Evaluation of different macular degenerations. Function, morphology and clinical outcomes.

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Function, morphology and clinical outcomes

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Marion Schroeder is a consultant ophthalmologist at the Department of Ophthalmology at Skåne University Hospital. This thesis explores morphology, function, and clinical outcomes of the two most common retinal degenerations in Sweden.

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LUND
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Evaluation of different macular degenerations - Function, morphology and clinical outcomes

Abstract

In the Western world, age-related macular degeneration (AMD) is the most common cause of severe visual decline in people over the age of fifty. The condition is divided into the untreatable dry AMD and treatable wet AMD. The established treatment consists of repeated intravitreal administration of anti-vascular endothelial growth factor (VEGF). Worldwide regimens are being overseen to gain effectiveness and to minimize undertreatment. *ABCA4*-associated retinal degenerations are recessively hereditary disorders with a wide range of variation and progression, including the decline of visual acuity (VA) and the visual field. It presents the most common form of inherited macular degeneration in young people, and, today, there is no treatment.

Concerning predictive factors, we evaluated data from two Swedish registries, the national Swedish Macula Registry (SMR) and the Swedish Retinitis Pigmentosa (RP) register from Lund that also includes patients from all over Sweden. Furthermore, in two clinical studies we compared anti-VEGF treatment outcomes. One survey followed two time cohorts from the beginning of the anti-VEGF era, where patients with treatment-naïve wet AMD received ranibizumab. The other study, a prospective, randomized clinical trial, compared the functional and morphological outcomes of two different regimens of treatment-naïve wet AMD with aflibercept.

We identified baseline characteristics of wet AMD patients from the SMR that were predictive for a VA below the national treatment criteria during a two-year treatment follow-up. In patients with *ABCA4*-associated retinal degenerations from the RP register it was seen that functional and morphological changes correlated well, and we found a promising electrophysiological predictor for progression. Of our two time cohorts, the second presented with no increase in the number of injections or VA but a tendency for the overall quality of life (QoL) score to improve compared to a decline in the first cohort. The other clinical study, evaluating wet AMD, revealed two equally effective aflibercept treatment approaches, without differences in the number of injections or treatment intervals. The electrophysiological results showed stable or improved values for the cones in the macula. In contrast, the rod response of the total retina decreased significantly.

To conclude, registries are a valuable source for evaluating the real-life clinical situation and treatment outcomes of patients, thereby supplementing the prospective, randomized clinical trials. Our established risk factors enable physicians and patients to more accurately assess the future outcome of two of the most common retinal degenerations in the Swedish population. We also show that a new treatment approach takes more time than expected to implement in a real-life clinical setting. But when improved, we will be able to choose from not only one effective regimen for wet AMD. Furthermore, our evaluation of retinal function during anti-VEGF treatment shows no toxicity concerning cones in the macula but a generally reduced rod response that needs to be further evaluated.

Key words: age-related macular degeneration, *ABCA4*-associated retinal degeneration, anti-VEGF, Swedish Macula Registry, electroretinography

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Thich Nhat Hanh

Table of Contents

Abstract

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List of papers

This thesis is based on the following four papers, which will be referred to in the text by their Roman numerals.

I. Schroeder M, Westborg I, Lövestam-Adrian M: Twelve per cent of 6142 eyes treated for neovascular age-related macular degeneration (nAMD) presented with low visual outcome within 2 years. Analysis from the Swedish Macula Registry (SMR). Acta Ophthalmol. 2019 Sep 13. doi: 10.1111/aos.14239. [Epub ahead of print]

II. Schroeder M, Rung L, Lövestam-Adrian M: No improvement in injection frequency or in visual outcome over time in two cohorts of patients from the same Swedish county treated for wet age-related macular degeneration. Clin Ophthalmol. 2017 Jun 9;11:1105-1111.

III. Schroeder M, Kjellström U, Lövestam-Adrian M: Electrophysiological evaluation and morphological follow-up of two regimens using Aflibercept for neovascular age-related macular degeneration. Submitted 2020.

IV. Schroeder M, Kjellström U: Full-field ERG as a predictor of the natural course of *ABCA4*-associated retinal degenerations. Mol Vis. 2018 Jan 4;24:1-16.

Abbreviations

AF	autofluorescence
Anti-VEGF	anti-vascular endothelial growth factor
A2E	N-retinylidene-N-retinylethanolamine
AMD	age-related macular degeneration
CRD	cone-rod dystrophy
CRT	central retinal thickness
dB	decibel
ERG	electroretinography
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	fluorescein angiography
FAF	fundus autofluorescence
FDA	food and drug administration
FfERG	full-field electroretinography
Hz	Hertz
ICG	indocyanine green
IT	implicit time
MfERG	multifocal electroretinography
NEI VFQ-25	National Eye Institute Visual Function Questionnaire 25
nAMD	neovascular age-related macular degeneration
OCT	optical coherence tomography
PIL	photoreceptor integrity line
PRN	pro re nata
QoL	quality of life
RPE	retinal pigment epithelium
RP	retinitis pigmentosa
TAE	treat-and-extend
SMR	Swedish macula registry
STGD	Stargardt disease
VA	visual acuity
VEGF	vascular endothelial growth factor

Introduction

Different macular diseases present with similar morphological features, some earlier and some later in life. Correct and early diagnoses are important to apply available treatment options, if there are any. Furthermore, the evaluation of patients is essential in finding the most effective regimen. In addition, large multicenter studies, even national register data or prospective clinical comparisons of different treatment regimens provide valuable information. Unfortunately, it has been shown that results from randomized clinical trials are not easily implemented in clinical real-world settings. And what if we have no treatment possibilities to choose from? In the case of mutations in the *ABCA4* gene, both clinical and electrophysiological assessment and genetic testing play an important role in differentiating phenotypes and genotypes with varying prognosis for the degree of visual impairment. With or without treatment possibilities, the assessment of degenerative macular diseases does impact the quality of life of patients of all ages.

Macular degenerations

The macula is the central part of the retina, with the highest resolution of VA. Macular degenerations can lead to central visual loss resulting in grave visual impairment. Characteristic degenerative changes in the macula begin with abnormalities in the retinal pigment epithelium (RPE) such as drusen, hyperpigmentation, atrophy, choroidal neovascularization, and fibrosis. The most common cause of macular degeneration in the elderly population is age-related macular degeneration (AMD), while in the younger population it is Stargardt disease (STGD), due to *ABCA4* gene mutations.

Age-related macular degeneration

Age-related macular degeneration (AMD) is the leading cause of central vision loss in the elderly Caucasian population above 50 years of age (Klaver et al. 1998). The origin is multifactorial, including heredity and environmental factors (Smith et al. 2001; Seddon et al. 2003; Clemons et al. 2005; Knudtson et al. 2006; Fletcher et al.

2008; Hogg et al. 2008; Evans & Lawrenson 2014; Broadhead et al. 2015). The majority of people who have AMD suffer from dry AMD, and only 8-12% develop neovascular AMD (nAMD) (Leibowitz et al. 1980; Jonasson et al. 2014). Dry AMD is characterized by drusen, hyperpigmentation and atrophic changes (Figure 1). Additionally, in nAMD you find choroidal or retinal neovascularization, triggered, for example, by VEGF, and secondary fibrosis, often referred to as disciform scarring (Figure 2). Only the neovascularizations are treatable with consecutive intravitreal injections inhibiting VEGF.

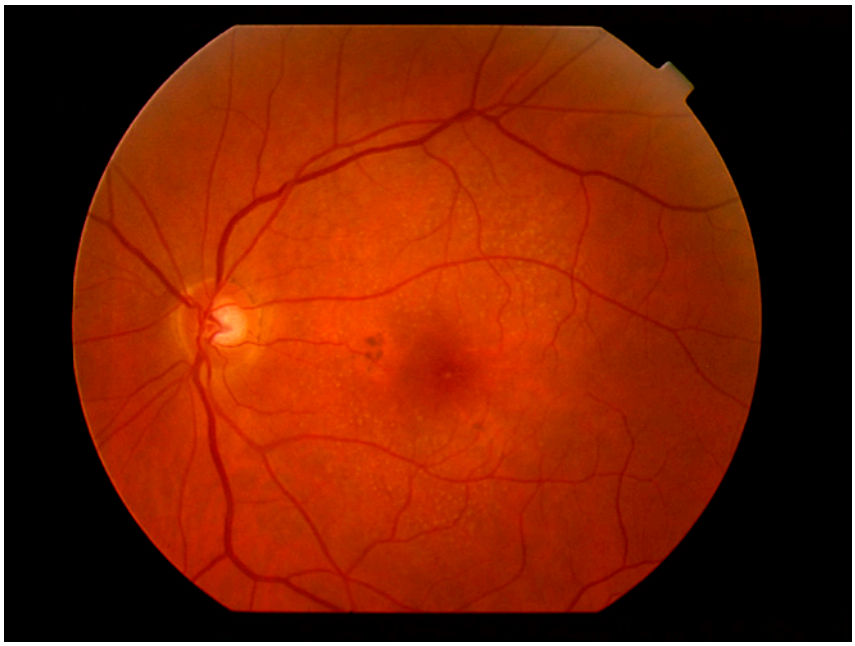


Figure 1.
Colour image of dry AMD with drusen och pigmentation.

