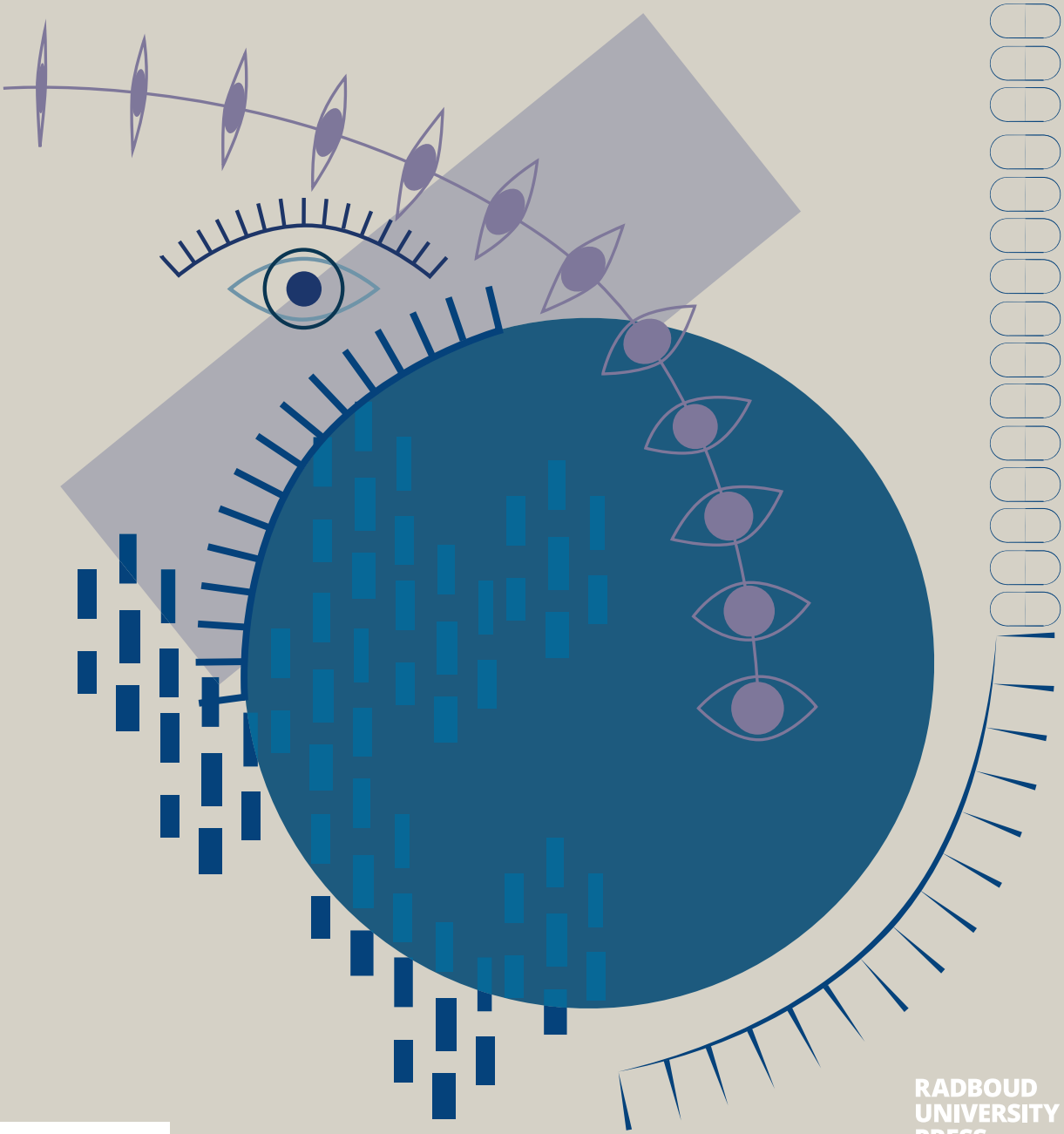
Prognostics of age-related macular degeneration



Francesco Cinque

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General Introduction

Age-related macular degeneration (AMD) is a highly prevalent ophthalmic disease with potentially severe consequences for patients' wellbeing. It represents the primary cause of irreversible blindness among the elderly in the West.(1) AMD is characterised by small, yellowish deposits named drusen which lie underneath the retinal pigment epithelium (RPE).(2) Drusen themselves typically do not cause visual disturbances and may as such go unnoticed. However, AMD is a progressive disease and in term might affect normal function of the retina leading to visual impairment and poor quality of life.

Classification

Within AMD there exists a spectrum of features visible on imaging modalities such as colour fundus photography which change according to disease severity. Different interpretations of cut off points delineating its stages have been proposed. This thesis embraces the stage definitions employed by the Rotterdam Study which is an ongoing cohort study in Rotterdam in the Netherlands.(3)

The definitions are as follows: RS stage 0 corresponds to no signs of age-related macular degeneration (AMD) at all or hard drusen <63 μm) only. RS stage 1: Soft distinct drusen (≥63 μm) only or pigmentary irregularities only, no soft drusen (≥63 μm). RS stage 2: Soft indistinct drusen (≥125 μm) or reticular drusen only, soft distinct drusen (≥63 μm). RS stage 3: Soft indistinct (≥125 μm) or reticular drusen with pigmentary irregularities. RS stage 4: Atrophic or neovascular AMD.(4) A further distinction is made between advanced (RS stage 4) and non-advanced disease (RS Stage 1 to RS stage 3).

Epidemiology

AMD is highly prevalent disease, especially within the elderly. It is important to note that estimates of prevalence and incidence are affected by the choice of grading system.(5) The numbers below take this into account and are consistent with the Rotterdam criteria.(4) First, prevalences for non-advanced AMD in the Western-European population range from 3.5% (95% confidence interval [CI] 2.1%–5.0%) at 55–59 years to 17.6% (95% CI 13.6%–21.5%) in people aged ≥85.(1). In contrast, prevalence of advanced AMD (RS stage 4) ranges from 0 to 9.8% (95% CI 6.3%–13.3%) in people aged 85 and older.(1) In general, among those who are affected with advanced disease, approximately

two-thirds is affected by neovascular AMD. Globally the prevalence of any AMD is between 8 and 8.7%.(6)

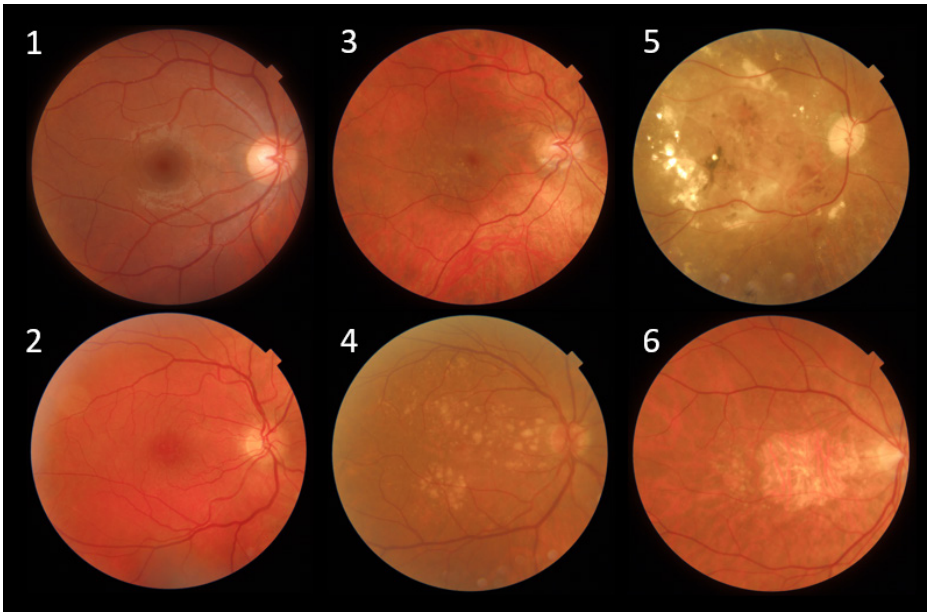

Figure 1. Example of Rotterdam Classification

RS stage 0: no drusen or hard drusen only.
RS stage 1: Soft distinct drusen (≥63 µm) only or pigmentary irregularities only, no soft drusen (≥63 µm).
RS stage 2: Soft indistinct drusen (≥125 µm) or reticular drusen only, soft distinct drusen (≥63 µm).
RS stage 3: Soft indistinct (≥125 µm) or reticular drusen with pigmentary irregularities.
RS stage 4: Neovascular age-related macular degeneration.
RS stage 4: Atrophic age-related macular degeneration.

The 2-year cumulative incidence of advanced AMD is .2% for people of 55 and older and increases with age.(7) Pooled results indicate a 5-year incidence of .7%.(8) Lastly, projected estimates of affected people in 2040 in Europe show an almost doubling of 14.9 to 21.5 million non-advanced AMD and 3.9 to 4.8 million advanced AMD cases.(1) This increase is due in large part to an ageing population.

Impact of AMD on vision-related quality of life

AMD has a pronounced negative impact on vision-related quality of life (VRQoL). VRQoL is a multifaceted or i.e. multidimensional concept which includes domains

such as emotional well-being, social participation, and economic considerations.(9) VRQoL is typically though not necessarily measured via a psychometric instrument, commonly referred to as a patient-reported outcome.(9-11) While VRQoL is a valuable measure, its multidimensionality might be challenging to interpret since it cannot be represented by a single scale.(9, 11)

Figure 2. Representation of Visual Limitations as Experienced by Patients with GA or CNV

Figure 2 shows a representation of how stage 4 (neovascular) AMD (1) and stage 4 (atrophic) AMD might be experienced by the patient. Figure 2.1 is a normal picture as experienced by someone with clear vision. Figure 2.2 shows metamorphopsia, a type of visual distortion, while 2.3 shows a central scotoma. Depending on the severity, visual acuity might be completely lost in later stages while peripheral vision is usually spared. Loss of central visual function limits the patient's ability to recognize faces amongst others (social blindness).

In AMD, patients affected by non-advanced disease typically experience mild visual disturbances or none at all.(12, 13) In contrast, the negative impact of advanced-AMD on VRQoL has been well studied. For example, AMD patients experience higher rates of depression compared to non-affected controls.(14) Moreover, AMD patients experience difficulty with many everyday tasks such as shopping, mobility, computer use, watching TV, reading, driving, self-care and face recognition.(14) Lastly, AMD patients who experience visual impairment are at an increased risk of falls and related injuries.(15) Loss of VRQoL extends to healthcare providers and health care systems around the world. Estimates of costs vary across countries on account of numerous reasons such diverse insurance systems and different populations. In the Netherlands, the costs of treatment with anti-VEGF agents alone was 50 million euro's in 2019.(16) Several measures correlate to VRQoL. Visual acuity of the best seeing eye in particular, is strongly associated with VRQoL.(17)

Neovascular AMD

The term 'neovascular AMD' comprises a host of terms that are sometimes erroneously used interchangeably. With the advent of increasingly sophisticated imaging techniques historical terms have become ambiguous or are now reserved for very specific phenomena. Therefore, this thesis makes use of the Consensus on Neovascular AMD Nomenclature (CONAN) which is a relevant effort to define terms associated with neovascular AMD.(18) Below is a partial list of the most important terms used in this thesis as defined by CONAN:

> *Macular neovascularisation (MNV) denotes neovascular disease in the macula from many causes. In AMD, the neovascularization may start in the outer retina, and therefore, the term choroidal neovascularization (historical term) is not appropriate for the class. MNV's might disrupt the blood-retina barrier and may leak deleterious protein material into their surroundings thereby disrupting and ultimately destroying the sensitive overlying neurosensory retina. The term 'leakage' is synonymous with 'exudation' and refers to the process of leaking this material.*

Intraretinal fluid is the result of leakage. In case of leakage, local retinal fluid removal capability is exceeded leading to accumulation of the fluid in retinal thickening and formation of cystoid spaces. The fluid in the retina may come from retinal vessels or a subretinal source if the external limiting membrane is not intact. Subretinal fluid is fluid resulting from leakage. Fibrosis is the build-up, in any layers of the retina, the subretinal space, the RPE monolayer, or the sub-RPE space, of tissue with significant collagen deposition.

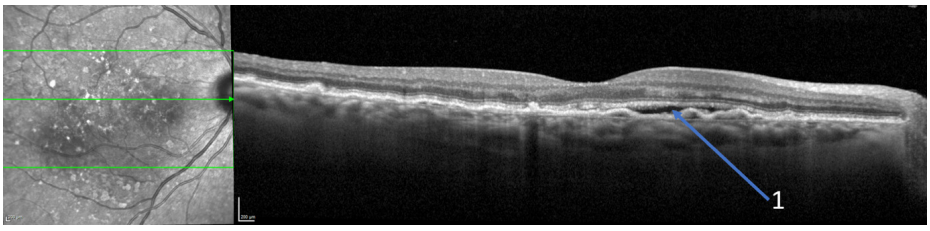

Figure 3. Subretinal fluid

Figure 3 shows an spectral-domain optical coherence tomography of patient with an MNV. The arrow (1) shows subretinal fluid.

MNV may be further differentiated according to anatomical properties of the neovascularisation. A type 1 MNV originates from the choriocapillaris and grows below the RPE. A type 2 MNV originates from the choroid and invades the subretinal space, thus traversing Bruch's membrane and the RPE. A type 3 lesion originates from the retinal circulation grows downwards to the outer retina. Regarding the clinical utility of differentiating types of MNV, it should be noted that all MNV, regardless of type are preferably treated with anti-vascular endothelial growth factor (anti-VEGF) agents. Clinically, over a 12 and 24 month period visual outcomes between types differ.(19, 20) However, the differences are small, and it is unclear if they persist over time. Of note, other vascular structures within the AMD-spectrum exists but they are less important for the present work.

Neovascular AMD is experienced as acute loss of visual acuity and metamorphopsia. If left untreated, visual acuity will continue to deteriorate. In fact, untreated neovascular AMD progresses to legal blindness in 80% of cases within two years.(21)

Atrophic AMD

To recapitulate, advanced AMD encompasses two distinct phenotypes which sometimes exist in tandem. Patients with atrophic AMD suffer distinct complaints compared to neovascular AMD. Similar to neovascular AMD, the term 'atrophic AMD' comprises various terms that are sometimes used interchangeably. In particular 'geographic atrophy' (GA) is often used synonymously with atrophic AMD. It may be thought of by some to exclusively refer to eyes with no signs and or history with neovascular AMD.(22) In this thesis, we follow the definitions set by the Classification of Atrophy Meetings (CAM).

Atrophy means a shrinking or withering, particularly because of poor nutrition or disuse. In the context of AMD, the term atrophy means either loss of tissue (typically) or the irreversible attenuation of tissue.(23) CAM proposes that the main term should be complete retinal pigment epithelium and outer retinal atrophy (cRORA). cRORA is defined using optical-coherence tomography - a vital imaging modality - as follows: (1) a region of hypertransmission of at least 250 µm in diameter, (2) a zone of attenuation or disruption of the RPE of at least 250 µm in diameter, (3) evidence of overlying photoreceptor degeneration, and (4) absence of scrolled RPE or other signs of an RPE tear.(23) GA, following

CAM, is reserved for eyes without MNV and is thus a subset of cRORA. Although it should be noted that this distinction cannot always be made conclusive since GA and neovascular AMD follow from the same disease process.(22)

Clinically, atrophic AMD is marked by the advent of an absolute scotoma that increases over time. The scotoma corresponds to the size of the atrophic lesion in the macula. Visual acuity is often not affected until much later because, importantly, the fovea is often not involved until very late in the disease (foveal sparing).(24, 25) In both atrophic and neovascular AMD, the patient experiences an absolute scotoma in end-stage disease and peripheral vision is often spared.

Risk factors for AMD

The term 'risk' is ubiquitous in medical science. In epidemiology 'risk' or 'risk factor' usually refers to a statistical relationship between an independent variable and a dependent variable. Inference about the causal nature of the relationship is suspended. In fact, the term risk ratio is reserved for calculating the strength of association between two variables within a cohort study as opposed to odds which are calculated in cross-sectional studies. However, it should be noted that while technical interpretations of risk exists, everyday practice including in medical science is resistant to strict technical usage of the term.

The following is a summary of the most significant clinical risk factors for AMD which is considered multifactorial. Age is the most consistently reported independent risk factor. (7, 26-29)

A systematic review found an additional association between current smoking and cataract surgery. (25) However, the association between cataract surgery and advanced AMD is likely due to confounding factors such as advanced, as both share similar risk factors. (30)

In contrast, pooled ORs for sex, race, iris colour, body mass index, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, serum cholesterol, serum triglycerides, and plasma fibrinogen were insignificant.(27) Notably, there are no systematic reviews that specifically differentiated clinical risk factors for atrophic versus neovascular AMD. There is also increasing evidence that a diet rich in fibre and omega-3 fatty acids may protect against advanced AMD.(31, 32)

Genetics

Genetic susceptibility plays an important aetiological role in AMD. Genome-wide association studies (GWAS) have played a key role in discovering genetic variants associated with AMD. GWAS uses a dataset of cases and controls which have been genotyped using a DNA-array. It compares the allele frequency of a certain genomic region across groups. A landmark GWAS by Fritsche, *et al.* identified 52 genetic variants at 34 loci that are independently associated with AMD. Nineteen of these variants are located in or near genes involved in the complement pathway.(33) Other pathways that are implicated include extracellular matrix remodelling, lipoprotein metabolism, and angiogenesis.

We may define heritability as the extent that a phenotypical variance in a population is explained by genetic variation.(34) Narrow-sense heritability (h^2) is the proportion of *additive* genetic variation.(35) Broad-sense heritability includes dominance and epistasis which are genetic terms describing interactions. Earlier estimates of AMD's narrow-sense heritability were estimated between 46 and 71 percent.(36) A 2020 study, using *in silico* pathway analyses estimated h^2 at 42% while the 52 variants identified by Fritsche, *et al.* account for 14%.(35)

Explanations abound for the missing heritability. For one, not all genetic variation is loaded into the arrays used for GWAS. Arrays are predefined and may not feature certain rare variants. Therefore, certain genetic variations may be missed as they are never tested for. Moreover, GWAS does not identify a single variant but rather a genomic region because many variants are inherited together (linkage disequilibrium). Therefore genotyping might be imprecise. Lastly, some common variants might contribute a small signal which is missed due to too low power.(34)

Genetic risks and AMD

Genetic risk scores (GRS) may be used to quantify risk for AMD using variants discovered through GWAS.(37) A GRS is calculated by multiplying the genetic number of alleles times the strength of its association with a particular outcome such as late AMD.(38, 39)

Colijn, *et al.* showed that in a cohort of 17 174 individuals, GRS distributions between cases and controls overlap substantially except for the tails of the normal distributions.(39) Thus, GRS are unsuitable as diagnostic but might be

used clinically in populations with a high *a priori* risk for disease (such as in early stages) or for example, to coach patients towards a better lifestyle.(40, 41)

Pathogenesis

While AMD is caused by a combination of genetic and environmental factors, its pathogenesis is poorly understood. Considering how ageing is paramount in AMD, there is a fundamental distinction that must be made regarding 'age' in AMD. Age might refer to chronological age or biological ageing. Its first use reflects an empirical observation that a certain eye phenotype with drusen is simply observed more frequently in the elderly. If age, however, is interpreted as biological ageing then we are in fact making the realist claim that there is a distinct, ageing-related process going on which is causing the phenotype. However, AMD is not a necessary companion to chronological age, i.e. there are relatively young patients with an AMD phenotype and not all elderly exhibit drusen. Therefore, age-related refers to biological ageing.(42)

Biological ageing is the progressive loss of physiological integrity which leads to function loss and increases the risk of death.(43) Ageing can be further subdivided on a cellular-molecular level as genomic instability, telomere erosion, epigenetic changes and, loss of protein homeostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and alteration in intercellular communications.(44) Biological ageing contributes to AMD since it lowers the threshold for AMD-related pathophysiological processes to take effect.

The macula contains many different cells which do not necessarily age at the same rate. An important distinction is the neuroretina which contains post-mitotic neurons and glial cells, and the RPE which is a single layer of post-mitotic which function as one part of the blood-retina barrier. The retina is among the most metabolically active tissues in the human body and its function is sustained by the RPE. In short, the RPE plays a key role in maintaining retinal homeostasis.

