use of intravitreal antiangiogenic drugs, namely Bevacizumab, for the treatment of PCV. Although a reduction in leakage was shown, this appears to be ineffective in reducing choroidal vascular changes. The EVEREST study (part of the SUMMIT programme) is being performed in Asia and is designed to evaluate whether verteporfin PDT monotherapy, or combination with ranibizumab, is superior to ranibizumab alone in symptomatic PCV. Until more evidence emerges, PDT, alone or in combination with antiangiogenic drugs, remains the first choice for treating PCV.
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<tbody>
<tr>
<td>Type</td>
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<td>Prospective</td>
<td>Retrospective</td>
<td>Prospective</td>
<td>Retrospective</td>
<td>Prospective</td>
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<tr>
<td>N</td>
<td>16</td>
<td>22</td>
<td>21</td>
<td>9</td>
<td>30</td>
<td>30</td>
<td>36</td>
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<tr>
<td>Age (average)</td>
<td>70.5</td>
<td>66.6</td>
<td>75.6</td>
<td>67.2</td>
<td>67</td>
<td>75</td>
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<tr>
<td>VA increase 12M</td>
<td>56.3%</td>
<td>45.5%</td>
<td>28.6%</td>
<td>0.0%</td>
<td>-</td>
<td>50.0%</td>
<td>25.0%</td>
<td>12.0%</td>
</tr>
<tr>
<td>VA stabilization 12M</td>
<td>31.3%</td>
<td>50.0%</td>
<td>57.1%</td>
<td>100.0%</td>
<td>-</td>
<td>30.0%</td>
<td>67.0%</td>
<td>77.0%</td>
</tr>
<tr>
<td>VA decrease 12M</td>
<td>12.5%</td>
<td>4.5%</td>
<td>14.3%</td>
<td>0.0%</td>
<td>-</td>
<td>20.0%</td>
<td>8.0%</td>
<td>11.0%</td>
</tr>
<tr>
<td>VA increase 24M</td>
<td>0.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.0%</td>
</tr>
<tr>
<td>VA stabilization 24M</td>
<td>100.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>70.0%</td>
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<tr>
<td>VA decrease 24M</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22.0%</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Comments</th>
<th>Average follow-up 12M</th>
<th>6 eyes at 24M</th>
<th>Mean VA improved from 0.50 to 0.38 logMAR</th>
</tr>
</thead>
</table>

Table 4 - PDT and PCV. Results from different studies.

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Ante-VEGF in the treatment of AMD

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1. Background

Since the beginning of the last century much attention has been focused on tumour vascularization. Warren Lewis(1), in 1922, and Gordon Ide, in 1939(2), had already considered the hypothesis of synthesis of a vascular growth factor by tumour cells. Synthesis of a vascular growth factor in the retina was proposed for the first time in 1948(3), in diabetic eyes by Isaac Michelson. In the beginning of 1970, Folkman and his research group demonstrated that tumour growth is directly related to tumour vascularization, which, in turn, depends on the expression of certain growth factors(4).

2. Vascular endothelial growth – VEGF

VEGF was first identified in 1983 by Donald Senger and Harold Dvorak’s group(5), who identified the Tumour Vascular Permeability Factor (VPF), which causes vascular hyperpermeability, in tumour cells from guinea pigs. In 1989, 3 groups, including Ferrara et al, published articles highlighting a molecule with pro-mitotic properties in endothelial cells. Ferrara et al. identified this protein in bovines, having named it Vascular Endothelial Growth Factor (VEGF), the term by which it has been known since then(6,7,8).

VEGF-A, a molecule involved in eye diseases such as Age-related Macular Degeneration (AMD) and diabetic retinopathy, is part of a family of genes that also includes VEGF-B, C and D, and the viral homologue VEGF-E, in addition to the Placental Growth Factor – PIGF.

VEGF-A, which has been extensively studied, is a dimeric 36-46 kd glycosylated protein with an N-terminal signal sequence and a heparin-binding domain(9,10).

Four different VEGF-A isoforms have been identified in humans with varying numbers of amino acids: VEGF121, VEGF 165, VEGF 189 and VEGF 206. They arise from alternative splicing of mRNA. The longer forms are matrix-bound and the shorter forms are freely diffusible. VEGF 165 is the dominant isoform in ocular neovascularization processes(11).

2.1 VEGF receptors

Three VEGF receptors have been identified: VEGFR-1 (fms-like tyrosine kinase-1 or Flt-1), VEGFR-2 (kinase insert domain-containing receptor or KDR) and VEGFR-3 (fms-like tyrosine kinase-4 or Flt-4), which is a receptor for VEGF-C and VEGF-D. VEGF-A binds both to the R1 and R2 receptors.

VEGFR-2 is considered the main VEGF mediator in endothelial cells. Its activation induces NO (nitric oxide) production, cell membrane and cytoskeleton reorganisation and proliferation and migration of endothelial cells. It is also involved in the activation of the phosphatidylinositol 3-kinase (PI3)Akt pathway, which is a crucial signal transduction pathway in the process leading to endothelial cell survival induced by VEGF-A(12).

2.2 Physiology of VEGF

VEGF-A is an important permeability inducer and is about 50,000 times more potent than histamine. It is also a potent mitogen in endothelial cells and may have an important role in maturing of new blood vessels through pericytes(13).

VEGF-A is involved in physiological angiogenesis in adults, for example, in the female reproduction cycle(14). In addition, VEGF-A mRNA is expressed in various healthy human adult tissues that do not show
angiogenesis, such as the epithelium of the choroid plexus in the brain, the glomerular epithelium in the kidney, the gastrointestinal mucosa and hair follicles\(^{(15)}\). It has been suggested that VEGF-A maintains the integrity of endothelial cells via anti-apoptotic signalling\(^{(15)}\). VEGF-A has been recognised as an important neuroprotectant in the central nervous system. VEGF-A exposure resulted in a dose-dependent reduction in retinal neuronal apoptosis\(^{(16)}\). Although mechanistic studies have suggested that VEGF-induced volumetric blood flow to the retina may be partially responsible for neuroprotection, ex vivo retinal cultures have revealed a direct neuroprotective effect for VEGF-A. VEGF receptor-2 expression has been detected in several neuronal cell layers of the retina, and functional analyses have shown that VEGFR-2 is involved in retinal neuroprotection\(^{(16)}\).

It has been shown that VEGF-A is secreted by Retinal Pigment Epithelial (RPE) cells, on their basal side, i.e. the side adjacent to the choriocapillaris, and the 3 VEGFRs are expressed in choriocapillaris endothelial cells, on the side facing retinal pigment epithelial cells. It has long been known that loss of RPE cells in the human eye causes atrophy of the choriocapillaris. These findings are consistent with a role of VEGF-A secreted by RPE cells as a permeability/survival factor for quiescent choriocapillaris endothelium\(^{(17)}\). Since VEGF is highly regulated by hypoxia, a feedback mechanism must exist in these epithelia to promote physiological formation of new blood vessels when tissue oxygenation is low. Unbalances in this mechanism may cause serious diseases, such as Exudative Age-related Macular Degeneration (AMD)\(^{(15)}\).

### 2.3 VEGF and pathology

The predominant role of VEGF-A in the development of pathological angiogenesis, such as that occurring in tumours and ischaemic and inflammatory processes was widely demonstrated in the last decade\(^{(18)}\). In hypoxic states, VEGF is secreted by RPE cells\(^{(19)}\). This factor induces endothelial cell proliferation and increases vascular permeability. It has been shown in several models that VEGF-A is required and sufficient for development of new blood vessels in the retina and the iris. As already mentioned, VEGF-A has been identified as a primordial factor in the neovascular response induced by retinal ischaemia. Therefore, VEGF-A levels are increased in the vitreous and retina of patients with neovascularization secondary to proliferative diabetic retinopathy, venous occlusion or retinopathy of prematurity\(^{(20-23)}\).

In clinical practice, observed blood VEGF levels are increased in AMD patients\(^{(24)}\). Many studies have revealed VEGF overexpression in neovascular membranes during autopsy procedures or after surgical extraction\(^{(25,26)}\). Since 1996, immunohistochemistry studies of frozen sections of neovascular membranes have shown significant VEGF levels in highly vascularized regions, although lower immunoreactivity has been observed in fibrotic membrane regions\(^{(28,29)}\). Drusens and basal linear deposits have also been associated with high VEGF levels\(^{(30)}\). Therefore, vascular endothelial growth factor A (VEGF-A) regulates angiogenesis and vascular permeability in the eye, both in physiological and pathological processes. This growth factor selectively influences endothelial cell growth, being particularly responsible for increased vascular permeability. It also plays a role in the survival of many cells. Inhibition of neoangiogenesis – the cause of exudative or neovascular AMD – was the basis of some disease-modifying therapies, since anti-VEGFs may delay or even halt disease progression. The vascular endothelial growth factor is a secreted protein that induces angiogenesis and increases vascular permeability and inflammation, which appear to contribute to neovascular AMD progression. Naturally, VEGF is the target of investigational drugs for the treatment of AMD\(^{(31,32)}\).

It is possible to inhibit every step of the angiogenesis cascade induced by VEGF: VEGF synthesis may be inhibited by inhibiting the synthesis of the corresponding mRNA or by inhibiting transcription\(^{(33)}\). The effect of VEGF may also be directly inhibited, by inhibiting protein action. This is the mechanism used in anti-VEGF therapies\(^{(34,35)}\). Angiogenesis may also be inhibited after VEGF binding, as occurs with anecortave acetate and squalamine lactate\(^{(36-38)}\).

Treatment of AMD with anti-VEGFs is thus considered to be a turning point since its emergence has allowed a more direct approach to choroidal neovascularization and its selective inhibition. Therefore, anti-VEGF treatments offer new hope to thousands of neovascular AMD patients, a disease that used to be understood as an untreatable condition associated with ageing before the emergence of anti-VEGF drugs.

These drugs are particularly effective in the early stages of the disease, when newly formed blood vessels are less mature: inhibition of their growth allows photoreceptors to remain viable, as well as reducing the risk of central fibrosis and delaying progressive loss of vision. Three drugs in this class are currently used in the treatment of AMD: pegaptanib (Macugen\(^{\circ}\)), ranibizumab
(Lucentis®) and bevacizumab (Avastin®), of which only the first two have been approved for this therapeutic indication.

3. Ranibizumab (Lucentis®)

Ranibizumab is a Fab fragment of a recombinant humanized monoclonal antibody with high affinity for VEGF-A (the ranibizumab binding site has an affinity for binding VEGF-A 140-fold higher than that displayed by the bevacizumab binding site) specifically studied for the treatment of AMD (39, 40, 41). Ranibizumab has a solid clinical development program for this therapeutic indication, involving over 7,000 patients. Ranibizumab binds to an amino acid chain common to all VEGF-A isoforms, thus rendering them inactive, reducing retinal and choroidal angiogenesis and halting the increase in capillary permeability. It has been shown in animal models that ranibizumab effectively penetrates the retina and the subretinal space after intravitreal injection. Its systemic half-life is short (2-3 hours, following intravitreal administration) and systemic clearance is fast, which makes its administration safe. The average vitreous elimination half-life is approximately 10 days (42, 43).

Ranibizumab has been approved for all types of exudative/neovascular AMD lesions: classic, predominantly classic, minimally classic and occult lesions with no classic/neovascular AMD lesions: classic, predominantly classic, minimally classic and occult lesions with no classic component, up to 12 disc areas (DA), where the neovascular component is ≥ 50% of the entire lesion. The recommended dose is 0.5 mg. Treatment includes a loading phase, consisting of 3 monthly injections, in the first 3 months, and a maintenance phase, where retreatment is decided according to disease progression, mostly evaluated in monthly visits through VA and OCT criteria, at least during the initial stage or recent neovascularization activity (44).

Phase III clinical trials MARINA and ANCHOR, which supported ranibizumab approval for the treatment of AMD, demonstrated that treatment with monthly intravitreal injections for a 12 months period was associated with a significant increase in visual acuity, compared to photodynamic therapy and placebo (45). After 12 months, 25-40% of patients treated with ranibizumab showed gains of ≥ 15 letters (ETDRS), compared to 5-6% of the control group patients (p < 0.001). Similar results were confirmed after 2 years. Both these studies have established ranibizumab as the first therapy not only capable of preventing loss of vision but also of improving vision in a substantial percentage of patients: 33% of the patients treated with ranibizumab in the MARINA study and 41% in the ANCHOR study showed visual gains of at least 15 letters (46, 47, 48, 49).

Subsequent studies (PIER, SUSTAIN, EXCITE) were aimed at defining flexible and individual dose regimes for the maintenance stage of treatment with ranibizumab, allowing an effective approach to maintaining visual gains, practical in terms of hospital follow-up and with maximum systemic and ocular safety (50, 51, 52). These visual gains translate into real benefits for patients. This effect was evaluated through 3 VFQ-25 sub-scales (near vision, distance vision and vision-related dependency); in fact, patients treated with ranibizumab showed improvements in these 3 sub-scales (MARINA and ANCHOR endpoints). Specifically regarding dependency, ranibizumab allowed patients to become more independent in their daily activities. Overall average VFQ scores increased by 4.6 points in the Lucentis® 0.5 group, compared to a 4.4-point decrease observed in the placebo group (53).

The PIER study evaluated an alternative therapeutic regime consisting of monthly injections, in the first 3 months, followed by quarterly injections, corresponding to a total of 6 injections within a year. After an initial gain of 4.8 letters at month 3, patients treated with ranibizumab had lost an average of 0.2 letters at month 12, whereas patients in the control group lost 16.3 letters. These results indicate that individual treatment criteria should be adopted during the maintenance stage, allowing an effective approach to maintaining visual gains, as well as allowing follow-up in clinical practice, with maximum systemic and ocular safety.

Vision is expected to be maintained in 90-95% of patients; a minimum gain of 3 lines should be observed in 30-40% of patients treated with ranibizumab (50). In the EXCITE study, the quarterly treatment regime used in the PIER study (0.3 mg and 0.5 mg) was directly compared with a monthly regime (0.3 mg). An average increase in VA was observed in all treatment groups during the 12 months of study duration. At month 12, compared to month 3, VA gains had decreased slightly with the quarterly regime (by -2.2 and -3.1 letters with ranibizumab 0.3 mg and 0.5 mg, respectively), having slightly increased (by +0.9 letters) with monthly administration of 0.3 mg of ranibizumab (52).

PrONTO, a small prospective, unicentric, open-label, non-randomized study sponsored by the investigator, evaluated the efficacy of 3 consecutive monthly injections, followed by individual retreatment based on OCT results (at intervals ≥ 1 month). Retreatment criteria
were: loss of 5 letters in VA, presence of fluid in the macula detected by OCT; increase ≥ 100 μm in central retinal thickness (CRT); de novo classic choroidal neovascularization; de novo macular haemorrhage; or persistent macular fluid detected by OCT. Despite similar VA outcomes to those observed in the MARINA and ANCHOR studies having been observed with a smaller number of intravitreal injections, comparisons are limited by substantial differences in study design. Although being a small, open-label trial, this study suggests that individual retreatment based on OCT results allows visual gains to be maintained with a smaller number of injections.

The SAILOR-cohort 1 study evaluated the efficacy and safety of 3 consecutive monthly injections followed by quarterly monitoring visits, injections according to VA criteria (loss of > 5 letters from the maximum previous VA score) and OCT, if available (increase > 100 μm in CRT from the lowest previous measurement). Additional visits/injections would take place if required. Average VA increased from baseline after the first 3 injections, having subsequently decreased to an average gain of 2.3 letters for both ranibizumab doses, a better outcome than that observed for the PIER study, albeit suboptimal compared to those observed in the ANCHOR and MARINA studies. These results indicate that quarterly visits are not sufficient to monitor and evaluate disease progression.

The objective of the SUSTAIN study was to evaluate the efficacy of 3 consecutive monthly injections followed by monthly monitoring and treatment according to the following criteria: loss of ≥ 5 letters from the maximum previous VA score, in the first 3 months; or increase ≥ 100 μm in CRT from the lowest previous measurement, in the first 3 months. It was observed at month 12 that the majority of visual gains achieved in the first 3 months had been maintained. Although this study consisted only of an interim analysis of 69 patients, the corresponding outcomes were consistent with previous pivotal clinical trials of Lucentis®, having thus been recommended as first line therapy by many Ophthalmological Societies (e.g., the Royal College of Ophthalmologists, the German Ophthalmologists Association, etc.) and NICE (National Institute for Health and Clinical Excellence).

Extension study HORIZON was performed in order to evaluate efficacy and safety after the first 2 years. This study was designed as a post-marketing surveillance to monitor the safety and tolerability of Lucentis®, with a follow-up period of up to 3 years. HORIZON enrolled 853 patients who had already completed one of the 2-year randomized Lucentis® trials, ANCHOR, MARINA or FOCUS. While participating in the ANCHOR, MARINA or FOCUS studies, patients received monthly injections (active treatment with Lucentis® or Visudyne®, or sham). During the HORIZON study, patients attended fixed quarterly visits; however, visit frequency could be increased by the investigator if they deemed it necessary to see the patient more often. Lucentis® 0.5 mg injections were given on an as-needed basis, when the investigator felt that the patient would benefit from Lucentis® treatment. The interval between injections was at least 30 days.

After 2 years (preliminary results), 69% of the 600 initial Lucentis®-treated patients received their injections. Visual Acuity was available for 384/600 patients. Among these 384 patients, median Snellen VA had increased by 3 lines, from 20/100 to 20/50, during the initial 2-year trial, having subsequently decreased by 2 lines from the HORIZON baseline to 20/80, at year 2 of the HORIZON study. Overall, the safety profile of Lucentis® was very good and consistent with previous pivotal clinical trials of Lucentis®. In general, better VA and anatomical outcomes after the first 2 years delayed the need for subsequent retreatment. Additionally, the need for early AMD treatment was somewhat confirmed. Some loss of previously achieved VA gains occurred, eventually related to sub-treatment during the extension period.