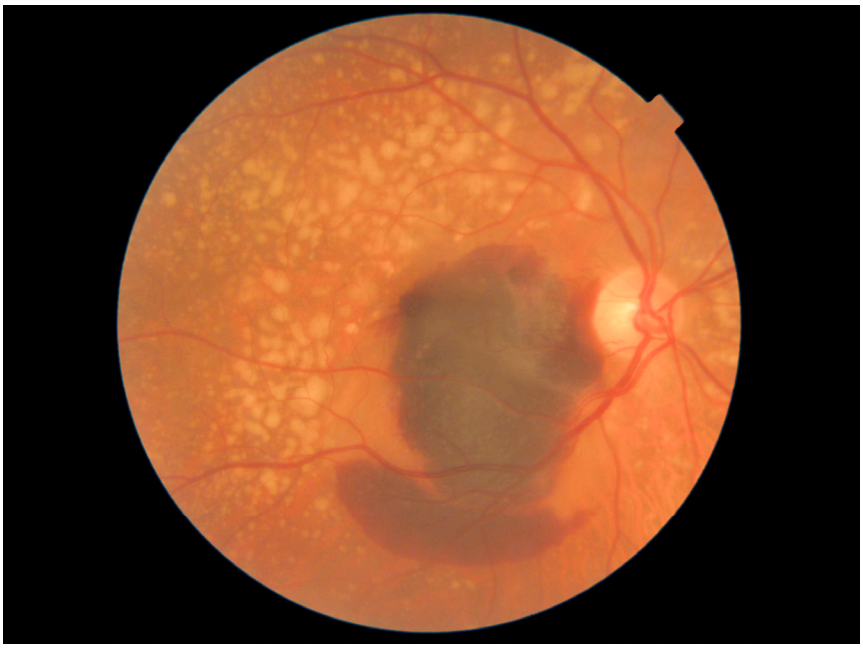


Figure 2.
Colour image of neovascular AMD with drusen and secondary subretinal hemorrhage.

In 2006, ranibizumab, a monoclonal antibody fragment (Steinbrook 2006), was approved by the Food and Drug Administration (FDA) for intravitreal treatment of nAMD. Bevacizumab is a monoclonal antibody that is used as an off-label treatment (Steinbrook 2006). Another anti-VEGF agent was approved by the FDA in 2011, aflibercept, a fusion protein with VEGF receptors 1 and 2 key domains (Heier et al. 2012). It binds VEGF-A, VEGF-B (Holash et al. 2002) and placental growth factor. Different agents and treatment protocols have been established and compared to improve the outcomes in the form of the most effective treatment accompanied by the best quality of life (QoL). But in a real-life setting, a widely known problem becomes apparent. Real-world data has shown to be inferior compared to randomized clinical trial outcomes with worse final VA and a lower number of injections (Holz et al. 2015). Randomized clinical trials only represent a narrowed spectrum of patients, whereas in a clinical setting we see a broader variety of patients and often have less rigorous inclusion/exclusion criteria and treatment adherence. Consequently, it is important to look at large real-world populations to follow and improve their treatment outcomes.

One possible setting is in the form of a registry. In Sweden, we are fortunate to have the SMR, a national quality registry that follows and evaluates the use of the various drugs and treatment regimens. In 2003, SMR was originally established to register

and follow Swedish patients nationwide under treatment for exudative neovascular macular changes. In 2008, it became a web-based registry. By the end of December 2018, the SMR had registered 41 735 eyes of 34 699 patients, and a total of 673 713 visits which include 426 397 treatments. More recently, in 2019, it has been enabled to also register the treatment of macular edema and neovascularization secondary to diabetic retinopathy and retinal vein occlusion. In 2018 the SMR covered 84% of all patients treated for nAMD, compared to the National Patient Register.

ABCA4-associated retinal degenerations

The *ABCA4* transporter protein is located in the disc membranes of the outer segments of the photoreceptors, the cones and rods. It transfers biological and toxic compounds across cell membranes. If the *ABCA4* protein is defect or missing, residues from the phototransduction cascade accumulate in RPE cells. Over time, this leads first to the death of RPE cells and later to the death of the overlying photoreceptors. Recently, however, there are studies proposing primary cell death of the photoreceptors prior to RPE pathologies (Gomes et al. 2009; Conley et al. 2012; Mullins et al. 2012; Fakin et al. 2016; Khan et al. 2018). The *ABCA4* gene, with 128 kbp, is a large gene and therefore has a vast potential for possible mutations, more than 1,000 known. The gene is located on chromosome 1 (1p22.1). *ABCA4* gene mutations include an array of inherited retinal degenerations such as cone-rod dystrophy (CRD), autosomal recessive RP, and the most common variant, STGD. The latter is the most common cause of macular degeneration in younger people (Figure 3). Mild and late debuting forms of STGD can present with phenotypes that may be confused with AMD. Important diagnostic tools, such as fundus autofluorescence (FAF) and electrophysiological evaluation help to differentiate the diagnosis. Currently, there is no treatment available. Therefore, it is important to be able to predict the natural course in these young patients, and help them to plan their future lives accordingly. In Lund, professor Sten Andréasson established the local RP register in the year 2000. The purpose was to collect and enable follow-up on patients with hereditary retinal degenerations. Another intention was that in case of a potential future treatment, it would be possible to directly contact and inform the patients. The registered patients have been referred to Lund for further evaluation of suspected retinal disease with uncertain origin, not only from the region, but also from all over Sweden. In 2020, the RP register holds 3400 patients.

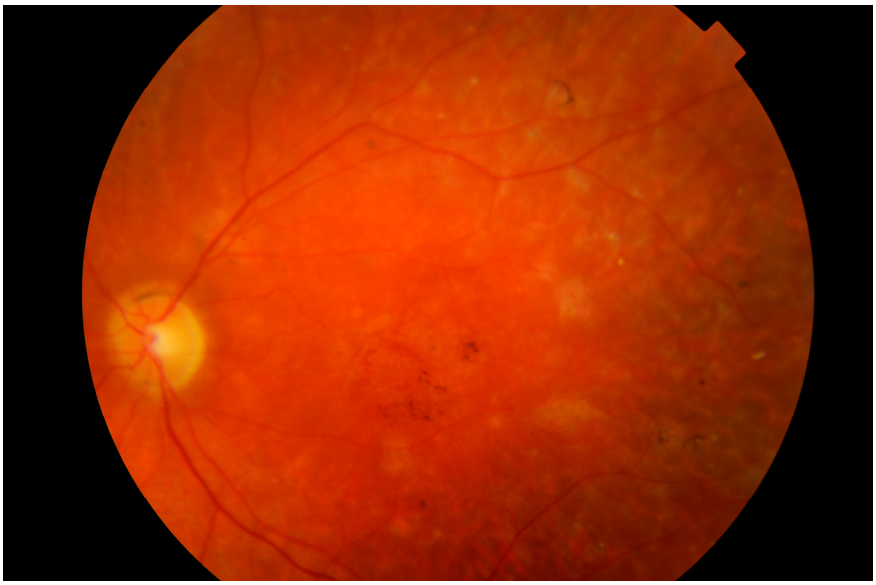


Figure 3.
Colour image of STGD with typical fundus changes as central atrophy, pigmentation and flecks.

Treatment

The development of nAMD anti-VEGF treatment regimens

The introduction of anti-VEGF treatment was the first intervention not only to temporarily halt the underlying process, but also to improve visual acuity. The pivotal ranibizumab studies, MARINA and ANCHOR, presented a monthly treatment regimen for neovascular macular degeneration (Rosenfeld et al. 2006; Brown et al. 2009). But this new treatment approach, which needed to be implemented in a real life clinical setting, proved to be challenging. It demanded funding and formation of a new branch of ophthalmology for a large group of patients who, until then, had been almost untreatable, apart from restricted laser or photodynamic therapy. Ophthalmologists faced an increasing burden of control visits and intravitreal procedures. A new approach, pro re nata (PRN), gained popularity. PRN represented an as needed regimen. Patients were supposed to be evaluated monthly and received treatment only in the event of signs of activity or recurrence, such as hemorrhage or new or increased intra- or subretinal fluid. This strategy lightened the treatment burden. But patients still did not receive their control visits and injections on time (Holz et al. 2015). The pivotal View 1 and View 2 aflibercept studies proposed fixed

interval dosing (Heier et al. 2012). After three consecutive monthly injections, patients received bimonthly injections in year one, without control visits between them. It became apparent that some patients could tolerate even longer treatment intervals, which would further reduce the treatment burden. The treat-and-extend (TAE) strategy (Berg et al. 2015) was introduced and widely embraced as an effective, more individualized and proactive treatment approach. Most commonly, TAE starts with three monthly, consecutive intravitreal injections. If presenting with no signs of activity, the interval can be extended by 2 weeks at a time until detectable signs of recurrence occur or the maximum treatment interval is reached. If there are signs of mild recurrence, the interval can be shortened by 2 weeks, or, in case of a more severe recurrence, directly reduced to the minimum interval of 4 weeks. TAE has been proven to be superior to PRN and comparable to monthly treatment outcomes, resulting in a positive, beneficial impact on patients and clinical resources (Kim et al. 2016; Johnston et al. 2017; Lee et al. 2017; Yang et al. 2017; Okada et al. 2018).