The RPE is susceptible to damage causes by oxidative stress. Exposure of lipofuscin and fatty acids to light results in the production of reactive oxygen species which cause mitochondrial dysfunction and hence energy deficiency. This leads to a vicious cycle whereby RPE cells either become senescent or

experience apoptosis thus burdening neighbouring RPE cells. In addition, stressed RPE cells induce an inflammatory response.(45) Stressed RPE cells release growth factors such as vascular endothelial growth factor (VEGF). VEGF induces vascular permeability and the formation of MNV which are responsible for retinal damage and visual loss.(46)

The complement system also plays an important role in the pathogenesis of AMD. The complement system is part of the innate immune system and fulfils a role in the early removal of pathogens, debris and dead cells. There are over fifty proteins that are either bound to cell membranes or circulate in inactive form in the blood.(47, 48) Activation into active proteins is rapid and occurs mainly through the alternative pathway. The alternative pathway is also most associated with AMD, it describes the continuous "tick-over" of complement factor 3 into C3(H2O) which when in contact with a microbe triggers further activation. The classical pathway functions through antibody-mediated binding of complement component 1q (C1q) to pathogen surfaces. The lectin pathway is the proteolytic cascade operates through of pathogen-associated molecular patterns (D-mannose, *N*-acetyl-D-glucosamine or acetyl groups), on the surface of pathogens or to apoptotic or necrotic cells, by the pattern-recognition molecules mannose-binding lectin.(49)

Evidence for the role of the complement system in AMD is provided through genetic analysis(33), analysis of the contents of drusen(47), higher markers of systemic activation(50, 51), and analysis of vitreous and aqueous humour contents.(52-55)

In sum, it seems clear that complement dysregulation contributes to AMD development. However, its role is not fully understood not least in part because the eye as an immune privileged site does not necessarily mirror systemic complement related changes.(56) A leading hypothesis is that complement overactivation leads to inflammation. Dampening inflammation through inhibition complement activation is a promising avenue for therapy development.(57)

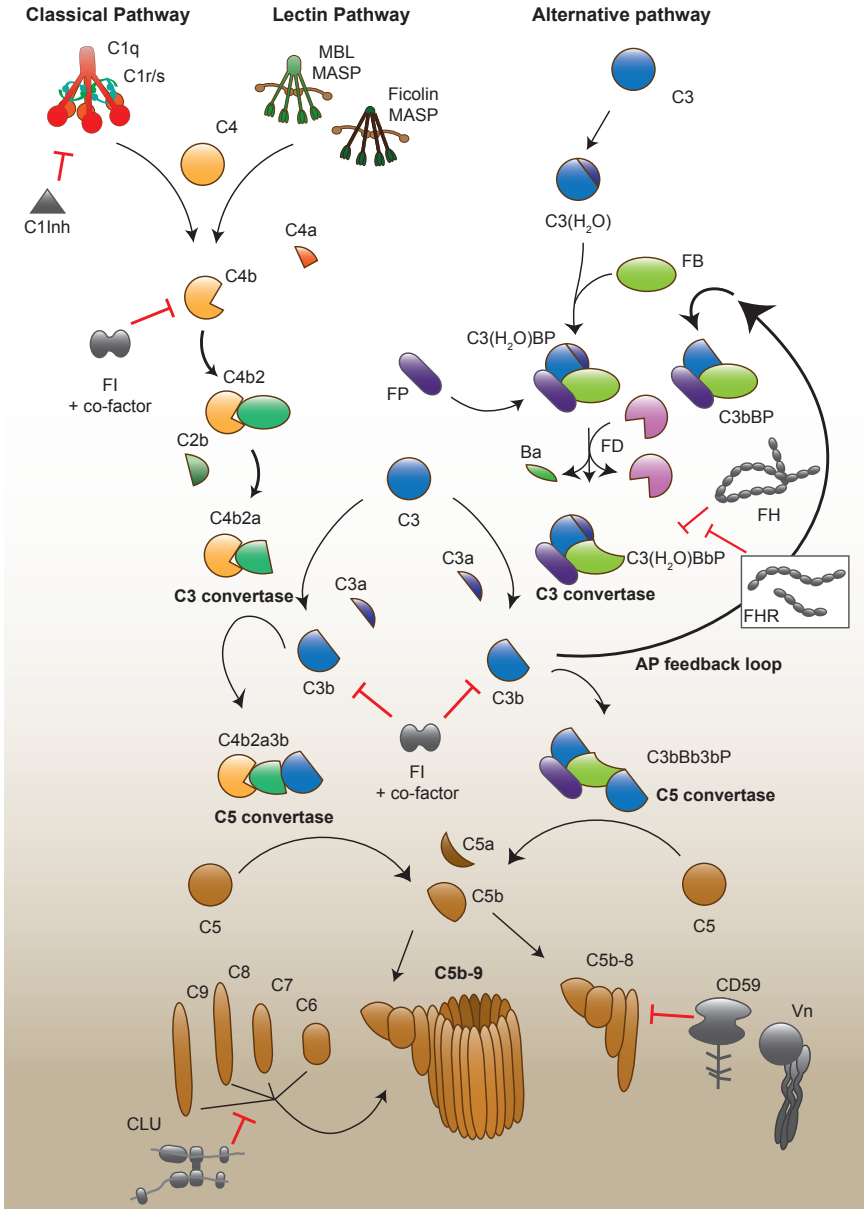

Figure 4. Overview of the Complement System

Figure 4 by De Jong, et al.(47) schematically illustrates the chain reaction that occurs upon activation of the complement system. Various complement factors, such as complement factor 5 (C5) and factor D (FD) shown in the figure, contribute to this activation. They do so by binding to other factors or by cleaving formed complexes. Additionally, there are factors that inhibit the system, such as factor H (FH) depicted in the figure. Disruption of this balance can occur due to changes in DNA that lead to increased or decreased production of certain factors. This results in the heightened activation of the complement system, as observed in AMD.

Prevention

The risk of developing age-related macular degeneration (AMD) can be influenced by lifestyle choices. All AMD patients are strongly advised to stop smoking, eat a diet high in fibre and Omega-3, and engage in regular exercise plenty.(58) The impact of these health-enhancing behaviours might particularly reveal themselves in individuals with high genetic susceptibility.(39)

Additional preventative strategies include supplementation. Lutein containing supplements are likely to diminish the risk of progression to advanced AMD especially in high risk patients.(59) AREDS group performed two large scale randomized studies which showed a decrease of progression to late AMD of 25% to 19%. The original AREDS formula has been altered slightly since its first inception. The AREDS-2 formula contains lutein (10mg), zeaxanthine (2 mg), zink (25-80 mg), vitamine C (500 mg) and vitamine E (400 IE).(60, 61).

Treatment of neovascular AMD

Neovascular AMD has since 15 years successfully been treated by intraocular injections that contain drugs which inhibit VEGF (anti-VEGF).(62) Treatment is initiated with a loading phase after which the clinician attempts to minimize the number of injections needed while preserving good visual acuity and absence of sub- and intraretinal fluid on OCT.

The first FDA-approved anti-VEGF, pegaptanib, has since fallen out of favour.(63) Currently there are four anti-VEGF drugs that are administered globally, namely, bevacizumab, aflibercept, ranibizumab and recently approved, brolucizumab. Bevacizumab, ranibizumab, and brolucizimab are humanised monoclonal antibodies that bind to VEGF and thus prevent VEGF to bind VEGFR-1 and VEGFR-2 expressed on the surface of endothelial cells. Aflibercept is a recombined fusion protein containing a fragment of human VEGF receptors combined with part of a human IgG1 receptor, which together bind VEGF with higher affinity than VEGF receptors.

Legal and financing disparities across countries influence the choice of drugs. All anti-VEGF show safe and effective treatment outcomes.(64, 65) Bevacizumab is officially off-label for AMD as it is registered for breast cancer.

However, bevacizumab is much cheaper than its counterparts and therefore remains the drug of first choice in the Netherlands.

The latest anti-VEGF is brolucizumab which was approved in the European union in 2022. The comparative advantage of brolucizimab is that relatively long treatment intervals can be achieved thereby diminishing the need for intraocular injections. All intraocular injections carry a small risk endophthalmitis (≈0.021%). The probability of endophthalmitis increases as the number of injections increases.(66) However, clinical trials have shown an increased risk of intraocular inflammation and vasculitis.(67) Therefore, it remains the fourth drug of choice in the Netherlands.

While anti-VEGF treatment is clearly a resounding success, not all patients benefit. Some are unresponsive to existing treatment while some become unresponsive over time. Clear figures are not available in part because treatment outcomes are shifting due to advancement of imaging techniques. (68) It seems clear however, from clinical experience that current treatment options for neovascular AMD might be improved upon. This is further underscored by the intensive nature of anti-VEGF treatment since successful treatment requires continual monitoring.(69)

Treatment of atrophic AMD

There is currently no treatment of atrophic AMD in the Netherlands. However, this may change; Following a string of failed randomized controlled trials which tested complement inhibitors on atrophic AMD, finally, two drugs have met their target. The US food and drug administration (FDA) approved pegcetocaplan in February 2023, a complement inhibitor targeting C3 following a 22% reduction lesion size growth in the DERBY-trial.(70, 71) An additional complement inhibitor (targeting C5), avancincaptad pegol, was approved following a 27 and 14% reduction in lesion growth from the GATHER1 and GATHER2 trials.(72, 73)

While FDA approval is a sure sign that atrophic AMD will be a treatable disease in the near future, some important challenges have yet to be met. There has been some controversy on whether the trials provide enough evidence of the drugs' efficacy. In fact, the European Medicines Agency did not approve pegcetacoplan citing lack of evidence of clinically meaningful benefits

for patients receiving intraocular injections.(71) In addition, intraocular complement inhibitors might lead to increased risk for MNV development and endophthalmitis and have been criticised precisely for that reason.(74)

Aims and outline of this thesis

The aim of this thesis is to study clinical and genetic prognostic factors for AMD. Accurate appraisal of prognostic factors will hopefully improve the accuracy of prognoses and thereby improve clinical care.

Prognostic factors may be patient-specific; they can vary from person to person and within the same person. This is important in medicine because in some cases, earlier or later could make all the difference. This idea of patient-specific windows of opportunity has been known as personalised medicine and is defined by the Horizon 2020 Advisory Group as 'a medical model using characterisation of individuals' phenotypes and genotypes (e.g. molecular profiling, medical imaging, lifestyle data) for tailoring the right therapeutic strategy for the right person at the right time, and/or to determine the predisposition to disease and/or to deliver timely and targeted prevention.'(75)

In **Research Chapter One** we studied the possible relationship between choroidal thickness and anti-VEGF treatment. Currently, neovascular AMD patients are treated chronically with anti-VEGF. Anti-VEGF might induce atrophy via thinning of the choroid, the major blood supply to the retina. Therefore, choroidal thinning has been hypothesized to be a major risk factor for atrophy.

In **Research Chapter Two** the risk of late AMD for patients carrying a rare, protein-changing variant in *CFI* or *CFH* was studied. Considering the key role of the complement system in AMD as outlined above, we hypothesized that carriers progress faster. In addition, several clinical outcomes related disease progression such as GA-size were studied

Research Chapter Three re-evaluates the risk of exudation in patients with a non-exudative MNV. Non-exudative MNV have been purported to carry a very high risk of exudation. In this study, we highlight how observation of non-exudative MNV in unilateral patients with longer young onset first eye involvement affects risk perception.

Research Chapter Four explores the ability of mesopic microperimetry to measure disease progression. Accurately and reliably measuring progression is key in prognostication and in future trials. In an effort to increase the sensitivity of mesopic microperimetry to disease progression, different calculations of mesopic microperimetry data have been performed. In this study we explore the effect of this calculations on the ability of mesopic microperimetry to capture pathological change over time.

Research Chapter Five explores different candidate outcomes from mesopic microperimetry, a psycho-functional test of retinal health in terms of visual function, or the extent through which visual deterioration hinders everyday activities such as reading. Since mesopic microperimetry measures a larger proportion of retinal surface, we hypothesized that that mesopic microperimetry outperforms visual acuity.

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Research Chapter One

Comparable choroidal thickness between eyes treated eyes and untreated fellow eyes in patients with unilateral neovascular age-related macular degeneration

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Abstract

Purpose

To investigate the potential effect of anti-VEGF treatment on choroidal thickness (CT) in unilateral neovascular age-related macular degeneration (AMD) patients.

Method

This is a cross-sectional study where patients were included as part of an ongoing prospective study which included patients with unilateral neovascular (n) AMD. The fellow eye served as control. All patients had spectral-domain optical coherence tomography (SD-OCT) with enhanced depth imaging (EDI) done at every visit. CT was measured independently by two graders at five locations: subfoveal, 1500 micron temporal and nasal, 3000 micron temporal and nasal. The average of the measurements was used after statistical verification of their accuracy. CT differences were initially analysed via a paired T-test and later via multiple linear regression. Variables such as number of injections were studied and presence of geographic atrophy (GA) in fellow eyes was evaluated via SD-OCT.

Results

A total of 112 patients met the inclusion criteria (Female 67%). The median (IQR) years of treatment was 2.6 (4.1). The subfoveal choroidal thickness (SFCT) in the neovascular (NV) eye appeared thinner in the NNV eye initially (-11.0 µm difference between NV and NNV SFCT (CI -23.4 to 1.3). However, after age-adjustment this trend disappeared (CI -29.8 to 4.6). In fact, apart from age (CI -6.2 to -0.1)), no other variable including number of anti-VEGF injections (CI -1.5 to 1.4) predicted SFCT. Presence of GA in fellow eyes did not influence the SFCT compared to non-GA fellow eyes, difference (CI -59.7 to 46.6).

Conclusions

This study shows no statistically significant CT difference in NV versus NNV eyes. There was no relationship between number of injections and CT.

Introduction

Age-related macular degeneration (AMD) is a progressive, neurodegenerative macular disease and the leading cause of visual impairment in the Western world. (1) Intravitreal injection with anti-vascular endothelial growth factors (anti-VEGF) is the mainstay treatment for exudation secondary to neovascular AMD. It is current practice in the western world to aim for a fluid-free optical-coherence tomography (OCT) image which has also resulted in a substantial treatment burden.(2) There is a high risk of disease-recurrence if anti-VEGF treatment is discontinued.(3) Considering our ageing population, the number of patients that receive continuous anti-VEGF injections will continue to grow.(1) Concerns have arisen as to whether chronic anti-VEGF treatment increases the risk of secondary macular atrophy.(4-6)

In the CATT-study, the percentage of patients with macular atrophy increased from 17 at year two to 38 at year five.(7) In the IVAN trial, a higher number of eyes with macular atrophy was observed in the monthly treated group compared to the as needed group (34% vs. 26% $P = .03$).(8) Overall one-quarter of neovascular AMD patients treated with various anti-VEGF developed macular atrophy within 1 to 2 years following treatment initiation.(4) Choroidal thinning is one of the main proposed mechanisms through which macular atrophy might be induced.(4) All commonly used anti-VEGF have been associated with decreasing of CT, particularly during the loading phase.(9-14) Koizumi, *et al.* for example reported a decrease of 35.8 µm in eyes receiving aflibercept treatment after 12 months.(10) There is limited evidence that choroidal thinning persists after long-term anti-VEGF treatment. One notable exception is Govetto, *et al.* who reported that choroids in NV-eyes were thinner than their fellow eye counterpart (subfoveal: 200.69 micron vs. 184.36 micron $P = .02$).(15)

Controversy with regard to the effect of anti-VEGF treatment on CT remains; in a recent large, prospective study there was no association with ranibizumab treatment and macular atrophy.(16) Moreover, a small pilot study of 10 patients with geographic atrophy with secondary macular neovascularisation did not observe an increase of geographic atrophy enlargement post-treatment.(17)

This study investigates the effect of anti-VEGF injections on CT and specifically addresses the potential concerns over long-term treatment with anti-VEGF for NV-AMD by comparing CT between these unilateral treated eyes and the fellow non-neovascular (NNV) AMD eyes.

Method

Design

Data was collected from Jan 2018 to jan 2022 as part of a prospective study at Radboud university medical centre. In this cross-sectional study, we measured choroidal thickness (CT) at baseline visit. All patients had neovascular AMD in one eye which was treated with, at minimum, 3 non-specified anti-VEGF injections. Exclusion criteria included previous photodynamic therapy, pathological myopia, advanced glaucoma, or other retinal diseases such as diabetic retinopathy that could interfere with the diagnosis of AMD. Patients were subjected to a comprehensive dilated eye-exam that included extensive imaging such as Spectral-Domain Optical Coherence Tomography (SD-OCT) with Enhanced Depth Imaging (EDI) [SpectralisTM HRA+OCT (Heidelberg Engineering, Heidelberg, Germany)]. In addition, visual acuity (VA) was measured using the Early Treatment Diabetic Retinopathy (ETDRS) letter chart. Ophthalmic history and medical history were assessed using a questionnaire. Ophthalmic history includes the time since first eye involvement to baseline and the number and type of anti-VEGF drugs. This study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the local ethics committee of the Radboud university medical centre, Nijmegen, The Netherlands. All participants provided written informed consent prior to participating.

Grading

CT was measured independently by two graders (FC) and (FT) using the calliper function in Heidelberg. CT was measured from BM to the outer border of the suprachoroid, subfoveally as well as 1500 and 3000 micron in the temporal, and nasal direction.(18) Measurement agreement was statistically analysed using Intraclass Coefficients Correlations (ICC). We used the mean of the graders as our final CT approximation. To increase measurement accuracy for subfoveal measurements, we additionally used Bland-Altman plots to visualise outliers (defined as exceeding a measurement discrepancy of two standard deviation [SD] from the mean SFCT). Outliers were revalued and corrected by experienced retinal specialists (SY and YL). Corrected values were then used for further analyses instead of the mean of the two graders. In addition, the Bland-Altman plots were used to assess the proportionality of measurement discrepancy and hence grader bias. Lastly, OCTs were graded by FC for the presence of complete retinal pigment epithelium and outer retinal atrophy (cRORA) as defined according to the consensus definition of atrophy associated with AMD. (19)

Outcome and Statistical analysis

We used a two-way random effects model for the ICC. We also computed a one-sample T-test for the mean difference of the measurement discrepancies to statistically assess grader bias and report the SD of the measurement discrepancies. Our main outcome is the difference in CT between NV and NNV fellow eyes which was assessed via a paired samples T-test. We also performed an age-corrected SFCT analysis using multiple linear regression. In addition, we modelled the influence of various clinical predictors on SFCT. For SFCT of NV eyes we investigated the relationship between SFCT (dependent variable) and, age, sex, visual acuity, cRORA, smoking, number of injections, type of anti-VEGF drug(s) (bevacizumab, ranibizumab, aflibercept), as independent variables using multiple linear regression. Similarly, for NNV eyes we performed multiple linear regression with SFCT as dependent variable and age, sex, visual acuity, cRORA, smoking as independent variables. We report adjusted R^2 as model performance. *P* values of < .05 were considered statistically significant and all statistical analyses were performed using IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA).

Results

Baseline

In total 116 patients met the criteria. However, in 4 cases the quality of the EDI-OCT was too weak to allow visualisation of the sclero-choroidal junction and thus these cases were excluded. Baseline characteristics of the remaining 112 patients are shown in table (1).

Table 1. Baseline Characteristics

Characteristics	Value N = 112
Time since first eye involvement, median (IQR), range, yrs	2.9 (4.8) 0 to 20
Age, m (SD), years	74.4 (8.2)
Female, No. (%)	75 (67%)
Caucasian, No. (%)	112(100%)
Visual acuity NV eye, m (SD), logMAR	.50 (.43)
Visual acuity NNV eye, m (SD), logMAR	.07 (.20)
cRORA NV eye, No. (%)	24/112 (21%)
cRORA NNV eye, No. (%)	46/112 (41%)
Supplement use, No. (%)	77 (69%)
Months since first eye developed MNV, median (IQR) range	35 (57) 0 - 245
Number of injections, median (IQR) range*	13 (23) 3 - 120
Type of injections, No. (%)	
One type of anti-VEGF	41
Bevacizumab	36 (88%)
Aflibercept	1 (2%)
Ranibizumab	4 (10%)
Combination of anti-VEGF medications	49
Bevacizumab + Aflibercept	21 (49%)
Bevacizumab + Ranibizumab	7 (14%)
Bevacizumab + Aflibercept + Ranibizumab	18 (37%)
Unknown type of injections	22

NV Neovascular, NNV non-neovascular, cRORA complete retinal pigment epithelium and outer retinal atrophy, MNV macular neovascularisation.
*n = 10 did not provide details about number of past injections,

Mean age (SD) was 74.2 (8.3) years. The median (IQR) time since first eye involvement with neovascular AMD was 2.9 (4.8) and ranged from 0 to 20 years. The median (interquartile range, IQR) number of injections received was 13 (23) during the median (IQR) 2.6 (4.1) years of treatment. Baseline visual acuity (SD) was 0.50 (.4) logMAR for NV eyes and .08 (.2) logMAR) for

NNV eyes. Of the 41 patients who were treated with one type of anti-VEGF, the majority (88%) was treated with bevacizumab. The 49 patients who were treated with a combination of anti-VEGF medications were most often treated with a combination of bevacizumab and aflibercept (49%). (Table 1.)

Grader agreement

The lowest ICC was measured at the 3000 micron temporal location (.941 NV and .944 in the NNV). Subfoveal ICC's were .991 and .993 for NV and NNV eyes. The other ICC's are reported in supplementary table (1). Bland-Altman plots showed approximately symmetrical measurement discrepancies, therefore not suggesting bias of either grader (see supplementary figure 1).

Statistical analysis confirmed this, as the mean (SD) difference of the measurement discrepancies was 0.0 µm (20.1) (*P* = 1) µm and 2.5 µm (18.6) (*P* =.1) for NV and NNV eyes. An example of the CT measurement as performed by the graders is visible in supplementary figure 2.

Evaluating CT differences

The SFCT difference between NV and NNV eyes was 11 µm (*P*=.08) (CI -23.4 to 1.3) as shown in Table (2) and figure (1). CT seemed thinner in the NV eye at every location, however no difference reached statistical significance. After correction for age, the CI for the difference between NV and NNV eyes widened of the CI (CI -29.8 to 4.6) (*P*=0.15). Also, we checked trends on the effect of age on the difference between NV and NNV SFCT and observed that the difference between NV and NNV SFCT diminished with advancing age (see Figure (2)).

Table 2. Choroidal Thickness at Every Measurement Location

Location	Mean CT NV eye (SD) µm	Mean CT NNV eye (SD) µm	Difference in µm (*P* value)	CI
Subfoveal	231.7 (109.3)	242.7 (110.3)	-11.0 (.08)	-23.4 to 1.3
1500 micron nasal	197.2 (103.0)	206.7 (105.9)	-9.5 (.1)	-21.2 to 2.1
3000 micron nasal	127.5 (70.1)	130.1 (72.7)	-2.6 (.5)	-10.6 to 5.3
1500 micron temporal	220.2 (89.1)	229.7 (87.0)	-9.4 (.1)	-21.1 to 2.2
3000 micron temporal	208.9 (67.3)	214.5 (70.7)	-5.6 (.3)	15.7 to 4.5

Table (2) shows choroidal thickness at every measurement location including statistical results.

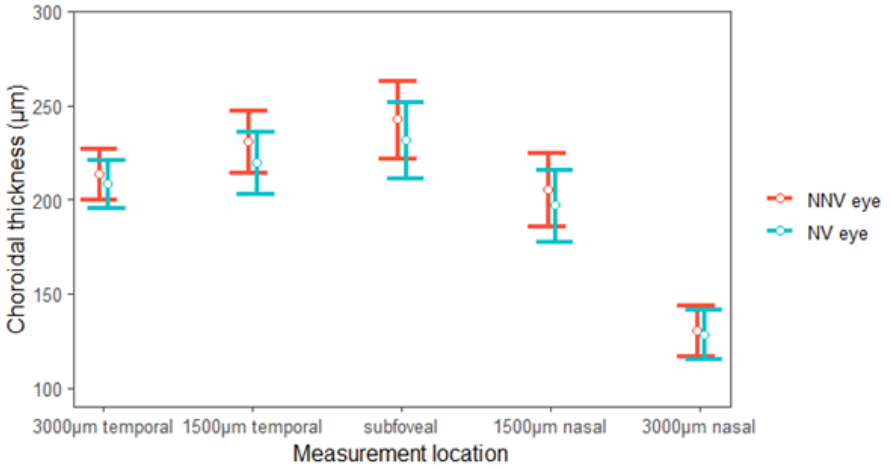

Figure 1. Choroidal Thickness in Neovascular Eyes and Non-neovascular Eyes

Figure 1 shows the choroidal thickness at all measurement locations for neovascular (NV) and non-neovascular (NNV) eyes. The error bars represent the 95% confidence interval

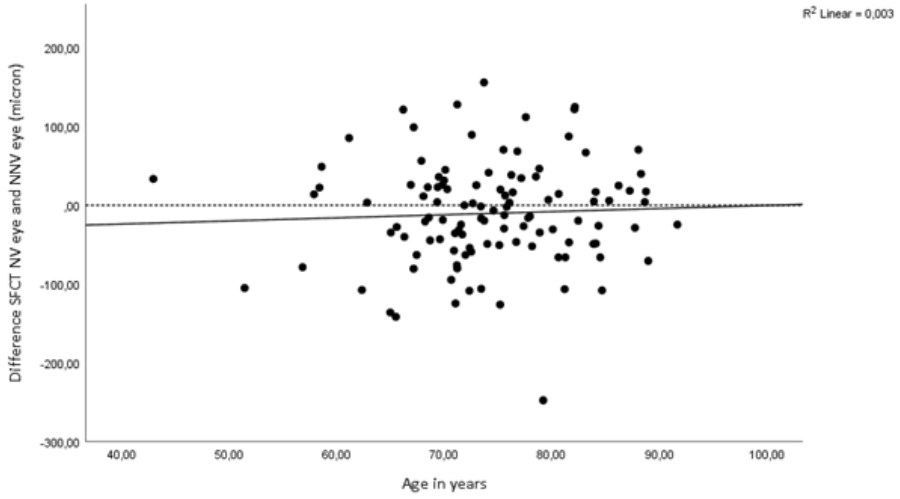

Figure 2. Difference between Subfoveal Choroidal Thickness and Age

The subfoveal choroidal thickness difference for NV and NNV eyes (y-axis), and age (x-axis).