Loss of visual acuity and the need for retreatment during the HORIZON study shows that the disease remains active after the first two years of monthly injections, evidencing the need for careful patient monitoring, as well as timely retreatment.
In clinical trials, the benefits of ranibizumab regarding visual acuity were independent of the type of CNV lesion. Additionally, these benefits were associated with a low rate (< 0.1%) of severe adverse events (endophthalmitis, retinal detachment, traumatic cataract). Less severe ocular adverse events occurred in less than 2% of patients, including intraocular inflammation and increase in intraocular pressure. In all clinical trials, Lucentis® revealed to be a well-tolerated drug, with no statistically significant differences observed in ocular adverse events between treatment arms. The results of the SAILOR study suggest a possible increase in the risk of de novo cardio vascular adverse events (CVA) in patients treated with ranibizumab with previous history of CVA or its risk factors (e.g., cardiac arrhythmias), although the differences observed were not statistically significant. Safety monitoring during the post-marketing period has confirmed the good ocular and systemic safety profile of ranibizumab, whose risk management plan has been strictly implemented. Other clinical trials are in course for other therapeutic indications, namely Diabetic Macular Oedema, Central Retinal Vein Occlusion and other ocular pathologies involving choroidal neovascularization, whose preliminary results have revealed to be promising.

4. Pegaptanib (Macugen®)

4.1 Introduction

Pegaptanib sodium (Macugen®, OSI-Eyetech Pharmaceuticals, Pfizer), was the first anti-VEGF inhibitor available for the treatment of choroidal neovascularization. This medicine is part of a new drug set called aptamers. The aptamers are synthetic oligonucleotides which acquire a specific tridimensional shape and allow high specificity and affinity to a great extent of therapeutic agents. These compounds are chemically synthetised with the use of nucleotide bases and the use of reverse transcription and PCR - polymerase chain reaction technology. Pegaptanib sodium is a 28-base ribonucleic acid (RNA) oligonucleotide with two branched 20KDa polyethylene glycol (PEG) moieties attached in order to increase the half-life of the drug in the vitreous cavity. The RNA sugar background is modified to prevent its degradation by endogenous endo and exo-nucleases. Pegaptanib sodium specifically targets the VEGF165 isoform. The pharmacokinetics of pegaptanib following intravitreal injection were profiled in a study of 147 subjects with exudative AMD (Apte RS, 2007). Either 1 or 3 mg of pegaptanib sodium per study eye was administered every 6 weeks for 54 weeks. For the 1 mg dose, mean maximal plasma concentrations were 20 – 24 ng/ml, and pegaptanib was measurable (> 8 ng/ml) in the plasma for up to 1 week after injection. The mean apparent terminal plasma half-life, determined from the 3 mg group, was 10 days. There was no plasma accumulation with administration of repeated doses. In addition, no serum antibodies against pegaptanib were detected.

In monkeys’ eyes, biologically active pegaptanib could be detected in the vitreous humor for at least 28 days, following a single 0.5 mg intravitreous injection dose.

4.2 Clinical trials with pegaptanib

4.2.1 Phases I and II studies

A phase IA safety study with 15 patients with exudative AMD, as well as a phase II study with 21 patients treated with pegaptanib associated or not to photodynamic treatment (PDT), with a follow up of 3 months, have shown that the intravitreous administration of pegaptanib with 6 week intervals was well tolerated and had anatomic and visual benefits.

4.2.2 Phase III study

The study VISION (VEGF Inhibition Study in Ocular Neovascularization) consists of two multicentric, randomized, prospective, controlled, dose-ranging and double-blinded phase III clinical trials, used for testing the safety and efficiency of pegaptanib sodium in the treatment of choroidal neovascularization secondary to AMD.

There were 1208 patients in this study, distributed by 117 centers and the main criteria for inclusion were: 50-year old or above with any kind of angiographic subtype of subfoveal choroidal neovascularization in the study eye secondary to AMD, with a lesion of 12 or below disc areas (including blood, scarring, atrophy and neovascularization). The best-corrected visual acuity varied between 20/320 and 20/40. Patients were randomized in four branches of the study: a group for simulation of pegaptanib intravitreous injections and one of three groups for administration of pegaptanib sodium intravitreous injections (with doses of 0.3 mg, 1mg or 3 mg). The injections (or simulations) were performed with 6-week intervals for 48 weeks, in a maximum of 8 injections per patient. All patients underwent...
the same procedures with exception of the scleral penetration performed in the group of intravitreous injection simulation. The ophthalmologist performing the injections was not authorized to undertake the patients’ follow up in order to guarantee the researcher’s concealment. For ethical reasons, treatment with PDT (Visudyn®) was allowed in some clinical centers in patients with mainly classic lesions, in all branches of the study and according to the researcher's criteria.

The primary study outcome measure was the proportion of patients who lost <15 letters of VA at the end of week 54. Additional efficacy end-points included: proportion of patients maintaining or gaining ≥ 0, 5, 10, or 15 letters, or losing ≥ 30 letters (severe vision loss); mean changes in VA from baseline to week 54, and the proportion of patients with VA of 20/200 or worse in the study eye at week 54. In total, 1186 subjects received at least one study treatment (mean, 8.5 of 9 possible injections)(61). All pegaptanib doses were superior to sham with regard to loss of < 15 letters of VA: 70, 71 and 65% for 0.3 mg (p < 0.001), 1 mg (p < 0.001) and 3 mg (p < 0.03) groups, respectively, versus 55% for sham. Overall, the 0.3 mg dose was found to be most effective and further discussion is limited to the 0.3 mg (approved) dose.

Pegaptanib was significant superior to sham in the percentage of subjects maintaining or gaining 0, 5, 10 or 15 lines of vision (p < 0.05) (71). Pegaptanib treated subjects were less likely to have severe vision loss (10 versus 22%, p < 0.001) or progress to VA ≤ 20/200 (38 versus 56%; < 0.001). Mean VA loss at week 54 was 7.95 letters for pegaptanib compared with 15.05 letters for sham (p < 0.05; 47% relative difference). Treatment effect was independent of angiographic subtype, baseline VA and lesion size, sex, age, race or iris color (71).

VISION trial had an extension for 48 additional weeks. Those patients receiving pegaptanib were randomized to either continue their pegaptanib dose or discontinue treatment. Subjects initially receiving sham were randomized to continue or discontinue sham or to receive one of the three pegaptanib doses. Overall, 1053 subjects were randomized; 941 (89%) were assessed at week 102 (mean, 15.7 of 17 possible total injections). Compared with sham (sham over 2 years or randomized to discontinue sham in year 2), more of those receiving pegaptanib 0.3 mg during 2 years lost < 15 letters (45 versus 59%; p < 0.05). Subjects continuing pegaptanib had the greatest benefits (72).

An exploratory analysis was conducted to assess the vision benefit of treating early subfoveal choroidal neovascularization secondary to AMD with pegaptanib in the VISION trials. Subjects were grouped according to two different definitions of early disease. Group 1 included those with lesions < 2 disc areas and a baseline VA of ≥ 54 letters, no prior PDT or laser photocoagulation and scarring or atrophy (n = 34 for pegaptanib 0.3 mg and n = 28 for sham). Group 2 included those with occult with no classic CNV, with an absence of lipid and worse VA in the study eye versus the fellow eye (n = 30 for pegaptanib 0.3 mg and n = 35 for sham) (70).

At week 54, the responder rates (lost < 15 letters) were significantly higher for pegaptanib versus sham (group 1: 76 versus 50%; p = 0.03; group 2: 80 versus 57%; p = 0.05). Pegaptanib-treated subjects in group 1 were approximately 10-times less likely to have severe vision loss than those receiving sham (3 versus 29%; p < 0.01); differences for group 2 were not as large (10 versus 17%; p = 0.17). On average, subjects in both pegaptanib-treated groups lost less VA (group 1: -5.6 versus -16.6 letters; p < 0.01; group 2: -4.0 versus -16.7 letters; p < 0.006). Notably, among those receiving pegaptanib 0.3 mg 12% of subjects in group 1 and 20% in group 2 gained ≥ 3 lines of vision, compared with 6% in the VISION study. These findings suggest that pegaptanib treatment early in the course of wet AMD may improve visual outcomes (65, 70).

### 4.3 Safety

During the VISION study and the second and third year extension no increased risk of systemic adverse events was identified, but patients with high risk of cardiovascular and cerebrovascular events were excluded from the clinical trials. Most adverse events reported in the study eyes were attributed to the injection procedure. The low risk of serious injection-related adverse events, such as endophthalmitis, traumatic cataract and retinal detachment were found to be modifiable with injection protocols changes during the study (Table 1).

Because VEGF is involved in a wide range of physiological processes, inhibition of this factor raises many safety concerns particularly in the context of extended treatment regimens (75-77).

The pegaptanib sodium selectively inhibits the most biologically active isoform of VEGF (VEGF 165), and according to some authors this quality allows a theoretical advantage in terms of safety comparing to the non-selective anti-VEGF like ranibizumab and bevacizumab. The systemic risks of non-selective VEGF inhibition have been illustrated with the use of intravenous injection of bevacizumab for the treatment of metastatic
colorectal and non-small-cell lung cancer, both approved indications for this agent. Nevertheless, the intravitreous administration of anti-VEGF agents for the treatment of exudative AMD results in much lower systemic exposures\(^6\). Although the theoretical superior safety of pegaptanib in comparison to other non-selective anti-VEGFs this has not been confirmed yet.

## 5. Bevacizumab (Avastin\(^*\))

### 5.1 Introduction

Bevacizumab (Avastin\(^*\), Genentech, Roche) is a recombinant, humanized, monoclonal immunoglobulin G1 antibody (149 kD) that binds to and inhibits the biologic activity of all isoforms of human VEGF. This molecule has 2 antigen-binding domains (ranibizumab has 1). In 2004, the FDA approved bevacizumab for use in patients with metastatic colorectal cancer. It has received additional approval for use in patients with non–small-cell lung cancer and those with metastatic breast cancer\(^7\). Though not formally studied or approved for any intraocular disease, Rosenfeld’s pioneering work and the unavailability of a related ocular drug, ranibizumab, led to rapid and wide use of bevacizumab all over the world\(^8,9\).

Using bevacizumab as an intravitreal injection to treat neovascular AMD is off-label at this time, however many ophthalmologists, appropriately offer intravitreal bevacizumab to AMD patients based on multiple forms of evidence: results from several retrospective case series, extrapolation from the magnitude of the outcomes reported with ranibizumab, the structural similarity between ranibizumab and bevacizumab, the individual, and the natural history of the disease if left untreated\(^9\). In the human retina, it is unclear if the molecule of bevacizumab fully distributes within the retinal layers or if localized inhibition of VEGF in the vitreous and inner retina is responsible for the clinical effects associated with administration\(^9,10\).

There are also theories that the larger size of bevacizumab relative to ranibizumab may result in bevacizumab not clearing as quickly from the eye, potentially resulting in longer duration of activity. To the knowledge of this author, this claim has not been confirmed\(^9\).

Full antibodies generally have longer systemic half-lives than antibody fragments. Therefore, it is assumed that the half-life of bevacizumab in the eye and in the circulation is longer than that of ranibizumab after intravitreal injection. Different half-lives for these 2 drugs may have implications for different dosing frequencies and different systemic toxicities\(^7,8,9,10\).

### 5.2 Experimental and clinical studies

Following the initial successful administration of this drug in the management of exudative AMD in May 2005, numerous case series were published illustrating the effectiveness of this treatment in a high proportion of patients\(^11\).

Almost all of the evidence supporting the use on neovascular AMD comes from off-label usage in short-term uncontrolled clinical case series, which suggests that intravitreal administration is apparently locally and systemically well tolerated and is associated with vision stabilization or improvement in most treated eyes\(^9,10,11,12\).

One of the earlier large retrospective case series in the

<table>
<thead>
<tr>
<th>Event</th>
<th>Year 1 n = 7545 injections</th>
<th>Year 2 n = 4091 injections</th>
<th>Year 3 n = 3227 injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endophthalmitis</td>
<td>0.16</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>Traumatic cataract</td>
<td>0.07</td>
<td>0.02</td>
<td>0</td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>0.08</td>
<td>0.17</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 1 – VISION study serious ocular adverse events rates (% per injection)\(^11\). Adapted from Rajendra S Apte, 2008.
literature included 81 consecutive eyes (79 patients) with subfoveal choroidal neovascularization treated with 1.25 mg (0.05 cc) intravitreal bevacizumab, at baseline and 1 month later if morphologic changes attributable to the CNV persisted (subretinal fluid, pigment epithelial detachment, retinal thickening). Seventy-eight percent had prior treatment with pegaptanib, photodynamic therapy (PDT), or both. After one IVB injection, 30 of 81 eyes had resolution of their subretinal fluid. At 2 months, 50% demonstrated resolution of leakage. The mean best corrected visual acuity (BCVA) improved from 20/200 to 20/125 at week 8 (p < 0.0001).

Spaide et al. in a subsequent study evaluated 266 eyes, 70% of which had prior treatment for exudative AMD (PDT or pegaptanib). At the 3-month follow-up (data available for 141 patients) 38.3% patients improved by 2 or more Snellen lines. Mean BCVA improved from 20/184 to 20/109 at 3 months (p<0.001). Central retinal thickness measured by OCT improved over 3 months from a mean of 340 microns to a mean of 213 microns (p<0.001).

A greater visual acuity effect has been reported in naïve eyes compared to those that have received previous treatment, for example in a study of 50 eyes (48 patients) treated with bevacizumab for exudative AMD found that naïve eyes responded more favorably than previously treated eyes. Six of the 14 (43%) of naïve eyes gained 3 lines or more of vision versus 17% of eyes that had undergone prior treatment. The naïve group's mean visual acuity improved from 20/160 at baseline to 20/63 (p<0.001) at week 24.

In another prospective case series, Bashshur et al. injected 2.5 mg (0.1ml) of bevacizumab (twice the dose most frequently used) into the vitreous in 17 eyes with wet AMD patients and followed by two additional injections at four-week intervals. Mean best-corrected visual acuity was 20/252 at baseline and 20/76 at week 12 (P < 0.001). Mean central subfield retinal thickness also improved between baseline and week 12 in all 17 patients. No systemic or ocular side effects were noted.

### 5.3 Safety

Data on the safety of intravitreal bevacizumab are more limited than data on ranibizumab or pegaptanib safety because there are no large, prospective, controlled safety studies with this treatment. Local side-effects are similar to those found for the other anti-VEGF agents.

A safety retrospective study evaluating the side effects of intravitreal bevacizumab reviewed 1265 patients for 12 months, with 92 lost to follow-up. Ocular complications included seven (0.16%) bacterial endophthalmitis, seven (0.16%) tractional retinal detachments, four (0.09%) uveitis, and a case (0.02%) of rhegmatogenous retinal detachment and another case (0.02%) of retinal detachment and vitreous hemorrhage.

In electrophysiological studies no negative side-effects were seen on the retina. In contrast, the results showed a recovery effect on photoreceptors even at the site of the CNV. Most of the in vitro, ex vivo and in vivo experiments excluded short-term negative effects on ocular cells and histology. A paper, however, discloses mitochondrial disruption in the inner segment of photoreceptors and apoptosis after high doses of intravitreal bevacizumab in the rabbit eye.

The electrophysiological investigation and light microscopy, in contrast appeared unaltered. This suggests that potential side-effects on the cellular level cannot be detected with the present diagnostic tools in clinical practice.

Intravenous use of bevacizumab in patients with colorectal cancer is associated with severe systemic side effects including arterial thromboembolism, gastrointestinal perforation, hemorrhage, hypertensive crisis and nephrotic syndrome. Initial studies using this therapy intravenously for ocular disease in a healthier population did not find nearly the same risks.

The dose of intravitreal bevacizumab is much lower (1/400th) of the dose used for intravenous treatment and has not been found to result in unexpected systemic side effects.

There are no studies adequately undertaken to identify rare systemic events. In a 3-month retrospective study of bevacizumab treatment in 266 patients, 1 (0.4%) developed a nonfatal myocardial infarction after the third injection. Two patients (0.8%) had apparent transient ischemic attacks (diagnosis was not definitive). There were 2 deaths, one from myocardial infarction. Nevertheless, that patient was a smoker with a history of emphysema. It is important to consider, however, that this population (mean age, 80.3 years) is at risk for
myocardial infraction regardless of treatment. Any potential safety concerns remain unknown and waiting for randomized and controlled clinical trials.

5.4 Discussion

The initial results of intravitreal bevacizumab for exudative AMD led to the acceptance of this off-label therapy by ophthalmologists around the world, assuming, based on case series evidence, that bevacizumab is at least almost as good as ranibizumab with respect to efficacy and safety. Some ophthalmologists might recommend bevacizumab instead of ranibizumab, even when it is available and affordable to the patient, because of the concerns regarding the treatment costs\(^{84, 92}\). Intravitreal bevacizumab accounts for more than 50% of all anti-VEGF therapy delivered for exudative AMD in the United States\(^{109}\).

The National Eye Institute is sponsoring a clinical trial to compare the safety and efficacy between bevacizumab and ranibizumab for the treatment of exudative AMD – CATT study. This study and other prospective, controlled and randomized trials in several countries (IVAN-UK, VIBERA-Germany, MANTA-Austria, LUCAS-Norway, GEFAL-France) will provide the best level of evidence regarding the efficacy and safety of bevacizumab. Some of these ongoing studies can give consistent information about the necessary dose-ranging and dosing-frequency to control AMD neovascularization.

6. Nice recommendations\(^{57}\) (National Institute for Health and Clinical Excellence; April 2008)

According to NICE, ranibizumab is the only anti-VEGF recommended for the treatment of Age-related Macular Degeneration (as per the NICE Guidelines, published in 2008).

Differences are clear when comparing the outcomes of clinical programs for both drugs (ranibizumab and pegaptanib). In clinical trials with ranibizumab, the percentage of patients who gained 15 letters or more was substantially higher, whereas in clinical trials with pegaptanib few patients gained 15 letters or more compared to the control group.

Regarding visual acuity outcomes (expressed as the average number of letters lost or gained by both treatment groups versus the control group), the observed results revealed that ranibizumab leads to statistically significant average gains, whereas pegaptanib only leads to a decrease in the average loss, i.e., ranibizumab is more effective than pegaptanib regarding improvements in visual acuity. Additionally, no benefits were observed in patients whose treatment with pegaptanib was discontinued after the first year, when compared to patients in the placebo group (VISION study results, published in 2006).

According to NICE, both drugs (ranibizumab and pegaptanib) have demonstrated clinical efficacy in the treatment of exudative AMD, although ranibizumab leads to increased clinical benefits and pegaptanib fails to represent a cost-effective example of healthcare resource use, thus not being recommended in the treatment of AMD. On the contrary, ranibizumab is referred as an option in the treatment of this condition, providing the following are observed for the treated eye:

- visual acuity between 6/12 and 6/96
- no permanent structural damage to the central fovea
- lesion size less than or equal to 12 disc areas in its greatest linear dimension
- evidence of recent disease progression (vessel proliferation, observed in fluorescein angiography, or recent changes in visual acuity).