Clinical tests

Visual acuity

The Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart has been developed to minimize inaccuracies from former visual acuity tests, such as Snellen (Bailey & Lovie 1976; Ferris et al. 1982; McGraw et al. 1995). Snellen charts contain a variable number of letters with disproportional spacing per line. In between the different lines, the increase in letter size is irregular. In contrast, ETDRS charts have a logarithmic scaling. Each line contains 5 letters with proportional spacing. ETDRS has become a widely acknowledged gold standard for visual acuity assessment, especially in randomized clinical trials.

Near visual acuity

In Sweden, in 1968 Carl-Hugo Björnsson introduced Lix (läsbarhetsindex), a readability index that can also be applied to languages other than Swedish (Anderson 1981). It is based on letter counting and determines the grade of difficulty of a text. The Lix score is calculated by adding the percentage of words with seven or more letters and the average number of words per sentence.

In the 1970s, the Tomteboda Resource Centre for Visually Handicapped Children in Solna, Sweden, developed the Swedish Tomteboda charts to evaluate near vision acuity in children and adults (Textskalor, Synträningsavdelning, Tomtebodaskolan,

1977). The charts are graded in typographical points from the smallest text size of 4 points to the largest size of 24 points (4, 5, 7, 8, 9, 10, 12, 14, 18, and 24 points). The near vision acuity is tested at 40 cm reading distance with added near vision correction if needed.

National Eye Institute Visual Function Questionnaire 25

The National Eye Institute Visual Function Questionnaire 25 (NEI VFQ-25) originates from a longer version with 51 items (Mangione et al. 2001; Mangione et al. 1998). The purpose of the NEI VFQ-25 is to enable patients with chronic eye disease, with special focus on AMD, glaucoma, cataracts, diabetic retinopathy, and cytomegalovirus retinitis, to self-report their vision-related health status. Researchers of the Early Manifest Glaucoma Trial have translated and validated the Swedish version (Parrish 1996; Parrish et al. 1997; Sherwood et al. 1998; Mills 1998; Hyman et al. 2005). NEI VFQ-25 consists of 25 questions (items) covering 11 vision-related subscales, and a separate item concerning general health, which is considered a robust predictor of future health and mortality (Table 1). The NEI VFQ-25 can be evaluated with the help of a two-step process (Mangione 2000). The first step is to transfer the acquired numeric values from the NEI VFQ-25 with the help of a scoring key into values between 0 and 100; with 0 presenting the lowest and 100 the highest possible score. At this stage, the recoded value represents the percentage of the highest possible score. In the second step, the score for each item with an available answer is divided by the total number of items with an available answer. The number of items and the items to be averaged are shown in table 1. The composite score is another extractable value with the aim of describing an overall score for the vision-related subscales by averaging 11 of the 12 subscales, excluding the general health item. Especially in studies that compare different groups with a small sample size, the error is more likely to be smaller for the overall composite score than for the specific subscales. The estimated duration is 5-10 minutes.

Table 1.
NEI VFQ-25 subscales with corresponding number of items and the specific items to be averaged.

Subscale	Number of items	Items to be averaged
General Health	1	1
General Vision	1	2
Ocular Pain	2	4, 19
Near Activities[1]	3	5, 6, 7
Distance Activities[2]	3	8, 9, 14
Social Functioning[3]	2	11, 13
Mental Health[4]	4	3, 21, 22, 25
Role Difficulties[5]	2	17, 18
Dependency	3	20, 23, 24
Driving	3	15c, 16, 16a
Color Vision[6]	1	12
Peripheral Vision	1	10

[1] Reading newspaper, seeing well at a close distance, finding things on a crowded shelf
[2] Street signs, shop names, steps, stairs in dim light, theater
[3] Social life, company
[4] Worrying, sense of frustration, "control over what I am doing"
[5] Limitations in daily life
[6] Matching clothes

Goldmann perimetry

Visual field testing with Goldmann perimetry can evaluate the complete visual field. The normal visual field includes 60 degrees superiorly, 110 degrees temporally, 75 degrees inferiorly, and 60 degrees nasally. At first, the test defines the peripheral borders and continues to the central field, also defining the blind spot. Stimuli vary in size and intensity. The size varies from the smallest, 0, to the largest stimuli, V. Intensity is defined by numbers and letters. From 1, the dimmest, to 4, the brightest, in 5 decibel (dB) steps. From a, the dimmest, to e, the brightest, for each 1 dB change. Generally, a brighter stimulus is used in the periphery compared to the center. The test is performed by moving a defined stimulus from the non-seeing periphery to the seeing area. Each point where the stimulus is first detected by the patient is marked and later, all points from the same stimulus are connected. The result composes a so called isopter for each stimulus. An isopter often resembles a circle. If an isopter shows an indented area that means the stimulus was not seen by the patient. Two of the most common stimuli are I4e for the periphery and I2e for the central part of the examined visual field. The test should not exceed more than 10 minutes per eye. Each eye is tested separately.

Optical coherence tomography

Optical coherence tomography (OCT) provides a noninvasive tool to assess different ocular tissues in vivo. Near-infrared emitted light is echoed, for example, from retinal structures. By using reference measurements, the delay of the reflected waves can be indirectly evaluated and define the depth at which the reflection occurred. OCT delivers cross-sectional images with high semi histological resolution. In the retina, OCT is able to visualize adjacent structures, different layers and measure retinal thickness, thus making possible to detect and follow pathological changes, such as intra- and subretinal fluid or tissue.

Fundus autofluorescence

Many fundus cameras can not only obtain color and red-free images but even autofluorescence images. Fundus autofluorescence (FAF) takes advantage of intrinsic fluorescence derived from different ocular tissues. We used the excitation wavelength of 530–580 nm and a barrier filter of 600–720 nm. FAF is mainly emitted by lipofuscin, more specific N-retinylidene- N-retinylethanolamine (A2E), a pigment within lipofuscin. A2E accumulates, for example, in RPE cells if the *ABCA4* protein is dysfunctional and that leads to increased fluorescence. On the contrary, a loss of RPE cells leads to decreased fluorescence and appears dark. That makes FAF a preferred, noninvasive tool to evaluate RPE function. A pattern of increased and reduced autofluorescence (AF), so-called flecks, are typical for Stargardt patients compared to AMD patients.

Fluorescein angiography

Fluorescein angiography (FA) visualizes the retinal circulation by using an intravenously administered dye, sodium fluorescein. Twenty percent of the fluorescein molecules are unbound, while the remaining 80% are protein-bound. They reach the retinal and choroidal circulation. If the RPE remains intact, the fluorescein molecule cannot pass through from the choroidal circulation. RPE pigment mainly blocks the choroidal fluorescence. After excitation of the unbound molecules by a wavelength in the blue spectrum (465–490 nm), they emit and fluoresces at 520–530 nm (yellow-green spectrum). This fluorescence can be recorded. Abnormal findings can be described as hyperfluorescence or hypofluorescence. Hyperfluorescence occurs in case of leakage from e.g. neovascularization, window defects from atrophies, pooling or staining. Vascular filling defects or blockage represent as hypofluorescence.

Indocyanine green angiography

Indocyanine green (ICG) angiography images the choroidal circulation. The dye is intravenously administered and is 98% protein-bound. Therefore, diffusion is limited and most of the dye remains in the choroidal circulation. ICG fluoresces at 790–805 nm, a longer wavelength than fluorescein. This makes it more effective in penetrating blockages, such as hemorrhages and pigment. The obtained image can show hyper- and hypofluoescence and, for example, choroidal neovascularizations are described as a plaque if the size is 1 disc diameter or larger, or as a hot spot if its size is less than 1 disc diameter.

Electrophysiology

Electrophysiology is a biomedical technique used to assess and measure the ion current flow in various biological tissues, such as the heart or the retina.

Full-field electroretinography

Full-field electroretinography (ffERG) objectively assesses the total retinal function, including rods, cones, and the combined response of both. It is measured in the dark- and light-adapted state. Usually, the pupil is dilated and, after topical anesthesia, a contact lens electrode is applied. We use a Burian Allen lens, a standard lens in electrophysiology. The response is shown as a wave pattern. The first negative wave, the a-wave, is the light-induced response predominantly from the photoreceptors, cones and rods. It is followed by a positive wave, the b-wave, representing the bipolar and Müller cells. Light-adapted 30 Hertz (Hz) flicker ERG is a specific isolated response from the cones. Implicit time (IT) is the time measured from the onset of the light stimulus until the maximum a- or, respectively, b-wave response. (Figure 4)

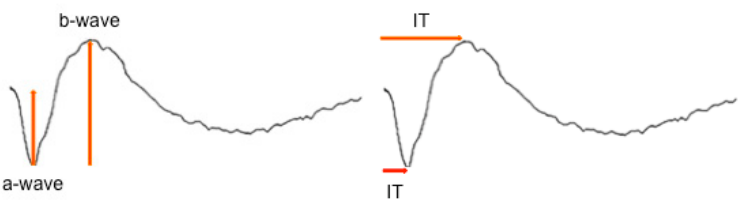


Figure 4.
FfERG amplitudes with a- and b-wave and implicit times (IT).