Clinical predictors of SFCT

For NV and NNV eyes, age had a modest effect on SFCT (CI -5.9 to -.4) (*P* =.02) (CI -6.5 to -1.2) (*P* =.01) (table 3). All other independent variables failed to predict SFCT for NV and NNV eyes. Adjusted R^2 was .06 and .06 for NV and NNV eyes. We visualised the relation of age and SFCT for NV and NNV eyes in supplementary figure (3) (R^2 was .08 and .07 for NV and NNV eyes). Neither model violated any of the following statistical assumptions: linear relation of independent and independent variables, normality of residuals, absence of multicollinearity.

Table 3. Clinical Predictors of SFCT in the Neovascular Eye

	SFCT NV			**SFCT NNV**		
Independent variables	*Estimates*	*CI*	*P*	*Estimates*	*CI*	*P*
(Intercept)	387.0			496,2		
Age	-3,2	-5.9 to -0.4	.02*	-3,8	-6.5 to -1.2	.01*
Sex	50.0	-1.6 to 96.0	.06	17,5	-29.2 to 64.1	.5
Visual acuity (logMAR)	-42.4	-86.0 to 34.0	.4	-14,2	-114.6 to 86.1	.8
cRORA	-7.9	-60.0 to 44.4	.8	7,7	-43.5 to 59.0	.8
Smoking (ever/ current)	23.3	-26.1 to 71.0	.4	-0,7	-47.4 to 46.0	.9
Year since first injection	5.4	-3.5 to 11.2	.3	4,6	-0.6 to 9.8	.1
Number of injections	-0.6	-1.4 to 1.3	.9			
One type of anti-VEGF Combination anti-VEGF		-65.4 to 54.1 -81.6 to 48.6	.9 .6			
Adjusted R^2	.06			.06		

*indicates statistical significance. SFCT subfoveal choroidal thickness, cRORA complete retinal pigment epithelium and outer retinal atrophy.

Table 3 shows estimates, CI and *P* values from two linear regression model with SFCT of NV eye and SFCT of NNV eye as dependent variable. We used pairwise deletion for our estimates.

Discussion

Main findings

In this cross-sectional paired eye study using data from a prospective cohort study, we observed a statistically insignificant 11 µm difference between SFCT of NV and NNV AMD eyes (*P* =.08). To appreciate this difference, note that the diameter of a human red blood cell is 7.5 to 8.7 µm.(20) Also, CT at other measurement locations in NV and NNV eyes did not differ significantly. In a similar vein, we attempted to elucidate the relationship with various clinical predictors and SFCT. In our analysis, only age had a marginal relationship with SFCT with an R^2 of .08 and .07. In fact, for NV SFCT, the combined variance explained of age, sex, visual acuity, cRORA, smoking, number of injections, type of injections: bevacizumab, ranibizumab, aflibercept, amounted to a paltry adjusted R^2 of .06. This selection of predictors represents some of the most cited associations with CT, in particular age(21), yet it leaves 94% of the variance unaccounted for in our analysis, suggesting that CT is either highly heterogenous or poorly understood.

Concerning the difference between NV CT and NNV CT we found an 11 micron difference while Govetto *et al.* reported a fifteen micron difference between SFCT (*P* =.02). One difference between our study and theirs is a power differential (N = 161). A second consideration is that the median (IQR) time since first eye involvement was 2.9 (4.8) years and ranged from 0 to 20 years in our sample while their sample was based on retrospective consecutive diagnoses of neovascular AMD in one eye.

Also, cRORA did not influence SFCT in NV or NNV eyes (NV eyes: *P*=.8)(NNV eyes: *P*=.8) (Table 3). Contradictory reports have emerged regarding disease stage and CT. A large study showed that intermediate AMD was associated with thicker CT while early AMD was not.(22) Govetto *et al* reported the largest CT difference in early staged eyes.(15) Eyes with GA seem to have thinner CT.(23)

Setting the issue of statistical significance aside, how does a 15 or 11 micron difference relate to our overarching hypothesis of CT and atrophy? Indeed, we performed this study since there are arguments that anti-VEGF injections could lead to macular atrophy through CT thinning. Due to the observational cross-sectional nature of our study it is perhaps impossible to attribute potential thinning strictly to anti-VEGF since progression of AMD co-occurs. The relationship between disease progression in neovascular

AMD is additionally complex as CT is shown to increase when exudation develops.(24, 25) Similarly, due to high recurrence, most neovascular AMD patients are continuously treated with anti-VEGF.(3) No cross-sectional or longitudinal study could therefore realistically separate the effects of progression of AMD and treatment with anti-VEGF. CT functions thus as an imperfect resultant measure of contrary and conflicting processes that either induce thinning or thickening. To provide definitive evidence of atrophy induction as a result of anti-VEGF treatment would require the unethical application of an anti-VEGF regiment in non-AMD patients. Alternatively, there is no evidence of GA-enlargement secondary to macular neovascularisation development regardless of anti-VEGF treatment intensity.(17) In patients with diabetic macular edema a retrospective analysis of 1437 patients showed that 4% developed a CT of less than 200 microns during the study period and no association with anti-VEGF treatment was found.(26) In sum, considering that our statistically non-significant CT difference was observed in this long-term treated cohort of nAMD patients, it seems unlikely that through persistent choroidal thinning our patients will have been exposed to an increased risk of macular atrophy.

Anti-VEGF

All the most commonly used anti-VEGF medications have at one point been linked to CT reduction.(14) Though effect sizes vary.(27) In our cohort, the majority of patients treated with a single anti-VEGF have been treated with bevacizumab reflecting current Dutch best-practice (table 1). Aflibercept in particular seems to bring about a clear effect as evinced by Koizumi *et al.* who reported a 35.8 μm decrease in CT in 12 months in 58 treatment-naïve neovascular AMD patients.(10). Of note, whilst our cohort did not feature treatment-naïve neovascular AMD patients, the majority of patients (86%) have at some point been treated with aflibercept (86%).(Table 1) Since no such CT diminution appears to have persisted in our cohort, perhaps aflibercepts effect on CT is limited to the loading phase.(28) Further complicating the comparison of these findings, apart from differing methodologies, are the many factors potentially modifying CT. These include but are not limited to: diurnal rhythm(29) intra-ocular pressure(30), axial length(31), certain medications (32), and coffee intake.(33)

Strengths and weaknesses

A major strength of our design is the cross-sectional within-patient comparison of both eyes as we thus control for many of the aforementioned factors that

seem to influence CT. A second major strength is our systematic approach to the measurement of CT. Our mean measurement difference of 0 and 2 µm ((*P*=.9)(*P*=.1) for NV and NNV eyes indicates that both graders interpretated choroidal borders similarly and that this variation is due to random fluctuation rather than systematic bias. By taking the mean of both graders, we hoped to correct for this random fluctuation and provided a good approximation of the true CT. However, we want to remark that the 20.0 and 18.0 µm SD of the mean differences is large in comparison to the effects studied. It seems likely that random error therefore still obfuscates the relations under investigation. Another limitation is the need for sufficient statistical power to identify small effects accurately. This concern extends to potentially fruitful analyses of CT in relation to MNV subtype which have not been performed for this reason. A study of comparable sample size did not find statistically significant differences in CT between MNV subtypes.(34) Additionally, the use of real-world data can hinder the precise disentanglement of certain associations being studied.

Future research

Moving forward, we welcome the more widespread use of automated choroidal segmentation.(35) Once this technology is demonstrated to clearly outperform humans, even in cases where the sclero-choroidal junction is poorly visible, employing it could aid the expansion of present data-sets without labour intensive human measurement. Automatic measurement would have the added benefit of homogenizing the measurement process which would aid cross-study evaluations. Lastly, local changes in the choriocapillaris have been observed at various AMD-stages and may be further quantified using OCT-angiography.(36, 37)

Conclusion

We did not find a statistically significant difference in CT between NV eyes treated with anti-VEGF versus NNV fellow eyes in a long term nAMD follow-up cohort. We therefore conclude that potentially induced choroidal thinning through the use of necessary anti-VEGF for NVAMD appears to be of limited concern as a risk factor for macular atrophy.

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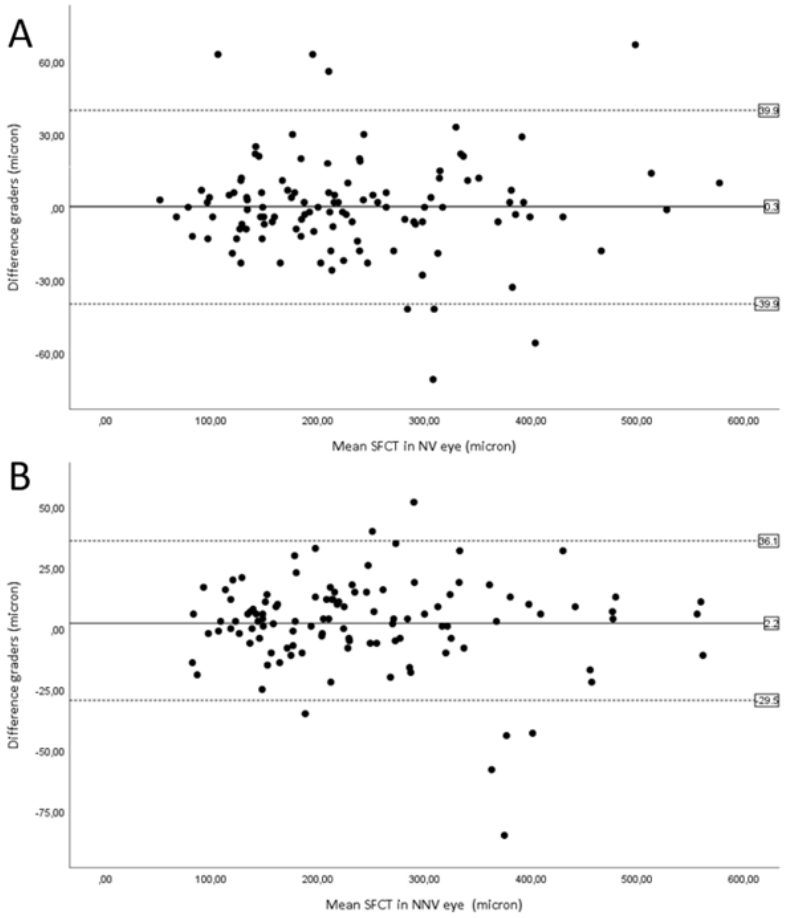
Supplementary material

Supplementary Table 1. Intraclass Correlation Coefficient

Location	Intraclass Correlation Coefficient	
	NV	NNV
Subfoveal	.991	.993
1500 µm nasal	.986	.991
3000 µm nasal	.971	.962
1500 µm temporal	.980	.981
3000 µm temporal	.949	.944

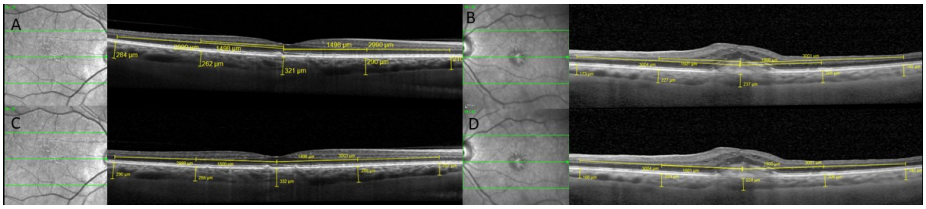
ICC, intra-class correlation coefficient.
For the ICC we used a two-random effects model, with consistency definition. We report the average measure.



Supplementary Figure 1. Bland-Altman plots

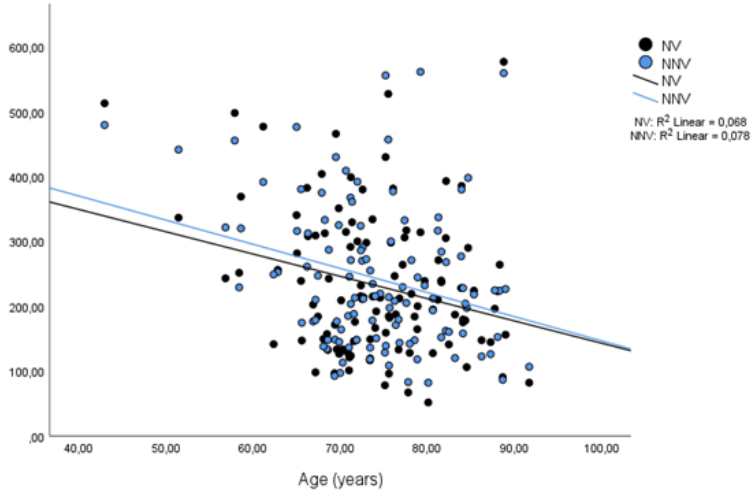
SFCT subfoveal choroidal thickness.

Bland-Altman plots showing agreement for subfoveal choroidal thickness between the two graders for NV (A) and NNV (B) eyes. The mean difference between the graders is visualised using a continuous line. The dotted lines represent the limits of agreement of 95% or 1.96 SD that contain 95% of all measurement differences between graders.



Supplementary Figure 2. Example of Choroidal Measurement

A. Measurement in non-neovascular eye by grader 1. B. Measurement of neovascular eye grader 1. C. Measurement of non-neovascular eye by grader 2. D. Measurement of neovascular eye grader 2.



Supplementary Figure 3. Subfoveal Choroidal Thickness and Age

NV Neovascular, NNV non-neovascular, SFCT subfoveal choroidal thickness.
The SFCT thickness for NV and NNV eyes (y-axis), and age (x-axis).

Research Chapter Two

Disease progression in age-related macular degeneration patients carrying rare variants in the complement factor H or complement factor I genes

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Abstract

Purpose

Rare variants in *CFI* and *CFH* genes are associated with age-related macular degeneration (AMD). This study aimed to compare the incidence of late AMD in carriers of these variants to a reference cohort using a long follow-up cohort (LF-cohort) and to examine short-term AMD progression in a short follow-up cohort (SF-cohort).

Method

This cohort study included two groups: the LF-cohort, observed for >5 years retrospectively and the SF-cohort, observed for 1 year prospectively, with patients attending in-hospital visits. 112 AMD patients with rare *CFH*/*CFI* or variants were invited from the European Genetic Database (EUGENDA). The LF-cohort's outcome was the incidence of late AMD per 100 person-years compared to a matched reference cohort. In the SF-cohort, geographic atrophy (GA), retinal sensitivity, and visual acuity were measured.

Results

The LF-cohort included 28 patients (median [IQR] age, 71.3 [24.3] years; 18 females [64%]) with an incidence rate of 6.2 per 100 person-years which was higher than the reference cohort (1.8 per 100 person years (P = .01)). The SF-cohort consisted of 44 patients (median [IQR] age 70.5 [16.5] years; 29 (65% female). Mean annual GA growth (SD) was 0.22 mm (0.13) in 19 eyes of 12 patients. Retinal sensitivity changed for late staged eyes (right eye: 17.2 dB to 15.7 dB, P = .03; left eye 17.3 dB to 16.4 dB, P = .06) whilst visual acuity did not.

Conclusions

Carriers of rare *CFI* or *CFH* variants show a higher incidence of late AMD. These patients may benefit from personalized gene therapy and complement inhibition in future trials.

Introduction

Age-related macular degeneration (AMD) is a progressive, multifactorial, neurodegenerative macular disease and the leading cause of visual impairment in the Western world.(1) Initially, patients may suffer mild visual disturbances but the disease can progress to late-stage AMD, characterized by geographic atrophy (GA) or choroidal neovascularization (CNV), significantly reducing quality of life.(2, 3) In Europe 67 million patients are affected by any stage AMD with an annual incidence of late AMD of 1.4 per 1 000 individuals (95% CI 0.8 to 2.6).(4) Age ranks among the most consistent independent risk factors.(5, 6) Modifiable risk factors, such as smoking and diet, are also strongly linked to disease progression.(7-9),[10] The link between the complement system and AMD was demonstrated through elevated complement activation in the eye and in the bloodstream of AMD patients.(11, 12) Genetics were subsequently found to play a major role with a genome wide association study identifying 52genetic variants at 34 loci associated with late AMD. The variants cluster in the complement system, lipoprotein metabolism, angiogenesis and extracellular matrix remodelling.(13)

Complement factor I (*CFI*) and complement factor H (*CFH*) are two key genes coding for proteins that regulate complement activation.(14) Complement factor I is a serine protease which acts as an inhibitor of the complement system while complement factor H, amongst others, functions as for factor I mediated degradation of C3b.(11) Multiple rare variants (minor allele frequency [MAF] <1%) in either *CFI* or *CFH* have been found to alter protein function or reduce factor I and factor H levels indicating low level inflammation.(15-19) Rare variants in *CFI* and *CFH* have been implicated as possible drivers for specific AMD phenotypes such as drusen nasal to optic disc,(20-23) and have been hypothesized to confer a higher risk for a more severe disease phenotype than common variants.(24) Moreover, rare variants in *CFI* and *CFH* have been found more frequently in patients with late AMD suggesting a high risk of disease progression.[14,(25, 26)] Likewise, rare *CFH* variants were observed in patients who developed AMD characteristics at a young age.[22,(27)] Based on these findings, we hypothesize that rare genetic variants in *CFH* and *CFI* could alter disease course by increasing the risk of AMD development and also the rate of progression over time. In this cohort study we aim to explore long-term and short-term disease progression in AMD patients carrying one or more rare variants in *CFH* or *CFI*. A better understanding of the role of rare variants in disease progression may have implications for therapy development.

Methods

Study population and study design

All Dutch AMD patients with a rare (MAF < 1%), protein-altering or splice-site variant in *CFH* or *CFI* in the European Genetic Database (EUGENDA) (entered between2004 and 2014) were invited for one or more follow-up visits between January 2018 and October 2019.

Out of a common pool of rare variants carriers, we created the Long Follow-up Cohort (LF-Cohort) including all patients without late AMD at baseline (the date of inclusion in EUGENDA) with a follow-up of at least five years. Drawing from the common pool in 2018 we studied short term disease progression (SF-cohort) in a prospective cohort including all patients, irrespective of disease stage at baseline, who completed at least one year of follow-up between January 2018 and October 2019. The SF-cohort was enriched with eligible patients who were seen in the outpatient clinic between January 2018 and September 2018 and agreed to participate in the prospective follow-up study. A flowchart of the patients included in the study is shown in Supplementary Figure 1.

At each visit we measured visual acuity (VA) using Early-Treatment Diabetic Retinopathy Study charts (ETDRS) and performed multimodal retinal imaging. We assessed retinal function via mesopic microperimetry (MMP) with the Macular Integrity Assessment [MAIA]; Centervue, San Jose, California, USA). We used a 4-2 strategy with the standard 10-degree stimulus grid containing 37 test points with one central point 12 points at 1°, 12 at 3°, and 12 at 5° degree eccentricity from the fovea on nondilated eyes. Smoking habits (ever, never, present) were assessed through our in-house developed questionnaire.

As a reference of disease progression in the general AMD population, we studied patients from the Rotterdam Study (RS). RS is a large ongoing population-based cohort study set in the city of Rotterdam in the Netherlands. These participants were matched randomly 3:1 on age, sex, smoking status (ever/never), and the square root of drusen area of the highest drusen area eye. An exact match was required for sex and smoking status, while a tolerance of ±5 years was allowed for age and ±0.5 mm for the square root of drusen area. If three suitable matches were not found, the matching ratio was loosened to 1:1 and matching requirement could be minimally relaxed Detailed information on the study procedures has been published previously.(28)

Both studies were conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the local ethics committee. All participants provided written informed consent prior to participating.

Outcome

Our main outcome for the LF-cohort was conversion to late AMD, which allowed us to study annual incidence. A modified version of the Wisconsin Age-Related Maculopathy Grading System (WARMGS)(29) was used to grade all AMD features, and subsequently classified these into the Rotterdam Study (RS) classification. Late AMD is defined as exudative or non-exudative neovascular or any GA.(30) To prevent overestimation of the progression rate, the date of conversion to late AMD was considered equal to the date of the study visit during which this stage was first observed. Only when more detailed information on conversion was available (e.g. date initiation of treatment for exudative AMD), did we adjust the date. We also compared potential determinants for progression including phenotype(stage, predominant drusen type, drusen area, extramacular drusen (yes/no), pigment and RPE degeneration per eye), genotype (GRS, pathogenicity), and smoking (ever, never, presently) between progressors to late AMD and non-progressors.

For the SF-cohort cohort, study outcomes were growth of GA and changes in VA and mean retinal sensitivity after one year.

Retinal imaging acquisition and Grading

We obtained Colour Fundus Photography (CFP) (DRI Triton [Topcon corporation, Tokyo, Japan]), Spectral-Domain Optical Coherence Tomography (SD-OCT) and, Fundus Auto-Fluorescence (FAF) (SpectralisTM HRA+OCT [Heidelberg Engineering, Heidelberg, Germany].

Grading of CFP was performed by experienced graders of the Eye-NED Reading Center. Automated CFP analysis was performed using an in-house developed deep learning algorithm that quantified drusen area in square millimetres (mm^2).(31) GA growth was measured on FAF with semiautomated software in eyes that showed signs of GA at baseline without CNV (RegionFinder, version 2.6.4.0, Heidelberg Engineering, Heidelberg, Germany).(32) In cases where GA borders were unclearly defined, SD-OCT was used to increase delineation accuracy. The GA surface area was expressed in mm^2. Eyes with GA extending outside the FAF borders were excluded. Grading of GA growth was performed by AB and FC jointly and disagreements were solved by open adjudication.

Genotyping

Genotyping of EUGENDA had been performed using whole exome sequencing (WES), single-molecule molecular inversion probes (smMIPs) or exome chip analysis, as described previously.11,(33),(34) Genotyping data were filtered for the presence of rare protein altering and splice-cite variants (MAF < 1%) in *CFH* or *CFI*, based on the non-Finnish European population as reported in the Genome Aggregation Database [gnomAD], http://gnomad.broadinstitue.org). When a rare variant was identified in one of the genotyping platforms, its presence was confirmed using Sanger sequencing. Variants were classified as benign, likely benign, likely pathogenic, and pathogenic based on functional and genetic data and evaluated by SdJ.