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joaofigueira@oftalmologia.co.pt
References:


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the intraocular tissue distribution, pharmacokinetics, and safety of 125I-labeled full-length and Fab antibodies in rhesus monkeys following intravitreal administration. Toxicol Pathol 1999;27:536–44.


Choroidal neovascularization in AMD has become a serious medical and social problem. One of the reasons for this problem is ageing of the population. However, a better understanding of this disease and the emergence of new treatment options have been witnessed in recent years. We combine agents that target angiogenesis-promoting cytokines with capillary occlusion therapies, such as photodynamic therapy (PDT) and, more recently, PDT combined with an anti-VEGF and a corticoid, with a view to achieve a synergistic action, with improved outcomes, reduced retreatment frequency and more sustained effects(1,2). This additive effect allows patients to be treated with lower doses, entailing added value through increased tolerability and decreased costs(3).

2. Health economics in AMD treatment: efficacy versus efficiency and the importance of equity

2.1 Seeking efficacy and efficiency – resource saving

The efficacy of a given drug or technique is assessed when comparative studies of visual outcomes are performed. If the observed outcomes are identical to those observed in previous studies it is concluded that no apparent advantages result from using the technique or drug in question. However, if the number of treatment sessions decreases, a smaller number of medicine vials is used or patients visit the hospital less frequently, it is concluded that the efficiency of the drug or method is greater. Drugs and methods that are equivalent in terms of efficacy may vary widely in terms of efficiency. We are thus faced with efficiency gains and better use of resources – more patients are treated with the same budget!

This theoretical improvement in clinical efficiency has the advantage of reducing the number of treatment sessions, with a consequent decrease in the cost of drugs used to treat each patient. Since each patient will make less visits to the hospital, the number of medical, nursing and technical staff hours required will decrease, the same occurring for equipment operation times and time spent at hospital premises(4).

It is referred in recent studies that the cost of drugs represents the greatest percentage of AMD treatment costs, as opposed to usual health cost distribution, where the largest percentage, of approximately 40%, corresponds to staff costs. Calculated cost percentages for treatment with pegaptanib correspond to 17% for staff and 70% for the drug; for treatment with the ranibizumab protocol, 83% of costs are associated to the drug(5,6).

2.2 Combined treatments – visual outcomes and efficiency results

Although it has not been definitely proved (large multicentric, randomized, controlled studies are in course) that the visual outcomes of combined treatments, double or triple, are better than those observed for the gold standard treatment, consolidation of published studies referring a reduction in the number of retreatment sessions would be desirable. However, improved treatment efficacy evidenced by better visual outcomes and an eventual reduction in the number of complications would also be desirable(5,7).

Calculated costs of 1 year of treatment per line of visual acuity were $84 for treatment with bevacizumab, following a regimen of treatment when necessary (PRN), and $766 for treatment with ranibizumab, following the gold standard protocol. Combined treatment costs varied between $71 and $269(6).
Due to their synergistic effect, combined treatments potentially lead to a decrease in the number of retreatment sessions, as well as sustained long-term visual benefits\(^{(1)}\), with gains and better clinical efficiency.

**3. Synergistic action and increased treatment effect**

How can we explain the fact that a synergistic effect is theoretically achieved by using various mechanisms of action, sometimes more effective than the sum of their separate effects? (Fig. 1)

### 3.1 Anti-VEGFs

The primary need to act on the key mechanism of the neovascularization process – VEGF – is widely known. By acting on this mechanism not only do we inhibit neovascularization but also act on oedema and the inflammatory mechanism, to a certain extent\(^{(8)}\).

### 3.2 Synergistic action of corticoids

When steroids are added, a synergistic action is achieved, since steroids act on various levels of the inflammatory process and angiogenesis regulation.

The complexity and diversity of glucocorticoid (GC) receptors in human tissue is highlighted by evidence that up to 6,000 genes are expressed or suppressed within hours of GC exposure. The enormous potential of using exogenous GC agents to downregulate processes involved in age-related macular degeneration must be balanced against a similar potential for counterproductive effects\(^{(9)}\).

Steroids activate receptors that induce the synthesis of specific proteins from DNA. Various mechanisms of action are proposed for steroids.

It is known that steroids act on local inflammatory mediators, stabilizing blood-retinal barrier function by increasing gap junction density and activity in capillary endothelial cells.

It is thought that triamcinolone decreases VEGF, which is a potent agent in increasing capillary permeability by increasing phosphorylation of proteins involved in tight intercellular junctions, such as occludin and Zonula Occludens-1 (ZO-1).

These agents also have an anti-inflammatory effect by inhibiting phospholipase A2, an enzyme that metabolizes cell membrane phospholipids to free arachidonic acid, which, in turn, originates thromboxane, leukot-

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**Figure 1 - Angiogenesis** - New blood vessels are formed in response to various physiological and/or pathological stimuli, of which hypoxia, is one of the most relevant. Hypoxia activates multiple cellular response cascades, with special emphasis on activation of extracellular matrix metalloproteases and increased synthesis and release of growth factors, including VEGF. The latter acts on membrane receptors, activating intracellular enzyme pathways through intracellular signalling, which results in response amplification. This ultimately leads to cellular proliferation, migration and differentiation, with formation of new blood vessels. Different drug categories act on different stages of the neovascularization process\(^{(9)}\). Joint action on various cascade levels should theoretically lead to an increase in treatment effect and/or a decrease in the effective dose and/or a more prolonged effect.
rienes and prostaglandins that cause vasodilatation, increased permeability and oedema. They also have an angiostatic effect by promoting a decrease in extracellular matrix (ECM) turnover through inhibition of plasmin activation. Plasmin activates matrix collagenases and metalloproteinases (MMPs) that dissolve the capillary basement membrane and trigger angiogenesis, with endothelial cell differentiation, migration and proliferation (Fig. 2). These agents also act on the interaction between ICAM-1 (Intercellular Adhesion Molecule-1) and leukocytes, inhibiting recruitment of the latter, thereby contributing to reduce the inflammatory component. It is also thought steroids may act on SDF-1 (Stromal-cell Derived Factor-1), inhibiting its action (Fig. 3)\(^9,10\). Steroids also decrease the expression of Major Histocompatibility Complex (MHC).
compatibility Complex Class II (MHC-II) molecules involved in the inflammatory process\(^{(12)}\). Therefore, we have scientific grounds supporting the combined action of treatment with corticoids\(^{(13,14)}\) (Fig. 4).

### 3.3 Associated FGF-2 inhibition and PEDF action

It is also possible to inhibit other factors, such as FGF-2, or induce PEDF locally, which has antiangiogenic effects that counteract the angiogenic effect of VEGF\(^{(15,16,17)}\).

### 3.4 Acting on the structural level by damaging newly formed blood vessels – PDT

The actions already referred involve blocking or inhibiting neovascularization and inflammatory mediators. However, it is also possible to act on a structural level, by damaging newly formed blood vessels. This is achieved through cellular damage and death mediated by free radicals induced by the action of laser on a photosensitizing agent – verteporfin\(^{(18,19)}\).

Standard fluence values of 50-J/cm\(^2\) or lower fluence values of 25-J/cm\(^2\) or 12-J/cm\(^2\) are normally used in combined treatment. Photodynamic therapy causes vascular occlusion but is associated to an inflammatory response that may be minimized by using corticoids and an anti-VEGF agent. Both agents may also inhibit the angiogenic stimulus represented by a VEGF rebound effect following occlusion of new blood vessels\(^{(20)}\). Capillary occlusion induced by PDT leads to hypoperfusion of the treated area, a condition that is theoretically worsened by concomitant use of anti-VEGF agents that prevent recapillarization. This effect has not been shown as negative; on the contrary, it appears that this recapillarization delay promotes neuronal recovery by decreasing oxygen and free radical concentrations\(^{(20)}\).

### 3.5 Other sites of action – associated surgical therapy

We shall not elaborate on this treatment combination, as it will be referred in a chapter dedicated to surgery.

### 4. Main studies

#### 4.1 Gold standard treatment

The efficacy and safety of combined treatments are evaluated in studies where drug combinations are compared with the gold standard treatment, which, according to the results of the MARINA\(^{(21)}\) and ANCHOR\(^{(22)}\) studies, consists of twelve consecutive monthly intravitreal injections of an antiangiogenic agent. Use of this treatment regime led to outcomes of 90% in vision stabilization and approximately 30-40% of significant improvement after one year.

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**Corticoids**

Angiogenesis and Macular Oedema

- **MMP** - matrix metalloproteinases
- **Extracellular matrix turnover**
- **Adhesion molecules expression**
  - **MHC-II expression** - major histocompatibility complex II (inflammatory cells)
  - **ICAM-1** - Intracellular adhesion molecule-I (leucocyte adhesion and transmigration)
  - **SDF-1** - Stromal-cell Derived Factor

Figure 4 - Corticoids inhibit angiogenesis and macular oedema throughout a lot of action pathways. See text.
4.2 Combined treatments: double and triple treatments

Most combined treatments include PDT, with or without reduction of the standard dose of 50 Joules/600mWatts/cm², associated to an anti-VEGF agent. PDT, antiangiogenic agents and steroids are often used in triple treatments.

According to the US National Institute of Health Clinical Trials Registry, at least 17 studies using combined treatments are currently in course. Some of these studies should be referred for their relevance, albeit in a summarized manner.

4.2.1 SUMMIT programme

This programme includes three large randomized clinical trials - DENALI (USA), MONT BLANC (Europe) and EVEREST (Asia) – whose objective is to evaluate the efficacy and safety of combining PDT (Visudyne) and ranibizumab, compared to monotherapy with this antiangiogenic agent, in patients with neovascular AMD and polypoidal disease (Everest). Primary analysis results from the Mont Blanc study at twelve months have shown no significant differences between the two groups.

4.2.2 RADICAL study (23)

This is a Phase II, multicenter, randomized study, using double and triple treatments, with ranibizumab (Lucentis), PDT (Visudyne) and dexamethasone, compared to monotherapy with Lucentis. Results analysis at 12 months was positive for the four study groups. The best outcomes with the smaller number of treatment sessions were observed for the triple treatment group with half the fluence.

4.2.3 LuceDex study (24)

This study researches the role of dexamethasone in neovascular AMD treatment. This is a prospective, randomized clinical trial comparing two treatment groups, one treated with a combination of ranibizumab and dexamethasone and the other undergoing ranibizumab monotherapy.

4.2.4 PDEX II study (25)

This is a prospective, multicentric, randomized, non-inferiority study comparing the relative advantages of treatment with PDT in a reduced dose (reduced fluence), dexamethasone and ranibizumab versus monotherapy with ranibizumab.

4.2.5 CABERNET study (26)

The objective of this study is to evaluate the efficacy of epiretinal brachytherapy with strontium 90 combined with two doses of Lucentis® administered one month apart, the first being injected upon vitrectomy.

4.2.6 VIA study (27).

The objective of this study is to determine whether a combination of a reduced dose of PDT and bevacizumab leads to a decrease in the number of treatment sessions required within a 6-month period, compared to monotherapy with bevacizumab.

This randomized, double-blind, controlled clinical trial revealed that a combination of bevacizumab and 25-J/cm² or 12-J/cm² PDT led to a decrease of approximately 50% in the number of treatment sessions required within a 6-month period. Favourable outcomes were also observed for visual acuity, although evaluation of this parameter was not the main objective of this study.

4.3 Considerations

The FOCUS study was one of the first multicentric studies performed using combined treatment with PDT (Visudyne) and ranibizumab (Lucentis), comparing its therapeutic efficacy with that of PDT monotherapy. This study’s shortcoming was failing to compare the efficacy of combined treatment with that of monotherapy with the antiangiogenic agent (27).

Although it is a not correct practice to compare results of two different studies, visual outcomes in this study (considering the combined treatment arm) were inferior to the outcomes observed for monotherapy with the antiangiogenic agent in the ANCHOR study.

Most combined treatment studies performed were either non-controlled studies or only monotherapy with PDT was used in the control group. This is the case of the FOCUS study.

An important question is to determine whether combining anti-VEGF agents with PDT results in a real increase in treatment efficacy regarding the primary endpoint, visual acuity, in addition to reducing the number of retreatment sessions required to stabilize vision.
trap-Eye in wet AMD was initiated in August 2007. Two phase III trials conducted by two pharmaceutical companies (Regeneron Pharmaceuticals, Inc. and Bayer HealthCare AG) are evaluating treatment with VEGF trap-Eye, at doses of 0.5mg every 4 weeks, 2mg every 4 weeks, or 2mg every 8 weeks (following three monthly doses), compared with treatment with 0.5mg of ranibizumab (Lucentis®, a registered trademark of Genentech, Inc.), administered every 4 weeks, according to its U.S. label, during the first year of the studies. PRN dosing will be evaluated during the second year of each study. The VIEW1 study is currently enrolling patients in the United States and Canada; the VIEW2 study is currently enrolling patients in Europe, Asia-Pacific, Japan, and Latin America(33).

5.2 FGF-2 inhibition

RPE from CNV patients expresses angiogenic growth factors whose action is partly independent from VEGF. In a study, Sthal concluded that anti-VEGF treatment (bevacizumab) inactivated all RPE-derived VEGF in a 3D collagen matrix culture of RPE isolated from surgically excised CNV-membranes (CNV-RPE) used to stimulate sprouting of endothelial cell (EC) spheroids, but was unable to fully inhibit EC sprouting induced by CNV-RPE. Combined anti-VEGF/anti-FGF treatment inactivated both growth factors and reduced EC sprouting significantly. In a comparison between the antiangiogenic effect of solitary anti-VEGF antibodies and combination treatment with anti-VEGF and anti-FGF-2 antibodies, greater inhibition was achieved for the latter. Targeted combined therapy can be superior to solitary anti-VEGF therapy. One possible candidate for combined therapy is FGF-2(34).
AdPEDF11 doses greater than 10(8) PU. This study provides evidence that adenoviral vector-mediated ocular gene transfer is a viable approach in the treatment of ocular disorders, suggesting that further studies of the efficacy of AdPEDF11 in the treatment of patients with neovascular AMD, as well as promising combined treatments, should be performed\(^{(36)}\).

### 5.4 Ranibizumab and Sorafenib

In two cases of recurrent exudative AMD in which intravitreal ranibizumab was used in combination with oral sorafenib, a tyrosine kinase inhibitor, improvements were observed in optical coherence tomography\(^{(37)}\), indicating this might also be a promising combination treatment.

### 5.5 Hydroxymethylglutaryl-coenzyme A reductase inhibitors, ACE inhibitors, trimethazine and third-generation beta-blockers

Statins have been referred as hypolipidemic, anti-inflammatory and antioxidant agents, improving endothelial function by increasing nitric oxide synthesis and release. Therefore, they might influence AMD pathogenesis. In fact, some studies refer a favourable effect on AMD, suggesting a role for these substances in combined treatment\(^{(38)}\).

A recent article refers treatment with ACE-inhibitors and/or AR blockers, combined with a statin, aspirin and third-generation beta-adrenergic receptor blockers or trimethazine can be used with some advantage in certain forms of AMD\(^{(39)}\).

### 6. The future of combined treatment

Only time will tell, whether double and triple treatments, will display similar efficacy results as the gold standard treatment.

One-year results (May 2009) of a prospective, randomized study of triple treatment with PDT, bevacizumab and triamcinolone in three patient groups were recently published. Study results were not superior to those observed for monotherapy with the antiangiogenic agent, although the number of retreatment sessions needed to stabilize vision after one year was smaller\(^{(40)}\).

It is known that some patients respond better to monotherapy, while others respond better to combined treatment; this is very likely due to individual patient and disease characteristics.

In the near future, development of non-anti-VEGF treatments with neuroprotective, antifibrotic and anti-inflammatory actions may contribute to the increased efficacy of new combined treatments.

Subtenon injection of long acting cortisones (anecortave) in combination with other procedures, namely anti-VEGF agents, should be studied\(^{(41)}\). Despite having been abandoned in monotherapy, these agents might prove useful in the combined treatment of AMD\(^{(42)}\).

Intensive research is currently in course regarding alternative actions on crucial neovascularization cascade steps and mechanisms that trigger this process (signalling), since these have the potential to become alternative strategies for the combined treatment of AMD. Emerging therapies will be described in a separate chapter. It shall be referred that the complexity of signalling pathways supports the concept of combined therapy as a way of achieving more adequate control of biological functions in general and neovascularization in particular\(^{(43, 44)}\).

### 7. Practical aspects

Combined therapies have long been used in the treatment of oncological and numerous other systemic diseases. In neovascular AMD, the objective of combined treatments acting upon different stages of the physiopathological process or the signalling pathway that triggers its mechanisms of action is to achieve synergistic action; therefore, an increased treatment effect and/or a decrease in the number of retreatment sessions required to stabilize vision are to be expected, as well as a more prolonged effect, smaller doses and increased drug tolerance\(^{(1)}\).

Treatment of this disease focuses on three main targets\(^{(5)}\).
1. Neovascularization.
2. The angiogenic process.
3. The inflammatory, cicatricial and exudative process.

In clinical practice, the following therapies are currently used in combined treatment:

1. Photodynamic therapy with verteporfin, with standard, low or very low fluence.
2. Antiangiogenic agents ranibizumab 0.5mg (Lucentis®), bevacizumab 1.25mg (Avastin®) and pegaptanib 0.3mg (Macugen®) (and VEGF Trap – aflibercept – in a phase III study).
3. Anti-inflammatory treatment with intravitreal dexamethasone and triamcinolone, with or without core vitrectomy to allow injection of a greater volume of an anti-VEGF (0.5mg/0.5ml) and dexamethasone (0.5mg/0.5ml) solution.
Based on efficacy in terms of visual acuity and considering the level of clinical evidence for the studies performed, it is possible to conclude that no level I evidence exists to recommend combined treatment instead of monotherapy. However, if both clinical efficacy and efficiency (smaller number of treatment sessions required; longer absence of active disease between treatments; smaller drug, staff and structural costs; smaller doses/increased tolerance) criteria are considered, combined treatment based on anti-VEGF agents should be favoured in most forms of exudative AMD, as early as possible, as suggested also by several studies with levels of evidence II-1 and II-2 and numerous studies with levels of evidence II-3 and III. Clinical criteria and the doctor’s experience should also weight significantly in deciding whether or not to opt for combined treatment.