Multifocal electroretinography

The area of the macula is too small in relation to the total retina and, therefore, not distinguishable in the total ffERG response. In contrast, multifocal electroretinography (mfERG) separately assesses the cone function in the central retina by recording the responses simultaneously from different retinal locations. It usually uses 61 or 103 local responses under light-adapted conditions. The hexagonal stimuli of each sector are scaled with eccentricity to produce equal amplitudes of the responses all over the matrix and change in a pseudo-random sequence every 13.3 ms. That means the illumination debut in each sector has another time-specific starting point. But the following sequence is the same for each sector. Each hexagon has a 50% chance of appearing white or black, being illuminated or not. At a distance of 30 cm the covered area is approximately the central 50° of the retina. The characteristic response is a biphasic wave. The first is a negative peak, N1, followed by a positive peak, P1, and finally a second negative peak, N2. The mfERG responses are not actual electrical potentials but resemble a mathematical extraction from the on, off sequence of each hexagon correlated to the continuous ERG signal. (Figure 5)

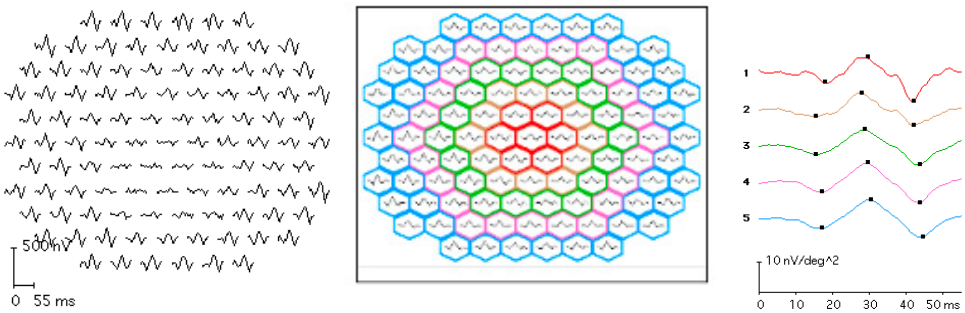


Figure 5.
Left: MfERG trace array with 103 local responses. Middle: 103 focal ERG waves divided into five different coloured rings. Right: The combined response for each ring.

Aims of the thesis

The purpose of this thesis was to identify prognostic factors for visual outcomes in patients with macular degenerations, and to evaluate the outcomes of different treatment regimens for nAMD at various points in time since the introduction of anti-VEGF treatment.

Paper I

To detect the baseline risk factors, extractable from the SMR, in patients with treatment-naïve nAMD that lead to VA values at or below treatment threshold within two years of anti-VEGF treatment.

Paper II

To compare the outcome factors for VA, injection frequency, and QoL in two time cohorts in real clinical life settings from the beginning of the anti-VEGF era with ranibizumab for treatment-naïve nAMD.

Paper III

To evaluate the electrophysiological function of photoreceptors and to compare two aflibercept treatment regimens in treatment-naïve nAMD over 18 months.

Paper IV

To assess electrophysiological function and retinal structure in *ABCA4*-associated retinal degenerations and their possible predictive value concerning the natural course of these disorders.

Subjects and methods

Paper I

All eyes of newly diagnosed nAMD patients, registered prior to their anti-VEGF treatment in the SMR between 1 January 2013 and 31 December 2014, were monitored over a period of two years. We evaluated baseline variables from the SMR of 6142 eyes with special focus on the subgroup of 780 eyes of 774 patients with a final VA of ≤ 35 letters, as well as the registered number of injections and which anti-VEGF drugs were used during the 2-year follow-up. The available baseline characteristics from the SMR were gender, age, VA, lesion type, lesion size, lesion location, symptom duration, and choice of drug.

Paper II

During the years 2007 and 2009, 50 eyes of 50 patients, respectively 26 eyes of 26 patients, from the Department of Ophthalmology at Helsingborg Hospital in Sweden, that presented with treatment-naïve nAMD, classic or occult lesions, were included into two cohorts. Both cohorts were followed in a real-life clinical setting, cohort 1 over 37 ± 7 months, and cohort 2 over 45 ± 4 months. The two time cohorts received anti-VEGF treatment with ranibizumab according to a PRN approach (Figure 6). PRN was defined as three consecutive monthly intravitreal injections as a loading dose, followed by clinical controls every 4-6 weeks. Reinjection was considered in case of reactivity signs on OCT, during the biomicroscopic examination or a visual decline of 5 or more letters. VA and NEI VFQ-25 results at baseline and at the final visit, and number of received injections were compared.

Pro re nata

Week	0	4	8	12	16	20	24	28	32	...
Injection	x	x	x	?	?	?	?	?	?	...

Figure 6.
PRN regimen. Initially, 3 consecutive monthly controls and injections (X), followed by monthly controls and retreatment as needed (?).

Paper III

In this prospective, randomized, clinical study, we compared the functional and morphological outcome of two different treatment regimens for treatment-naïve nAMD with aflibercept, an anti-VEGF drug, over 18 months. 41 eyes of 41 patients from the province of Skåne were randomized 1:1 either to arm 1 or 2. Arm 1 received three consecutive monthly aflibercept injections, followed by bimonthly treatment until week 52, according to the fixed-label interval dosing. Thereafter, the TAE regimen was applied, a more individualized and proactive approach (Figure 7). Arm 2 was treated following the TAE protocol throughout the entire 18-month follow-up period (Figure 7). The shortest possible treatment interval was 4 weeks, the longest allowed interval was 12 weeks. We assessed VA, central retinal thickness (CRT), injection rate and interval, and evaluated cone and rod function with ffERG and mfERG.

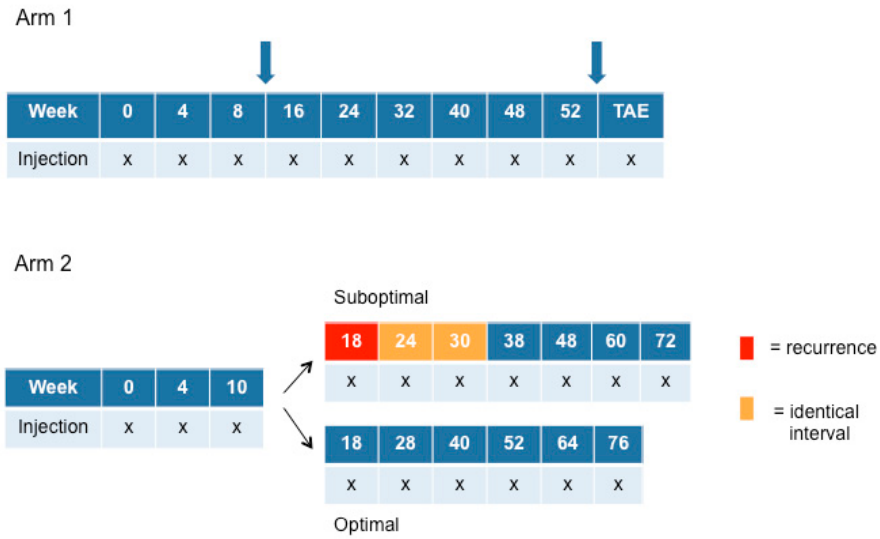


Figure 7.
Study treatment regimens with aflibercept. Arm 1 with the fixed-label interval dosing, followed by the treat-and-extend (TAE) regimen. Arm 2 with the TAE regimen from the beginning showing an optimal and suboptimal course of treatment.

Paper IV

We screened the RP register in Sweden for patients with the diagnosis STDG or CRD. 34 patients with genetically confirmed *ABCA4* compound heterozygous or homozygous *ABCA4* mutations were divided into three groups depending on their latest available visual fields with Goldmann perimetry. Ten patients were included in group 1, which was defined by a scotoma area restrained to the central 10°. In group 2, we included 19 patients with a more extended scotoma with outer limits of 10–35°. Group 3 included five patients with the largest scotomas. They presented with remaining functional visual fields restricted to the temporal areas. In addition to biomicroscopic examination, we obtained VA, OCT, and fundus images including FAF, ffERG, and mfERG. In 23 patients, previous ffERG results were available for comparison with the latest examinations.

Results

Registries as a source of prognostic values of estimating future outcomes

Papers I + IV

Register data has become an important tool for evaluation of real-world clinical treatment outcomes in a larger perspective. Another aim can be to establish a database, such as the RP register, where it is possible to continuously add and evaluate information over time in order to specifically define a disease, for example through sequence analysis of a specific gene, changes over time in visual fields, or electrophysiological examinations. This register data can help to predict the future outcome or probable prognosis of a yet untreatable disease, as shown in papers I and IV.

In paper I, we used the data from the SMR to establish which available baseline characteristics predicted a visual outcome of a final VA of ≤ 35 letters during a 2-year follow-up. The result of a logistic regression analysis showed that the eyes of patients in the low visual outcome group presented with the following baseline factors: a lower VA than the group with VA outcome of > 35 letters; $p < .0001$, older age; $p < .0001$, worse-seeing eye treated; $p = .0007$, and a larger lesion size; $p = .005$ as presented in table 2.

Table 2.
Baseline characteristics from the SMR as risk factors to predict a visual outcome of ≤ 35 letters.