In order to quantify the genetic load, we calculated a genetic risk (GRS) score based on 52 AMD associated variants[11] as described previously.[31] The GRS was considered missing if one of the following high-impact variants were not available: *CFH* rs10922109, *CFH* 570618, *CFB* rs429608, *ARMS2/HTRA1* (rs3750846) and *C3* rs2230199. In the event that both smMIPs and exome chip genotyping was available in a patient, smMIPs genotyping was used.

Importantly, in the reference cohort absence of a rare variant in *CFH* or *CFH* was not verified but assumed.

Statistical analysis

We calculated the incidence rate per 100 person years by dividing the total number of incident late AMD cases by the sum of follow-up durations and multiplying this number by 100. We compared the incidence rate in our LF(35)-cohort to the incidence rate in the reference cohort using an exact two-sided Poisson test We compared the determinants of progressors vs. non progressors via binary logistic regression with adjustment of each determinant for age and sex. In the SF- cohort, we used a one sample T-test for changes in VA and mean retinal sensitivity. Square root transformation was applied on GA growth to limit the impact of baseline GA area differences, and to drusen area to account for the non-linear scaling of area measurements and reduce heteroscedasticity.(35) Differences between participants and non-participants were evaluated using Students T-tests. *P* values < .05 were considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA).

Results

Cohort description

In total, 112 patients met the inclusion criteria and 42/112 participated in one or more follow-up visits. Major grounds for non-participation were death (n = 24) and unwillingness to participate (n = 22). We provided a summary of other reasons for non-participation in Supplementary Figure1. Compared to the participants, non-participants were older (70.0 years and 81.1 years, respectively; $P < .001$) and were more likely to have late AMD at baseline (16 of 42 vs. 47 of 70; $P < .001$) (Supplementary Table1). An additional 12 patients were included from our outpatient clinic for the prospective follow up study. In sum, 34 patients carried a rare variant in *CFH* and 20 patients carried a rare variant in *CFI* (Supplementary Table2). These patients carried 27 unique rare variants (20 in *CFH* and 7 in *CFI*) (Supplementary Table2).(13-16, 19, 20, 23, 25, 36-60)

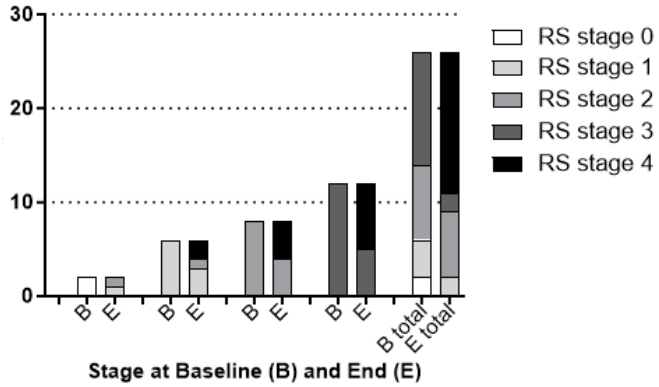

Figure 1. Progression per Stage in Long Follow-up Cohort

Abbreviations: RS, Rotterdam Study. AMD, age-related macular degeneration. RS stage 0 corresponds to no signs of AMD at all or hard drusen <63 µm) only. RS stage 1: Soft distinct drusen (≥63 µm) only or pigmentary irregularities only, no soft drusen (≥63 µm). RS stage 2: Soft indistinct drusen (≥125 µm) or reticular drusen only, soft distinct drusen (≥63 µm). RS stage 3: Soft indistinct (≥125 µm) or reticular drusen with pigmentary irregularities. RS stage 4: Atrophic or neovascular AMD.

Long-term follow-up (LF) cohort

The LF-cohort consisted of 28 patients without late AMD at baseline (median [IQR] age, 71.3 [24.3] years; 18 females [64.3%]). The median (IQR) follow-up duration was 8.9 (3.2) years. All baseline characteristics for the LF-cohort are shown in Table 1. Of note, 2 patients were graded as RS stage 0, but presented with extramacular drusen. Fifteen patients (54%) progressed to late AMD(total

person-years was 240).This led to an incidence rate of 6.2 per 100 person-years, or an annual cumulative incidence of 6.2%. Figure 1 illustrates the stage graded at baseline and at the end of follow up. The number of patients per stage at baseline and end date is detailed in Supplementary Table 2. We compared the incidence rate of the LF-cohort to that in the reference cohort; 18 were successfully matched at a 3:1 ratio, 3 were matched 1:1. For six participants, matching on age was not possible due to their younger age (below 45 years at baseline); therefore, the these individuals were matched 1:1 with the youngest possible referent within .5 mm square root drusen area. Additionally, for one individual with a high square root of drusen area, the standard matching tolerance of ±0.5 mm was relaxed to ±1.0 mm to enable a suitable match. Resultingly, in the reference cohort, the incidence rate of late AMD was 1.8 per 100 person years (annual cumulative incidence 1.8%) (455 total person-years, 8 events) which was different (P = .01) compared to the LF-cohort.

Table 1. Baseline Characteristics

Characteristics	Long follow-up cohort	Prospective cohort	Rotterdam Study cohort
	n = 28	n = 44	n =64
Age (at baseline and start prospective study resp.), median (IQR), years	62.6 (24.3)	70.5 (16.6)	66.2 (17.6)
Follow-up, median (IQR), years	8.9 (3.2)	1.1 (.1)	6.2 (5.3)
Female, No. (%)	18 (64)	29 (66)	48 (75)
Smoking, No. (%)			
Never	13 (46)	15 (34)	32 (50)
Ever	14 (50)	26 (59)	12 (18.75)
Current	1(4)	2 (5)[a]	20 (31.25)
Rare variant gene, No. (%)			
CFI	13 (46)	15 (34)	
CFH	15 (54)	29 (66)	
GRS, median (IQR)	.84 (1.4)	1.08 (2.2)	
Stage, No. (%)			
RS stage 0	2 (7)	0 (0)	30 (46.9)
RS stage 1	6 (21)	3 (7)	21 (32.8)
RS Stage 2	8 (29)	6 (14)	11 (17.2)
RS stage 3	12 (43)	6 (14)	2 (3.1)
RS stage 4	0 (0)	29 (66)	0 (0)

Abbreviations: NA, not applicable. RS, Rotterdam Study. *CFI,* complement factor I. *CFH,* complement factor H. GRS, genetic risk score. RS stage 0 corresponds to no signs of AMD at all or hard drusen <63 µm) only. RS stage 1: Soft distinct drusen (≥63 µm) only or pigmentary irregularities only, no soft drusen (≥63 µm). RS stage 2: Soft indistinct drusen (≥125 µm) or reticular drusen only, soft distinct drusen (≥63 µm). RS stage 3: Soft indistinct (≥125 µm) or reticular drusen with pigmentary irregularities. RS stage 4: Atrophic or neovascular age-related macular degeneration

*one patient did not provide smoking details, therefore numbers do not add up to 44.

Table 2. Comparison of Progressors and Non-Progressors adjusted for Sex and Age

Characteristics	Progressors n = 15	Non-Progressors n = 13	*P* value
Follow-up, median (IQR), years	10.3 (2.9)	7.4 (2.4)	.1
AMD stage eye baseline, No. (%)			
RS stage 0-1	2 (13)	6 (46)	.2
RS Stage 2	4 (27)	4 (31)	.8
RS stage 3	9 (60)	3 (23)	.1
Predominant drusen type per eye*, No. (%)			
Hard-Soft < C1	1 (7)	4 (31)	.4
Soft distinct-indistinct	14 (93)	7 (54)	
Drusen area in grid per eye, No. (%)			.1
0 – 10 %	7 (48)	11 (85)	.1
– 25 %	5 (33)	1 (8)	.1
25 – 50 %	3 (20)	1 (8)	
Extramacular drusen per eye, No. (%)**			
Yes	11 (76)	10 (77)	.2
No	4 (27)	2 (15)	
Pigment and RPE degeneration per eye, No (%)			.3
No pigment	7 (47)	10 (77)	.5
Increased pigment	1 (7)	1 (8)	.1
Increased pigment and RPE degeneration	7 (47)	2 (15)	
Smoking, No. (%)			
Never	6 (40.)	7 (54)	.3
Ever	9 (53)	6 (47)	
GRS, median (IQR)	0.99 (1.71)	0.74 (1.34)	.9
Rare variant gene, No.	7 *CFH* 8 *CFI*	8 *CFH* 5 *CFI*	.7
CADD score ≥ 20, No. (%),	11 (73)	9 (69)	.5
Pathogenicity†			
Pathogenic or likely pathogenic	10 (83)	8 (80)	.4
Benign or likely benign	2	2	

Abbreviations: GRS, genetic risk score. RS, Rotterdam Study. MAF, minor allele frequency. CADD, combined annotation dependent depletion. RPE, retinal pigment epithelium. AMD, age-related macular degeneration. RS stage 0 corresponds to no signs of AMD at all or hard drusen <63 µm) only. RS stage 1: Soft distinct drusen (≥63 µm) only or pigmentary irregularities only, no soft drusen (≥63 µm). RS stage 2: Soft indistinct drusen (≥125 µm) or reticular drusen only, soft distinct drusen (≥63 µm). RS stage 3: Soft indistinct (≥125 µm) or reticular drusen with pigmentary irregularities. RS stage 4: Atrophic or neovascular AMD.

*in 2 eyes of the non-progressors predominant drusen type could not be determined due to inconclusive grading. †Likely pathogenic and pathogenic have been collapsed into one category, 6 variants were of unknown significance and excluded. ** one missing due to inconclusive grading.

We compared demographic, phenotypic, and genetic characteristics between progressors and non-progressors. No determinant was statistically significant after adjusting for age and sex. Mean GRS (SD) did not differ significantly between progressors (0.99 (1.71) vs. non progressors 0.74 (1.34)) (*P* = .9), nor did the proportion (%) of pathogenic or likely pathogenic (83% vs. 80%) (*P* = .4). (Table 2) The distribution of GRS scores for progressors vs. non-progressors is visualized in Supplementary Figure 2.

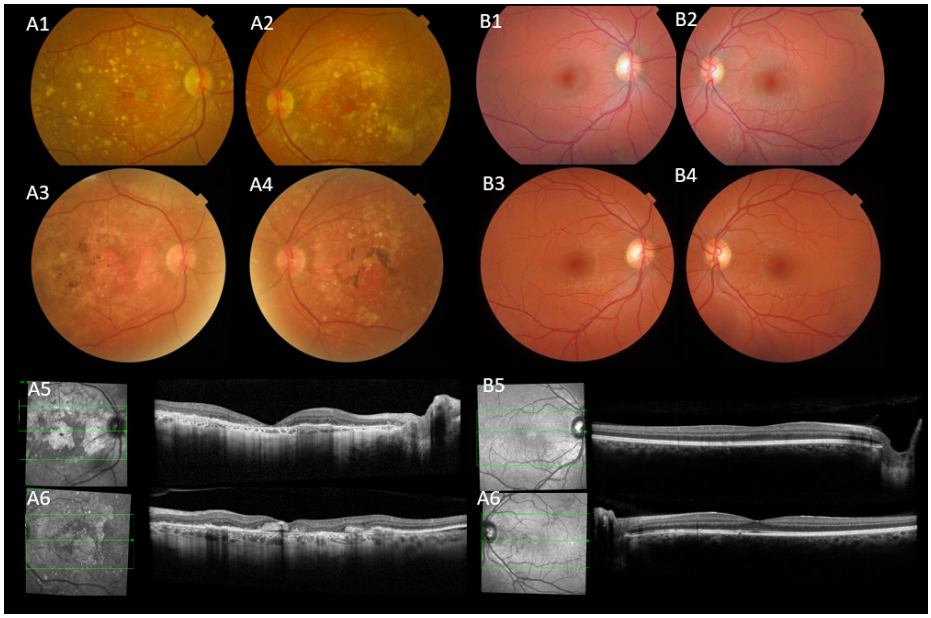

Figure 2. Example of Two Patients (Father and Son)

Figure 2 shows imaging of father and son, both carry the c.550delA (p.Ile184Leufs*33) variant in the complement factor H gene. A1-2 shows color fundus photography (CFP) of the father with bilateral advanced age related macular degeneration at age 62. A34, shows CFP of the father who is now aged 72. A5-6 advanced disease on spectral-domain optical coherence tomography (SD-OCT). B1-2 is CFP of the son aged 19 years without macular drusen. B3-4 CFP of the son, now aged 29 years with bilateral macular drusen. B5-6 We show small macular drusen on SD-OCT.

Example of two noteworthy patients

One patient had been included in EUGENDA database at age 18 after genetic testing revealed that he carried the same frameshift variant in *CFH* (c.550delA (p.Ile184Leufs*33) as his father.(20, 61, 62) The father visited the outpatient clinic from age 61 to 72. During this time both eyes, which showed signs of both CNV and GA at baseline showed worsening of GA. Visual acuity worsened from 0.1 (logMAR) in the right eye and 0.2 (logMAR) in the left eye to 0.4

logMAR and 1.0 logMAR. The son showed a small number of hard drusen in the peripheral retina already at his age. He progressed to RS stage 2 at the age of 29 (Figure 2). Both patients, and in particular the son, were advised to take supplements according to the AREDS2 formula(63), and to adhere to a healthy lifestyle. Notably, the GRS differed between father (2.93) and son (0.17).

Short-term follow-up (SF-cohort)

The SF-cohort consisted of 44 patients with all stages at baseline (median [IQR] age 70.5 [16.5] years; 29 (65%) females. Twenty-one patients of the LF-cohort also participated in the SF-cohort.

GA growth was measured in 19 eyes of 12 patients. Mean GA growth (SD) was 0.22 mm (0.13) per year. (Supplementary Figure 3). An example of a patient with follow-up of GA with multimodal imaging is provided in figure 3.

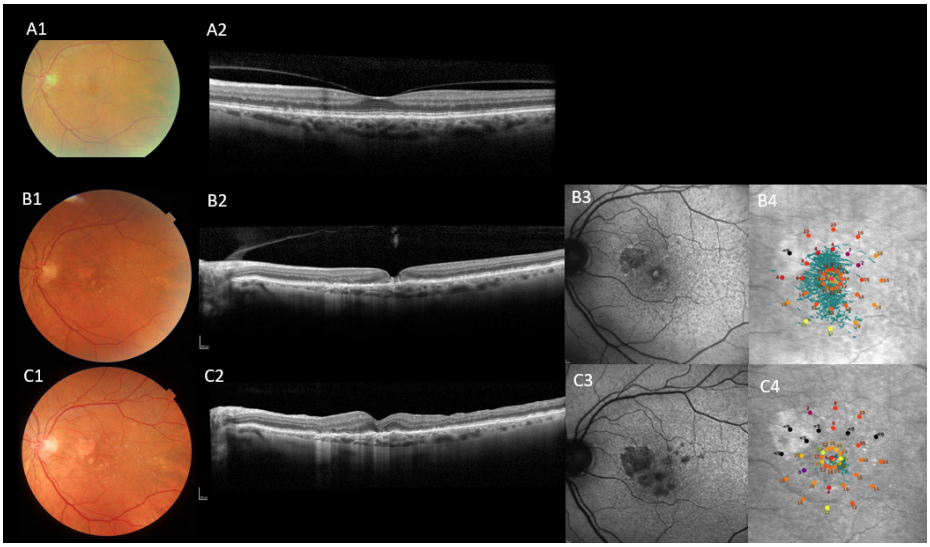

Figure 3. Multimodal Imaging of Geographic Atrophy Growth

Figure 3: Panel ABC multimodal imaging of a female patient with a rare variant in the complement factor H, c.2572T>A, p.W858R. A1-2 age 68. A1 color fundus photography (CFP) of left eye graded as RC 3 with corresponding spectral domain optical coherence tomography (SD-OCT) (A2) showing drusen. B1-4 now aged 75 years, CFP (B1) shows geographic atrophy. The SD-OCT (B2) shows some hypertransmission and ellipsoid zone disruption. B3 shows the fundus autofluorescence (FAF) with atrophy. B4 shows the mesopic microperimetry with functional abnormalities. C1-4. One year later, there is an increase in the amount of drusen on CFP (C1), the hypertransmission area has clearly increased on SD-OCT (C2). Atrophy growth as measured via FAF (C3) was 1.55 mm^2. Lastly, there is an increase in abnormal thresholds on the microperimetry grid (C4).

Functional assessment

Next, we evaluated functional measures of progression using MMP and VA. Some patients were unable to perform MMP, this was case for 14 eyes of 9 patients. Of these, 9 eyes had late AMD. In sum, 74/88 eyes were measured twice with an interval of one year. Mean retinal sensitivity did not change when we included all stages (right eyes: 21.4 dB to 20.8 dB, $P = .07$; left eyes: 20.2 dB to 19.6 dB, $P = .1$). However, mean retinal sensitivity did change when stratified for baseline stage (right eye: 17.2 dB to 15.7 dB, $P = .03$; left eye 17.3 dB to 16.4 dB, $P = .06$) (Supplementary Table 5). For a smaller subset of patients (n = 49) 20 months of microperimetry data were available. During this extended follow-up mean retinal sensitivity decreased significantly for late AMD eyes (right eye 19.5 dB to 17.2 dB, $P = .05$; left eye 19.5 dB to 17.1 dB, $P = .01$). VA did not change during 12 or 20 months of follow-up (Supplementary Table 6)

Discussion

In this study with variable follow-up time, we investigated incidence of late AMD and short-term progression of AMD in carriers of rare *CFI* or *CFH* variants.

Long term follow-up

We observed an incidence rate to late AMD of 6.2 per 100 person-years which significantly exceeded that of patients in the reference cohort (1.8 per 100 person-years) of the Rotterdam study ($P = .01$) reflecting faster progression for carriers. Conversion to cumulative incidence yielded a 5-year cumulative incidence of 27% for the LF-cohort. This exceeds the 5-year risk for late AMD (RS stage 4) provided by the Rotterdam Study for patients aged 60-69 years (3.0% and 17.5% for RS stage 2 and RS stage 3, respectively)(5), thereby further underscoring the relatively high rate of progression. A large retrospective cohort-study found that the incidence rate for GA and CNV for patients with either intermediate or early AMD was 2.0 and 3.2 per 100 person-years.(64) However, this calculation was based on a much older population with a mean age of 78 years. Our patients progressed at a higher rate while being much younger. Future efforts could parse out diverse incidence rates across diverse ages of onset for rare variant carriers vs. non-carriers.

In select cases, we suggest a causative role for particular *CFI* or *CFI* variants in AMD disease course. We illustrated this by the father and son both carrying a *CFH* c.550delA frameshift mutation.(20, 61, 62) This mutation is associated

with low levels of CFH protein.*(62)* The son showed alarming development of macular drusen at the age 29 after first presenting at age 19 with peripheral drusen only. The GRS of 0.17 is not strongly associated with late AMD, (33) and does not explain the very early onset of disease. Similarly, the father showed considerable worsening of late AMD features during the approximate 10-year follow-up. In sum, our findings of the higher progression rate in conjunction with specific cases of a very early onset, suggest that rare variants can be strong drivers of disease.

Short term follow-up

During the short term progression study 44 patients were studied in terms of retinal function and phenotypical characteristics. Annual growth rate (SD) of GA was 0.22 mm (0.13) In contrast, annual GA (SD) growth has been estimated as approximately 0.33mm (0.17) in the general AMD population.(65) Preclusion of eyes with GA extending outside FAF-borders limited complete growth measurement. However, our sample size constrains substantive speculation on this point. Moving on to functional outcomes, we observed that mean retinal sensitivity changed significantly during a period of 12 and 20 months for eyes with late AMD whereas no such change was observed for VA. This could serve as an argument for further research into mesopic microperimetry as an endpoint in clinical trials. We could not however, objectify any significant decrease in mean retinal sensitivity for non-late eyes, which is likely a result of the small number of non-late eyes. Hsu et al for example reported a 12 month difference of −3.0 (3.3) dB decrease for 39 patients with intermediate AMD.(66) Lastly, the short-term progression analysis could benefit from reanalysis with novel biomarkers that are currently being developed by MACUSTAR.(67)

Limitations

Our study is primarily limited by the small sample size particularly of non-late carriers and the variable follow-up duration. A second limitation is that rare variant carriers were sampled from EUGENDA, a clinic based case-control-study, instead of a population-based cohort which might have enriched our sample with more advanced AMD cases within the bounds of the matching criteria Also, our rare variants sample consisted of variants of varying pathogenicity. Additionally, a more precise estimate of the progression per person-years could not be calculated due to unavailable images at intermediate dates. However, since we conservatively estimated progression we are more likely to have underestimated true progression rates. Lastly, we could not verify non-carrier status of patients in the reference cohort. This is

however, unlikely to significantly impact our findings. While no exact estimates of protein, changing *CFI*/*CFH* variants in non-late AMD exist. Previous work finds a rare (MAF<1%), protein-changing *CFH* variant in 7.7% of late-AMD cases.(27) In addition only 1% of late AMD cases were previously found to carry a rare (MAF<1%) *CFI* variant with associated low serum factor I levels.(40) It is reasonable to assume that rare variants occur infrequently in our reference-cohort and do not therefore, explain potential incidence rate difference.

In conclusion, our results suggest a higher incidence rate in AMD patients carrying rare variants in *CFI* or *CFH*. Therefore, rare variant carriers are interesting candidates for future trials as they may serve as a proof of concept in novel complement factor target studies, but this must be balanced against the initial costs of genetic screening." Lastly, the impact of rare variants on disease progression is significant. Therefore, regardless of their low prevalence, genetic analyses should not be limited to GRS alone but should also involve sequence analysis of the *CFH* and *CFI* genes to identify rare coding variants.(33)

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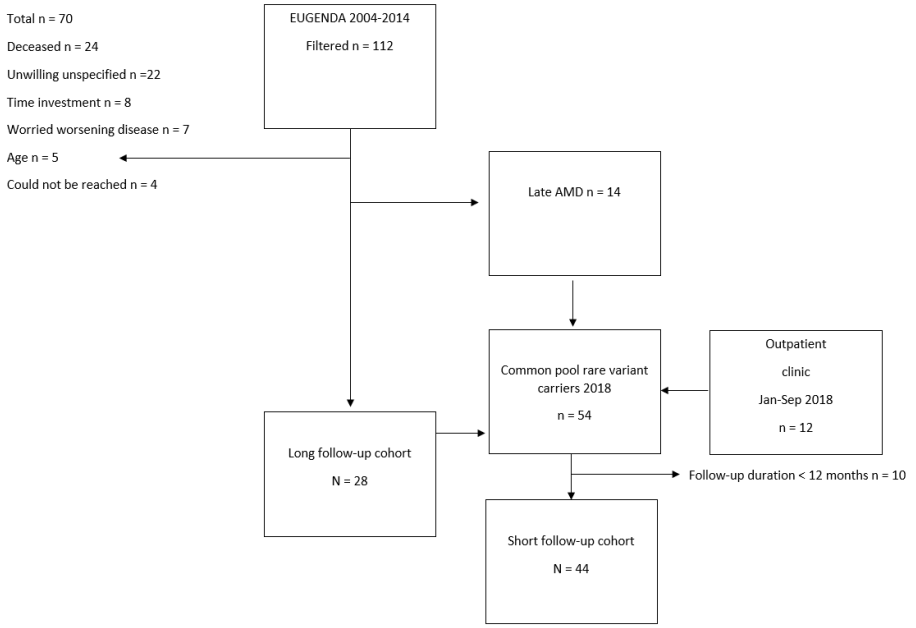
Supplementary Material

Supplementary Figure 1. Flowchart of Patient Inclusion



Supplementary Table 1. Characteristics of Participants and Non-participants

Characteristics	Participants n = 42	Non-participants n = 70	*P* value
Female, No. (%)	24 (38)	45 (62)	.5
Age in 2018, mean (SD), years	70.0 (13.6)	81.1 (12.7)	<.001
Advanced AMD at baseline, No. (%)	16 (38)	47(67)	<.001
Smoking status,[a] No. (%)			
Never	14 (33)	29 (41)	.3
Ever	24 (57)	26 (37)	
Current	3 (7)	5 (7)	

Abbreviations: AMD, age related-macular degeneration.
[a]The variable 'smoking' was missing for 11 patients.