7.1 Particular cases of AMD

Due to their poor response to monotherapy, cases of Retinal Angiomatous Proliferation (RAP) and Polypoid Choroidal Vasculopathy should be primary candidates for anti-VEGF-based combined treatment.

7.2 Improved healthcare and increased equity

If combined treatments are proved to lead to better outcomes (greater VA line gains) or more sustained gains, as referred in most studies, the superior clinical efficacy of this treatment approach will be established. Although the costs of two or three different treatments need to be considered when calculating combined treatment costs. Costs per patient will be reduced if fewer overall resources are used. This is a more efficient strategy, as well as a principle to follow in health economics: to manage scarce resources so that health investments may benefit more patients, instead of necessarily making expense cuts. It is also about increasing equity – increasing the number of patients benefiting from treatment[1].

Levels of clinical evidence

Level I – At least one well-designed study – randomized, controlled studies.
Level II-1 – High-quality, non-randomized, controlled studies.
Level II-2 – Studies with a control group involving more than one research centre or group.
Level II-3 – Studies with no control group; series studies, with or without intervention.
Level III – Opinion of respected authorities, based on clinical experience, descriptive studies or specialised committee reports.

Abbreviations

ACE - angiotensin converting enzyme
AR - angiotensin receptor
ICAM-1 - Intercellular Adhesion Molecule-1
SDF-1-CR XR4 Axis – Stromal-Derived Factor-1 and its CRXR-4 receptor
PEDF - Pigment Epithelium-Derived Factor
VEGF - Vascular Endothelial Growth Factor
References:


23. ClinicalTrials.gov Identifier: NCT00492284

24. ClinicalTrials.gov Identifier: NCT00793923

25. ClinicalTrials.gov Identifier: NCT00390208

26. ClinicalTrials.gov Identifier: NCT00454389


In recent years, new treatment modalities such as photodynamic therapy and intravitreal anti-VEGF injections have been added to the treatment armamentarium for age-related macular degeneration (AMD). Prior to the introduction of these therapies, only laser photocoagulation had been shown in a large randomized controlled trial to confer a statistically significant benefit in the treatment of subfoveal choroidal neovascularization (CNV) secondary to AMD regarding to long-term visual acuity (VA) when compared to the natural history of the condition(1-3). Unfortunately, the Macular Photocoagulation Study also showed that laser photocoagulation was associated with immediate average reduction of VA, with benefits over no treatment apparent only six months after treatment(1-3). In fact, recovery of good vision rarely occurred in these patients.

As a result of the limited treatment options, alternative therapies, such as submacular surgery for CNV removal, were pursued with limited or no success. Meanwhile a different management paradigm for AMD was established with macular translocation. However, because of its higher risk of complications, its popularity has waned with the wider availability of photodynamic therapy and the introduction of intravitreal anti-VEGF agents. Nonetheless, they remain potentially useful treatment options, even if their role in the management of AMD is neither established nor consensual.

In the meantime, vitreoretinal surgery and vitrectomy have had important developments, mostly due to advances in sutureless transconjunctival vitrectomy, use of dyes, tamponade agents and new equipments. Novel surgical approaches for AMD are under scrutiny: the association of submacular surgery with pigment cell transplantation, the use of adjuncts, such as recombinant tissue plasminogen activator (r-TPA) for subretinal hemorrhages displacement combined with anti-VEGF treatment.

Recently, a new insight involving the vitreoretinal surface has been proposed for the pathophysiologic mechanisms underlying the development of CNV in AMD. Accordingly, posterior vitreomacular adhesion may be another risk factor in a subpopulation of patients with wet AMD, opening another path for a surgical approach in the treatment algorithm for this condition(4-7). Future studies are needed to define the role of vitreoretinal surgery in such cases.

2. Macular translocation

The first experiments on retinal relocation were conducted and reported in the beginning of the 80’s. Their aim was to study the anatomic dependency of the foveal retina on foveal retinal pigment epithelium (RPE) and choroid. The assumption that visual function could be preserved with foveal displacement has originated from cases of ectopic macula from retinal traction or after surgery in patients with retinopathy of prematurity and retinal detachments with giant tears(8). They ended up showing the feasibility of rotating the macula around the optic disc with reattachment of the fovea in animal eyes(8). One might say that this was the starting point for the idea of rotating the macula of eyes with subfoveal CNV to a new area of underlying RPE-Bruch’s membrane-choriocapillaris complex – macular translocation (MT) - as a treatment for the condition.

Although the exact pathogenesis of CNV secondary to AMD is not known, the natural history of this condition is progressive loss of central vision over time. The initial retinal dysfunction responsible for impaired vision in eyes with subfoveal CNV may be attributable to factors
such as subretinal fluid, subretinal hemorrhage, and retinal edema. When fibrous proliferation and degeneration of the overlying photoreceptors occur during the later stages of the disease, the visual loss becomes irreversible. The rationale of macular translocation is that moving the neurosensory retina of the fovea in one eye with recent-onset subfoveal CNV to a new location before the occurrence of permanent retinal damage, may allow it to recover or to maintain its visual function over a healthier bed of RPE-Bruch’s membrane-choriocapillaris complex.

In addition, relocating the fovea to an area outside the CNV allows the ablation of the latter, by laser photocoagulation without destroying the fovea, in an attempt to preserve central vision. On the other hand, some surgeons have combined macular translocation with CNV removal, allowing the fovea to be relocated to an area outside the RPE defect created during submacular surgery. Several different surgical techniques for macular translocation have been described and are currently in use. These techniques produce different degrees of postoperative foveal displacement, and can broadly be classified into two categories: (2.1) full macular translocation, and (2.2) limited macular translocation.

### 2.1 Full macular translocation

After developing their surgical techniques in rabbit eyes\(^3\), Machemer and Steinhorst became, in 1993, the first surgeons to demonstrate the feasibility of macular translocation in humans\(^10\). Their technique involved lensectomy, complete vitrectomy, planned total retinal detachment by transretinal infusion of fluid under the retina, \(360^\circ\) peripheral circumferential retinotomy, rotation of the retina around the optic disc, and reattachment of the retina with silicone oil tamponade. Besides allowing retinal rotation to occur, the retinotomy also made way to the subretinal space for synchronous blood and CNV removal. Since then, a number of investigators have subsequently modified this technique\(^12\). Corrective extraocular muscle surgery for globe counter-rotation, due to frequent postoperative cyclovertical diplopia or awareness of a tilted image, may be done during the primary surgery or at a later stage\(^11\). While some surgeons have found the results of macular translocation encouraging\(^13\), others found the surgery unpredictable\(^19\).

In a series of 50 consecutive eyes with subfoveal CNV from AMD that underwent full macular translocation followed-up for a median period of 21 months (12-36 months), Pertile and Claes reported an improvement of \(\geq 2\) Snellen lines in 66%, while 28% remained stable (\(\pm 1\) line) and 6% decreased by \(\geq 2\) lines\(^20\). In another prospective, interventional, consecutive non-comparative case series of 61 AMD patients who underwent the same procedure and followed-up for 12 months, the visual acuity improved by \(\geq 1\) Snellen lines in 52%\(^21\). Toth et al. also showed improvements in distance and near visual acuity, contrast sensitivity and reading speed in a series of 25 consecutive AMD patients\(^22\).

In addition to exceptional surgical technique, avoiding intra and postoperative complications, the key for success after macular translocation seems to be patient selection\(^23,24\). If this procedure is performed on a patient without viable foveal photoreceptors, there is no chance for visual improvement.

Still, the main drawback of macular translocation lies on its high rate of complications. Rhegmatogenous retinal detachment, with or without proliferative vitreoretinopathy development, is the most common serious complication of macular translocation. Rates up to 19% have been reported\(^25\). Persistent or recurrent subfoveal CNV has been described in up to 30% of patients undergoing this procedure\(^25\). With a longer follow-up, cystoid macular edema and subfoveal RPE atrophy may be limiting factors for improved postoperative visual acuity. In a long-term follow-up of full macular translocation (14 to 79 months – mean 38.2 months), 28% of patients developed subfoveal RPE atrophy associated with loss of visual function by the third year postoperatively\(^18\).

In figures 1 to 4, we present two anecdotal cases in which full macular translocation was performed. They presented with extensive submacular hemorrhages that were considered inadequate for anti-VEGF therapy. Visual rehabilitation was achieved, enabling the patient’s independence for daily activities.

### 2.2 Limited macular translocation

In an effort to overcome the major complication following macular translocation with large retinotomy: proliferative vitreoretinopathy (PVR), de Juan developed a new technique in 1998\(^26\). His technique involved transretinal hydrodissection using small posterior retinotomies to induce a subtotal retinal detachment, a complete vitrectomy, anterior-posterior shortening near the equator and retinal reattachment. As no large retinal break was created, the likelihood of developing PVR was thought to be lower. As more experience was gained with this surgery, additional modifications were made to the original technique.
Figure 1 - Case I – Patient with severe visual acuity loss in his left eye 3 days before admission (right eye lost to advanced AMD). He presented with a submacular hemorrhage complicating wet AMD. Full macular translocation was performed (António Travassos – Coimbra Surgical Centre, Coimbra Portugal). Three years after surgery, left eye visual acuity was 20/200.
Figure 2 - Case II - A 85-year-old man with an extensive submacular hemorrhage associated with subfoveal CNV.

Figure 3 - Case II - Four months after a full macular translocation (Claus Eckardt-Staedtische Kliniken Frankfurt A.M., Germany), his visual acuity was 20/80.
Surgery in AMD

This is essentially a five-step procedure, that starts with the placement of scleral imbricating sutures (either in the superotemporal or inferotemporal quadrants for inferior and superior translocations, respectively) for antero-posterior shortening of the eyeball. This is followed by a pars plana vitrectomy with posterior vitreous detachment induction. The third step should be an internal subretinal hydrotension through small self-sealing retinotomies for creation of a partial neurosensory retinal detachment. After tightening the scleral imbricating sutures, a redundancy of the neurosensory retina relative to the eyeball is created that should allow the desired foveal displacement, after retinal reattachment with fluid-air exchange. Limited macular translocation may be either inferior or superior, depending on the movement of the neurosensory macula relative to the underlying tissues.

The largest series of limited macular translocation by Pieramici et al., in a retrospective review, analyzed the outcome of 102 consecutive eyes of 101 patients that underwent inferior limited macular translocation for AMD-related subfoveal CNV. At three and six months postoperatively, 37% and 48% of the eyes, respectively, experienced ≥ 2 Snellen lines of visual improvement. After one year of follow-up, 39.5% still maintained a ≥ 2 lines of improvement, while 29.0% remained unchanged and 31.4% lost ≥ 2 lines of visual acuity. Recurrent CNV developed in 34.6% of the eyes, being subfoveal in most of the cases (65%).

Besides the usual risks inherent to pars plana vitrectomy, limited macular translocation is associated with additional risks similar to those of scleral buckling surgery. As with full macular translocation, the rate of complications won its popularity. Although conceived in an effort to reduce the rate of PVR development, this has remained the most common serious complication after limited macular translocation. Rates of retinal detachment up to 17.4% have been reported. Insufficient macular translocation is another important limitation of this procedure, restricting its indications to smaller CNV.

Is there a role for macular translocation in the current era of anti-VEGF therapy and combined treatments?

In the latest edition of Vitreous Microsurgery (4th Edition, 2007 - Lippincott Williams & Wilkins), Steve Charles et al. stated that, at the present time, macular translocation has no place in the treatment of age-related macular degeneration. They argued that these procedures still come with an unacceptable number of serious complications.

In a recent review of the functional outcomes of macular translocation for wet-AMD, Eandi et al. concluded that there wasn’t enough evidence in randomized trials to support the benefit of surgery. Future studies should involve patients with small neovascular membranes that are non-responsive to the present medical armamentarium, and that accept the risks of surgery in an effort to improve their visual function.
This is a procedure that demands a high level of surgical skill and experience, with a rather flat learning curve. The number of complications is unacceptably high. At the present time, the very small incidence of cases that could benefit from this procedure does not allow every Ophthalmology department with vitreoretinal surgical capability to gain expertise on macular translocation. Referral of few cases to selected surgical units with the proper human and technical resources and experience, seems the best choice.

### 3. Submacular surgery

As already has been said, a variety of surgical treatments have been developed for exudative AMD disease and one of those are the different modalities of submacular surgery. We will review these surgical approaches and their actual role in the AMD treatment.

#### 3.1 Surgery with subfoveal neovascular membrane removal

In 1988, Juan and Machemer(31) published the first results regarding removal of blood or fibrous submacular complications in four AMD patients. Countless publications of retrospective studies in small numbers of patients ensued, with no control group, describing the benefits of this technique in stabilizing the disease, albeit displaying reduced functional benefits(32-34). The need to determine the actual benefits of submacular surgery in managing choroidal neovascularization led to the Submacular Surgery Trials (SSTs). One of the objectives of this study was to determine whether surgery not only stabilizes vision for the various types of AMD lesions but also increases vision with actual repercussion in the quality of life of these patients(35).

In the pilot trial used as a test to determine the best method to be used in this multicentre, randomized, controlled study no reason was found to prefer submacular surgery instead of laser photocoagulation in AMD patients with similar lesions to those displayed by study patients(36).

Several surgical techniques are described in the literature. In summary, these techniques include standard pars plana vitrectomy, with or without posterior hyaloid membrane removal; posterior retinotomy followed by infusion of subretinal saline solution or r-TPA into lesions with a large haemorrhagic component, membrane mobilization and its removal with surgical forceps, followed by eventual aspiration of blood or clot aspiration, depending on the situation. Possible intraocular haemorrhages may be controlled by increasing the intraocular pressure, either by raising the irrigation bottle or using heavy perfluorocarbon liquids. The procedure is finished with a fluid-air exchange, followed by gas buffering, maintaining the patient in the prone position until gas reabsorption(37-40).

Due to the physiopathology of this disease, it was observed in histopathological studies that inadvertent and undesired removal of the pigment epithelium often occurs during membrane removal, especially for type 1 membranes. The absence of the pigment epithelium leads to loss or atrophy of photoreceptors and choriocapillaries, which unfavorable visual recovery(41). The percentage of removed epithelium is variable but may reach significantly high values, as observed in the SSTs, where the pigment epithelium was involved in 84% of removed membranes(42). Therefore, both the functional results and the impact on the quality of life observed for the various subgroups considered in the SSTs, compared to natural disease progression, led the authors not to recommend submacular surgery as a treatment option(43-46).

#### 3.2 Autologous pigment epithelium transplants

As previously referred, the poor results achieved with surgical removal of subfoveal membranes, largely due to resulting atrophy or rupture of the photoreceptor-retinal pigment epithelium complex, led some groups to combine neovascular membrane removal with simultaneous transplant of iris or retinal pigment epithelium, with the objective of restoring normal subretinal conditions(47-49). The surgical technique used is similar to that used in submacular surgery; in addition to the aspiration and subsequent pigmented epithelial cell transplant procedures.

I had the good fortune and the pleasure of assisting in a surgical procedure performed during my training at the Eye Hospital of Rotterdam with Dr. van Meurs, a pioneer in this field (A.M.); therefore, it is his technique which is described in general terms: complete pars plana vitrectomy after inducing posterior hyaloid membrane detachment, paramacular retinotomy and removal of the subfoveal membrane, creation of a peripheral retinal detachment in the walled off inferior retina by the transtretinal injection of Ringer’s solution into the subretinal space, removal of the detached retina with the vitrectome, aspiration of pigment epithelial cells with a micropipette connected to an insulin syringe, centrifugation of aspirated material, reinjection through the retinotomy of...
a mixture of the cellular concentrate and an agent able to promote adhesion in the subretinal space and buffering using gas or silicone\textsuperscript{(49)}. Although good tolerability has been demonstrated for pigment cells in the subretinal space, as well as absence of membrane recurrence, the poor functional results obtained in most studies have been attributed to several factors, including lack of differentiation in pigment cell repopulation, failure in adhesion to Bruch’s membrane and failure to form a regular pigment epithelial cell monolayer\textsuperscript{(47-51)}. Also according to other authors, the behavior of transplanted cells depends essentially on the type of environment found at the seeding location\textsuperscript{(52)}.

In a continuous attempt to change this discouraging framework, Peyman performed the first homologous and autologous EPR-Bruch’s membrane transplants in 1991\textsuperscript{(53)}. Later, Aylward et al., of the Moorfields Eye Hospital, were the first team to describe the concept of total patch translocation of the EPR-choroid complex to the subfoveal area\textsuperscript{(54)}. The patches collected in the macular area near the EPR lesion were small; in follow-up, none of the nine transplant patients displayed any function after 5 years\textsuperscript{(55)}. van Meurs in Rotterdam described a modification to this technique in which a full-thickness patch of retinal pigment epithelium-choroid of approximately 1.5x2mm is harvested from within a circular zone isolated by heavy diathermia in the superior midperiphery\textsuperscript{(56)}. Subsequently, other groups have been publishing small case series presenting not only expressive and sustained improvement in visual acuity and reading ability but also recovery of central fixation, evidence for graft revascularization (also demonstrated histologically in animals experiments) and a normal autofluorescence over the patch in patients with exudative or dry AMD\textsuperscript{(56-64)}.