Baseline risk factors	p-value
Lower baseline VA	< .0001
Older age	< .0001
Worse-seeing eye treated	.0007
Larger lesion size	.005

During the 2-year follow-up, 12.7% of all eyes presented with a final VA of ≤ 35 letters (Figure 8). If patients presented with a VA of ≥ 70 letters at baseline, only 4% of those eyes presented with a final VA of ≤ 35 letters (Figure 9). Of eyes with a baseline VA of ≤ 35 letters, 1% remained in the low VA group.

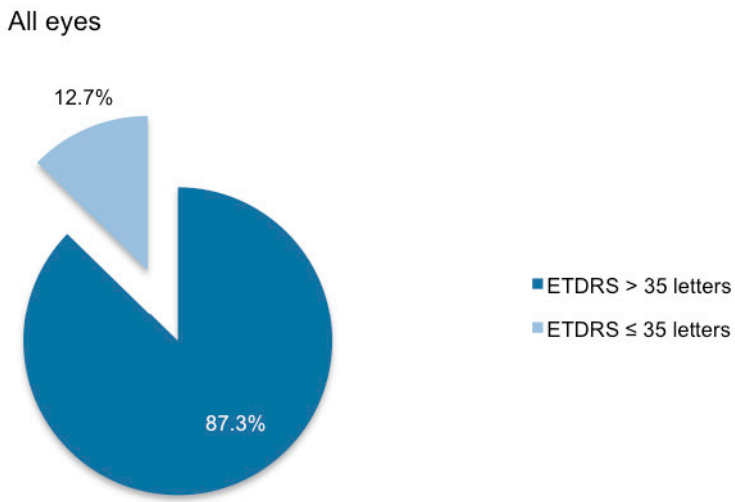


Figure 8.
The distribution of final VA of all patients at 2-year follow-up.

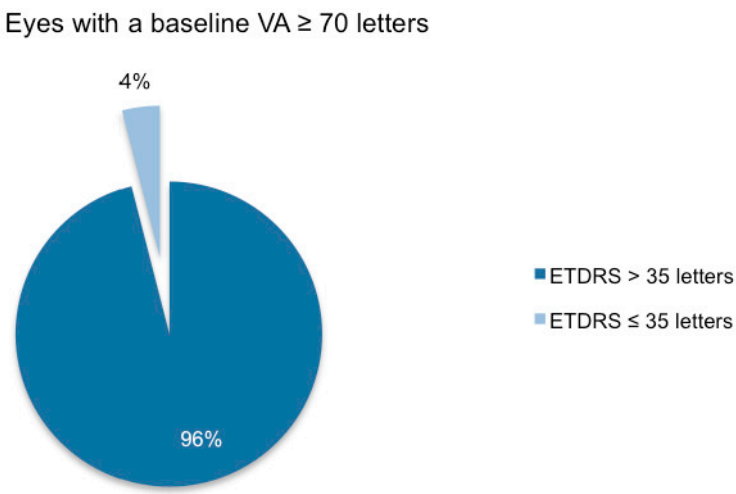


Figure 9.
The distribution of final VA of patients with a baseline VA ≥ 70 letters at 2-year follow-up.

Furthermore, eyes with a final VA of ≤ 35 letters received fewer injections, 6.2 ± 3.8 vs. 8.7 ± 5.4; $p < .00001$ during the 2-year follow-up. Out of all 6142 eyes, 77% received monotherapy with either aflibercept (31%), ranibizumab (24%), or bevacizumab (22%). The choice of anti-VEGF drug did not significantly influence the total number of injections until a VA of ≤ 35; 4.9 ± 3 mean number of injections with aflibercept, 4.3 ± 2.5 with ranibizumab, and 4.8 ± 3 with bevacizumab.

In paper IV, we found significant differences between three groups that were defined by their different size of visual field defects of 34 patients from the RP register.

FAF changes grew more severe from group 1 to group 3 (Table 3). In group 1, we saw minor central areas in the macula with often absent AF, and/or reduced or increased AF restricted to the posterior pole. All patients in group 1 showed flecks and peripapillary sparing. In group 2, the affected areas were generally larger than in group 1. The main differences were the predominant presence of flecks with low autofluorescence and that most of the patients presented without peripapillary sparing. In group 3, almost all patients had completely lost their autofluorescence in enlarged areas of the posterior pole and beyond and showed no peripapillary sparing.

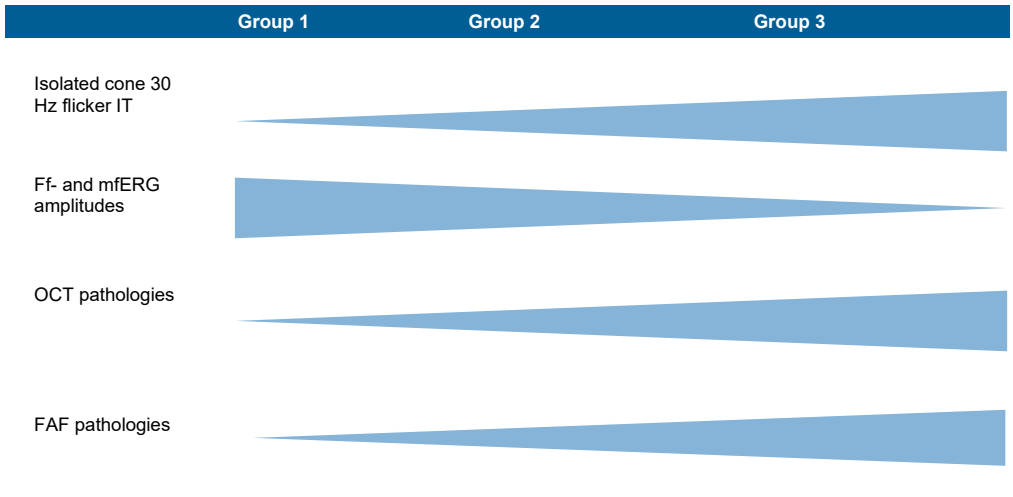
Between the three groups, for both eyes, ffERG values showed statistically significant differences concerning amplitudes of the isolated rod b-wave, the combined rod-cone a-wave, the combined rod-cone b-wave, and the isolated cone 30 Hz flicker amplitude, as well as the isolated cone 30 Hz flicker IT; $p < .0001$. The amplitudes decreased from group 1 to group 3, and accordingly, the IT worsened with prolonged values from group 1 to group 3 (Table 3). Compared to the control group, groups 2 and 3 presented with reduced amplitudes of the isolated rod b-wave, the combined rod-cone a-wave, the combined rod-cone b-wave, the isolated cone 30 Hz flicker, and the prolonged isolated cone 30 Hz flicker IT; $p < .0001$. We also compared the control group with the prior ffERG results of 23 of our patients. Already, these earlier results showed significantly increased values of the cone 30 Hz flicker ITs in group 2; $p = .009$ (right eye), $p < .0001$ (left eye), and group 3; $p = .001$ (right and left eye).

In general, the mfERG amplitudes of the 5 rings demonstrated the highest values in group 1, the second highest in group 2, and the lowest values in group 3 (Table 3). The amplitudes were lower for group 2 only for rings 2 and 3 of the right eye compared to group 3. Among the three groups, the mfERG amplitudes of all rings differed significantly. When compared to the control group, measurements were also statistically significantly reduced, except for ring 5 of the left eye in group 1.

OCT scans revealed areas of retinal thinning and changes to the junction of the inner and outer segments of the photoreceptors, the so-called photoreceptor integrity line (PIL) (Table 3). Group 1 to group 3 showed an increasing number of segments with retinal attenuations. All patients showed disturbances or absence of PIL.

We could not establish a direct correlation of *ABCA4* gene mutations and a specific phenotype.

Table 3.
Visualization of characteristic differences between groups 1-3 concerning isolated cone 30 Hz flicker IT, ff- and mfERG amplitudes, OCT and FAF pathologies in paper IV.



Perspective of nAMD treatment over time

Papers I- III

The evaluated PRN approach in paper II resulted in apparently inferior treatment outcomes compared to the fixed interval and TAE regimens in paper III.

In paper II with two different time cohorts and a PRN regimen, the mean VA declined over a 3-year period; in cohort 1 from 53 ± 14 letters to 45 ± 24 letters; p = .01, in cohort 2 from 52 ± 15 letters to 46 ± 22 letters; p = .17. Patients received 7.8 ± 5 injections in cohort 1, and 8.5 ± 7 injections in cohort 2 over three years; p = .66. In both cohorts, the worse-seeing eye received less injections over time than the better-seeing eye. As we have seen in paper I, the worse-seeing eye treated was one of the prognostic factors in the SMR for a worse visual outcome.

The mean composite score from the NEI VFQ-25 showed a statistically significant decrease over time in cohort 1, from a 64 ± 21 score to a 59 ± 25 score; p = .04. In cohort 2, we could see an improvement but it was not statistically significant. The baseline composite score was 64 ± 28 and the final score 67 ± 23; p = .38.