Supplementary Table 2. Overview of rare variants (abridged)

Patient	Gene	Nucleotide Change	Protein change	Pathogenicity category	Comment
1	*CFH*	7C>G	Leu3Val	Likely benign	
2	*CFH*	428-14T>C	-	VUS	
3	*CFH*	481G>T	Ala161Ser	Likely benign	
	CFH	2850G>T	Gln950His	Benign	
4	*CFH*	496C>T	Arg166Trp	Likely pathogenic	
5	*CFH*	524G>A	Arg175Gln	Pathogenic	Individuals 5 to 8 belong to the same family
6	*CFH*	524G>A	Arg175Gln	Pathogenic	Individuals 5 to 8 belong to the same family
7	*CFH*	524G>A	Arg175Gln	Pathogenic	Individuals 5 to 8 belong to the same family
8	*CFH*	524G>A	Arg175Gln	Pathogenic	Individuals5 to 8 belong to the same family
9	*CFH*	550delA	Ile184Leufs*33	Pathogenic	Individuals 9 to 10 belong to the same family
10	*CFH*	550delA	Ile184Leufs*33	Pathogenic	Individuals 9 to 10 belong to the same family
11	*CFH*	578C>T	Ser193Leu	VUS	Individuals11 to 13 belong to the same family
	CFH	908G>A	Arg303Gln	VUS	
12	*CFH*	578C>T	Ser193Leu	VUS	Individuals 11 to 13 belong to the same family
	CFH	908G>A	Arg303Gln	VUS	
13	*CFH*	578C>T	Ser193Leu	VUS	Individuals11 to 13 belong to the same family
	CFH	908G>A	Arg303Gln	VUS	
14	*CFH*	578C>T	Ser193Leu	VUS	
	CFH	908G>A	Arg303Gln	VUS	
15	*CFH*	607_610dupCCAA	Lys204Thrfs*26	Pathogenic	Individuals 15 to 16 belong to the same family
16	*CFH*	607_610dupCCAA	Lys204Thrfs*26	Pathogenic	Individuals 15 to 16 belong to the same family

Supplementary Table 2. Continued

Patient	Gene	Nucleotide Change	Protein change	Pathogenicity category	Comment
17	*CFH*	764G>A	Gly255Glu	Likely pathogenic	
18	*CFH*	764G>A	Gly255Glu	Likely pathogenic	
19	*CFH*	1069T>A	Cys357Ser	Likely pathogenic	
20	*CFH*	1126C>T	Gln376*	Pathogenic	
21	*CFH*	1135T>C	Trp379Arg	Likely pathogenic	
22	*CFH*	1198C>A	Gln400Lys	VUS	
23	*CFH*	1215G>T	Lyn405Asn	Likely benign	
24	*CFH*	1222C>T	Gln408*	Pathogenic	
25	*CFH*	1222C>T	Gln408*	Pathogenic	Individuals 25 to 26 belong to the same family
26	*CFH*	1222C>T	Gln408*	Pathogenic	Individuals 25 to 26 belong to the same family
27	*CFH*	2329A>G	Ile777Val	Likely benign	
28	*CFH*	2572T>A	Trp858Arg	Likely pathogenic	Individuals 28 to 30 belong to the same family
29	*CFH*	2572T>A	Trp858Arg	Likely pathogenic	Individuals 28 to 30 belong to the same family
30	*CFH*	2572T>A	Trp858Arg	Likely pathogenic	Individuals 28 to 30 belong to the same family
31	*CFH*	2596+8G>T	-	VUS	
32	*CFH*	2748C>G	Tyr916*	Pathogenic	
33	*CFH*	3234G>T	Arg1078Ser	Likely benign	Individuals 33 to 34 belong to the same family
34	*CFH*	3234G>T	Arg1078Ser	Likely benign	Individuals 33 to 34 belong to the same family
35	*CFI*	1709G>C	Ser570Thr	Benign	
36	*CFI*	1657C>T	Pro553Ser	Likely pathogenic	
37	*CFI*	1657C>T	Pro553Ser	Likely pathogenic	

Supplementary Table 2. Continued

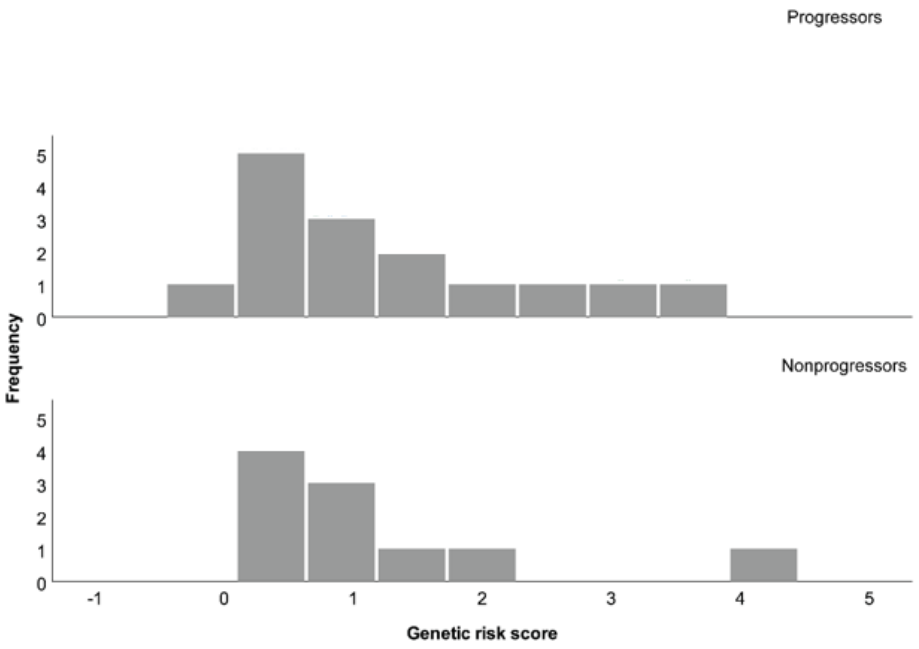
Patient	Gene	Nucleotide Change	Protein change	Pathogenicity category	Comment
38	*CFI*	1657C>T	Pro553Ser	Likely pathogenic	
39	*CFI*	1342C>T	Arg448Cys	VUS	
40	*CFI*	563G>C	Gly188Ala	Pathogenic	
41	*CFI*	392T>G	Leu131Arg	Pathogenic	
42	*CFI*	355G>A	Gly119Arg	Pathogenic	
43	*CFI*	355G>A	Gly119Arg	Pathogenic	Individuals 43 to 44 belong to the same family
44	*CFI*	355G>A	Gly119Arg	Pathogenic	Individuals 43 to 44 belong to the same family
45	*CFI*	355G>A	Gly119Arg	Pathogenic	
46	*CFI*	355G>A	Gly119Arg	Pathogenic	
47	*CFI*	355G>A	Gly119Arg	Pathogenic	
48	*CFI*	355G>A	Gly119Arg	Pathogenic	
49	*CFI*	355G>A	Gly119Arg	Pathogenic	
50	*CFI*	355G>A	Gly119Arg	Pathogenic	Individuals 50 to 52 belong to the same family
51	*CFI*	355G>A	Gly119Arg	Pathogenic	Individuals 50 to 52 belong to the same family
52	*CFI*	355G>A	Gly119Arg	Pathogenic	Individuals 50 to 52 belong to the same family
53	*CFI*	148C>G	Pro50Ala	Pathogenic	
54	*CFI*	148C>G	Pro50Ala	Pathogenic	

CFI or complement factor I, CFH or complement factor H, VUS or variant of unknown significance. Full version is available at DOI 10.34973/qzr7-j052 (repository).

Supplementary Table 3. Number of Carriers per Stage at Baseline and End Date in Long follow-up-cohort

Stage No. (%)	Baseline No. (%)	End No. (%)
RS stage 0	2 (7)	0 (0)
RS stage 1	6 (21)	4 (14)
RS Stage 2	8 (29)	7 (25)
RS stage 3	12 (43)	2 (7)
RS stage 4	0 (0)	15 (54)

Abbreviations: RS, Rotterdam Study. RS stage 0 corresponds to no signs of age-related macular degeneration (AMD) at all or hard drusen <63 µm) only. RS stage 1: Soft distinct drusen (≥63 µm) only or pigmentary irregularities only, no soft drusen (≥63 µm). RS stage 2: Soft indistinct drusen (≥125 µm) or reticular drusen only, soft distinct drusen (≥63 µm). RS stage 3: Soft indistinct (≥125 µm) or reticular drusen with pigmentary irregularities. RS stage 4: Atrophic or neovascular AMD.



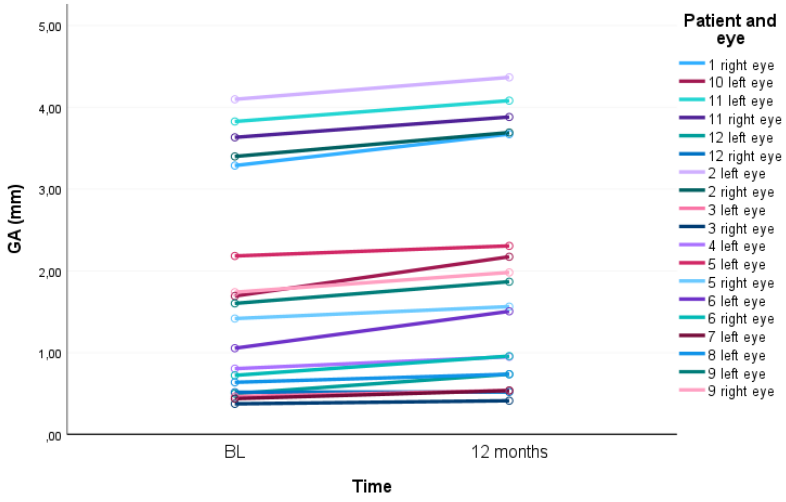
Supplementary Figure 2. Genetic Risk Score Distribution of Progressors vs. Non-Progressors

Supplementary Figure 2 compares the frequencies of genetic risk scores of progressors and non-progressors.

Supplementary Table 4. Geographic Atrophy Growth Rate in 12 Months

Patient, eye	Gene	Variant	Baseline GA area, mm	12 month GA area, mm	GA growth (mm/year)
1 right eye	*CFI*	355G>A	3.29	3.67	0.38
2 right eye	*CFI*	148C>G	3.40	3.69	0.29
2 left eye	*CFI*	148C>G	4.10	4.37	0.27
3 right eye	*CFI*	392T>G	0.38	0.41	0.04
3 left eye			0.46	0.54	0.07
4 left eye	*CFH*	2329A>G	0.81	0.95	0.15
5 right eye	*CFH*	496C>T	1.42	1.56	0.15
5 left eye			2.18	2.31	0.12
6 right eye	*CFH*	2572T>A	0.72	0.96	0.24
6 left eye			1.06	1.51	0.45
7 left eye	*CFH*	1222C>T	0.44	0.54	0.10
8 left eye	*CFH*	1126C>T	0.64	0.74	0.10
9 right eye	*CFI*	1657C>T	1.74	1.98	0.24
9 left eye			1.60	1.87	0.26
10 left eye	*CFI*	355G>A	1.69	2.17	0.48
11 right eye	*CFH*	7C>G	3.63	3.88	0.25
11 left eye			3,83	4.08	0.25
12 right eye	*CFH*	578C>T90	0.52	0.52	0.00
12 left eye			0.50	0.74	0.24

Abbreviations: GA, geographic atrophy. *CFI*, complement factor I. *CFH*, complement factor H.



Supplementary Figure 3. Geographic Atrophy Growth (square root transformed)
Baseline geographic atrophy lesion size and growth in 12 months.

Supplementary Table 5. Mean Retinal Sensitivity at 12 and 20 Months

	Baselinemean (SD), dB	End datemean (SD), dB	*P* Value
12 months			
Both eyes (n = 74)	20.5 (8.0)	20.2 (8.6)	ND
Right eye (n = 39)	21.4 (7.5)	20.8 (8.5)	.07
Left eye(n = 35)	20.2 (8.1)	19.6 (8.7)	.1
12 months stratified by disease stage			
Right eye (non-late AMD) (n = 18)	26.4 (2.4)	26.6 (2.0)	.5
Left eye (non-late AMD) (n = 11)	26.5 (2.5)	26.7 (2.1)	.5
Right eye (late AMD) (n = 21)	17.2 (7.8)	15.7 (8.6)	.03*
Left eye (late AMD) (n = 24)	17.3 (8.2)	16.4 (8.8)	.06
20 months			
Both eyes (n = 49)	22.3 (6.2)	20.8 (7.2)	ND
Right eye (n = 25)	22.4 (6.3)	20.9 (7.5)	.02*
Left eye (n = 24)	22.3 (6.2)	20.7 (7.0)	.01*
20 months stratified by disease stage			
Right eye (non-late AMD) n = 11	26.1 (2.0)	25.7 (2.3)	.1
Left eye (non-late AMD) n = 9	27.0 (2.4)	26.7 (2.1)	.6
Right eye (late AMD) n = 14	19.5 (7.1)	17.2 (8.0)	.05*
Left eye (late AMD) n = 15	19.5 (6.2)	17.1 (6.5)	.01*

Abbreviations: ND, not determined. AMD, age-related macular degeneration.
* indicates statistical significance at alfa = 5%.

Supplementary Table 6. Visual Acuity at 12 and 20 Months

	Baseline mean (SD), logMAR	End datemean (SD), logMAR	*P* Value
12 months			
Both eyes (n = 86)	0.33 (0.5)	0.36 (0.5)	ND
Right eye (n = 43)	0.27 (0.4)	0.32 (0.4)	.3
Left eye(n = 43)	0.39 (0.5)	0.41 (0.5)	.5
12 months stratified disease stage			
Right eye (non-late AMD) (n = 18)	0.31 (0.5)	0.33 (0.2)	.6
Left eye (non-late AMD) (n = 14)	0.33 (0.4)	0.41 (0.5)	.4
Right eye (late AMD) (n = 25)	0.23 (0.4)	0.27 (0.4)	.4
Left eye (late AMD) (n = 29)	0.41 (0.5)	0.41 (0.5)	.9
20 months			
Both eyes (n = 51)	0.27 (0.4)	0.36 (0.5)	ND
Right eye n = 25)	0.19 (0.4)	0.21 (0.3)	.6
Left eye (n = 26)	0.35 (0.4)	0.30 (0.4)	.3
20 months stratified disease stage			
Right eye (non-late AMD) n = 9	0.28 (0.5)	0.32 (0.5)	.6
Left eye (non-late AMD)) n = 8	0.40 (0.4)	0.45 (0.5)	.7
Right eye (late AMD) n = 16	0.15 (0.2)	0.15 (0.2)	.9
Left eye (late AMD) n = 18	0.33 (0.4)	0.24 (0.4)	.1

Abbreviations: ND, not determined. AMD, age-related macular degeneration.

Research Chapter Three

Clinical course of non-exudative macular neovascularisations in sustained unilateral neovascular age-related macular degeneration

Authors

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Under review Graefe's Archive for Clinical and Experimental Ophthalmology

Abstract

Purpose

To assess the risk of conversion of non-exudative macular neovascularisations (NEMNV) in relation to disease history in sustained unilateral neovascular age-related macular degeneration (AMD).

Methods

This case-only cohort study included six-monthly visits. Fellow-eye NEMNV were identified using fluorescein and indocyanine green angiography. Patients were stratified by time since first-eye exudation: <5 years (G1) or ≥5 years (G2). The primary outcome was the cumulative annual conversion rate of NEMNV, expressed as conversion rate ratios (CRR) with 95% confidence intervals (CI). Secondary outcomes were independent grading of foveal involvement, core vessel, central core vessel, round shape, sharp borders, and lesion growth

Results

A total of 134 patients (65.7% female) were included. Mean age at baseline was 74.2 years (SD 8.0). Median time since first-eye exudation was 2.9 years (IQR 1.0–6.3). Patients in G2 had an earlier age at first-eye exudation (66.0 [9.1] years) than G1 (72.7 [7.8]; P < .001). Seven NEMNV lesions were identified (G1:2, G2:5). Cumulative conversion rates were 22.1% overall, 14.3% in G1, and 27.1% in G2. CRR were 2.3 (95% CI 1.3–3.3), 1.3 (95% CI −0.2–3.1), and 4.0 (95% CI 0.8–7.1). All but one NEMNV showed growth

Conclusion

Most NEMNV show growth time regardless of conversion to exudative AMD and appear at a higher risk of conversion. The rate of conversion may depend on the age of onset of first-eye exudation. Clinicians should consider both disease history and vascular features when evaluating predictive signs of conversion.

Introduction

Age-related macular degeneration (AMD) is the most common cause of irreversible blindness in the western population.(1, 2) Neovascular, exudative AMD, one of the end stages of AMD, is characterized by exudation and intra- and subretinal fluid accumulation due to a macular neovascularisation (MNV).(3) The prognosis of exudative AMD has markedly improved with the advent of the anti-vascular endothelial growth factor treatment.(4)

Demographically, populations across the world are ageing and since increasing age is the most important risk factor for AMD(5, 6), a surge in cases is expected.(1) Therefore, there is a broad need to accurately assess who is most at risk of developing a sight threatening MNV. Among those most at risk are patients who already developed exudative AMD in one eye, because the risk for progression towards bilateral MNV is relatively high (10% annual).(7)

Evidence suggests that patients with a non-exudative macular neovascularisation (NEMNV) are at a particularly high risk for conversion to exudation.(8) NEMNV, defined as treatment-naïve type 1 neovascularisations without exudation, may be visualized non-invasively using dye based angiography and optical coherence tomography angiography (OCTA).(9) Other terms that have been used for these NEMNVs include subclinical choroidal neovascularisation and quiescent choroidal neovascularisation.

A study of unilateral neovascular AMD patients, reported that 34.5% of NEMNV became exudative over a period of 2 years, yielding a relative risk of 13.6 compared to non-NEMNV eyes.(10) However, estimates of the risk of annual NEMNV exudation range widely; from 21.1% to 52%.(11, 12) Similarly, the prevalence of NEMNV in unilateral neovascular AMD populations ranges from 6% to 24%.(11-17)

This uncertainty regarding timing of conversion hampers clinical management, as doctor's struggle with scheduling visits and fear the risk of missing early signs of exudation. To address this, we studied the clinical course of NEMNV in the fellow eye of patients with unilateral exudative AMD, specifically focusing on its relationship with the time elapsed since the first eye was diagnosed.

Method

Design, setting and participants

Data were collected as part of an ongoing monocentre prospective cohort-study with cross-sectional sampling conducted at the Radboud University Medical Centre in Nijmegen, the Netherlands, aimed at studying progression of AMD in the fellow eye of patients with unilateral neovascular AMD. The inclusion criteria were unilateral MNV secondary to AMD, while ophthalmic conditions in the fellow eye that would interfere with the diagnosis and study procedures were grounds for exclusion, including diabetic retinopathy, severe cataract, advanced glaucoma, and retinal vein occlusion. Patients were included from January 2018 to May 2023. Potential participants were identified through ophthalmologists in neighbouring clinics, contact-advertisements in patient association newsletters, and our own clinical population. Patients were followed up for two years or until the fellow eye developed exudative MNV. If patients discontinued before the two-year mark, they were asked to provide a reason. Where possible, post-study follow-up data were collected either by telephone consultations or review of the electronic patient files. This study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the local ethics committee. All provided written informed consent prior to participating.

Study protocol

At each visit presenting visual acuity (VA) was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart. Demographic and ophthalmic history was assessed through questionnaires. Multimodal imaging was performed in mydriasis.

Image acquisition

Colour fundus photography was obtained (CFP) (DRI Triton (Topcon corporation, Tokyo, Japan), along with spectral-domain optical coherence tomography (SD-OCT) and optical coherence tomography angiography (OCTA) with 3x3mm, 512 B-scans (Spectralis HRA+OCT (version 6.9.54.0; Heidelberg Engineering, Heidelberg, Germany)).(18, 19) In the case of a suspected MNV, fluorescein and indocyanine green angiography (FA-ICGA) was performed to confirm its presence and exudation.(9)

Outcomes

The main outcome was the annual cumulative conversion rate for NEMNV. Also, the 95% CI conversion rate ratio (CRR) was calculated. Secondary outcomes

were independently graded (yes/no) foveal involvement, core vessel, central core vessel, round shape, sharp borders, growth of NEMNV.

Stratification

To account for the effect cross-sectional sampling which includes patients at various points in their disease course, all patients were stratified in two groups based on the time between the onset of exudative MNV in the first eye and the study baseline. The difference between onset and study baseline was less than 5 years for group one (G1) and more than 5 years for group two (G2).

Detection of NEMNV

Detection of NEMNV on OCTA was achieved through a combination of methods. The first method involved assessment of an *en face* avascular zone slab using the device-specific fully automated segmentation algorithms and projection artifact removal algorithms. Additionally, a custom slab was created by segmenting a layer with Bruch's membrane as reference with a thickness of 30 microns. Additional settings such as contrast were optimised for appearance of MNV structures. Optimal settings were configured per NEMNV at first appearance and maintained throughout the analysis.

NEMNV qualitative analysis

Two graders (FC)(YL) independently graded NEMNV according to criteria described by Carnevali, *et al.* at three time points.(20) The following aspects were evaluated: foveal involvement (yes/no), presence of core vessel traveling in the choroid (yes/no), shape of NEMNV (round/irregular), borders (sharp/ill-defined), growth over time (yes/no). Converted NEMNV were graded at first detection, the last available scan before conversion to exudative MNV, and at conversion to exudative MNV. Non-converting NEMNV were graded at first detection, midway between first detection and the last available scan, and at the last available scan. Disagreements were solved by open adjucation.