3.3 Management of submacular haemorrhage: displacement with or without r-TPA

In addition to having a poor prognosis, exudative forms of AMD, which display a relevant haemorrhagic component, are difficult to diagnose (concerning membrane location and extension) and to treat\textsuperscript{(65,66)}. In the era of antiangiogenic agents, this is possibly the only form of AMD for which surgery is indicated, with the main objectives of avoiding damages caused by blood (mechanical, metabolic and toxic damages to the photoreceptor-RPE complex) and allowing subsequent treatment (laser or PDT)\textsuperscript{(37,67,68)}. As previously referred, blood was initially removed by aspiration or mechanically extracting. On a later date, Lewis was the first researcher to report the fibrinolysis properties of r-TPA, which make this agent useful in removing blood clots\textsuperscript{(38-40)}. A procedure involving displacement of submacular blood by intravitreal injection of r-TPA and gas, followed by prone position, was described for the first time by Herriot in 1997\textsuperscript{(69)}. Blood is normally displaced temporally or infero-temporally, with a significant increase in visual acuity occurring immediately after the aforementioned procedure, as described in countless published outcomes. Duration of haemorrhage has been pointed out by some authors as the main predictive factor for the aforementioned improvement\textsuperscript{(70-76)}. The usefulness of intravitreal r-TPA as an adjuvant to this technique has been questioned, not only because r-TPA diffusion to the subretinal space has not been proved in experimental studies, but also because some studies demonstrated the success of pneumatic displacement of subretinal blood without concomitant injection of r-TPA\textsuperscript{(75-77)}. Therefore, a hybrid technique was introduced by Haupert in 2001, combining submacular surgery with pneumatic displacement\textsuperscript{(78)}. After a few changes, this technique is currently used in some centers, as described: pars plana vitrectomy, removal of the posterior hyaloid, injection of r-TPA (12.5 μg/0.1 mL) into the subretinal clot using a 39-gauge flexible translocation cannula and fluid-air exchange followed by prone position. The advantage of this technique is its smaller percentage of associated intra and post-operative complications, which is probably due to the smaller extent of tissue manipulation involved and consequent reduction in retinal injury\textsuperscript{(78,79)}.
References:


57. MacLaren RE, Uppal GS, Balagga KS et al. RPE Patch Graft Auto-Transplantation in Macular Degeneration: A Prospective Cohort Study. ARVO. Fort Lauderdale, USA, Apr 30-May 4, 2006. E-Abstract 2693.


As researchers and clinicians are beginning to understand that wet age-related macular degeneration (AMD) is more than simply a vascular disease that includes angiogenic, vascular and inflammatory components, they are exploring new agents with different mechanisms of action addressing multiple targets in this complex pathophysiology. Some of them are already available in human trials or even approved. Others are still under laboratory investigation and few is known about them.

We will discuss, in this review, promising emerging therapies for neovascular AMD that aim to improve outcomes, safety and treatment burden through novel mechanisms of action. For the new promising research components an Internet research was made and preliminary therapeutic strategies and results are presented.

There are two aspects that must be addressed: the first is related with the platform therapy, corresponds to how the product is developed and correlates directly with its internal structure; the second is related to the “targeting of action”, is correlated with the mechanism of action and its effects.

Figure 1 - Three potential therapeutic targets: 1- inhibition of angiogenic proteins production; 2- angiogenic protein neutralization; 3- angiogenic proteins receptors inhibition or endothelial cell apoptosis induction (Fig. 1).

Almost every ophthalmic drug either currently available or under development for the treatment of AMD began life as a cancer therapy.
Related to the endothelial cell membrane there are four potential anti-angiogenic agents:

Those that inhibit the production of angiogenic factors (Bevasiranib, PF-04523655) or stimulate the production of antiangiogenic agents (adPEDE11, Lentivirus).

Those that neutralize the action of angiogenic agents in the extracellular environment (Ranibizumab, Bevacizumab, Pegaptanib, VEGF trap and E10030).

Those that reduce the expression of membrane cellular receptors (AGN745) or interact with Integrins (Voloximab, JSM6427).

Those that interact in the inner space of the cell (Valatanib, TG100801/TG101095, Pazopanib, AGO13958, AL39324 Sirolimus, and Zybrestat).
3. Vascular endothelial growth factor (VEGF) blockers:

3.1 Monoclonal antibodies

3.1.1 Lucentis® and Avastin® - Wet AMD Intravitreal

These two drugs emerged from the promising technological platforms of monoclonal antibodies. Technology platforms of monoclonal antibodies are promising and allow the development of many new drugs, given the ease to develop drugs for mediators or receptors that were previously identified. Current conditions for these drugs are exposed in the corresponding chapter. Future prospects about their use in AMD are essentially made by process innovation, with adoption of optimized therapeutic schemes of combined therapy and improvement of intra-eye drug delivery.

3.2 Aptamers

3.2.1 Macugen® - Wet AMD Intravitreal

Aptamers are oligonucleotide ligands that are selected for their high-affinity to bind molecular targets. Pegaptanib sodium (Macugen®; Eyetech Pharmaceuticals/Pfizer) is an RNA Aptamer directed against vascular endothelial growth factor (VEGF)-165, the VEGF isoform primarily responsible for pathological ocular neovascularization and vascular permeability.

“Pegaptanib therefore has the notable distinction of being the first aptamer therapeutic approved for use in humans, paving the way for future aptamer applications.”

Like the use of Lucentis® and Avastin® future prospects with the use of Macugen® are essentially made by process innovation, with adoption of optimized therapeutic schemes of combined therapy.

3.3 Fusion proteins

3.3.1 VEGF Trap-Eye - Wet AMD Intravitreal

The aflibercept, VEGF-Trap, results of a process of bioengineering where extramembrane fragments of receptors 1 and 2 of VEGF are merged to IgG1 FC fragment. This recombinant fusion protein is a composite decoy receptor based on VEGF receptors VEGFR1 and VEGFR2. The VEGF Trap (Regeneron Pharmaceuticals, Tarrytown, NY, USA) is an 110kDa soluble recombinant protein with the binding portions of VEGF receptor 1 and 2 fused to the Fc region of human IgG that binds all VEGF isoforms with a very high affinity (about 140 times that one of ranibizumab). Aflibercept is a fully human soluble fusion protein that binds all forms of VEGF-A along with the related Placental Growth Factor (PlGF).

This high affinity fusion protein is used to block the biological activities of VEGF by preventing it to bind to its receptors. VEGF-Trap effectively suppresses tumor growth and vascularization in vivo.

In phase 1, randomized, placebo-controlled trial of VEGF Trap administered intravenously for treatment of choroidal neovascularization, the Clinical Evaluation of Antiangiogenesis in the Retina (CLEAR)-AMD 1 group found a dose-dependent increase in systemic blood pressure with a maximum tolerated dose of 1mg/kg. This dose resulted in the elimination of about 60% of excess retinal thickness after either single or multiple administrations. CLEAR IT-1 was a phase I dose escalation study of a single intravitreal injection of various doses of VEGF Trap (0.05, 0.15, 0.5, 1, 2, and 4mg). At 6 weeks, mean visual acuity gain was 4.8 letters and mean OCT central retinal thickness decreased from 298μm to 208μm across all groups. Higher doses resulted in gaining more letters. The potential benefit of VEGF Trap is its longer duration of action compared with single injections of other anti-VEGF agents because of its higher affinity and longer intravitreal half-life. It seems that VEGF Trap would offer less frequent dosing resulting in fewer injections, lower cost and reduced risk of complications.

From August 2007 it’s initiated a phase III global development program for VEGF Trap-Eye in wet AMD. During the first year of the two phase III trials, the companies (Regeneron Pharmaceuticals, Inc. and Bayer HealthCare AG ) are evaluating VEGF Trap-Eye dosed 0.5 mg every 4 weeks, 2 mg every 4 weeks, or 2 mg every 8 weeks (following three monthly doses) in direct comparison with ranibizumab (Lucentis® Genentech, Inc.) administered 0.5 mg every 4 weeks according to its U.S. label. PRN dosing will be evaluated during the second year of each study.

Currently phase III clinical trials, VIEW 1 and 2 study will assess its efficacy and safety in patients with neovascular AMD. The VIEW1 study (in the United States and Canada) and the VIEW2 study (in Europe, Asia Pacific, Japan, and Latin America) enrolled 1200 patients.
3.4 siRNA

3.4.1 Bevasiranib (Cand5) - Wet AMD Discontinued

Despite Bevasiranib has been discontinued it’s worth mentioning because it was the first therapy based on the Nobel Prize-winning RNA interference (RNAi) technology to advance to phase III clinical trials. Bevasiranib was a first-in-class small interfering RNA (siRNA) drug designed to silence the genes that produce vascular endothelial growth factor (VEGF).

“The decision to conclude the clinical program follows a review of preliminary trial data by the Independent Data Monitoring Committee, which found that although Bevasiranib showed activity when used in conjunction with Lucentis® (ranibizumab, Genentech), the trial was unlikely to meet its primary endpoint.”

The trial was COBALT for “Combination of Bevasiranib and Lucentis” Therapies” for AMD. It was a phase III, randomized, double-blinded, parallel-assignment study of the RNAi drug administered either every 8 or 12 weeks as a maintenance therapy following three injections of Lucentis®.

3.4.2 PF-04523655 - Wet AMD Intravitreal

It is a siRNA, 19 nucleotides in length, that inhibits the expression of the hypoxia-inducible gene RTP801. This stress-response gene mediates the mammalian target of rapamycin (mTOR) pathway. PF-04523655 has been shown to reduce the volume of choroidal neovascularization in a mouse model. PF-04523655 has been shown to cause regression of CNV in experimental studies of mice and primates. Intravitreal small-interfering RNA (siRNA) PF-04523655 (Quark; licensed to Pfizer) used to treat choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD) drug administered every 8 or 12 weeks as a maintenance therapy following three injections of Lucentis®.

3.4.3 AGN-745 (Sirna-027) - Wet AMD Development was halted

Allergan has halted development of its siRNA-based wet age-related macular degeneration, Sirna-027 is the first chemically modified short interfering RNA (siRNA) targeting Vascular Endothelial Growth Factor Receptor-1 (VEGFR-1). VEGFR-1 is found primarily on vascular endothelial cells and is stimulated by both VEGF and placental growth factor (PIGF), resulting in the growth of new blood vessels. By targeting VEGFR-1, Sirna-027 is designed to reduce pathologic angiogenesis mediated by both VEGF and PIGF. Development was halted for AGN-745 after the drug failed to meet a key efficacy endpoint in a phase II study.

The trial compared the effect of three different monthly doses of AGN-745 with Genentech’s antibody drug Lucentis®, the standard of care for AMD, in treating the subfoveal choroidal neovascularization associated with the disease. Both drugs were administered via intravitreal injection. Apparently no safety issues were associated with AGN-745, a chemically modified siRNA. But since the drug did “not meet its efficacy hurdle” — improvement in visual acuity — Allergan opted to halt its development.

4. Anti-platelet derived growth factor

4.1 E10030 (Aptamer) - Wet AMD Intravitreal

One of them is E10030 (Ophthotech), an anti-platelet-derived growth factor (anti-PDGF-B) aptamer. E10030 strongly binds to PDGF-B. PDGF-B plays a key role in recruiting the pericytes that envelop the new vessels and make them more resistant to the anti-VEGF attack. In combination with anti-VEGF, this new agent could represent a breakthrough therapy.

A phase I study with an intravitreal anti-platelet-derived growth factor (PDGF) aptamer that targets pericytes, was evaluated in combination therapy with ranibizumab (Lucentis®, Genentech) in patients with neovascular age-related macular degeneration (AMD) with promising results regarding safety and efficacy.

5. Anti tyrosine kinase

Approximately 2000 kinases are known, and more than 90 Protein Tyrosine Kinases (PTKs) have been found in the human genome. They are divided into two classes, receptor and non-receptor PTKs.

5.1 Anti receptor kinase (suffix -nib):

“At present, 58 receptor tyrosine kinases (RTKs) are known, grouped into 20 subfamilies. They play pivotal roles in diverse
cellular activities including growth, differentiation, metabolism, adhesion, motility and death. RTKs are composed of an extracellular domain, which is able to bind a specific ligand, a transmembrane domain, and an intracellular catalytic domain, which is able to bind and phosphorylate selected substrates. Binding of a ligand to the extracellular region causes a series of structural rearrangements in the RTK that lead to its enzymatic activation. In particular, movement of some parts of the kinase domain gives free access to adenosine triphosphate (ATP) and the substrate to the active site. This triggers a cascade of events through phosphorylation of intracellular proteins that ultimately transmit ("transduce") the extracellular signal to the nucleus, causing changes in gene expression. Many RTKs are involved in oncogenesis, either by gene mutation, or chromosome translocation, or simply by over-expression. In every case, the result is a hyper-active kinase, that confers an aberrant, ligand-independent, non-regulated growth stimulus to the cancer cells."\textsuperscript{(16)}

From these 20 subfamilies of Receptor Tyrosine Kinases (RTK), seven families are promising field of investigation and only two families of RTK represent now the most promised field of drug development in AMD; fibroblast growth factor receptor (FGFR) family and vascular endothelial growth factor receptor (VEGFR) family.

<table>
<thead>
<tr>
<th>RTK Class I</th>
<th>Epidermal growth factor receptor family</th>
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<tr>
<td>RTK Class II</td>
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<td>RTK Class IV</td>
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<td>RTK Class V</td>
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<tr>
<td>RTK Class XII</td>
<td>RET receptor family (RET proto-oncogene)</td>
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<tr>
<td>RTK Class VIII</td>
<td>Eph receptor family</td>
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5.2 Fibroblast growth factor receptor (FGFR) family – Class IV

Fibroblast growth factors comprise the largest family of growth factor ligands\textsuperscript{(17)}. The natural alternate splicing of four fibroblast growth factor receptor (FGFR) genes results in the production of over 48 different isoforms of FGFR. These isoforms vary in their ligand binding properties and kinase domains, however all share a common extracellular region composed of three immunoglobulin (Ig) like domains (D1-D3), and thus belong to the immunoglobulin superfamily\textsuperscript{(18)}.

5.3 Vascular endothelial growth factor receptor (VEGFR) family – Class V

Vascular endothelial growth factor (VEGF) is one of the main inducers of endothelial cell proliferation and permeability of blood vessels. Two RTKs bind to VEGF at the cell surface, VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flik-1)\textsuperscript{(19)}. The VEGF receptors have an extracellular portion consisting of seven Ig-like domains so, like FGFRs, belong to the immunoglobulin superfamily. VEGFR-2 is the major mediator of endothelial cell proliferation, migration, survival, and permeability. The function of VEGFR-1 is less well defined, although it is thought to modulate VEGFR-2 signaling.

The therapeutic strategy is the blockade of VEGF effects by inhibition of the tyrosine kinase cascade downstream from the VEGF receptor. The concept of disrupted signaling appears to be effective in the pharmacological treatment of neovascularization\textsuperscript{(20)}.

5.4 Intracellular inhibition of the tyrosine kinase cascade:

This promising therapeutic strategy is the blockade of VEGF effects by inhibition of the tyrosine kinase cascade downstream from the VEGF receptor; such therapies currently in development include, Vatalanib, TG100801, Pazopanib, AG013958 and AL39324.

5.5 Vatalanib - Wet AMD

Oral administration of PTK787 (Vatalanib), a tyrosine kinase inhibitor that blocks phosphorylation of VEGF and PDGF receptors, provides inhibition of retinal neovascularization. The development of new vessels are prevented while there is no effect on mature retinal vessels in murine\textsuperscript{(21)}. Vatalanib (PTK787 or PTK/ZK) is a small molecule protein kinase inhibitor that orally administered inhibits angiogenesis. It is being studied as a possible treatment for several types of cancer. Vatalanib is being developed by Bayer Schering and Novartis. It inhibits all known VEGF receptors (VEGFR1, VEGFR2, and VEGFR3) as well as platelet-derived growth factor receptor-beta and c-kit, but is most selective for VEGFR-2. The “Safety and Efficacy of Oral PTK-787 in Patients With Subfoveal Choroidal Neovascularization Secondary to Age-related Macular Degeneration" (ADVANCE) study evaluate the tolerability and safety of 3 months treatment with PTK-787 tablets given daily\textsuperscript{(22)}. 
5.6 TG101095 - Wet AMD
Topical

It is a topical tyrosine kinase inhibitor that specifically targets VEGFR, only tested in animal models. JAK2 is a signalling kinase that acts downstream from erythropoietin, a glycoprotein hormone, which, along with VEGF, is involved in the pathogenesis of diabetic retinopathy. The VEGF receptor and JAK2 inhibitor TG101095 dosed topically bid for two weeks significantly reduced CNV area in a laser-induced CNV mouse model(23).

5.7 Multi-targeted kinase inhibitors

Multi-targeted kinase inhibitors have been shown to be effective in oncology. Newly developed small molecule kinase inhibitors (including TG100572 and the prodrug TG100801), which inhibits VEGF, PDGF, and FGF receptors in addition to Src family of kinases (sarcoma proto-oncogenic tyrosine kinases family). They act in intracellular environment(24).

5.8 TG100801 - Wet AMD
Topical

TG100801 a prodrug version of TG100572, is administered noninvasively as an eye drop and is designed to suppress VEGF mediated leakage and additionally the kinase targets associated with inflammation, edema, and angiogenesis which are the pathological hallmarks of AMD and of other back of the eye diseases including diabetic macular oedema and proliferative diabetic retinopathy(25). It is synthesized at TargeGen (TargeGen inc San Diego). Topical administration of TG100801 suppressed CNV in mice and reduced the retinal oedema induced by retinal vein occlusion in rats, without observable safety issues. Data have suggested that the delivery of these agents occur by local penetration through sclera rather than by systemic absorption as neither compound was detectable in the plasma(24). Therefore, TG100801 may offer equal efficacy to injectable agents, while offering the convenience and potential safety advantages due to a non-invasive route of delivery and eye penetration. Currently a multicentric, open-label, randomized, phase II study is evaluating the effects of 30 days of dosing with two dose levels of TG100801, instilled twice a day, on central retinal/lesion thickness, as measured by optical coherence tomography (OCT). The safety of TG100801 in patients with AMD will also be evaluated in this trial(26).

5.9 Pazopanib (GW786034) - Wet AMD
Topical

Pazopanib (GW786034), by GlaxoSmithKline, is a second-generation multi-targeted tyrosine kinase inhibitor against all VEGF receptors (VEGFR-1, VEGFR-2, VEGFR-3) PDGFR-a, PDGFRβ, and c-kit that blocks tumour growth & inhibits angiogenesis. An early phase trial is evaluating the safety, and pharmacokinetics of Pazopanib eye drops in patients with neovascular AMD(27).

5.10 AG013958: Wet AMD
Subtenon

AGO013958 (Pfizer Inc.) is a subtenon injectable Tyrosine kinase inhibitor. A phase I/II, randomised, masked, single and multiple dose, sequential dose-escalation study of the safety and efficacy of AG-013958 in subjects with subfoveal choroidal neovascularization associated with age-related macular degeneration has been completed(28).