In paper III, the baseline variables of age, gender, symptom duration, VA, and lesion type did not differ between the two treatment arms. At the final visit, both arms had increased in VA, in arm 1 from 63.5 ± 10.5 to 69.1 ± 9.2 letters; p = .098; and in arm 2 (n = 20) from 66.8 ± 13.6 to 73.9 ± 9.0 letters; p = .002. During the 18 months, CRT decreased in arm 1 from 327.8 ± 87.6 µm to 218.1 ± 34.4 µm; p < .000, in arm 2 from 303.7 ± 92.0 µm to 226.4 ± 32.3 µm; p < .000. Arm 1 had received 11.3 ± 1.7 injections vs. 10.9 ± 2.0 in arm 2 with no significantly statistical difference between the two arms. Nor did we see a significant difference in reached injection intervals at month 18, where the mean injection interval was 9.2 ± 3.4 weeks in arm 1 vs. 9.5 ± 3.1 in arm 2, or a difference in the maximum reached interval during the complete follow-up time, at 10.2 ± 2.0 weeks in arm 1 arm and 10.5 ± 2.1 weeks in arm 2.

In paper I, we were unable to compare the outcome of different treatment regimens because that variable was not registered yet.

Papers I-III presented baseline data from as early as 2007, with the latest data from 2016, where we could confirm the trend that the baseline VA increased over time (Figure 10). At the end of follow-up, we also registered a gain in the administered number of injections per year in papers I-III (Table 4).

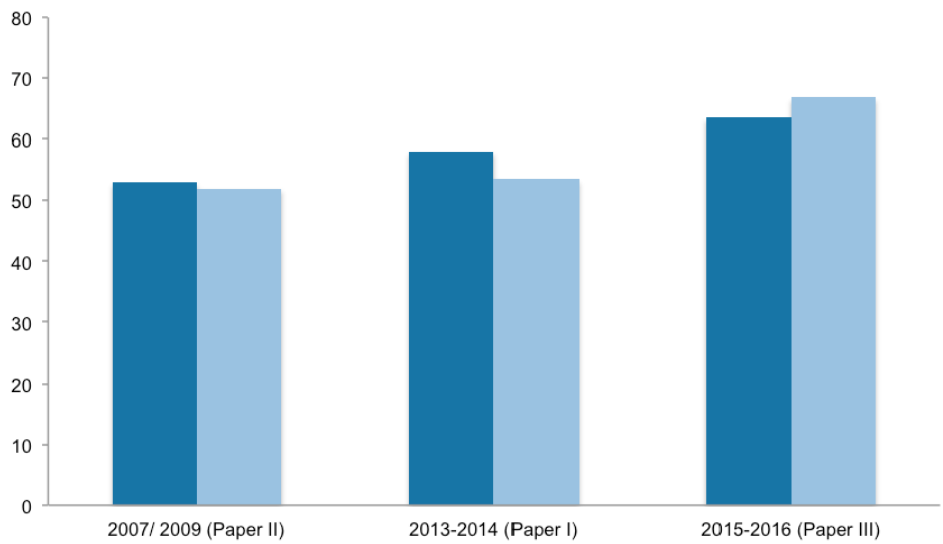


Figure 10.
Baseline VA of the two different groups in papers I-III. Paper II: cohort 1 (dark), cohort 2 (light). Paper I: Group with final VA > 35 letters (dark), group with final VA ≤ 35 (light). Paper III: arm 1 (dark), arm 2 (light).

Table 4.
Comparison of the two different groups in each of the papers I-III. Showing the treatment regimen, follow-up time, number of injections per follow-up period and per year, and the statistical significance level of differences in number of injections.

	Treatment regimen	Follow-up time in months	Number of injections (injections/ year)	Statistical difference in number of injections
Paper I				
Group with a VA outcome of > 35 letters	Not defined	24	8.7 ± 5.4 (4.4)	p < .00001
Group with a VA outcome of ≤ 35 letters	Not defined	24	6.2 ± 3.8 (3.1)	
Paper II				
Cohort 1	PRN	37 ± 7	7.8 ± 5 (2.6)	Not significant
Cohort 2	PRN	45 ± 4	8.5 ± 7 (2.3)	
Paper III				
Arm 1	Label + TAE	18	11.3 ± 1.7 (7.5)	Not significant
Arm 2	TAE	18	10. 9 ± 2.0 (7.3)	

Evaluation of aflibercept treatment effect on retinal function in nAMD

Paper III

When comparing baseline and 18-month ffERG results for the isolated 30-Hz flicker cone amplitudes and ITs, these showed no statistically significant change. In contrast, the combined rod-cone a-wave amplitude and the isolated rod b-wave amplitude significantly decreased over time; p = .043 and p = .026, respectively.

The summed mfERG values of rings 2-5, for amplitudes and ITs, remained stable with no significant change from baseline to the final follow-up after 18 months; p = .878 vs. p = .922. Only in ring 1 did the amplitude increase significantly during the study period; p = .041 (Figure 11).

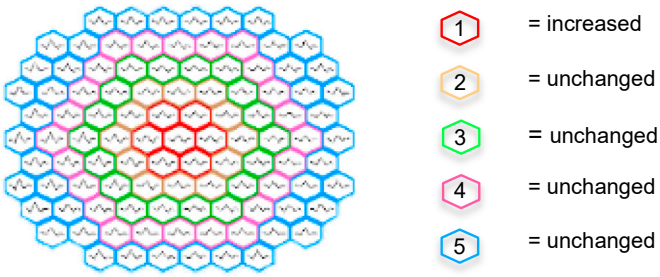


Figure 11.
Left: MfERG with its 103 focal ERG waves. Right: Each colour marks the focal waves used to calculate the combined response for each ring. Showing the amplitude changes in rings 1-5 over 18 months.

Discussion

Macular degenerations are a broad field. We decided to focus on the two most common types in Scandinavia. On the one hand, the macular degeneration that manifests in an older population, nAMD, and on the other hand, that which is seen mostly in a younger population, *ABCA4* retinal degenerations. AMD has multifactorial origins, such as lifestyle, genetic and environmental factors (Smith et al. 2001; Seddon et al. 2003; Clemons et al. 2005; Knudtson et al. 2006; Fletcher et al. 2008; Hogg et al. 2008; Evans & Lawrenson 2014; Broadhead et al. 2015), whereas *ABCA4*-associated retinal degenerations are heredity diseases. Besides their differences, their clinical appearance can sometimes be confused with each other, especially in overlapping age groups. Both groups of patients are concerned about their eyesight and appreciate access to as much information as possible about the future status of their chronic eye diseases; regardless of whether there is a promising treatment. Because the *ABCA4* degenerations cannot yet be treated, there is an extra focus on prediction of further progress, and finding possible parallels between different geno- and phenotypes. With our work, we wanted to specify and widen the spectrum of information for our patients.

AMD - Treatment recommendations and prognostic factors

As earlier emphasized, it is of eminent value not only to evaluate randomized clinical trials but also to compare them with outcomes in real-world clinical settings. A prospective approach is preferred, but even a retrospective data review can provide significant insight. Considering the amount of patients who develop nAMD, it is valuable for each patient and the health care system to be aware of the most probable prognosis for the individual treatment outcome. On the one hand, patients have to be informed about their future prospective outcomes in order to proceed with the continuous injection procedure and adhere to it. And on the other hand, physicians must to be able to calculate and effectively distribute the available resources. Since the first efforts, the intravitreal treatment of nAMD has experienced rapid development of different drugs and treatment regimens.

Especially in the early phase of the introduction of a new treatment approach, the clinical evaluation of its implementation is important and valuable. As shown by our two different time cohorts in paper II, the multi-step process with strictly interconnected elements, such as clinical follow-up and as-needed treatment (PRN) with ranibizumab, could not be implemented as effectively or as soon as expected. The most apparent problem was not the novel injection process but the timely clinical follow-up, which interfered with the treatment adherence, and the low injection frequency after the first loading dose of three consecutive injections. Despite our expectations, it resulted in inferior improvement of VA compared to monthly treatment in the pivotal trials (Rosenfeld et al. 2006; Brown et al. 2009), even in the second time cohort. Other studies pointed out that this is a widespread issue (Singer et al. 2012; Rofagha et al. 2013; Holz et al. 2015; Boulanger-Scemama et al. 2016). Still, the results were better than the natural course of nAMD (Bressler 2001; Rosenfeld et al. 2006). In any case, there was an apparent need for a change and the evolution of new treatment regimens. In paper II, we could also demonstrate that some patients increased by > 15 letters in VA despite a low number of injections, compared to other studies (Holz et al. 2011; Singer et al. 2012), suggesting that not all patients required monthly monitoring or treatments.