Grading

Experienced graders of the Eye-NED Reading Center graded CFP and SD-OCT multimodally for features AMD-features.(21) A modified version of the Wisconsin Age-Related Maculopathy Grading System (WARMGS)(22) was used to grade all AMD features, which were subsequently classified into the Rotterdam Study (RS) classification.(21) SD-OCT scans were graded by FC for presence of complete retinal pigment epithelium and outer retinal atrophy (cRORA) according to criteria by the Classification of Atrophy Meetings group.(23)

Statistics

Baseline characteristics for G1 to G2 were statistically evaluated as follows: categorical data via chi-square tests, continuous data using an independent samples T-test. The annual cumulative conversion for NEMNV per group was calculated through dividing the number of converted NEMNV by NEMNV person-time and applying subsequent Poisson approximation.(24) The conversion rate ratio per group was calculated with formula (1) derived from the incidence rate ratio.(25)

$$CRR = \frac{NEMNV_1/PY_1}{NEMNV_0/PY_0}. \quad (1)$$

CRR: conversion rate ratio.
$NEMNV_1$: no. of converted NEMNV.
PY_1: person-years NEMNV at risk.
$NEMNV_0$: no. of non-NEMNV conversions.
PY_0: person-years of non-NEMNV at risk.

A 95% CI of the CRR was calculated via the standard deviation of the log rate ratio. Upper and lower bounds are defined as CRR ± 1.96 times the standard deviation of the rate ratio.(25) In case the precise moment of exudation was unknown, the date of first anti-vascular endothelial growth factor injection was used. *P* values < .05 were considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA).

Results

Study cohort

In total, 148 applications were received for the present study of which 134 met the inclusion criteria. The patients who were not eligible were excluded based on the following revised diagnoses: idiopathic choroidal neovascular membrane (7) acquired vitelliform macular degeneration (3), central serous chorioretinopathy (3), myopia (1).

In total N = 134 (female: 88, 65.7%) were included. Median (IQR) time since first eye exudation was 2.9 (1.0-6.3). Age at baseline was 74.2 (8.0). Mean (SD) follow-up in study was 1.7 (1.3) years ranging 0 to 6.0. Fellow eye grading was

as follows; RS stage 0 (31, 23.1%), RS stage 1 (0, 0%), RS stage 2 (44, 32.8%), RS stage 3 (33 (24.6%), cRORA (20, 14.9%). (Table 1)

Table 1. Baseline Characteristics

	Total (N = 134)
Time since onset of exudative AMD, median (IQR),	2.9 (1.0-6.3)
Female, No. (%)	88 (65.7%)
Age diagnosis exudative AMD, mean (SD), years	70.0 (8.7)
Baseline age, mean (SD), years	74.2 (8.0)
Lifestyle and ophthalmic history	
Smoking (yes), No. (%)	10 (7.5%)
Ex-smokers (yes), No. (%)	81 (61.2%)
Smoking(ever), No. (%)	92 (68.7%)
Lutein and zeaxanthin supplements (yes), No. (%)	32 (58%)
University level education* (yes), No. (%)	48 (35.8%)
Number of injections, median (IQR)†	13 (7-30)
Fellow eye‡	
RS stage 0	31 (23.1%)
RS stage 1	0 (0%)
RS stage 2	44 (32.8%)
RS stage 3	33 (24.6%)
cRORA, No. (%)	20 (14.9%)
Visual acuity	
Visual acuity first eye, mean (SD), letters	58.5 (24.1)
Visual acuity fellow eye, mean (SD), letters	81.7 (7.6)

Abbreviations: NA, not applicable. cRORA, complete retinal pigment epithelium and outer retinal atrophy. RS, Rotterdam Study. RS stage 2: Rotterdam Study stage 2; Soft indistinct drusen (≥125 µm) or reticular drusen only, soft distinct drusen (≥63 µm). nAMD or neovascular age-related macular degeneration.
*university refers to having a degree from a practical university (in Dutch: *hoger beroeps onderwijs*) or higher, missing = 8, †missing = 25. ‡missing = 4.

In total, 32 left the study before the 2-year study period was completed. The reasons for premature discontinuation were: death (8), finding the study too burdensome (7), other non-ophthalmic illnesses (6), travel distance (5), participating in a rival ophthalmic study (2), other (4). The study design is represented in a flowchart (Supplemental Figure 1).

Group 1 and 2 characteristics

G1 featured 92 patient, G2 featured 42. Age at baseline did not differ for both groups (G1: mean [SD] age 73.8 [7.8], G2: 75.0 [8.3], *P* = .4) whereas age of exudative AMD onset did (G1: mean [SD] age 72.7 [7.8], G2: 66.0 [9.1], *P* < .001). (Table 2).

Table 2. Group 1 and 2 characteristics

	Group 1 Less than 5 years	Group 2 More than 5 years	*P* value
	(n = 92)	(n = 42)	
Time since onset of exudative AMD, median (IQR), years	1.6 (.6-3.1)	9.0 (6.4-10.8)	NA
Female, No. (%)	62 (67.4%)	26 (61.9%)	.5
Age at onset exudative AMD, mean (SD), years	72.2 (7.8)	66.0 (9.1)	<.001*
Baseline age, mean (SD), years	73.8 (7.8)	75.0 (8.3)	.4
Lifestyle and ophthalmic history			
Smoking (yes), No. (%)	8 (9.8%)	1 (2.4%)	.1
Ex-smokers (yes), No. (%)	49 (53.3%)	33 (78.6%)	.01*
Smoking(ever), No. (%)	58 (63.0%)	34 (81.0%)	.04*
Lutein and zeaxanthin supplements (yes), No. (%)	55 (59.8%)	22 (52.4%)	.4
University level education* (yes), No. (%)	33 (35.9%)	15 (35.7%)	.8
Number of injections, median (IQR)†	11 (6-20)	32 (14-51)	.01*
Fellow eye‡			
RS stage 0	21 (22.8%)	10 (23.8%)	.9
RS stage 1	0 (0%)	0 (0%)	NA
RS stage 2	37 (40.2%)	7 (16.7%)	.01*
RS stage 3	20 (21.7%)	13 (31.0%)	.3
cRORA, No. (%)	9 (9.8%)	11 (26.2%)	.01*
Visual acuity			
Visual acuity first eye, mean (SD), letters	65.1 (19.5)	44.6 (26.8)	<.001*
Visual acuity fellow eye, mean (SD), letters	82.4 (5.6)	80.3 (10.3)	.1

Abbreviations: NA, not applicable. cRORA, complete retinal pigment epithelium and outer retinal atrophy. RS, Rotterdam Study. RS stage 2: Rotterdam Study stage 2; Soft indistinct drusen (≥125 µm) or reticular drusen only, soft distinct drusen (≥63 µm). nAMD or neovascular age-related macular degeneration.

*Indicates *P* <.05. *university refers to having a degree from a practical university (in Dutch: *hoger beroeps onderwijs*) or higher, missing = 4,4. †missing = 13, 12. ‡missing = 4,0.

G2 contained more ex-smokers (G1: 49 (53.3%), G2: 22 (78.6%), *P* = .01) and ever-smokers than G1 (G1: 58 (63.0%), G2: 34 (81.0%), *P* = .04). Other lifestyle factors were similar across groups. G2 had the highest proportion of cRORA (G1: 9 (9.8%), G2: 11 (26.2%), *P* = .01) and the lowest proportion of RS stage 2 (G1:37 (40.2%), G2: 7 (16.7%), *P* = .01). G2 had worse visual acuity in the first eye (G1: mean [SD] letters 65.1 [19.5], G2: 44.6 [26.8], *P* < .001) while mean visual acuity for the fellow eye did not differ. (Table 2)

Main results

Seven NEMNV were detected, of which 2 were in G1. Notably, 2 NEMNV were initially missed and were detected during follow-up. However, all NEMNVs were appeared present at baseline after in-depth examination of images. No incident NEMV were detected during follow-up. Four out of the 7 NEMNV became exudative during follow-up with a median conversion time of 1.5 ranging from 10 months to 3 years. For the total group (N = 134) annual cumulative conversion of NEMNV was 22.1% with a CRR of 2.3 (95% CI 1.3 – 3.3). (Table 3) In G1, annual cumulative conversion of NEMNV was 14.3% with a CRR of 1.3 (95% CI -0.2 – 3.1) while in G2 this was 27.1% with a CRR 4.0 (95% CI 0.8 – 7.1).

Table 3. NEMNV and Conversion Rate Ratio

	Total (N = 134)		G1: Less than 5 years (n = 92)		G2: More than 5 years (n = 42)	
	NEMNV	**No NEMNV**	**NEMNV**	**No NEMNV**	**NEMNV**	**No NEMNV**
Converted	4	31	1	23	3	8
Person-years	16.0	290.1	6.5	188.2	9.5	101.9

Abbreviations: NEMNV, non-exudative macular neovascularisation; CRR, conversion rate ratio

Descriptive and qualitative analysis of NEMNVs

All NEMNV patients were female, the median age at baseline was 72 and ranged from 43 to 92 years. All NEMNV-eyes showed drusen and none showed cRORA. Three of 7 were graded as RS stage 3, the others were graded as RS stage 2. All NEMNVs displayed sharply defined borders at baseline (figure 1). We identified a core vessel in all cases, whereas a central core vessel was visible in 3 cases. Three NEMNV displayed foveal involvement. Only one NEMNV was shaped roundly. For growth analysis, 6 out of 7 had available follow up. Of these NEMNV, 5/6 (83%) displayed growth between baseline and visit 2. Of the NEMNV that became exudative (patient I-IV) all (100%) showed growth. Non-

converting NEMNV showed growth in most cases (2 of 3 or 66%)Between visit 2 and 3, 3 NEMNV showed growth. (Figure 2) (Supplemental Table 1)

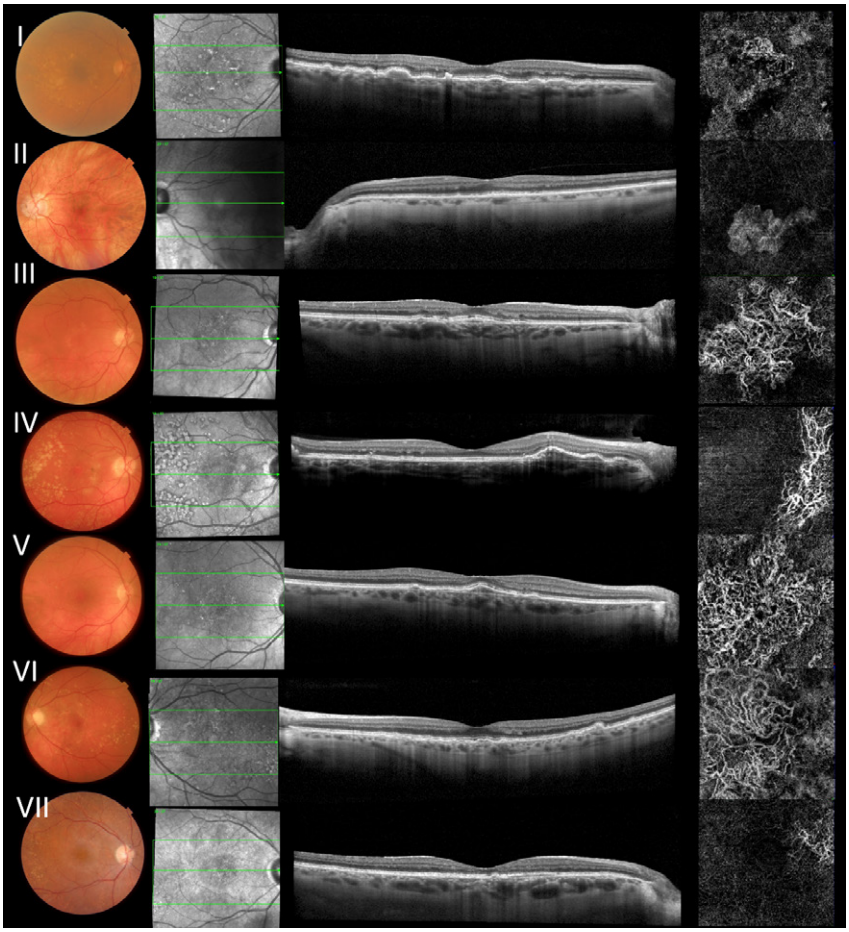

Figure 1. Baseline Imaging of NEMNV

Baseline imaging of all non-exudative macular neovascularisations (NEMNV) for patient I-VII, right to left panel: colour fundus photography (CFP), infra-red image, spectral domain optical coherence tomography (SD-OCT), optical coherence angiography (OCTA). I. Female, 78 years, settings: Bruch's membrane with depth of 40 micron. II. female, age 92 years, settings: avascular zone. III. Female, age 74 years, settings: artifact removal, contrast 1:5, retinal pigment epithelium to Bruch's membrane. IV. Female, age 43 years, settings: avascular zone. V. Female, age 72 years, settings: Bruch's membrane with a thickness of 40 micron. VI. Female, age 67 years, settings: projection artifact removal, contrast 1:7, reference retinal pigment epithelium with a depth of -27 micron and thickness of 40 micron. VII. Female, age 70, settings: avascular zone.

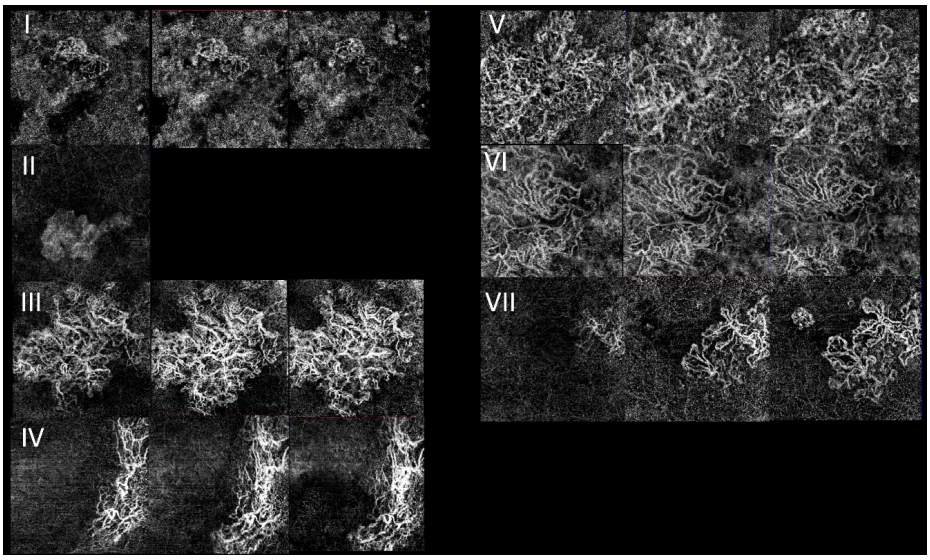

Figure 2. OCTA of NEMNV at different visits

Optical coherence angiography (OCTA) of non-exudative macular neovascularizations (NEMNV) in patients I-VII was analyzed. Patients I-IV developed exudative age-related macular degeneration (AMD). The left panels represent images baseline, the middle panels show the penultimate OCTA images, and the right panels display the OCTA images at the moment of exudation. For patients V-VII, the left panels represent images at diagnosis, the middle panels show images from midway visits, and the right panels display baseline from the last visit. The settings for all images are the same and are detailed below.

Patient I: A sharply defined, non-foveal lesion with a core vessel showed growth over time between the first and penultimate visits. The time between the first and middle panels is 7 months, and 4 months between the middle and last panels.

Patient II: A sharply defined, non-foveal lesion with a core vessel was observed. Growth could not be determined due to unavailable images.

Patient III: A sharply defined, foveal lesion with a central core vessel showed growth between all visits. The time between the first and middle panels is 2.8 years, and 2 months between the middle and last panels.

Patient IV: A sharply defined, non-foveal lesion with a central core vessel showed growth between the first and penultimate visits. The time between images is 10 months from the first to middle panel and 2 weeks from the middle to last panel.

Patient V: A sharply defined, foveal lesion with a central core vessel showed growth across visits. The time between images is 1 year 3 months from the first to middle panel and 1 year 1 month from the middle to last panel.

Patient VI: A sharply defined, foveal lesion with a core vessel did not show growth across visits. The time between images is 1 year from the first to middle panel and 1 year 1 month from the middle to last panel.

Patient VII: A sharply defined lesion with a core vessel showed growth across visits, involving the fovea over time. The time between images is 2 years from the first to middle panel and 1 year 10 months from the middle to last panel.

Discussion

In this case-only cohort study of N =134 patients with unilateral exudative AMD, annual conversion of NEMNV was 22.1% with a CRR of 2.3 (95% CI 1.3 – 3.3). However, risk of conversion varied with time since onset of exudative AMD in the first eye. The annual conversion rate was 27.1% with a CRR of 4.0 (95% CI 0.8 – 7.1) for patients with more than five years since first eye onset of exudative AMD (G2). In contrast, these figures were 14.3% with a CRR of 1.3 (95% CI -0.2 – 3.1) for patients whose first eye onset of exudative AMD was less than five years before baseline (G1), This discrepancy raises two important questions: First, how might we explain this contrast and, second, what is the actual risk of conversion for NEMNV?

Groups 1 and 2 were stratified based on the time since onset of exudative AMD in the first eye, leading to important differences. G2 had more severe phenotypes in the fellow eye and poorer visual acuity in the first eye, reflecting a longer disease duration. Although baseline age and lifestyle factors were broadly comparable across groups, G2 had a younger age of onset of exudative AMD, which may indicate a greater genetic burden (Table 2). (26, 27) However, genetic factors and age at onset alone are unlikely to fully account for the observed differences in CRRs.

Instead, we hypothesize that differences in CRRs are, at least in part, due to the timing of observation. In this study, NEMNV may have been detected at a relatively late stage in their development, contributing to the impression that they are at particularly high risk of conversion. Additionally, length-biased sampling likely played a role: patients with a longer duration since exudation onset were more likely to be included in the study, leading to an overrepresentation of such cases (28) (Supplemental Figure 2). Given that most NEMNV cases were observed in G2—the group most susceptible to this bias, risk perception may have been influenced accordingly.

This hypothesis has important consequences for the appraisal of current literature. Most if not all on the natural history or predictive factors of NEMNV or subclinical CNV or quiescent CNV – all related terms - make no mention of the effect of length-biased sampling.(11-13, 15, 16, 20) As stated, estimates of the prevalence of NEMNV in AMD populations range from 6 to 24%.(11-16) While existing studies vary in regards to sample size, fellow eye inclusion

criteria, definition and detection method of NEMNV, oversampling patients with longer disease durations could impact prevalence estimates.

Qualitative analysis shows that most NEMNV show change over time regardless of conversion (e.g. 83% of NEMNV with available follow-up showed growth). All converted NEMNV showed growth and most non-converted (66%). (supplemental table 2) However, it is challenging to appreciate these findings since age of first eye exudation appears to influence conversion rates (the highest conversion rate was observed for those with earlier age of onsets in the first eye). To fully appreciate potential predictive signs therefore, future analysis should analyse potential predictive vascular changes in conjunction with duration of disease history.

Compounding the aforementioned difficulty of assessing potential predictive signs, is the fact that our sample shows selection. G2 in particular does not include NEMNV which may have formed after onset of first eye exudation but that converted before baseline. It is tenuous to speculate on the behaviour of these NEMNV.

Considerations for future studies include the non-standard imaging protocols and imaging devices.(29) As OCTA devices improve and become more widespread, NEMNV conversion risk may decrease due to earlier detection. Additionally, definitions vary and might be further standardised; subclinical choroidal neovascularisation, quiescent choroidal neovascularisation, or NEMNV are related but not equal terms. Sacconi, *et al.* proposed to reserve the term 'quiescent' for lesions not becoming exudative for a period of six months.(9) In this study none of the NEMNV converted before six months so the term quiescent equally applies. However, being observed to not-converge for any length of time should not be confused with the notion of being "stable" which is what the term quiescent aims to convey. Therefore, we opted to not use this term. In sum, future studies seeking to improve understanding of conversion risk might do so by following up all NEMNV from the moment of first eye exudation or using an otherwise specified, standardised starting point.

Strengths and limitations

A major strength of this study is the verification of NEMNV with ICG in combination with the systematic multimodal visits. Stratification on time since first eye exudation results in lower power with subsequent high uncertainty which is reflected in the wide 95% CI of CRR's. However, stratification is

necessary to curtail survivor effects which are a limitation of the present design. As is customary for exploratory studies, post-hoc correction for multiple testing was not applied. Lastly, length-biased sampling might affect conversion risks ratio however, since stratification was performed its impact is limited.

In conclusion, most NEMNV show change over time regardless of conversion to exudative AMD and NEMNV appear at a higher risk of conversion. However, the rate of conversion appears to depend on the age of onset of first eye exudation. Therefore clinicians should assess disease history in conjunction with vascular change. Future and ongoing efforts to study predictive signs of exudation in the non-neovascular eye may benefit from analysing risks of NEMVN conversion from the onset of first eye exudation.(30)

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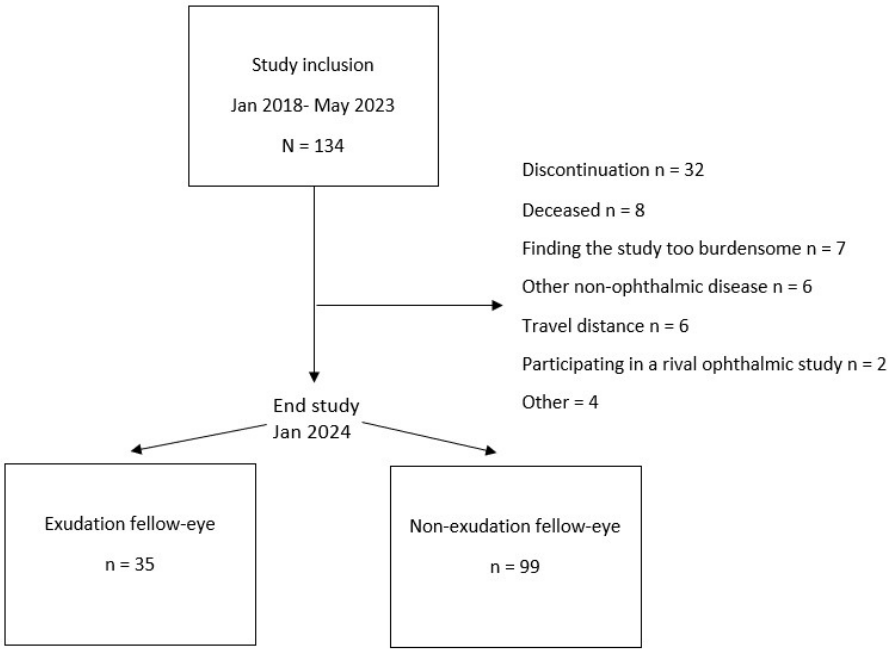
Supplemental Material

Supplemental Figure 1. Flow-chart of Study

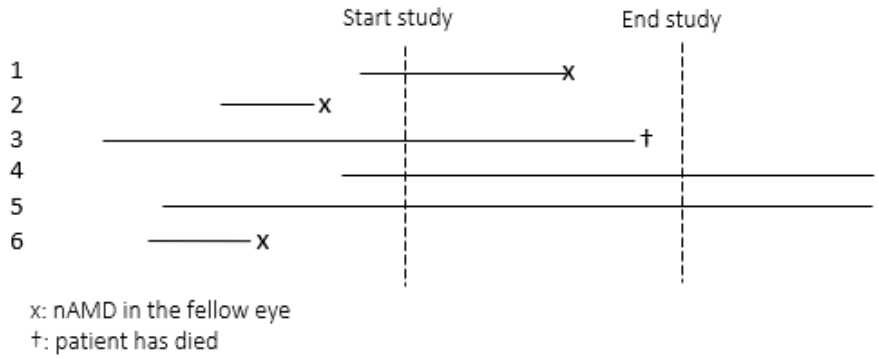


Supplemental Figure 1 shows a flow-chart of this study and its main events.