6. mTOR inhibitor

6.1 Sirolimus - Wet AMD
Subconjunctival and intravitreal

Sirolimus (MacuSight inc.), also known as rapamycin, is an immunosuppressant drug used to prevent rejection in organ transplantation. It was originally developed as an antifungal agent and has potent immunosuppressive and antiproliferative properties. Sirolimus inhibits the mammalian target of rapamycin (mTOR). The mammalian target of rapamycin is a protein kinase that regulates cell growth and metabolism in response to changes in the environment. Sirolimus administered via subconjunctival injections was as effective as sirolimus administered via intravitreal injection. A phase II, randomized, multicentric study in wet AMD is now taking place; (EMERALD) is currently recruiting patients with wet AMD for a phase II study of an ocular sirolimus formulation in combination with Lucentis®. This is a randomized, multicentric study and is taking place throughout the United States(29).
7. Anti integrins therapy

7.1 JSM6427 inhibitor of integrin α5β1 - Wet AMD Intravitreal

Intravitreal JSM6427 (Jerini Inc) is a potent and selective inhibitor of integrin α5β1. Animal studies have shown an inhibition of choroidal neovascularization (CNV). This suggests that JSM6427 may provide a new approach for the treatment of age-related macular degeneration in humans. Integrins are transmembrane receptors composed of α and β subunits that mediate binding to extracellular matrix or other cellular receptors (Fig. 3). Blocking angiogenesis through inhibition of integrin-mediated signaling has the potential to inhibit the cellular responses to growth factors as well as to cytokines and other inflammatory mediators(30).

7.2 Volociximab: α5β1 Antagonist - Wet AMD Intravitreal

Volociximab (Ophthotech) is a high-affinity chimeric monoclonal antibody (Mab) that inhibits the functional activity of alpha-5 beta-1 integrin found on the endothelial cells involved in the formation of blood vessels. "Volociximab binds to α5β1 integrin and blocks the binding of α5β1 integrin to fibronectin, thereby inhibiting a pivotal interaction required for angiogenesis. Volociximab administration has resulted in strong inhibition of rabbit and primate retinal neovascularization. In monkeys with laser-induced choroidal neovascularization (CNV), volociximab significantly inhibited CNV proliferation and reduced the degree of lesion formation. In a rabbit model, volociximab administered either intravenously or intravitreally prior to the onset of neovascularization significantly reduced angiogenesis as compared to control. Similar antiangiogenic efficacy with volociximab has also been shown in multiple preclinical models of tumor angiogenesis." (31)

A phase I open-label, multicenter study of volociximab is currently on going. The objectives of this study are to evaluate the safety, tolerability, and pharmacokinetic profile of volociximab intravitreous injection in subjects with subfoveal choroidal neovascularization secondary to age-related macular degeneration (AMD)32).

8. Vascular disrupting agents

Zybrestat (OXIGENE Inc.) is a synthetic prodrug, Combretastatin A4 Phosphate that is converted to Combretastatin inside endothelial cell. It was originally derived from the root bark of the South African Bushwillow tree (Combretum caffrum).

The mechanism of action is a vascular disrupting agent (VDA) by a dual action: tubulin depolymerizing agent and cell junction disruption (Fig. 4). These actions upset the physical structure of the existing blood vessels. VE-cadherin disrupts the VE-cadherin/b-catenin complex interfering with cell-cell contact and induces loss of cell-cell contact that increases vascular permeability, leading to increased interstitial pressure and additional loss of blood flow. Tubulin depolymerization acts at the colchicines-binding site of the b-subunit of endothelial tubulin, inducing disruption of the endothelial cytoskeleton that results in shape changes. Normally flat, the endothelial cells become more spherical, and this decreases the size of the blood vessel lumen, causing decreased blood flow and thrombosis. It seems that the
cytoskeleton of newly formed cells is sensitive to CA-4-P, whereas the cytoskeleton of mature cells is not. This appears to underlie the selective shutdown of neovascular vessels compared to that of normal vessels. Currently Zybrestat has been tested intravenously-administered in clinical studies in patients with forms of macular degeneration. The topical administration cells identical to those induced by VEGF with increase in endothelial cell proliferation and reduction of apoptosis what leads to increase in capillary network formation(34). Antagonists of nAChR abolish the proangiogenic effect of nicotine nAChR and VEGF: Two distinct but interdependent angiogenesis pathways(35). Neutralization of VEGF resulted in a significant but

is being tested in animal studies. A phase II study in patients with polypoidal choroidal vasculopathy (PCV) is initiated(33). Current therapies active against wet AMD appear to have limited benefits in patients with PCV, and OXIGENE (Oxigene inc. San Francisco) believes the abnormal vasculature in the retina and choroid that contributes to PCV patients loss of vision may be susceptible to treatment with Zybrestat.

9. Anti-nicotine agents

Nicotine has a potent angiogenic effect. It has two distinct but interdependent pathways for angiogenesis; nAChRs are involved in the native angiogenic response, and this pathway is distinct from those triggered by VEGF or FGF. Nicotine induces morphological changes in endothelial not complete inhibition of nAChR-mediated network formation.

9.1 Nicotinic acetylcholine receptor antagonists

Non-selective cholinergic agonists such as nicotine have been shown to induce angiogenesis, enhancing tumor progression. Moreover, α7 AChR (nicotinic acetylcholine receptor) selective antagonists such as α-bungarotoxin and methyllycaconitine as well as the non-specific antagonist mecamylamine have been shown to inhibit endothelial cell proliferation and ultimately blood vessel formation. Such pharmacologic properties can lead to the discovery of new specific cholinergic antagonists as anti-AMD therapies. Conversely, the pro-angiogenic effect of specific agonists can be used to treat diseases that respond to revascularization such as diabetic ischemia and atherosclerosis, as well as to accelerate wound healing(36).
9.2 ATG003: nAChR antagonist - Wet AMD

Topical

ATG003 (Mecamylamine) topical delivery in animal models significantly inhibits laser induced CNV in a mouse model of AMD.

Currently a phase IIa study and phase IIb study is terminated, 330 subjects are enrolled with dose ranging; 0.3% and 1% topical solutions and the data is not published\(^\text{(37)}\).

Phase IIb study, with 60 subjects, was design to study the safety of 1% topical mecamylamine bid for 48 weeks in patients receiving maintenance injections of Lucentis\(^*\) or Avastin\(^*\); this study is a double-masked, randomized, placebo-controlled study. The study is ongoing, but not recruiting participants\(^\text{(38)}\). In this phase the patients receive either a dose of ATG003 or a placebo, while continuing the Lucentis\(^*\) or Avastin\(^*\) treatment. All patients will be treated for up to 48 weeks, during which time they will be monitored to assess the drug's safety, tolerability and efficacy.

10. Gene therapy

10.1 AdPEDF - Wet AMD

Intravitreal

Pigment epithelium-derived factor (PEDF) is one of the most potent antiangiogenic proteins. It inhibits VEGF-induced proliferation, migration of endothelial cells, reduces VEGF-induced hypermeability and causes vessel regression in established neovascularization\(^\text{(39, 40)}\). PEDF is also a neurotrophic factor\(^\text{(41)}\) and also inhibits/antagonizes other angiogenic factors such as platelet-derived growth factor and fibroblastic growth factor. GenVec (GenVec inc, Gaithersburg, Maryland) delivers AdPEDF utilizing a modified adenoviral vector delivery system.

In phase I studies testing the safety and feasibility of intravitreal injection AdPEDF was safe and generally well-tolerated at all dose levels tested in 28 patients\(^\text{(42,43)}\). Other phase I studies\(^\text{(44,45)}\) in 22 patients with less severe wet AMD find similar results; there were no dose-limiting toxicities or drug-related severe adverse events. Further studies for AdPEDF in patients with wet AMD are under way.

10.2 Lentivirus: Wet and Dry AMD

The LentiVector (Oxford BioMedica plc, Oxford, England) has been shown to efficiently and effectively deliver genes to the specialized, nondividing cells of the retina. The system is based on the lentivirus equine infectious anemia virus (EIAV). Applications for the gene vector system include gene therapy, transgenesis, stem cell manipulation, somatic disease models, target validation and gene discovery. For AMD, RetinoStat aims to preserve and improve the vision of patients with wet AMD through antiangiogenesis by delivering endostatin and angiostatin genes directly to the retina\(^\text{(46, 47)}\).

11. Anti-complement inhibitors

Complement activation has been implicated in a number of acute and chronic conditions. There is strong evidence that AMD is an inflammatory disease; Aberrant activation of the complement system is implicated in the wet and dry forms of AMD\(^\text{(48-49)}\). Patients with AMD demonstrate elevated systemic inflammatory biomarkers of inflammation (CRP, IL-6 and homocysteine). Histopathologic analyses of human AMD neovascular complex specimens demonstrate inflammatory infiltrates. Recent studies implicated local inflammation and activation of the complement cascade in the formation of drusen\(^\text{(50)}\). Complement-mediated inflammation in AMD is also reinforced by multiple genetic linkage and association studies published in Science\(^\text{(48-51)}\) and in New England Journal of Medicine\(^\text{(52)}\).

All this, strong support for the complement-mediated disease in wet and dry forms of AMD.

11.1 POT-4 inhibits C3 - Dry AMD

Intravitreal

POT-4 developed by Potentia Pharmaceutical (Potencia Pharmaceutical, Inc., Louisville) affects the body’s “complement” system. Inflammation plays a role in developing macular degeneration. Eight complement proteins are associated with AMD and POT-4 affects C3. The problem is that inflammation is useful in fighting infections. If the complement system is completely shut down, there is an increase risk for the development of bacterial infection.

Potentia has completed a phase I trial for POT-4 (called ASap) in patients with wet AMD. The trial was designed to determine the safety and tolerability of an intravitreal injection of POT-4, as well as its stability and depot-forming properties. In the study investigators observed only minimal and mild local adverse events related to the injection with no serious adverse events related to the drug itself\(^\text{(53, 54)}\).
11.2 JPE1375 inhibits C5 - Dry AMD

Intravitreal

JPE-1375 (Jerini AG, Berlin Germany) is a small molecule peptidomimetic antagonist targeting the complement pathway, a highly validated target for dry AMD. It targets C5aR, the receptor for complement factor C5a, which is a key component involved in the activation of inflammatory cells. JPE-1375 has shown significant efficacy in multiple preclinical models.

11.3 ARC1905 inhibits C5 - Dry/Wet AMD

Intravitreal

ARC1905 (Ophthotech Corp. Princeton, NJ) is a PEGylated, stabilized aptamer targeting complement factor C5. ARC1905 inhibits activation of the downstream proinflammatory complement cascade (including generation of C5a and the membrane attack complex). "Ophthotech's anti-C5 aptamer, ARC1905, is a potent and selective inhibitor of factor C5 of the complement cascade. Inhibition of the complement cascade at the level of C5 prevents the formation of the key terminal fragments responsible for tissue pathology, C5a and the membrane attack complex (MAC; C5b-9). The C5a fragment is pro-inflammatory, while the membrane attack complex initiates cell lysis and releases proangiogenic molecules (eg. PDGF and VEGF). Histopathologic specimens of human dry AMD lesions strongly stain for C5 and MAC at the key sites of pathology. ARC1905 spares the formation of upstream complement components such as C3b, which are important in host defense mechanisms. By inhibiting C5-mediated inflammatory and MAC activities, therapeutic benefit may be achieved in both dry and wet AMD while sparing the immunoprotective functions of the complement system. A phase I open-label, multicenter study of ARC1905 in patients with dry AMD will be initiated in Q2 2009."

11.4 Eculizumab: Inhibits C5 - Dry AMD

Intravitreal

Eculizumab (Soliris, Alexion Pharmaceuticals) is a humanized monoclonal antibody derived from a murine anti-human C5 antibody. Eculizumab specifically inhibits the terminal complement protein C5, thereby preventing its cleavage to C5a and C5b during complement activation. The strategic blockade of the complement cascade at C5 prevents the release of the downstream anaphylatoxin C5a and prevents the formation of the lytic membrane attack complex (MAC). Eculizumab is FDA-approved for the intravenous treatment of another complement-mediated disease known as paroxysmal nocturnal hemoglobinuria. Currently a phase II study with eculizumab for the treatment of patients with dry AMD, known as the Complement Inhibition with Eculizumab for the Treatment of Non-Exudative Age-related Macular Degeneration (COMPLETE) Study. Patients with GA or high-risk drusen are being randomized 2:1 to receive intravenous infusions of eculizumab or placebo.

12. Radiation

12.1 Strontium-90 beta radiation -Wet AMD

CABERNET is a multicenter, randomized, controlled phase III study that enrolls 450 subjects at 45 clinical centers worldwide. In this research, patients receive either the standard injection of Lucentis® (ranibizumab) or the radiation plus Lucentis®.

A tiny source of radiation is placed inside the eye near the macula, held there for about 4 minutes and then removed. The radiation destroys the abnormal blood vessels and prevents the growth of blood vessels to stop the progression of wet macular degeneration vision loss. The system treats neovascularization of retinal tissue by means of a focal, directional delivery of radiation to the target tissues in the retina. Using standard vitreoretinal surgical techniques, the sealed radiation source is placed temporarily over the retinal lesion by means of a handheld medical device. If epiretinal brachytherapy proves successful, it can reduce the number of injections needed to just two injections over a period of 12 months.

12.2 TheraSightTM ocular brachytherapy System: Wet AMD

A trial sponsored by Theragenics Corporation will investigate the safety and ability of using the TheraSightTM Ocular Brachytherapy System to treat wet AMD. A radioactive button mounted on an applicator wand is positioned behind the eye and held in place touching the outer surface of the eye for 5 to 20 minutes. A study will take place at 6 clinical sites and will compare 3 different dosages or amounts of radiation, so all participants will
receive treatment. Enrollment is still underway for this clinical trial.

### 13. Immunomodulator

#### 13.1 Glatiramer Acetate: T helper 2 inducer - Dry AMD Subcutaneous

Another proposed new treatment of dry AMD is a subcutaneous injection of glatiramer acetate (Copaxone, Teva Pharmaceutical Industries). Glatiramer acetate has been shown to reduce cognitive decline, eliminate plaque formation, and induce neuron survival and neurogenesis in a mouse model for Alzheimer’s disease (AD).

Drusen formation in age-related macular degeneration (AMD) shares some similarities with Alzheimer’s disease (AD), which is associated with amyloid deposits. Aggregated beta-amyloid induces microglia to become cytotoxic and block neurogenesis. This medication, increases the proportion of T helper 2 lymphocytes (these T cells are anti-inflammatory in nature). It seems that these glatiramer-acetate-specific T helper 2 cells would produce cytokines such as interleukin (IL)-4 and reduce amyloid-induced retinal microglial cytotoxicity in AMD.

Copaxone is administered as a subcutaneous injection. Two double blind, randomized clinical trials at the New York Eye & Ear Infirmary and the Kaplan Medical Center, Rehovot, Israel, have been initiated in 2006 and 2007 respectively, and are enrolling up to 60 patients combined. The primary outcome tested in these trials is the reduction in the total area of drusen. Results have not been published yet.

### 14. Prevent injury (anti-oxidants)

#### 14.1 OT-551 - Dry AMD Topical

OT-551 is administered topically, developed by Othera Pharmaceuticals, Inc. (Exton, PA). This eyedrop contains a small molecule that downregulates the overexpression of the protein complex nuclear factor (NF)-B. NF-B is a transcription factor that is highly activated when oxidative stress, inflammation, and angiogenesis occurs. In preclinical models, OT-551 has demonstrated anti-oxidative, anti-inflammatory and anti-angiogenic activity. These results support the development in diseases such as AMD and cataract. OT-551 is the first eye drop to be tested in a clinical trial as a treatment for dry AMD. There are 2 phase II, 2-year trials ongoing for patients with GA secondary to AMD.

#### 14.2 Fenretinide (Compound ST-602) - Dry AMD Oral

Fenretinide, or (N-[4-hydroxyphenylretinamide), is an oral compound that decreases serum retinol by binding to retinol-binding protein, and promotes renal clearance of retinol. This in turn decreases the bioavailability of retinol for the retinal pigment epithelium (RPE) and photoreceptors. A2E (N-retinylidene-N-retinylethanolamine), a retinoid byproduct, is a major fluorophore in lipofuscin and a significant source of RPE cytotoxicity. It is hypothesized that by reducing toxic retinoid byproducts of visual cycling, there will be a slowing of GA progression.

Sirion Therapeutics, Inc. (Tampa, FL), is sponsoring a phase II trial to assess the benefit of fenretinide in the treatment of GA. The study group is ongoing. Patients have been randomized to 1 of 2 doses (100 mg or 300 mg) or placebo, and they are being followed for 2 years.

### 15. RPE transplantation

Another potential treatment for AMD is replacing damaged and unhealthy RPE by healthy tissue. In a study series of ten patients (four had AMD), human neural retinal progenitor cell layers and RPE were transplanted. All four patients with AMD had vision of 20/200 or worse and experienced improved visual acuity, none improved to better than 20/200. There was no graft rejection during a follow-up time of up to six years.

### 16. sFlt-1

Soluble (s)Flt-1 is a naturally occurring protein antagonist of VEGF formed by alternative splicing of the pre-mRNA for the full length VEGFR-1. The angiostatic activity of sFlt-1 results from inhibition of VEGF. It is not clear if sFlt-1 has a role in normal eyes, but several studies show the evidence of the effect of overexpression of sFlt-1 in ocular neovascularization models.
16.1 Recombinant sFlt-1 chimeric proteins - Wet AMD Intravitreal

Inhibition of VEGF by intravitreal injections of recombinant sFlt-1 chimeric proteins and antisense oligodeoxynucleotides have been shown to prevent retinal neovascularization in mouse models(74).

16.2 AdsFlt-1 - Wet AMD Intravitreal

Intraocular injection of AdsFlt-1 suppressed retinal and choroidal neovascularization(72-73,75). Periocular injection of AdsFlt-1 resulted in transduction of episcleral cells, penetration of the sclera and high levels of AdsFlt-1 in the choroid, which markedly suppressed CNV(70). Long-term suppression of CNV was achieved with intraocular injection of AAVsFlt-1 in mice and monkeys(75).

17. Others

17.1 Ciliary neurotrophic factor - Dry AMD Implant

Ciliary neurotrophic factor (CNTF) is being investigated as a treatment for dry AMD because it has a potent neuroprotective action; it has been shown to inhibit photoreceptor apoptosis in an animal model of retinal degeneration. CNTF has been shown to slow photoreceptor degeneration in animal models of retinal degenerations and thus may be effective in protecting photoreceptors in AMD(76). A phase II trial utilizes an encapsulated cell technology (ECT) to deliver CNTF to the retina. The implant is a small capsule that contains human retinal pigment epithelium cells. These cells have been given the ability to make CNTF and release it through the capsule membrane into the surrounding fluid. In this study, two different CNTF dose levels will be used: a high dose and a low dose, as well as a sham surgery (or placebo) group(77). The cells can survive for approximately 18 months following implantation into the vitreous cavity with a single scleral suture(78). The trial, sponsored by Neurotech Pharmaceuticals USA (Lincoln, RI), is ongoing and not recruiting.