As aflibercept, another anti-VEGF agent, entered the market, it introduced a new label regimen with a loading dose of three consecutive injections, followed by a bimonthly treatment during year one. Soon, the more proactive and individualized TAE regimen (Berg et al. 2015) began to replace PRN, and partly also the aflibercept label regimen. Clinical treatment adherence improved and the emphasis now lay on which regimen achieved the best results in order to minimize under- or overtreatment. In paper III, we compared the TAE regimen to fixed interval dosing, according to the original label, by using aflibercept. The two compared treatment arms, with similar baseline characteristics, showed no difference in the final number of received injections, final injection interval, or maximum injection interval during 18 months. When looking at the numbers of given injections per year in papers I-III, we clearly showed that in the latter study, for the first time, the cohorts are not undertreated. We could not compare the results directly due to different study designs, with also partly prospective, partly retrospective data. The results were representative for the Swedish population. Interestingly, the two arms in paper III started with a high VA, the highest of papers I-III. As we could demonstrate in papers I, II, and III, the baseline VA had increased between the different recruitment periods which extended over the years 2007-2016. This is in accordance with other study outcomes (Rosenfeld et al. 2006; Brown et al. 2009; Heier et al. 2012; Gillies et al. 2019). Nonetheless, we would prefer an even higher baseline VA to be able to improve long-term visual acuity as seen in various long-term follow-up studies (Gillies et al. 2015; Holz et al. 2019). The impact of VA deficiencies on daily life are often underestimated. Data from the Blue Mountains Eye Study documented the impact

from visual impairment of best-corrected <0.5 to >0.1 Snellen VA (approximately <70 to >35 ETDRS letters) in the better eye. Independent of other known risk factors for each outcome, they showed a 3-fold increase in the number of falls (Hong et al. 2014), an 8-fold higher hip fracture risk in 2 years (Ivers et al. 2003), more use of community support services (Wang et al. 1999), earlier nursing home care (Wang et al. 2003), reduced quality of life with an impact similar to major systemic disease (Chia et al. 2006), and a 2-fold higher relative risk for greater depression (Hong et al. 2015) and higher mortality (Karpa et al. 2009). A high baseline VA usually does not improve much due to the ceiling effect, but it could possibly contribute to better preservation of the VA level over a longer time period (Williams & Blyth 2011; Ross et al. 2013; Writing Committee for the UK AMD EMR Users Group 2014; Gillies et al. 2015). As we established from the SMR in paper I, a lower baseline VA was a predictor for worse visual outcome in nAMD. In this study, only 4% of eyes with a baseline VA of ≥ 70 letters declined to a final VA of ≤ 35 letters, compared to 12.7% of all included eyes. In accordance with the INSIGHT study (Lövestam-Adrian et al. 2019), we found that patients with a baseline VA of ≤ 35 letters had potential to improve over time. In our study, only 1% remained in that low vision group, meaning that 99% of eyes showed improvement in their VA level above the threshold where it was recommended to continue the intravitreal treatment. It is debatable when to stop the treatment. Especially in patients with only one functioning eye left, even a minor improvement in visual acuity might enable that individual to become or remain more independent and self-sufficient.

We did not find the variable, macular lesion type, to be predictive as proposed by for example Grunwald et al. (Grunwald et al. 2014), who found a better visual outcome for occult lesions and a worse prognosis for retinal angiomatous proliferations. This might be due to the fact that macular lesion size was registered in only 75% of the eyes in our study. Rasmussen et al. (Rasmussen et al. 2015) emphasized the importance of a short time-to-treatment to improve VA outcome, but we could not confirm the variable of symptom duration as a prognostic factor for a better or worse visual outcome in paper I.

Not only the morphological and visual outcome is important. We consider it necessary to also evaluate the impact on retinal function of upcoming intravitreally applied drugs, such as aflibercept, that are intended to influence retinal cells. Electrophysiological tools, such as ffERG and mfERG, reflect the retinal function of different retinal cell types. This enables us to monitor and evaluate treatment effects, as well as detect possible toxicity.

In paper III, we were the first to electrophysiologically assess, with both ffERG and mfERG, and prospectively follow up on a larger cohort of 41 nAMD patients treated with aflibercept under the period of 18 months. To our knowledge, there is only one other published electrophysiological study with aflibercept (Takayama et al. 2017).

Their survey presented 42 eyes of 42 treatment-naïve nAMD patients. The eyes had received three monthly aflibercept injections and only the results of the macular function after three injections compared to baseline were retrospectively evaluated. They showed improved amplitudes and ITs at 3-month follow-up. Studies of other anti-VEGF drugs, ranibizumab and bevacizumab, with various, mostly shorter follow-up times, showed mainly improved or unchanged mfERG amplitudes and ITs (Pedersen et al. 2010; Campa et al. 2011; Moschos et al. 2011; Park et al. 2011; Torres-Soriano et al. 2012; Pedersen et al. 2016; Ju et al. 2017; Reinsberg et al. 2017). Two surveys, with only a few patients each, reported reduced mfERG amplitudes as the result of the follow-up after three monthly ranibizumab injections (Feigl et al. 2007; Almeida et al. 2015). Our aflibercept data completed the information that the majority of patients, intravitreally treated with the three clinically available anti-VEGF agents, showed stable or improved mfERG values with no signs of toxicity for the cones, over a certain time period. Corresponding to the mfERG results, the dark- and light-adapted 30-Hz flicker cone responses for the ffERG were stable. In contrast, we measured a decrease in the combined rod-cone a-wave amplitude and an even larger decline for the isolated rod b-wave amplitude. Rods dominate the combined ffERG response thus the noted decline was probably due to rods alterations and not to the cones. We can speculate that these ffERG results might be due to described features of the natural course of AMD and aging (Birch & Anderson 1992; Holopigian et al. 1997; Owsley et al. 2016) or to retinal toxicity.

ABCA4-associated retinal degenerations - Prognostic factors

AMD is a well-known disease. But there are other retinal degenerations that can be mistaken for AMD. Especially, smaller atrophic lesions in the macula that not primarily involve the fovea and do not directly present with grave visual impairment can remain asymptomatic under a longer period of time. Sometimes they are an accidental finding or have developed into symptom-giving central visual field defects. Even elderly patients can present with atrophic changes that do not represent AMD. In particular, patients around 50 years of age with findings resembling dry AMD but actually originating from another degenerative macular disease are in the risk zone to be falsely diagnosed with dry AMD. The younger the patient, the more likely it is that another origin of the retinal degeneration is suspected. FAF can be applied to differentiate between *ABCA4*-associated retinal degeneration and age-related macular degeneration. FAF changes can even visualize the severity of different *ABCA4*-associated retinal degenerations. As in AMD, ERG is a valuable follow-up tool in

ABCA4-associated retinal diseases. In the latter, ffERG and mfERG are even irreplaceable diagnostic tools. Because the *ABCA4* degenerations cannot yet be treated, there is an extra focus on finding possible connections between different genetic mutations and phenotypes, and also on discovering parameters that can predict further progress.

We could not associate specific *ABCA4* gene mutations to certain retinal function values. In the literature, there are published surveys about existing correlations (Cremers et al. 1998; Van Driel et al. 1998; Shroyer et al. 1999; Klevering et al. 2005; Fakin et al. 2016; Fakin, Chiang et al. 2016), and no correlations (Yatsenko et al. 2001; Gerth et al. 2002; Burke & Tsang 2011). But as almost expected, we detected the homozygous mutations more frequently in group 3 with the most severe defects in the visual field, OCT, FAF, the largest decline in the ff- and mfERG amplitudes, and the most increased IT. Interestingly, 71% of our patients presented a more progressive form of degeneration exceeding the central 10° of their visual fields. In accordance with our data, other studies have reported normal ffERG in STGD (Lois et al. 2001; Cella et al. 2009) but increasing ffERG impairment in CRG (Maugeri et al. 2000; Lois et al. 2001; Campa et al. 2011), which represents the more aggressively progressing retinal degeneration of the two. Especially, surveys of patients with RP had earlier presented prolonged IT as a sign of progression (Berson et al. 1969; Berson 1993; Hood et al. 1998; Seeliger et al. 1998), whereas fewer had published that result in patients with *ABCA4* retinal degenerations (Eksandh et al. 2001; Kjellstrom 2014; Fakin et al. 2016). The obtained changes in retinal function correlated well with changes in the retinal structure, as also reported by others concerning OCT and ffERG (Testa et al. 2012; Fakin et al. 2016).

Future perspectives

Since the introduction of the intravitreal anti-VEGF treatment, we have seen impressive developments in treatment approaches and improved outcomes for our patients. Our future goal is to become even more efficient. One important issue is to increase the awareness of nAMD among the public, including opticians and primary care personal. This would shorten the time to referral and the time to treatment (Rasmussen et al. 2015) for these patients, thereby improving important factors for treatment success. Electronic devices are also under evaluation, such as an application with a self-test to uncover low degrees of visual changes in nAMD (Winther & Frisén 2015), or an OCT home device with a self-measuring software to reduce clinical visits and hopefully detect a treatment-requiring recurrence in time (Maloca et al. 2018). Moreover, machine learning approaches are being tested to predict the required future number of injections (Bogunovic et al. 2017) or to assist us physicians in

analyzing the increasing volume of OCT images (Chakravarthy et al. 2016; Venhuizen et al. 2017). Nonetheless, I am convinced that it holds potential to improve efficiency in patient care and in the use of resources and costs in the health care system. I look forward to taking part in this progress.

For patients with *ABCA4*-associated retinal degenerations, we have no treatment yet. Gene therapy is still at an early stage, such as the ongoing StarGen study for patients with STGD (NCT01736592). There are also drug trials, for example with the aim of interfering with A2E and lipofuscin accumulation, ALK-001 (NCT02402660). Stem cell transplantation is also considered a promising treatment but is still in its preclinical stage (Claassen et al. 2019). In the future, I would like to be able to not only look for predictors of progression but also compare different treatment outcomes in macular degenerations, or in the best of all cases to prevent the development of any pathological changes.

Overall, it is an exciting era for retinal researchers.