Supplemental Table 1. Qualitative analysis NEMNV

Patient	I	II	III	IV	V	VI	VII
Foveal involvement	No	No	Yes	No	Yes	Yes	No
Core vessel	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Central core vessel	No	No	Yes	Yes	Yes	Unclear	No
Round shape	No	No	No	No	Yes	Unclear	Unclear
Sharp borders	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Growth visit 1-2	Yes	NA	Yes	Yes	Yes	No	Yes
Growth visit 2-3	No	NA	Yes	No	Yes	No	Yes

Abbreviations: NEMNV, non-exudative macular neovascularisation. 3 Supplemental figure 2 shows the grading results which have also been summarized below figure 2



Supplemental Figure 2

Supplemental figure 2 shows how length-biased sampling results in in overinclusion patients with longer disease histories.

Research Chapter Four

Power and clinical utility of mesopic microperimetry analysis strategies in age-related macular degeneration

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Abstract

Purpose This study evaluates whether Mesopic Microperimetry (MMP) provides a more robust measure of retinal function compared to visual acuity (VA) in age-related macular degeneration (AMD) clinical trials, with a focus on optimal analysis strategies.

Method Fellow eyes of unilateral neovascular AMD were prospectively studied. Presenting VA was measured. The Macular Integrity Assessment Microperimeter (MAIA), was used with a 4-2 staircase strategy with a 10° diameter grid containing 37 loci. Three analysis strategies were calculated: the mean of 37 sensitivity thresholds (MS), the percent-reduced threshold (PRT), and the log-transformed candela mean (MS cd log). Sample size requirements were calculated for 12- and 24-month follow-ups using a paired one-sided T-test ($\alpha = .05$, power = 0.80).

Results N = 123 were analysed (82 (65.5%) females; mean age 74.2 (7.8) years). Ranked high to low, the required sample size at 12 months was: MS (n =51), MS cd log (n = 52), PRT (n = 139), and VA (n = 203). Similar trends were seen at 24 months, with MS requiring the smallest sample size (n = 85) and VA the largest (n = 1673).

Conclusion All MMP analysis strategies outperformed VA, and MS required the least number of patients to show significant changes. This trend was consistent for both 12 and 24 months. These findings provide strong statistical arguments for the use of MMP in longitudinal within-subjects clinical trials and suggest that averaging decibels is optimal.

Introduction

Age-related macular degeneration (AMD) is a progressive retinal disease and the major cause of irreversible visual impairment in elderly in the Western world.(1) Presently, natural history studies and clinical trials evaluating potential new therapies are being conducted.(2) To interpret outcomes of these studies, and to translate them to daily clinical practice, in-depth understanding of the clinical endpoints is essential.

Current AMD trials use best-corrected visual acuity (BCVA) as a functional endpoint. (3) However, BCVA has limitations as both a primary and secondary measure. (4) It shows minimal change over typical trial durations due to its limited sensitivity to early and intermediate AMD, extra-foveal geographic atrophy (GA), and even late AMD with foveal involvement. (4-6) BCVA cannot capture relevant extra-foveal improvements in exudative AMD or adequately reflect slow foveal changes in GA, which often exceed trial durations. (7, 8) Its inability to distinguish between AMD stages without foveal involvement may overlook critical pathology, hindering the evaluation of potential therapies. (9)

MMP – a relatively new functional retinal test – involves presenting light stimuli of varying intensity to predefined retinal locations, with the sensitivity threshold (candela/m^2 expressed as decibels) algorithmically determined for each location.(5) MMP has shown to be sensitive to progression while BCVA remained stable even in non-advanced AMD.(10-12) Based on these findings, MMP has been used as an secondary endpoint in clinical trials evaluating therapies for patients with GA secondary to AMD.(4, 13, 14)

There has been growing interest into the optimal analysis strategy of MMP venturing beyond either the mean of al sensitivity thresholds or a point-wise cut-off approach using absolute (0 db) or relative scotoma (e.g. <10 dB).(4, 5, 14) The latter is criticized for obscuring true change, while the former might 'miss' localised pathology.(4) While some proposed analysis strategy changes are specifically designed for GA, such as limiting the number of analysed sensitivity threshold values to those bordering GA, further general investigation is warranted for the use of MMP in other AMD stages.(2, 10, 14, 15)

The issue of random measurement error transcends disease stage and is relevant considering that the test-retest repeatability of the Macular Integrity Assessment microperimetry (MAIA; CentreVue, Padova, Italy) is approximately 4 dB and, in

the case of deep scotoma including GA, 6 dB (95% coefficient of variation). These figures might be considered substantial relative to its 36 decibel scale.(5)

We hypothesize that random measurement error affects analysis strategies (mean or cut-off) differentially. This will have consequences for statistical power and calculations of estimated annual change, both of which are essential for clinical trial design and interpretation of results. In this exploratory study, we explore three analysis strategies including the aforementioned mean, cut-off, and a novel approach in a group of AMD patients from a prospective cohort study. The novel approach averages raw candela/m^2 prior to log transformation. We hypothesize that the novel approach is more robust to random variation resulting in a higher power to detect change over time while retaining sensitivity to local pathology.

Method

This secondary analysis was performed using patients with available follow-up at 12±2 months and 24±2 months from the primary, ongoing, prospective cohort study with unilateral neovascular AMD patients. Details of the primary study are outlined below.

Primary Study characteristics; Design, Setting, Participants & Design

Participants were included between Jan 2018 to Nov 2022 and were evaluated every six months at the Radboud university outpatient clinic in Nijmegen. Participants left the study if the fellow eye became exudative and/or received anti-vascular endothelial growth factor secondary to macular neovascularisation. The study period encompassed two years after which participants were given the opportunity to continue or leave the study. Participants who left before two years without CNV in the fellow eye were asked to provide a reason. The aim of our study was to investigate AMD-related changes prior to exudation in the fellow eye.

Patients with the following criteria were included: unilateral macular neovascularisation (MNV) secondary to AMD without any ophthalmic complications in the fellow eye that would interfere with the study procedures, such as: diabetic retinopathy, severe cataract, glaucoma, retinal vein occlusion. Potential participants were selected from referrals from ophthalmologists from neighbouring clinics, contact-advertisements in patient association newsletters and our own clinical population. This study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the local ethics committee. All participants provided written informed consent prior to participating.

Medical and demographic information of each patient was collected using questionnaires. At each visit, visual acuity (VA) was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart. MMP was performed with the MAIA using a 4–2 staircase strategy with a grid of 10° diameter containing 37 radially oriented points centred on the fovea. A test-run prior to the actual examination was performed. Participants were not in mydriasis.(5, 16) The grid was carefully aligned on the fovea. After mydriasis, multimodal imaging was performed, including colour fundus photography (CFP) (DRI Triton (Topcon corporation, Tokyo, Japan) and spectral-domain

optical coherence tomography (SD-OCT) (SpectralisTM HRA+OCT (Heidelberg Engineering, Heidelberg, Germany)).

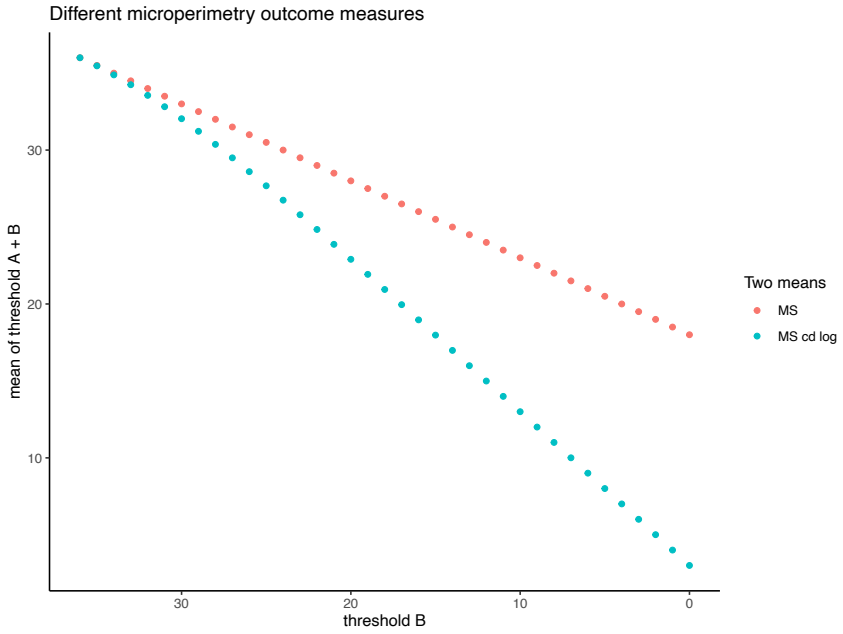

Figure 1. Demonstration of Difference between MS and MS cd log

In figure 2 we demonstrate the difference between mean sensitivity or the mean of all 37 thresholds (MS) and MS cd log or the log transformed mean of candela values of 37 thresholds. Suppose we have a grid containing thresholds A and B. In our example threshold A is fixated while threshold B decreases with a step of 1 db. MS and MS cd log will behave differently as threshold B decreases. MS cd log will diverge from MS for lower dB values

MMP Analysis Strategies

For the present analysis three analysis strategies of MMP were calculated:

- Mean sensitivity (MS): this is the average of all 37 sensitivity thresholds in dB.
- Mean sensitivity candela log (MS cd log): MS cd log is calculated by first converting each sensitivity threshold into its corresponding candela/m^2 value.(5) In step 2, all thresholds in candela/m^2 are then averaged. The third step is to retransform the product of step 2 back to a dB value. The effect of this transformation relative to MS is outlined in Figure 1.
- Percent reduced threshold (PRT): the number of sensitivity thresholds below 25 dB divided by 37 (the total number of thresholds), expressed as a decimal value.

Grading

CFP were graded by EYE-NED grading centre (Rotterdam, the Netherlands) according to the Wisconsin age-related maculopathy grading system (WARMGS) (17) and then converted to the Rotterdam study criteria (RS stage 0-4).(18)

Outcomes

The main outcome is the estimated sample size (power 80%) required to detect a statistically significant decline (at 0.05 level) in VA, MS, MS cd log and, PRT at 12 and 24 months. A relatively small sample size indicates that a decline in a certain analysis strategy is easier to detect than a decline in others. Similarly in a fixed dataset an analysis strategy with a smaller estimated sample size will a lower chance of type II error.

Secondary outcomes were predicted annual change (95% CI) for each RS stage for VA, MS, MS cd log and, PRT using a mixed linear model

Statistical methods

As a first, simple analysis, the average and variance of the paired differences at two different time points (12 and 24 months) was used to estimate the required sample size in VA, MS, MS cd log, and PRT. This was done for a power of 80% at a 0.05 significance level using a one-sided T-test.

Then, given the repeated measures design of the data, all timepoints were used in a mixed linear model. Estimated annual change of VA, MS, MS cd log, and PRT was modelled using a two-level multilevel model as outlined by Singer and Willet. (19) We report our statistical output following the recommendations of Monsalves, *et al*. (20) Level 1 represents individual change of Y_{ij} in VA, MS, MS cd log, and PRT for time in years Y_{ij}. (1) Level 2 models the patient-specific intercept β_{0i} (2.1) and the patient-specific slope β_{1i} (2.2) for interindividual predictors RS-stage 2 to 4 (S_{i2}, S_{i3}, S_{i4}) with RS-stage 0 as reference. The full model is given by:

$$Y_{ij} = \beta_{0i} + \beta_{1i}t_{ij} + \epsilon_{ij}, \quad (1)$$

and

$$\beta_{0i} = \gamma_{00} + \gamma_{01}S_{i2} + \gamma_{02}S_{i3} + \gamma_{03}S_{i4} + u_{0i}, \quad (2.1)$$

$$\beta_{1i} = \gamma_{10} + \gamma_{11}S_{i2} + \gamma_{12}S_{i3} + \gamma_{13}S_{i4} + u_{1i}. \quad (2.2)$$

γ_{00}: average baseline value for patients with RS-stage 0.

γ_{10}: average slope for patients with RS-stage 0.

γ_{01}, γ_{02}, γ_{03}: Differences in intercepts for RS stage 2,3, and 4 compared to RS-stage 0.

γ_{11}, γ_{12}, γ_{13}: Changes for the slope for RS-stage 2, 3, and 3 compared to RS-stage 0.

The 95% CI and *P* values are reported. Goodness of fit was provided via marginal R^2 which assigns a percentage score to the variance explained of all fixed effects: time, RS-stage, and time*RS-stage, and secondly, conditional R^2 which represents the combined fixed and random effects.(21) The variance partition coefficient was provided (variance between and within subjects) using a null-model. All analyses were conducted in R 4.1.3 (2022-03-10) (R Core Team, Boston, MA, 2014). The lmer (1.1-35.1), lmertest (3.1-3), and the tidyverse packages (2.0.0) were used.(22, 23)

Results

Participants

During Jan 2018 and Nov 2022 132 patients met the inclusion criteria for the primary study. Four patients developed bilateral exudative neovascular AMD between registration and first visit and were not invited for further participation. Also, MMP for 5 additional patients was not performed. The reasons were as follows: cognitive impairment/failure to grasp instructions (2), hemianopsia (1), bilateral leg amputation (1), unable to fixate (1). In total, N = 123 patients were selected for the present study. During follow-up 18 patients developed an MNV in the fellow eye. Twenty-nine patients discontinued prematurely. The reasons were: death (7), travel distance (5), finding the study too burdensome (5), other non-ophthalmic illnesses (4), participating in a rival ophthalmic study (2), other (6). Some patients failed to visit at the appointed intervals on account of covid-restrictions but did not leave the study. Therefore, n = 68 patients were selected at 12 months and n = 31 at 24 months.

Table 1. Baseline Variables

Variable	Value N = 123
Time since first eye involvement, mean (SD), years	4.4 (4.1)
Age baseline, mean (SD), years	74.2 (7.8)
Female, No. (%)	82 (67.5%)
Smoking, No. (%)	
Currently	8 (6.5%)
Ever	76 (61%)
Never	39 (33.5%)
University level education*	47/116 (38.2%)
Phakic , No. (%)	84 (68.9%)
RS stage 0-4, No. (%)	
RS stage 0	29 (23.6%)
RS stage 1	0 (0%)
RS stage 2	41 (33,3%)
RS stage 3	32 (26.0%)
RS stage 4	21 (17.1%)

*n = 7 missing values,

Abbreviations: RS stage 0 corresponds to no signs of age-related macular degeneration at all or hard drusen <63 µm) only; RS stage 1, Soft distinct drusen (≥63 µm) only or pigmentary irregularities only, no soft drusen (≥63 µm); RS stage 2, Soft indistinct drusen (≥125 µm) or reticular drusen only, soft distinct drusen (≥63 µm); RS stage 3, Soft indistinct (≥125 µm) or reticular drusen with pigmentary irregularities; RS stage 4, Atrophic.

Descriptives

Of total (N = 123) mean (SD) age was 74.2 (7.8) years and 82 (67.5%) were female. The mean (SD) time since first eye involvement with neovascular AMD was 4.4 (4.1) years while the mean (SD) follow-up time was 1.9 (1.2) years as of august 2023. At baseline, the fellow eye of 29 (23.6%) patients was graded as RS stage 0 corresponding to no signs of AMD at all or hard drusen (<63 µm) only, 0 (0%) as RS stage 1), 41 (33.3%) as RS stage 2, 32 (26.0%) as RS stage 3, and 21 (17.1%) as RS stage 4 or atrophic AMD. Additional demographic information can be found in table 1.

Change in VA and retinal sensitivity (MS, MS cd log, PRT)

At baseline mean (SD) VA was 81.9 (7.0) letters. Retinal sensitivity (mean (SD)) was 23.9 (4.9) dB, 22.5 (6.6) dB, and .4 (.3) decimal value for MS, MS cd log, and PRT, respectively. (Table 2) Mean loss (SD) of VA was .87 (5.0) and -.45 (7.5) letters at 12 and 24 months. Retinal sensitivity change was -.7 (2.0) dB for MS, -1.15 (3.3) dB for MS cd log, and .04 (.2) decimal value for PRT at 12 months. At 24 months the change was -1.05 (3.9) dB, -1.10 (5.4) dB, and .04 (.3) decimal value for MS, MS cd log, and PRT, respectively. (Table 2) Longitudinal changes for VA, MS, MS cd log, and PRT are visualised in figure 2 showing increasing instability for each subsequent analysis strategy. PRT clearly demonstrates the highest variability.

Sample size

The estimated required sample size (Table 2) for VA is n = 203 at 12 months. This was n = 51 for MS, 52 for MS cd log, and 139 for PRT. At 24 months n was 1673 for VA, n = 85 for MS, n = 152 for MS cd log, and n = 312 for PRT.

Table 2. Uncorrected Changes and Required Sample Size

All stages	VA, letters mean (SD)	MS, dB mean (SD)	MS cd log, dB mean (SD)	PRT, dec. v. mean (SD)
Baseline	81.9 (7.0)	23.9 (4.9)	22.5 (6.6)	.4 (.3)
Δ 12 months	-0.87 (5.0)	-0.70 (2.0)	-1.15 (3.3)	.04 (.2)
Δ 24 months	-0.45 (7.4)	-1.05 (3.9)	-1.10(5.4)	.04 (.3)
Required sample size, 12 months (n = 68)	203	51	52	139
Required sample size, 24 months (n = 31)	1673	85	152	318

Abbreviations: dec. v., decimal value; VA, visual acuity; MS, mean sensitivity; MS cd log; mean sensitivity candela log; PRT, percent reduced threshold. Required sample size indicates the number of patients based on 12±2 months, 24±2 months of available patients, we expect to need to include to have an 80% chance to detect a decline at .05 significance level using a one-sided T-test

Estimated annual change

Intercepts for RS-stage 4 were statistically different for MS (*P*<.001), MS cd log (*P*<.001), and PRT (*P*<.001) compared to all other stages. Similarly, intercepts for RS-stage 3 were statistically different for MS (*P*=.01), MS cd log (*P*=.02), and PRT (*P*<.001) compared to all other stages. (Table 3)Boxplots showing the distributions of VA and retinal sensitivity across RS stages are presented supplemental Figure 1.

In the MS model, annual change for RS-stage 4 was estimated to be significantly higher compared to average change (-1.11 dB, 95%CI (-2.12 to -.09), *P*<.03). No other MMP output variable showed a statistically significant interaction effect. Estimated annual change for RS stage-4 was -1.07 dB 95%CI(-2.42 to .028) for MS cd log and in PRT this was .03 95%CI(-.03 to 10). (Table 3) For VA the estimated annual loss for RS stage 4 was 5.5 letters 95%CI(-7.53 to -2.58), *P*<.001).

The variance partition coefficient indicated that 69% of the variance for VA existed between patients, similarly these numbers were 80% for MS, 83% for MS cd log and 73% for PRT. (Supplemental Table 1) All models converged. Ranked highest to lowest, marginal R^2 was .394, .352, .272, .180 for MS cd log, MS, PRT, and VA respectively.

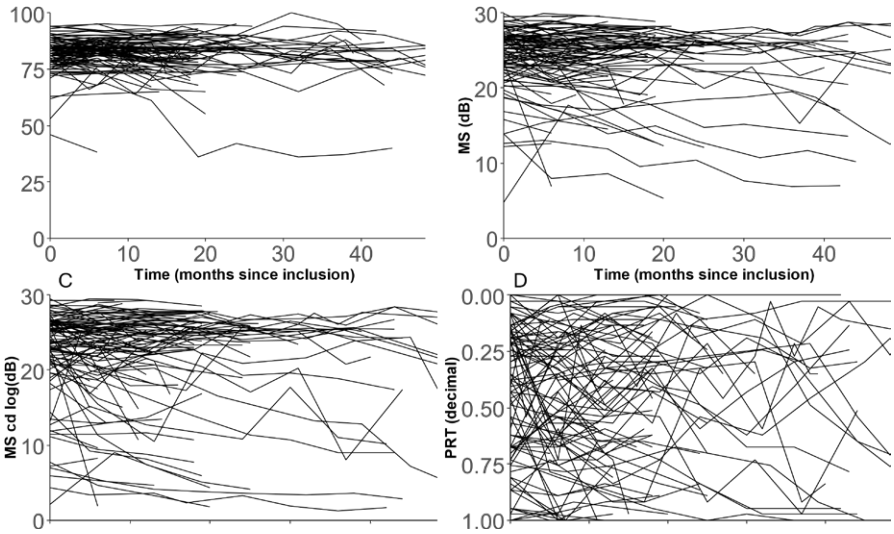

Figure 2. Longitudinal changes of VA, MS, MS cd log, and PRT

Abbreviations: VA, visual acuity; MS, mean sensitivity; MS cd log or mean sensitivity candela log; PRT or percent reduced threshold.

Spaghetti-plots showing longitudinal change for each analysis strategy.

Table 3. Predicted changes of VA, MS, MS cd log, and PRT per Stage (abridged)

	VA		MS		MS cd log		PRT	
Predictors	*Estimates*	*P*	*Estimates*	*P*	*Estimates*	*P*	*Estimates*	*P*
Intercept	83.46		26.39		25.42		.23	
Time (years)	0.9	.2	-0.26	.4	-0.29	.5	0.02	.4
RS stage 4	-3.53	.08	-6.83	<.001	-10.96	<.001	0.47	<.001
RS stage 3	-2.63	.1	-2.72	.01	-3.24	.02	0.28	<.001
RS stage 2	0.15	.9	-1.93	.06	-2.15	.1	0.13	.06
Time *RS stage 4	-5.05	<.001	-1.11	.03	-1.07	.1	0.03	.3
Time * RS stage 3	-2.19	.05	-0.27	.6	-0.97	.1	0.02	.4
Time * RS stage 2	-1.82	.09	0.08	.9	0.08	.9	-0.01	.8
R^2/ Conditional R^2	0.180/0.782		0.352/0.889		0.394/0.884		0.274/0.821	

Abbreviations: VA, visual acuity; MS, mean sensitivity; MS cd log; mean sensitivity candela log; PRT, percent reduced threshold; RS stage 0 corresponds to no signs of age-related macular degeneration at all or hard drusen <63 µm) only; RS stage 1, Soft distinct drusen (≥63 µm) only or pigmentary irregularities only, no soft drusen (≥63 µm); RS stage 2, Soft indistinct drusen (≥125 µm) or reticular drusen only, soft distinct drusen (≥63 µm); RS stage 3, Soft indistinct (≥125 µm) or reticular drusen with pigmentary irregularities; RS stage 4, Atrophic. Abridged: CI have been left out for editing purposes and may be found at DOI: 10.34973/t8kb-x644 (repository).