17.2 Rheopheresis - Dry AMD

Rheopheresis is still an unproven therapy for dry macular degeneration. Rheopheresis is a form of therapeutic plasmapheresis designed to remove species circulating in the blood that are larger than 25 nm (about 500 kilodaltons) using a doublestaged membrane filtration system. The intended targets include immune complexes, immunoglobulin M, beta 2-macroglobulin, fibrinogen, von Willebrand factor, low density lipoprotein cholesterol, and others(79).

“This procedure has been proposed as a possible treatment to prevent the progression of dry AMD by improving the retinal and choroidal microcirculation. The largest study performed to assess the effectiveness of rheopheresis in dry AMD is the Multicenter Investigation of Rheopheresis for Age-related macular degeneration (MIRA-1) trial. Study patients had at least 10 soft drusen within 2 disc diameters from the foveal center and/or GA. Interpretation of the results from the MIRA-1 trial has been controversial. The sole outcome measure was LogMAR VA. At 1 year, the treated group had a LogMAR VA of 0.02 ±0.213, and the placebo patients had a VA of 0.02 ±0.20 (P=.977). This may have implied that the treatment was not effective in improving VA. However, a post hoc analysis showed that a large proportion of the subjects (37% of treated and 29% of placebo) were mistakenly included in the trial and that a number of the subjects did not receive the required number of rheopheresis treatments. When reanalyzed, the treatment arm of this “modified per protocol” group of subjects did have a statistically significant improvement in visual acuity (treated improved 0.08 ±0.166, placebo decreased 0.01 ±0.164, P=.001). Furthermore, a larger proportion of treated subjects experienced an adverse event requiring intervention (24.0%) compared to those receiving placebo (5.8%)(80). The Occulogix (Waltham, MA) phase II study was suspended.”(82-83)

17.3 Improvements in ophthalmic drug delivery

“Ophthalmic drugs have traditionally been administered topically, which in general provides therapeutic levels to the anterior chamber of the eye but not to the posterior segment. Therefore, topical administration of drugs has been largely infeasible for posterior segment diseases such as AMD and diabetic macular oedema. In contrast, intravitreous injection provides direct delivery to the posterior segment and allows therapeutic levels to be attained. However, this route of administration can require repeated injections for chronic disorders and is associated with a small risk of complications. Several alternative strategies for drug delivery have therefore been developed, such as implantable devices that deliver small,
highly potent, lipophilic therapeutics intraocularly. The small size of these implants precludes long-term (>30 days) delivery of large, water-soluble compounds, but they have been used to deliver corticosteroids. In addition, these implantable devices generally cannot be used to deliver proteins, antibodies and other high-molecular-mass biotherapeutics. Administration of compounds to the eye by approaches that do not involve injection through the sclera also remain attractive alternatives to intravitreal injection. High-molecular-mass compounds such as immunoglobulins, and oligonucleotides as large as 24 nucleotides have been found to be capable of diffusing through the sclera when deposited on or within the sclera.”(84)

Nanotechnology and Iontophoresis represent promising drug delivery systems.

“Iontophoresis is a technique that consists of the administration of drugs to the body through tissues using an electric field involving a small potential difference. The active electrode, which is in contact with the drug, is placed at the site to be treated, and a second electrode, with the purpose to close the electric circuit, is placed at another site of the body. The electric field facilitates the transport of the drug that should be mainly ionized.”(85)

17.4 Wet AMD pipeline: most promising candidates

adPEDF.11: Gene therapy, intravitreal injection, phase I
AGN211745: siRNA, intravitreal injection, phase II
Zybrestat: vascular disrupting agent, topical, animal studies
Sirolimus: Multi-mechanism, Subconjunctival or intravitreal injection or oral, phase II
ATG003: nAChR antagonist, topical , phase II
Avastin™ and Lucentis™: anti-VEGF, intravitreal injection, available
Macugen™: anti-VEGF, intravitreal injection, available
VEGF Trap: VEGF receptor decoy, intravitreal injection, phase III
Vatalanib: Tyrosine kinase inhibitor, oral, phase II
Pazopanib: Tyrosine kinase inhibitor, , topical , phase II
TG101095 / TG100801: Tyrosine kinase inhibitor, topical, phase II
AL-39324: Tyrosine kinase inhibitor, intravitreal injection, animal studies
AG013958: Tyrosine kinase inhibitor, subtenon injection, animal studies
JSM6427: integrin antagonist, intravitreal injection, phase I
PF-04523655 (REDD14NP) Wet AMD; phase I (Quark/Pfizer)

17.5 Dry AMD pipeline: most promising candidates

Ciliary Neurotrophic Factor: Neuroprotective implant, phase II
Fenretinide: Decreases serum retinol, oral, phase II
OT-551: Anti-oxidant, anti inflammatory, topical, phase II
POT-4: Complement C3 inhibitor, phase II
Glatiramer Acetate: Immunomodulator, subcutaneous, phase II
RPE transplantation: animal studies and phase I
Rheopheresis: Plasmapheresis, phase III
Gene transfer: Intravitreous and subtenon, phase I

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References:

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OPTICAL COHERENCE TOMOGRAPHY IN AGE-RELATED MACULAR DEGENERATION

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1. Introduction

Optical coherence tomography (OCT) acquires cross-sectional images with semihistologic resolution. The technology precisely defines the location and nature of the changes in the retina and adjacent structures and objectively evaluates the thickness of the retina and surrounding structures. These capabilities allow detection of newly emerging fluid and/or intraretinal or subretinal tissue and tissue below the retinal pigment epithelium (RPE). OCT provides important information on serous retinal detachments, hemorrhages, and subretinal neovascular membranes that are components of exudative macular degeneration and more precise analysis of anatomic structures and subtypes of neovascular membrane lesions. These capabilities facilitate an understanding of the differences among the classic membranes, hidden membranes, retinal angiomatous proliferation (RAP), and disciform scars in the natural course of the disease and assess the treatment response. The images are presented in a scale of colors (or gray), such that tissues that reflect more light or disperse more light are shown in red and white, respectively, while the ones that reflect or disperse the least light are shown in blue and black. Tissues that moderately reflect light are shown as green or yellow. The color represents the optical properties of the tissues and not the tissues themselves. Therefore, the image is not real but represents the true dimensions of the measured structures. 

The vitreous transmits light without reflecting it and is depicted in black in OCT images (Figure 1). The posterior hyaloid usually is indistinguishable from the retinal surface except when the posterior vitreous detaches and appears as a weakly reflective band. The choriocapillaris and choroid are highly reflective layers, because they are vascular and limit light penetration into the deeper layers. Blood vessels are identified by their high reflectivity and the screening effect generated on adjacent tissues (Table 1).
Figure 1. A normal eye.

Table 1. Reflectivity Patterns of the Retinal Structures in Normal Eyes

<table>
<thead>
<tr>
<th>Reflectivity Pattern</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperreflectivity pattern</td>
<td>Nerve fiber layer, thicker in the nasal portion (red), RPE and choriocapillaris (red)</td>
</tr>
<tr>
<td>Normalreflectivity pattern</td>
<td>Inner and outer plexiform layer (yellow-green), inner and outer nuclear layers (blue)</td>
</tr>
<tr>
<td>Hyporeflectivity pattern</td>
<td>Photoreceptor layer</td>
</tr>
</tbody>
</table>
2. Age-Related Maculopathy

Age-related maculopathy, considered the precursor of age-related macular degeneration (AMD), is defined as the presence of areas of hyperpigmentation or hypopigmentation of the RPE and/or confluent or soft drusen. When soft drusen in the macular region are associated with focal areas of mobilization of pigment (hypopigmentation and hyperpigmentation), there is an increased risk of progression to AMD. Drusen, which are degenerative nodular formations located mainly in Bruch’s membrane, are comprised of proteins, lipids, mucopolysaccharides, and other components that appear in adulthood and tend to increase in size and number over time.

Drusen is the AMD characteristic that is detected early in the clinical examinations. On OCT, drusen appear as RPE deformation or thickening that may form irregularities and undulations (Figure 2).

Drusen are classified histologically as hard formations and defined as small hyaline deposits with delimited margins that are considered age-related low-risk changes, and soft drusen, which are deposits of granular or amorphous material considered to be precursors of AMD. Drusen can progress to an atrophic form (dry) or exudative form (wet) of AMD. The risk is based on the lesion number, size, and confluence.4,6,8,1-15

Figure 2. Drusen are seen as undulations and elevations in the hyperreflective band of the RPE with less reflective material beneath them, while the inner retinal layers remain generally intact.
3. Atrophic Macular Degeneration

In the macular region, progressive atrophy of the RPE, the outer retinal layers, the choriocapillaris, and dense clusters of drusen can be seen. OCT can detect decreases in retinal thickness and increases in the reflectivity of the RPE detachment, which results from the decreased ability of the atrophic retinal tissue to attenuate light. The reduced retinal thickness and volume can be determined by the retinal maps that display the areas of greatest atrophy, identify the extent of the atrophy, and monitor progression. Geographic atrophy (GA) represents the final stage of dry AMD.6,8,10,16,17

Figure 3. In a case of retinal atrophy, the OCT shows a highly reflective choroidal signal due to retinal thinning and RPE hypopigmentation, which allows greater beam penetration into the choroid and greater reflectivity. The retinal map allows quantification of the decreased retinal thickness.
4. Exudative Macular Degeneration

OCT, a fundamental tool in the diagnosis and management of patients with choroidal neovascularization (CNV), allows identification of active neovascular membranes and determination of the extent of the membranes in many cases. The technology is useful for assessing subfoveal involvement and also helps diagnose hidden choroidal neovascular membranes, for which fluorescein angiography (FA) shows confusing patterns. OCT also can monitor treatment responses by providing information about the need for retreatment. 3,8,15,18-23

Well-defined classic CNV appears on OCT as hyper-reflective areas in contact with or in front of the RPE; the pathology may be dome-shaped or appear as a thin formation (fusiform or nodular) (Figure 4). Retinal edema is always present to some extent in front of the active membrane; if the retina is thinner than normal, new vessels may be latent. CNV is less evident a few weeks after onset, and only interruption, breakdown, and pronounced thickening of the RPE can be seen.

![Figure 4](image)

*Figure 4.* OCT visualizes the components of the neovascular membrane, a retinal pigment epithelial detachment (PED), detachment of the neuroepithelium (NED), intraretinal fluid, and subretinal hemorrhage.
5. RPE Detachment

a. Hemorrhagic Detachment

An RPE detachment can hemorrhagic in the presence of neovascular membranes. Retinal hemorrhages often are seen under the RPE. Detachment of the RPE forms a steep angle to the choriocapillaris, and the accumulated blood cells block penetration of the light rays from OCT in the area of the detachment, so the penetration is minimal and forms a shadow that hides the choriocapillaris and other subsequent layers (Figure 5).

b. Serous Detachment

During development of exudative AMD, OCT shows serous detachments of the RPE as optically clear areas between the RPE and the choriocapillaris (Figure 6). The detached pigment epithelium forms a steep angle with the underlying choriocapillaris, which may be initially clear and then contains cells and becomes blurred. A detachment also can be single, multiple, dome-shaped, or bilobed.6,8,24

c. Neurosensory Retinal Detachment

Active choroidal neovascular membranes cause edema and small serous detachments of the neurosensory retina. The membranes also can occur frequently in cases of pigment epithelial tears (Figure 7).

Figure 5. Hemorrhagic detachment of the RPE.
Figure 6. Serous Detachment of the RPE.

Figure 7. Neurosensory retinal detachment.
6. Intraretinal Fluid

Intraretinal fluid either can occur diffusely and cause increased retinal thickness and reduced retinal reflectivity or be localized or non-reflective cysts (cystic edema) (Figure 8).

Figure 8. Intraretinal cysts.
RPE tears are serious complications of AMD and appear during the evolution of a serous or serohemorrhagic detachment of the epithelium. RPE tears also can be secondary to photodynamic therapy (PDT) and laser photocoagulation. Patients with occult CNV membranes and pigmented epithelium detachments are at high risk of developing pigmented epithelium tears.6,8

Figure 9. RPE tears, sudden disruptions in the continuity of the hyperreflectivity layer of the pigment epithelium, show strong reflectivity from the choroid in the absence of the pigment epithelium, followed by sudden interruption of the band’s pigment epithelial hyperreflectivity.
Polypoidal choroidopathy is considered a variant of neovascular membranes with serous or hemorrhagic detachment of the retina and RPE in the posterior pole. The pathology is seen on FA and especially by videoangiography with green indocyanine green (ICG). Both tests show dilated vessels in the choroid layer with the characteristic presence of polypoidal structures which usually occur at the termini of vessels at the edge of the vascular network. (Figure 10).\textsuperscript{5,24-26}

\textbf{Figure 10.} OCT shows small cup-shaped RPE elevations and choroidal angiomatous lesions. Hemorrhagic and serous detachments of the retina and the RPE also can be seen.
RAP is a particular type of intraretinal neovascularization consisting of vascular anastomosis of aberrant retinal vessels. In choroidal neovascular membranes, new vessels proliferate through the RPE and infiltrate the retina and eventually communicate with the retinal circulation to produce retinochoroidal anastomosis. In RAP, the neovascularization originates in the same retinal vessels. The diagnosis is based on the presence of hyperfluorescence corresponding to the neovascularization (hot spot) in ICGA images, and the presence of dilated retinal vessels associated with multiple intraretinal, preretinal, or subretinal hemorrhages, with varying degrees of intraretinal edema in the fundus examination. A proposed three-stage classification is seen in Table 2.

<table>
<thead>
<tr>
<th>Table 2. RAP Stages</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
<td>Proliferation of intraretinal capillaries originating from the deep capillary network in the macular area</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Presence of retinal-retinal anastomosis and intraretinal edema and a possible association with a PED and/or subretinal neovascularization</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Retinal-choroidal anastomosis and emergence of new choroidal vessels</td>
</tr>
</tbody>
</table>
Although the initial stages are only visible by fundus examination and angiographic examinations due to the small size of the initial injury, OCT is extremely useful for identifying RAP and its associated manifestations.6-8,19,27-29

The initial signs that correspond to stage 1 (intraretinal vascularization) consist of a focal area, usually extrafoveal, with increased retinal reflectivity that are not associated with epiretinal, intraretinal, or subretinal changes or changes in the retinal thickness. Progression of RAP on OCT is characterized by the presence of intraretinal or subretinal fluid, the former characterized by well-defined confluent hypo-reflective spaces and the latter (neurosensory detachment) characterized by a well-defined hypo-reflective space between the neurosensory foveal retina and other highly reflective bands corresponding to the RPE. When RAP reaches the subretinal space and merges with the RPE, a serous detachment of the RPE usually develops (stage 2 or CNV). In well-developed cases, there may be retinal choroidal anastomoses (stage 3 or CNV) and it is impossible to clearly differentiate stage 2 from stage 3 on OCT.

Figure 11. A and B, OCT shows RAP associated with edema, intraretinal cysts, and subretinal fluid. C, OCT shows the RPE and the retinal neurosensory layer detachments.
10. Disciform scars

Disciform scars form the stage of exudative AMD in which the retinal tissue is replaced by scar tissue (usually vascular). Disciform scars develop with regression of subretinal hemorrhages and retinal edema and hyperplastic elements of the RPE. Disciform scars are usually dry and show marked destruction of all retinal layers. It is less common to find diffuse exudation with elevation of the entire sensory retina at the posterior pole (Figure 12).

Figure 12. OCT shows a hyperreflective fibrotic nodular area that corresponds to the fibrotic area and easily detects diffuse or cystoid edema. In the wet form, OCT shows diffuse or cystoid edema involving the RPE detachment.
11. Time-Domain vs. Spectral-Domain OCT in AMD

The new spectral-domain (SD) OCT devices include a spectrometer in the receiver that analyzes the spectrum of reflected light on the retina and transforms it into information about the depth of the structures according to the Fourier principle. This technology eliminates the need to mechanically move the reference arm with the consequent increase in the speed with which images are received and axial resolution in time-domain (TD) OCT. In addition to greater speed in capturing images and higher definition, the algorithms used by SD-OCT differ from those of TD-OCT, and the retinal thickness measurements are not comparable between the two. While TD-OCT determines the total retinal thickness by measuring the distance from the internal limiting membrane to the highest hyperreflective band, i.e., that combining the inner and outer segments of the photoreceptors, SD-OCT set this threshold in the RPE hyperreflective band, so the retinal thickness values are higher than those obtained by TD-OCT (Figure 13).

These differences justify the results of studies that compared the two devices in patients with wet AMD. Thus, Mylonas and colleagues found that in a number of patients with wet AMD, the retinal thickness measurements obtained by three SD-OCT devices were higher than those obtained by TD-OCT instruments. The authors also emphasized the importance of segmentation analysis as the main source of errors with both devices. The effect of this parameter had already been studied widely for Stratus OCT (Carl Zeiss Meditec Inc., Dublin, CA), and the proposed solution to avoid these errors was the manual correction of segmentation lines in each image, especially in clinical trials of neovascular AMD, in which the difficulty establishing the limits in cases with subretinal and intraretinal fluid was automatically higher. To assess the incidence of errors at this level in the SD-OCT apparatus, Krebs and colleagues assessed 104 patients with neovascular AMD with both TD-OCT and SD-OCT and analyzed the position of the lines drawn automatically by segmentation analysis in each case. The results showed differences between the devices, with TD-OCT committing errors in 69.2% of cases and SD-OCT in 25%. These data suggested that SD-OCT makes fewer errors in automatic segmentation analysis, and these can be corrected manually identical to TD-OCT and therefore constitute a marked improvement in the main source of erroneous measurements. Finally, some studies have compared both devices in a series of patients treated with antiangiogenic agents. Sayanagi and colleagues compared tomographic findings with TD-OCT and SD-OCT in 58 patients with wet AMD treated with ranibizumab (Lucentis, Novartis) and concluded that SD-OCT is better than the TD linear mode B and mode 3D cube for detecting intraretinal cysts and intraretinal and subretinal fluid or fluid under the RPE, making it a more effective tool for managing these patients.