Conclusions

Caucasians in particular represent a group of patients predisposed to develop AMD and to be exposed to the increasing prevalence of AMD, with about 10% of those developing nAMD. Even if the progress is slow, we should work continuously to swiftly implement and adapt data from randomized clinical trials into real-life settings, not only with the aim of improving treatment outcomes but also to point out difficulties in the process of doing so. Our data emphasized the value of consistency and adherence to treatment regimens, and of evaluation and improvement of treatment approaches over time. In addition to randomized clinical trials, we looked at real-world data from the SMR that enables us in Sweden to compare our local outcomes nationally and with international results. We pointed out that low baseline visual acuity, worse-seeing eye treated, older age and a larger membrane size were the baseline characteristics from the SMR that had significantly predicted a lower visual outcome of ≤ 35 letters during a two-year follow-up.

The electrophysiological evaluation of retinal diseases adds valuable tools with different purposes but of equal importance for the affected patients.

In nAMD, we demonstrated that cones were not negatively affected by the intravitreally treatment with aflibercept over 18 months, whereas we found a decrease in rod and combined rod-cone function that needs to be further investigated.

In *ABCA4*-associated retinal degenerations, specifically patients with Stargardt disease and cone-rod dystrophy, OCT, FAF, ffERG, and mfERG helped us to evaluate the morphological and functional features of a uniquely large cohort of Swedish patients. We also proposed values of the cone 30 Hz flicker IT as a possible predictor of the future disease progression and visual outcome prior to severe measurable signs of disease. Electrophysiology might be used to follow up and evaluate future treatments for these hereditary diseases, as currently in nAMD.

Hopefully, we were able to raise your awareness of nAMD and its different treatment regimens, the need to start early and persistent treatment, and the possible predictive values in nAMD and *ABCA4*-associated retinal diseases to improve the lives of our patients.

Populärvetenskaplig sammanfattning

Bakgrund

I västervärlden är åldersrelaterad makuladegeneration (AMD) den vanligaste orsaken till grav synnedsättning hos människor över 50 år. Förekomsten av AMD ökar med åldern och 30% av personer över 75 år har någon typ av AMD, den torra eller våta formen, som drabbar det centrala seendet. Våt AMD utvecklas hos ungefär 10% av alla AMD patienter och orsakas av nya, läckande blodkärl i gula fläcken som obehandlade kan leda till en snabb synförsämring. Tidigt i förloppet kan patienter uppleva olika synfenomen, exempelvis en suddig fläck eller krokseende, där raka linjer uppfattas som krokiga, i det centrala synfältet.

Däremot utgör ärftliga makulaförändringar pga förändringar i *ABCA4* genen en vanligare orsak till varierande grader av synnedsättning och synfältsdefekter hos personer i arbetsför ålder. Det finns över 1000 kända mutationer i genen och man kan idag utifrån genotyp inte avgöra hur sjukdomen kommer utvecklas. Symptomen som nedsatt synskärpa och ökande ljuskänslighet debuterar för de flesta patienter smygande redan i barndomen i 6- till 20-års åldern. Sjukdomen kan påverka näthinnefunktionen i olika grad från enbart defekter i det centrala synfältet till synförlust av det centrala och perifera synfältet. *ABCA4*-associerade sjukdomar är i dagsläget inte behandlingsbara men kan förhoppningsvis inom snar framtid bli föremål för genterapi. Genom elektrofysiologiska och andra undersökningsmetoder kan man kartlägga de olika formerna och på så sätt bättre förutsäga prognosen för patienten, samt vilka som kan lämpa sig för genterapi. Dessutom är det viktigt för dessa unga patienter att utifrån prognosen kunna planera sin framtid.

Våt AMD har de senaste åren fått nya behandlingsmöjligheter genom antitillväxtfaktor läkemedel (anti-VEGF). Det finns två godkända läkemedel, ranibizumab och aflibercept, och ett icke-godkänt preparat, bevacizumab, för ögonbruk. Dessa injiceras i ögats glaskropp med olika tidsintervall. Vi vet dock inte hur syncellerna påverkas av aflibercept vid kontinuerlig användning, då VEGF även behövs för gangliecellernas överlevnad. Därför är det viktigt att undersöka syncellernas funktion i centrala och perifera näthinnan. Under tiden har det också

utvecklats olika behandlingsstrategier som utvärderas via större eller mindre studier, prospektivt eller retrospektivt. Nationella register som det Svenska Makularegistret (SMR) hjälper till så att varje registrerande klinik kan följa, bedöma och jämföra sina egna eller landets behandlingsresultat av våt AMD och även andra näthinnesjukdomar som behandlas med anti-VEGF.

Syfte

Syftet med avhandlingen var att identifiera faktorer som kan bidra till att prediktera synutvecklingen hos patienter med retinala degenerativa sjukdomar i makula, samt att utvärdera resultaten av olika behandlingsschema för våt AMD vid olika tidpunkter sedan debut av anti-VEGF behandlingen.

Projektbeskrivning och resultat

I första projektet utvärderade vi behandlingsnaiva patienter med våt AMD från det Svenska Makularegistret under två års tid. Målet var att identifiera faktorer från registret som förknippades med lågt visusutfall under uppföljningsperioden. Riskfaktorer för ett sämre visusresultat var högre ålder, lägre synskärpa, större lesion, och att ögat med sämre syn behandlades. Utöver detta uppmärksammades att dessa patienter hade fått färre antal injektioner än patientgruppen med bättre slutvisus. Patienter som debuterade med ett högre utgångsvisus hade en lägre risk att sjunka till behandlingsgränsen.

I det andra projektet följde vi två olika tidskohorter (2007-2010; 2009-2013) av patienter med nydebuterad våt AMD och deras behandling med ranibizumab. Strax innan den första kohorten startade hade ranibizumab blivit godkänt i Sverige. Vi jämförde kohorternas visusutfall, erhållna antal injektioner samt livskvalitet via en enkät (NEI VFQ-25). Den senare kohorten visade ingen ökning i antal injektioner eller förbättrat visusutfall men en tendens till förbättring av den generella livskvaliteten i jämförelse med den första kohorten.

Det tredje projektet var en klinisk prospektiv studie av behandlingsnaiva patienter med våt AMD som behandlades med aflibercept och två olika behandlingsstrategier med 18 månaders uppföljningstid. Vi bedömde behandlingsresultatet utifrån visusutfall, retinal tjocklek, elektrofysiologi, antal injektioner samt injektionsintervall. Synskärpa och retinal tjocklek förbättrades i båda grupperna. Mellan arm 1 och arm 2 fanns ingen signifikant skillnad avseende antal injektioner över tid, 11.3 ± 1.7 injektioner i arm 1, 10.9 ± 2.0 i arm 2. Arm 1 och arm 2 visade inte heller någon

signifikant skillnad vid bedömning av injektionsintervallet vid månad 18, 9.2 ± 3.4 veckor i arm 1 mot 9.5 ± 3.1 i arm 2, eller det maximala injektionsintervallet under uppföljningsperioden, 10.2 ± 2.0 veckor i arm 1 och 10.5 ± 2.1 veckor i arm 2. Den elektrofysiologiska undersökningen av gula fläcken kunde inte detektera några toxiska näthinneförändringar i samband med aflibercept behandlingen över 18 månader. Däremot visade elektrofysiologiska undersökningar av hela näthinnan att det kombinerade stav-tappsvaret samt det isolerade stavsvaret hade blivit sämre över tid.

I projekt nummer fyra delades patienter med *ABCA4*-associerade retinala degenerationer i tre grupper beroende på graden av synfältsinskränkning. Vi jämförde tidiga och sena morfologiska samt elektrofysiologiska undersökningsfynd. Patienter visade en tydlig korrelation mellan synfältsdefekter och näthinnas funktion samt morfologi. De elektrofysiologiska undersökningarna mätte en förlängd överledningstid av det isolerade tappsvaret för hela näthinnan som verkade vara prediktiv för den långsiktiga progressen jämförd med tidigare undersökningssvar.

Slutsatser

Vi har visat på vikten av att använda registerdata vid uppföljning av olika näthinnesjukdomar. Hos patienter med våt AMD kan man redan vid behandlingsstart ta hänsyn till prognostiska faktorer som är karakteristiska för den svenska befolkningen. Genom data från ett annat svenskt register har vi hittat en möjlig elektrofysiologisk prediktor, en tidigt förlängd överledningstid, för att förutse den individuella degenerationsprogressen hos patienter med ärftliga *ABCA4* förändringar i näthinnan.

Processen att implementera och successivt förbättra en ny behandling inom den kliniska vardagen har inte kunnat förbättras signifikant på några år som jämförelsen mellan våra två svenska tidskohorter visar. Det bekräftar att man tidigt bör fokusera på och följa upp att ett nytt system används så effektivt som möjligt, samt att alla nödvändiga resurser används på bästa sätt.

Under en 18 månaders uppföljning av intravitreal aflibercept behandling har den retinala funktionen i gula fläcken varit stabil och inte visat några toxiska effekter. Däremot har stavarnas funktion påverkats under tiden, och den möjliga orsaken måste utredas vidare. Utöver detta har vi jämfört två populära behandlingsregimer med aflibercept och kan presentera likvärdiga behandlingsresultat för nAMD patienter under uppföljningsperioden. Det är värdefullt för våra, oftast äldre, nAMD patienter att ha två effektiva behandlingssätt då det ena kan passa bättre till den aktuella levnadssituationen än det andra.

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