12. Correlation with Other Techniques in AMD

OCT retinal studies in patients with AMD can complement information obtained with other conventional examinations, such as angiography, or other more modern techniques such as autofluorescence. We discuss combining these techniques in recent studies.

OCT and Angiography

Because of the widespread use of OCT, the use of FA has dropped to second place in routine consultations, but it is
still an essential examination for studying CNV in patients with AMD, and FA and ICGA are especially important for identifying occult disease. When comparing both techniques, because OCT is gaining definition with new versions and devices, the non-invasive nature of FA and ICGA takes precedence over conventional angiographic techniques, to the extent that some studies have reported similar results for both examinations. Krebs and colleagues and Saddu and colleagues proposed that OCT can detect signs of activity similar to FA in a study in which two independent observers analyzed the agreement. The authors reported that even OCT was slightly more sensitive than FA for determining the activity of the membranes, while other authors stated that it was necessary to manually adjust the segmentation analysis of images to achieve similar results.22,38 More recently, Malamos and colleagues used high-definition OCT to analyze patients diagnosed by FA with different types of AMD (early, neovascular classic, minimally classic, and occult). They reported a significant correlation between different angiographic patterns and various parameters with OCT. The authors could discriminate different types of damage using OCT to the extent that they could identify their precise anatomic retinal maps.39 In cases with hidden membranes and some cases of RAP, the relevance of FA is undoubtedly even greater because of the difficulty of localizing the pathology in the tomography images (Figure 14). However, some authors have described changes associated with OCT membranes, such as exudation signs and the almost constant presence of RPE detachments, and changes within the polymorphic features of these lesions.3 Until the development of new techniques to visualize the choroid with OCT such as that proposed by Spaide and colleagues, the most informative method to explore these cases is FA.6-42 However, OCT seems to override FA in the identification of signs of activity in patients previously treated with PDT, in which interobserver agreement is much higher for OCT than for FA.43-46 Finally, the sequential use of both techniques for screening of wet AMD in patients with impaired visual acuity (VA) has been proposed. The screening was first performed with OCT and then based on the results, fluorescein and indocyanine FA, which obtained adequate sensitivity and specificity for the early detection of AMD in these patients.45

13. OCT and Autofluorescence

Autofluorescence allows gathering of information about the metabolic status of the retina. This technique based on the ability of tissues to emit more or less fluorescence depending on the amount of accumulated lipofuscin to be excited by light at specific wavelengths and provides the opportunity to evaluate the retinal functional status. Since OCT obtains a detailed picture of the retinal anatomy, several studies have evaluated the structure/function in patients with AMD and correlated the two techniques. Bearelly and colleagues reported that there was a significant association between OCT and autofluorescence findings and that marked hyperfluorescence at the edges of the plaques of GA correlated with hyper-reflective changes in the outer retinal layers identified by OCT that did not occur in healthy retinas with normal autofluorescence and OCT (Figure 15). These findings used a combination of both techniques to determine the patterns of progression of GA plaques in patients with AMD, which had been targeted previously by other authors as discussed below.37 Autofluorescence currently is the most frequently used test to assess disease progression in dry AMD; however, OCT provides additional information, so its role has not been ruled out in the study of these patients to the extent that some authors have proposed methods based on OCT to evaluate progression of atrophic plaques, as described in the following section.17
Figure 14. The retinography corresponds to a polypoidal choroidopathy (A) where you can observe the presence of intraretinal haemorrhages. In the red-free image (B) you can observe the tomographic cut line. The OCT shows a marked elevation of the cup-shaped RPE (C). By indocyanine green angiography you can observe a hot spot that confirms the diagnosis (DE).
Figure 15. (A) The retinography shows a juxtafoveal atrophic plaque. (B) The autofluorescence image defines the atrophic plaque as an area of complete absence of fluorescence. (C) The OCT shows an increased choroidal reflectance due to the window effect produced by the overlying atrophic plaque. (D) Infrared image captured by the OCT that shows the tomographic section of figure C.
14. Clinical and Therapeutic Implications of OCT in AMD

OCT is now a routine examination for patients with AMD. Although some studies have advocated a role for OCT in the dry forms of the disease, its use is greater in patients with the neovascular forms, and it is now a main method of study in these patients. From the first studies of PDT, the different patterns of response to treatment were assessed with OCT, and the development of antiangiogenic therapies made OCT a fundamental tool in routine patient management. In recent years, OCT has become increasingly important, to the extent that recent studies have based the need for retreatment on the findings in OCT images.

15. Dry AMD

The better definition achieved with the OCT devices that include SD technology allows more detailed study of the outer retinal layers and RPE, which are important structures in the development of dry AMD. Thus, new SD-OCT instruments can accurately distinguish the presence and size of drusen and RPE changes, making it possible to differentiate the different retinal layers to identify changes at that level.48 Thus, in a group of patients with dry AMD, Schuman and colleagues49 found localized thinning of the photoreceptor layer immediately above the drusen compared to healthy controls, suggesting a degenerative process with cell loss to explain the decreased visual function in this group (Figure 16).

Bearelly and colleagues17 studied the thinning at the edges of the plaques of GA to establish a gradient in the thickness of the photoreceptors layer from the healthy retina to the atrophic plaque. Although they had a small sample size in their study (n = 17), they concluded that SD-OCT allows quantitative measurement of disease progression and postponed for future study the application of the technique. Moreover, to study progression of the dry forms, other studies have been designed to correlate the findings with the SD-OCT scans with other techniques such as the autofluorescence mentioned previously. To this end, Stopa and colleagues47 analyzed a series of patients with dry AMD and correlated SD-OCT images of areas of GA, and isolated hard drusen and soft coalescing drusen with retinographies and images with autofluorescence. Thus, in addition to finding different patterns of reflectivity for each type of drusen, they observed that certain patterns of hyperreflectivity in some drusen and the overlying retina corresponded to increased or decreased autofluorescence at these points. This established a certain morphology-function relationship with both scans (Figure 17).

Figure 16. (A) Central soft drusen may be seen in the retinography. (B) Drusen are observed as undulations and elevations of RPE hyperreflective band with less reflective material below them.
Retinal study using OCT is now a fundamental tool to manage patients with wet AMD. Since the introduction of PDT as an antiangiogenic agent a decade ago, OCT has provided information on the activity of neovascular membranes and determines the need to establish treatment, treatment response, and early signs of recurrence or resolution. The importance of this was that several multicenter studies were designed to evaluate treatment protocols of antiangiogenic therapies that rely on OCT as the main criteria for retreatment. This opened the door to development of treatment protocols determined by OCT such as those raised in the following studies to optimize treatment regimens in these patients.

PrONTO Study (Prospective Optical Coherence Tomography Imaging of Patients with Neovascular Age-Related Macular Degeneration Treated with intraOcular Ranibizumab): The injection protocols established in multicenter phase III studies of ranibizumab for neovascular AMD, the ANCHOR and MARINA trials, consisted of monthly intravitreal injections for 2 years for all study eyes that resulted in substantial gains in VA in various intermediate controls and at the final examination. However, the dose of 24 injections carried a human and economic burden that made it difficult and impractical to administer. In phase I and II studies of ranibizumab before these studies, an extension study was conducted in which administration...
of new additional doses were left to the discretion of the investigator based on the presence of diffusion on FA or intraretinal or subretinal fluid on OCT. This subanalysis showed that often the presence of retinal fluid could be detected much earlier by OCT than FA, leaving the door open for a greater role of OCT in treatment.

Considering this, Fung and colleagues at the Bascom Palmer Eye Institute in Miami, Florida, proposed the PrONTO Study. In that study, after three monthly loading doses, monthly retreatments of ranibizumab were administered for 2 years according to the criteria in Table 3. Three criteria are based on OCT analysis (decreases exceeding five letters of VA [ETDRS] with fluid in the macula detected by OCT, increases exceeding 100 microns in central retinal thickness compared to previous lowest value measured by OCT or the recurrence of fluid on OCT in a previously dry eye) (Figure 18). The VA results of the study were similar to those of previous phase III trials but required significantly fewer injections (an average of 5.6 vs. 12 injections during the first year of the study and 9.9 vs. 24 injections to complete the second year).

The good results obtained with the varying numbers of injections based on OCT led to the inclusion of OCT as a primary criteria in new multicenter studies such as the SAILOR or SUSTAIN studies. Those studies included a larger number of patients than in the original early study (n = 40) and confirmed the role of OCT in patient monitoring.

Figure 18. In the PrONTO clinical trial, three criteria of retreatment are based in the analysis by OCT: Decrease of more than 5 letters of VA (ETDRS) with fluid detected in the macula by OCT (A), increase of more than 100 µm in the central retinal thickness respect to the lowest previous value measured by OCT (B1, B2) or fluid reappearance in the OCT in a previous dry lesion (C1, C2)
Figure 19. In the second year of the study, the presence of qualitative changes in the OCT images that suggested fluid recurrence was included, such as intraretinal cysts, subretinal fluid, or progression of a PED.59

Table 3. PrONTO Study (1st year). Criteria for Reinjection after Three Loading Doses

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Details</th>
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<tbody>
<tr>
<td>1. Decrease of 5 letters of VA (ETDRS) and fluid on OCT</td>
<td></td>
</tr>
<tr>
<td>2. Increase of 100 microns in central retinal thickness on OCT</td>
<td></td>
</tr>
<tr>
<td>3. Macular hemorrhage</td>
<td></td>
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<tr>
<td>4. Classic CNV in emerging FA</td>
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<tr>
<td>5. Persistent fluid on OCT 1 month after the last injection</td>
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</table>

*Corrections for the second year of study: Qualitative changes on OCT images suggestive of recurrent macular fluid, including: intraretinal cysts, subretinal fluid, PEDs.*
OCT and Reviews: “Treat & Extend”

In patients with wet AMD treated with antiangiogenic drugs, the frequency of the injection was not the only parameter assessed. The high number of regular examinations also is a substantial burden for patients, families, and the health system. Several studies (PIER, EXCITE) proposed revisions to the fixed protocols after 1 month, but the results were worse than those obtained with monthly revisions, with both fixed reinjection protocols and those based on OCT.6,57 Therefore, it is necessary to establish specific criteria to control AMD and allow more time between retreatments without losing the treatment effectiveness. Once again, OCT had a fundamental role in this approach. In this sense, Spaide proposed a revision scheme called Treat and Extend; after three monthly loading injections, patient were reevaluated in 6 weeks, at which time the physicians determine whether there are signs of activity by funduscoppy and edema or intraretinal or subretinal fluid by OCT (Figure 20).58

The results depend not only on treatment but also the frequency of subsequent examinations by the protocol presented in this proposal, which takes a leading role in OCT. The use of this treatment regimen is being evaluated in a study conducted at Wills Eye Institute, Philadelphia, Pennsylvania. The preliminary results are promising, with a lower frequency of examinations, i.e., an average of 7.4 injections annually and similar VA outcomes to those obtained in the PRONTO study in a greater number of patients (n=92).59

Prognostic Factors in OCT

Several OCT parameters are associated with decreased VA and different responses to other antiangiogenic treatments regarding VA, central thickness findings, and prognosis after treatment. Singh and colleagues retrospectively analyzed a group of patients with wet AMD treated with bevacizumab (Avastin, Genentech, south San Francisco, CA) and concluded that in addition to VA pretreatment the total retinal thickness measured by OCT affected the final VA and the total decrease in the post-treatment retinal thickness, which was lower in patients who had received

Figure 20. Treatment schedule and revisions of Treat & Extend.
It appears that parallel to the development of new devices, structures assume special importance, such as the junction of the inner and outer segments of the photoreceptors, which until now was only identified, as shown by studies with prototypes with high-definition axial resolution of 3.5 microns as we will discuss later in this chapter. Lee and colleagues reported that the presence of posterior vitreomacular adhesion on OCT was associated with CNV in a large series of patients with AMD (n = 251). Those authors suggested that this finding is a possible risk factor for subretinal membrane development because of chronic vitreomacular traction on the retina, opening the door to a possible surgical approach in patients not responding or resistant to drug treatment (Figure 21). Keane and colleagues also found that the main factor associated with decreased VA was the volume of subretinal tissue, and in fewer cases, thickening of the neurosensory retina, without a significant association with the total volume of subretinal fluid and RPE detachment and VA. However, these parameters did not justify the variability found in the VA for similar values of subretinal tissue volume or thickening of the neurosensory retina. The authors pointed to the complex pathophysiology of the neovascular membranes and the limitations of the TD-OCT used to explain the results. Sayanagi and colleagues reported that SD-OCT is a superior generation of TD-OCT than its predecessors for assessing the activity of the neovascular membranes and changes in AMD after ranibizumab treatment, and Kiss and colleagues pointed to the RPE status of neovascular membranes as the main predictor of VA in patients treated with ranibizumab in addition to the conventional parameters such as central retinal thickness. Several studies also reported a significant correlation between a hyperreflective band indicating the integrity of the junction between the inner and outer segments of the photoreceptors and higher VA in patients treated with ranibizumab.

Figure 21. The chronic retinal vitreomacular traction has been considered as a possible risk factor for development of subretinal membranes. In the image, a subretinal membrane associated with a vitreomacular traction in the presence of an RPE detachment and subretinal fluid may be appreciated.
Ahlers and colleagues the Medical University of Vienna led by Ursula Schmidt-Erfurth, MD, pointed to a new parameter as a prognosis factor in patients with AMD, i.e., the optical density ratio of the subretinal fluid detected by OCT.\textsuperscript{69} The authors suggested that this ratio may be an indirect way to measure the integrity of the barrier and therefore be useful for differential diagnosis between different exudative macular diseases such as central serous chorioretinopathy and to assess the response to antiangiogenic drugs (Table 4).

**OCT and Wet AMD in Clinical Practice**

To reach a consensus on the criteria of treatment and frequency of revisions, daily management of these patients is individualized, and the decisions are based on clinical examination and qualitative analysis of OCT images.\textsuperscript{2} The main signs of activity of the neovascular membranes in the OCT are the presence of intraretinal or subretinal fluid and RPE detachments and tears. These OCT findings should be evaluated biomicroscopically for the presence of fibrosis in disciform scars, which are final and irreversible stages of the disease that sometimes can have show of these signs on OCT. The presence of any of these tomographic signs, the patient’s VA, and the ophthalmoscopic and angiographic appearance of the lesions should be evaluated by ophthalmologists to reach treatment decisions and revisions in each case until the current prospective multicenter studies shed light on results based on conclusive evidence.

**Table 4. Prognosis Factors According to OCT as Identified by Various Authors.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Author and year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total retinal thickness</td>
<td>Singh 2009</td>
</tr>
<tr>
<td>Subretinal tissue volume</td>
<td></td>
</tr>
<tr>
<td>Neurosensory retina thickening</td>
<td>Keane 2008</td>
</tr>
<tr>
<td>RPE status</td>
<td>Kiss 2009</td>
</tr>
<tr>
<td>Integrity of the union of outer and inner segments of photoreceptors</td>
<td>Sayanagi 2009, Kaluzny 2009, Chen 2009, Witkins 2009</td>
</tr>
<tr>
<td>Posterior vitreomacular adhesion</td>
<td>Lee 2009, Mojana 2008</td>
</tr>
<tr>
<td>Optical density ratio</td>
<td>Ahlers 2009</td>
</tr>
</tbody>
</table>

**17. New Techniques and Prototypes in AMD**

**OCT and Confocal Scanning Laser Ophthalmoscopy**

Some new SD-OCT instruments (Spectralis HRA-OCT, Heidelberg Engineering, Inc., Heidelberg, Germany; Spectral OCT/SLO, Opko/OTI, Inc., Miami, FL) are equipped with this method, which simultaneously captures digital funduscopic images (infrared, red free autofluorescence, FA and ICGA) and OCT images from the same light source. This enables clinicians to correlate the two images pixel by pixel and allows a detailed topographic survey and tomographic retinal structures.\textsuperscript{70} In AMD, these devices are used especially in studies attempting to correlating OCT and autofluorescence in GA.\textsuperscript{71-72}

**Displaying the Choroid with Depth-Enhanced Optical Imaging OCT**

The principle of OCT requires penetration of the emitted beam through the structures observed before being received for analysis. Thus, with the standard technique, images of the full-thickness retina to the RPE, Bruch’s
membrane and choroid immediately adjacent space can be obtained, but the remainder of the choroid is beyond the capabilities of the instrument. To study this layer, Spaide developed a system consisting of an approximating scanner to the eye for multiple inverted images that then are processed to obtain a higher quality image in which the choroid can be seen in greater depth. Several diseases have been studied using this technique. This technique may be relevant for studying wet AMD associated with RPE detachment and especially in occult CNV, which often cannot be visualized conventional OCT.40,41,75-76

**New Prototypes: High-Speed Resolution Ultrahigh OCT**

SD instruments reach axial resolutions close to 5 microns using a source that emits light at a certain wavelength and detection system based on the Fourier principle. To obtain even higher resolutions, several groups have designed devices that vary primarily based on the light source. A group at Tufts Medical Center, Boston, developed a prototype that uses a broadband laser superluminescent diode combined with a Fourier detection system that can achieve axial resolutions up to 3.5 microns and allows precise analysis of the outer retinal layers, such as the photoreceptors layer, the RPE, and Bruch’s membrane.77 The investigators have published several studies highlighting the importance of these strata in the pathogenesis of dry AMD, the ability for early detection of intraretinal and subretinal fluid, and the status of the photoreceptors before and after ranibizumab treatment in a series of patients with wet AMD.4,66,78,79

**RPE Study of Polarization-Sensitive OCT**

SD instruments identify external retinal structures but provide limited information on the region of the RPE, making it possible to assess only the status based on hyper-reflectivity. To explore differences in the reflectivity of the RPE in the various AMD subtypes, an OCT prototype has been designed that incorporates a receiver capable of obtaining measurements of intensity and polarization at each point of study. In a study conducted in 44 patients with drusen, GA, disciform scarring, and neovascular forms, the authors described the different patterns of involvement of the RPE and suggested future uses of the device to assess the status, progression, and even disease prognosis.80
References:


16. Sandhu SS, Talks SJ. Correlation of optical coherence tomography, with or without additional colour fundus photography, with stereo fundus fluorescein angiography in diagnosing choroidal neovascular membranes. Br J Ophthalmol 2005;89:967–970