Discussion

In this secondary analysis of fellow eyes of 123 unilateral neovascular AMD patients, all MMP analysis strategies outperformed VA in terms of power(10, 12), and MS was most powerful. (Table 2) This trend was consistent for both 12 and 24 months of follow-up.

Estimated annual change provides additional insights into stage-related change for each analysis strategy. All analysis strategies showed the fastest worsening for higher staged individuals over the course of the study as would be expected. However, only MS was statistically significant. For RS stage 4, estimated annual change was -1.11 dB 95%CI(-2.12 to – 0.09) for MS, while for MS cd log, this was -1.07 dB with a widened, insignificant 95%CI of -2.42 to .028. For PRT the 95%CI was even wider: -.03 to .10 with an estimated annual change of .03.

Of all three analysis strategies PRT was weakest in detecting longitudinal decline. Why is averaging optimal and why is PRT bad in terms of power? This is straightforwardly explained by the phenomenon that dichotomisation of a continuous variable results in loss of statistical power.(24) If the test value is erroneously measured below the true value and the cut-off, this creates false-positives and consequently inflates the number of "pathological" points. If however, the test value is in reality above the true value, this will result in false negatives. PRT therefore, is uniquely susceptible to random error thus weakening its ability to detect longitudinal decline.

Power issues likely persist when different cutoff definitions are applied, such as age- and retinal locus-specific reference thresholds.(25) Therefore, pointwise evaluation, as Pfau, *et al.* suggested, should only score values below the coefficient of variation as resulting from pathological change.(5) However, this approach would undermine the usefulness MMP in detecting change, given that the average annual loss for intermediate AMD is -3.0 (3.3) dB.(10) Scoring only below the coefficient of variation may therefore obscure pathological changes. However, age-specific PRT retains important advantages, particularly for between-subject comparisons. It offers an intuitive, standardized method to identify functional deficits relative to normative populations and thus remains useful for regulatory and clinical decision-making. The present critique is limited to its efficiency in detecting within-subject longitudinal change, where MS may offer greater statistical power.

MS cd log can be viewed as a middle ground between MS and PRT. To our knowledge, this is the first application of the transformation in this context. The theoretical appeal lies in its tendency to produce lower values than MS in areas of poor sensitivity, while retaining similar values in regions of good sensitivity (see Figure 1). However, our findings do not show that MS cd log outperforms MS in terms of statistical power or stage differentiation. While we expected the transformation to improve robustness to random error and enhance sensitivity to scotoma formation, these benefits may be offset by increased variance in regions of low sensitivity. Nonetheless, it may be premature to dismiss this strategy, as it could prove valuable in alternative applications, such as detecting subtle changes in intermediate AMD.

Limitations

This study has several limitations. It relies on a non-trial-specific dataset, and non-AMD-related factors, such as phakic changes, may influence MMP and VA measurements. However, the within-dataset comparisons ensure generalizability. A minor limitation is the use of presenting visual acuity instead of BCVA, resulting in a suboptimal comparison between MMP and VA-derived measurements. We acknowledge the use of different statistical approaches for power calculations (paired *t*-tests) and secondary exploratory analyses (mixed-effects linear models). While this could be seen as a methodological inconsistency, the power calculations were based on simple, conservative assumptions and are not the source of the large sample size estimates at later timepoints. Rather, these inflated estimates reflect the small number of available observations at 24 months (N = 31), a consequence of attrition and study design. Specifically, participants with longer follow-up were more likely to have stable disease and no incident events such as MNV, introducing a selection effect that reduces longitudinal differences thus inflating power requirements.

Future research

As part of MMP optimalisation, custom-grids are increasingly employed and developed.(15) Following our results, custom grids would similarly benefit from averaging decibels and appropriate number of test loci would result in increasing robustness and power. Future efforts should ascertain an appropriate and optimal number of test loci, balancing test feasibility with statistical considerations. Importantly, methodological choices for a specific analysis strategy should be aligned with clinical relevance. This requires linking MMP change to patient-reported outcomes, in order to establish

thresholds for what constitutes meaningful functional improvement or decline. While our findings support MS as the most efficient analysis strategy for detecting change, further research is needed to determine whether changes in MS reflect perceptible improvements in real-world visual function. (26) (27)

In sum, averaging threshold sensitivities results in the most stable outcome, detecting change with the least required sample size out of three analysis strategies. In addition, only MS was able to statistically discriminate higher-staged longitudinal decline compared to other MMP derived analysis strategies. When detecting within-subject change over time trial designers should consider an averaging approach such as MS. However, age-specific PRT may still be preferable for between-subjects comparisons especially against population norms. Secondarily, longitudinal trials should anticipate attrition and selection effects if events like MNV lead to study withdrawal.

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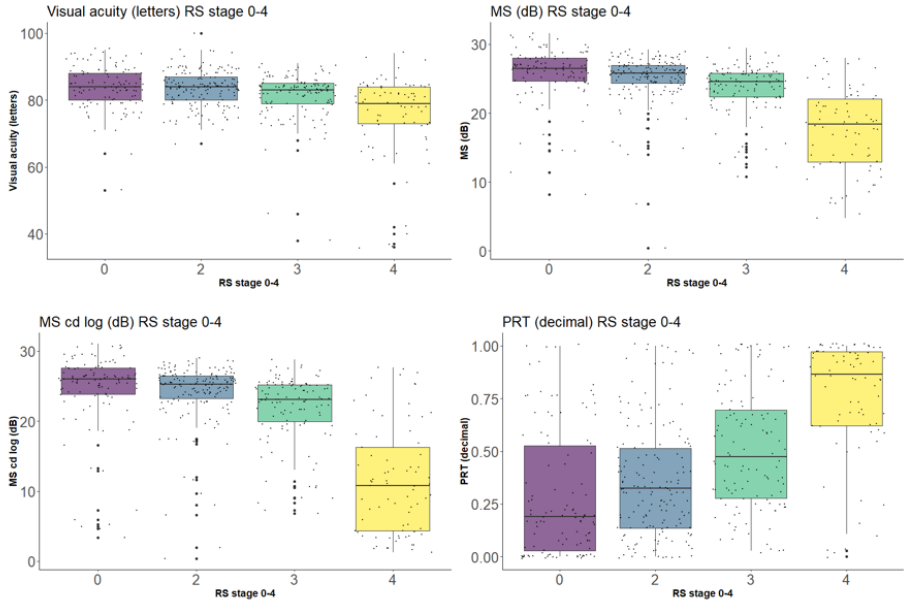
Supplementary Material



Supplemental Figure 1. VA, MS, MS cd log, PRT at Baseline

VA visual acuity, MS or mean sensitivity, MS cd log or mean sensitivity candela log, PRT or percent reduced threshold. In supplemental figure 4 baseline values for VA, MS, MS cd log, PRT, per stage (RS stage 0-4) are shown. RS stage 0 corresponds to no signs of AMD at all or hard drusen <63 µm) only. RS stage 1: Soft distinct drusen (≥63 µm) only or pigmentary irregularities only, no soft drusen (≥63 µm). RS stage 2: Soft indistinct drusen (≥125 µm) or reticular drusen only, soft distinct drusen (≥63 µm). RS stage 3: Soft indistinct (≥125 µm) or reticular drusen with pigmentary irregularities. RS stage 4: Atrophic or neovascular age-related macular degeneration.

Supplemental Table 1. Null models for VA, MS, MS, cd log, and PRT

	VA	MS	MS cd log	PRT
Variance between patients	44.8	17.3	37.7	.08
Variance within patients	20.1	4.3	7.9	.03
VPC (between/between + within)	69%	80%	83%	73%

VPC or variance partition coefficient for null models. VA visual acuity, MS or mean sensitivity, MS cd log or mean sensitivity candela log, PRT or percent reduced threshold.

Research Chapter Five

Visual acuity provides a more meaningful measure of vision-related functioning than mesopic microperimetry in age-related macular degeneration

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Abstract

Purpose Mesopic Microperimetry (MMP) is a promising tool to evaluate retinal function in clinical trials. While Visual Function (VF), the ability to perform vision-related tasks, relates strongly to Visual Acuity (VA) in patients with Age-related Macular Degeneration (AMD), the relationship between MMP and VF remains unclear.

Methods
A cross-sectional study in patients with AMD was performed. VF was measured by questionnaire using a subset of the NEI-VFQ33. The Macular Integrity Assessment Microperimeter (MAIA), was used with a 4–2 staircase strategy with a 10° diameter circular grid containing 37 loci. Three interpretations of the retinal sensitivity data were calculated: the mean of the 37 thresholds (MS), the percent-reduced threshold (PRT), and the log-transformed candela mean (MS cd log). MS, PRT, and MS cd log were tested via stepwise hierarchical linear regression and R^2.

Results
N = 102 patients were analysed (64 females (61%); mean (SD) age, 71.8 (11.2). VF was explained best by VA (MS; R^2 VA= .38, MS cd log; R^2 VA = .38, PRT; R^2 VA = .39). All retinal sensitivities contributed significantly to total R^2. In the MS cd log model, (Total adjusted R^2 = .52) the combined contribution of variance explained by VA and MS cd log was 46% (Partial adjusted R^2 =.46).

Conclusion
MMP is associated with VF but VA provides a more meaningful estimation of this same construct. These results suggest that VA provides stronger evidence of clinical efficacy.

Introduction

Age-related macular degeneration (AMD) is a progressive retinal disease that is currently the leading cause of irreversible vision loss in the world for elderly people.(1) In early AMD, patients may suffer mild visual disturbances but the disease can progress to late-stage AMD, characterized by geographic atrophy (GA) or choroidal neovascularization (CNV), significantly reducing quality of life.(2, 3)

Current trials in AMD use best-corrected visual acuity (BCVA) as functional endpoint. (4) However, BCVA, whether primary or secondary, has limitations in AMD.(5) BCVA shows little change over the course of most trial durations as it is inadequately sensitive to changes in early, intermediate AMD, and extra-foveal GA; and may fail to sufficiently discriminate in late AMD with foveal involvement.(5, 6) For instance, BCVA cannot measure relevant extra-foveal improvement in (exudative) AMD.(7) In GA, the slow development of foveal involvement might exceed the standard duration of most clinical trials.(8, 9) These shortcomings may negatively impact the measurability of a potential therapeutic

Mesopic Microperimetry (MMP) is a promising psycho-physical functional test that has already been implemented as a secondary outcome measure in GA clinical trials.(10-14) MMP entails controlled retinal sensitivity mapping of the macula and simultaneous measurement of fixation stability.(6) Patients are presented light stimuli of varying intensity in candela/m2, usually expressed in decibel (dB). The threshold, or retinal sensitivity, at which the patient can perceive this stimulus is then determined. BCVA is the ability to distinguish separate points at a distance over a high contrast background, in a high luminance environment, exclusively reflecting foveal function, while MMP measures retinal function beyond the fovea. (15, 16) MMP might be utilised in future trials because of its discriminatory ability in earlier staged AMD. (17, 18)

An important argument to justify use of MMP as a primary endpoint would be to show that MMP is related to vision-dependent tasks or visual function.(19) Visual function (VF) is a measure of everyday vision-dependent tasks such as reading and perceiving facial expressions.(20) While VF relates strongly to VA in AMD patients, the relationship between MMP and VF remains unclear.(21)

This study investigates how much VF is explained by MMP and VA. The output of the MMP can be interpreted using various methods, including the geometric

mean which is the average of all thresholds in dB, a percentage score of loci scoring below threshold, and the novel approach of averaging the candela/m2 scores before log transformation. Since, MMP output yields different variables, we aimed to explore these three distinct interpretations of retinal sensitivity in addition to fixation metrics in this study.

Method

Study design and participants

This secondary baseline analysis was performed with data collected from January 2018 through April 2019 at the Radboud university medical centre in Nijmegen (the Netherlands) as part of the European Genetic Database (EUGENDA). All patients had at least one eye with macular drusen secondary to AMD. The exclusion criterion was incorrect alignment of the microperimetry grid on the fovea to ensure valid measurement. Two cohorts were defined based on VA: the best-seeing eye cohort and the worse-seeing eye cohort. This study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the local ethics committee (2027-3535). All participants provided written informed consent prior to participating.

Study protocol

Presenting visual acuity was measured using the early-treatment-diabetic -retinopathy (ETDRS) fast method.(22-24) A print-out of the Dutch consensus translation of the NEI-VFQ39 (25) was handed out on site and completed using both eyes and reading glasses if necessary. MMP was performed with the macular integrity assessment microperimeter (MAIA) (CentreVue, Padova, Italy) using a 4–2 staircase strategy with a grid of 10° diameter containing 37 radially oriented points centred on the fovea. Trained personnel explained the procedure and performed a mock exam prior to actual exam at baseline to prevent a learning curve effect. MMP was not performed in mydriasis as the MAIA is able to operate with a pupil diameter of 2.5 millimetres.(6, 26) The following imaging modalities were performed: Colour Fundus Photography (CFP) (DRI Triton [Topcon corporation, Tokyo, Japan]), Spectral-Domain Optical Coherence Tomography (SD-OCT) and, Fundus Auto-Fluorescence (FAF) (SpectralisTM HRA+OCT [Heidelberg Engineering, Heidelberg, Germany]. All imaging was done in mydriasis. Ophthalmic history and medical history was assessed through questionnaires.

Main outcome: visual function

The long-form visual functioning scale (LFVFS-39) measuring VF was used.(27) A LFVS-39 score of 100 indicates no difficulty in performing activities due to vision, while 0 indicates complete inability to perform activities due to vision.(27) The LFVFS-39 averages the recoded responses to items 2, 5, 6, 7, 8, 9, 10, 12, 14, A3, A4, A5, A6, A7, and, A8 from the NEI-VFQ. Despite the LFVFS-39 still retaining differential item functioning, i.e. some items measure

differently for sex(27), its primary advantage is its better precision over the LFVFS-25 which is comparatively smaller scale using solely the NEI-VFQ25. Application of this Rasch-based revision has since been manyfold.(21, 28)

VF can be derived from the NEI-VFQ, the most used vision-related quality of life (VRQoL) questionnaire in ophthalmology.(29) Psychometric evaluation of the NEI-VFQ via Rasch-analysis has shown that the NEI-VFQ violates unidimensionality, i.e. it does not measure a single construct which is not uncommon in QOL measurements.(27) This can be resolved by segregating a subset of the original questions into two distinct measures of VRQoL: VF and socioemotional functioning.(27) VRQoL is a multifaceted i.e. multidimensional construct which includes extra domains such as emotional well-being, social participation, and economic considerations.(20) VF and VRQoL are similar in that they can be measured through psychometric instruments.(20, 29, 30) While VRQoL is a valuable measure, its multidimensionality renders it more challenging to interpret since it cannot be represented by a single scale.(20, 29)

MMP variables

Retinal sensitivity is measured at each of the 37 loci on a 0-36 dB scale such that 0 dB corresponds to the brightest stimulus at 318 cd/m^2.(6) Retinal sensitivity data can be analysed differently.(5, 6) We explored three different strategies:

1) mean sensitivity (MS); this is the average of all 37 thresholds in dB.
2) mean sensitivity candela log (MS cd log). MS cd log is calculated by first converting each threshold into its corresponding candela/m^2 value.(6) Next, all thresholds in candela/m^2 are averaged. Subsequently, all values are transformed back to a dB value.
3) percent reduced threshold (PRT); the number of thresholds below 25 dB divided by 37, expressed as a decimal value.(5, 6) (Figure 1)

Grading

Grading of CFP, SD-OCT and FAF was performed by experienced graders of the Eye-NED Reading Center using the modified version of the Wisconsin Age-Related Maculopathy Grading System (WARMGS).(32) This was then reclassified into the Rotterdam Study (RS) classification.(33) Patient stage is defined by the highest-staged eye.

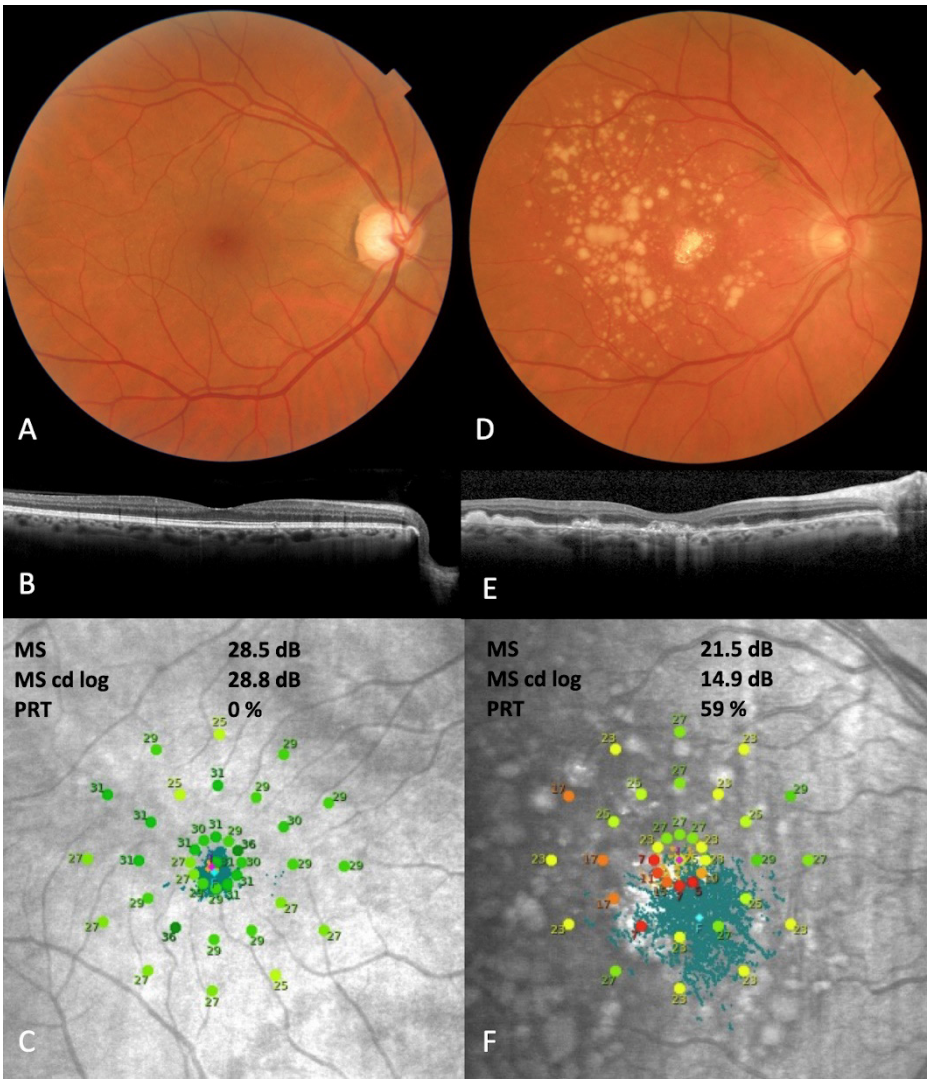

Figure 1. Example of Different Strategies

A. Colour fundus photography (CFP) of eye with early age-related macular degeneration (AMD). B. Corresponding spectral-domain optical coherence (SD-OCT) B-scan showing small drusen. C. Corresponding retinal sensitivity. D. eye with advanced AMD. E. corresponding SD-OCT with hypertransmission and large drusen. F. corresponding retinal sensitivity. In C and F we show the mean sensitivity (MS), Mean candela log (MS cd log), and percent reduced threshold (PRT) values.

Figure 1 demonstrates how different calculations of retinal sensitivity diverge in patients with varying stages of AMD.

Fixation metrics are provided in the form of P1, P2, BCEA-63, and BCEA-95.(31) In brief, P1 and P2 describe the percentage of fixation points within 1° and 2° radii circles. BCEA-63 and BCEA-95, or bivariate contour ellipse area, describes the area in degrees (°) which contain 63 or 95% of fixation points.(31)

Statistical analysis

Exploratory simple linear regression of VF (LFVFS-39) was performed with the following variables measured in the best-seeing eye and worse-seeing eye respectively; MS (dB), MS cd log (dB), PRT (decimal value), P1 (%), P2 (%), BCEA-63 (°), BCEA-95 (°). The coefficient of determination (R^2) of VF (LFVFS-39) and VA of the best-seeing eye (VABE) (letters) and VF (LFVFS-39) and VA of the worse-seeing eye (VAWE) (letters) served as benchmarks. Next, a three stepwise hierarchical multiple linear regression models was constructed with VF (LFVFS-39) as dependent variable. In each model a different retinal sensitivity measure; MS, MS cd log, and PRT served as retinal function measure. Candidate independent variables were limited to those variables with a statistically significant F-test score from the previous analysis and the following potential confounding variables based on the literature: AMD stage (6), sex (21, 27) and age. (34) This process was iterated three times, yielding nine different regression models. The first series of three models (MS, MS cd log, and PRT resp.) were constructed using variables measured in the best-seeing eye; the second series were based on the worse-seeing eye (worse-seeing eye cohort); the third series included both eyes. For all 9 models, the following assumptions were visually checked: 1) linearity, 2) multivariate normality, 3) absence of homoscedasticity, and 4) absence of multicollinearity (variance inflation factor, VIF <10). α was 5%. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA).

Results

Cohort description

In the best-seeing eye cohort, 4 patients had missing data and additionally the grid was misaligned in 2 additional patients. This resulted in N = 102 patients (64 females (61%); mean (SD) age, 71.8 (11.2) years). (Table 1) Mean (SD) VA was 79.6 (10.9) letters. In total, 40 eyes were graded as RS stage 4 of which 14 were neovascular, 21 Atrophic, and 5 showed a combination. The patient stage was RS stage 4 in 84 patients.

Table 1. Baseline Demographics

Characteristics	Best-seeing eye N = 102	Worse-seeing eye n = 84
Age, mean (SD), years	71.8 (11.2)	71.5 (11.5)
Female, No. (%)	64 (60.7%)	51 (61%)
VA, mean (SD), letters	79.6 (10.9)	63.5 (20.7)
Stage		
RS stage 0	3 (2.9%)	0 (0%)
RS stage 1	0 (0%)	0 (0%)
RS stage 2	29 (28.4%)	10 (12%)
RS stage 3	30 (29.4%)	13 (15%)
RS stage 4	40 (39.2%)	61 (73%)
Patient stage, No. (%)		
RS stage 0	0 (0%)	0 (0%)
RS stage 1	0 (0%)	0 (0%)
RS stage 2	9 (8.8%)	10 (10%)
RS stage 3	9 (8.8%)	9 (11%)
RS stage 4	84 (82.4%)	67 (80%)
RS stage 4 patient, No. (%)		
Neovascular	14 (13.7%)	22 (26%)
Atrophic	21 (20.6%)	8 (10%)
Combined	5 (4.9%)	31 (37%)

Abbreviations: RS stage 0 corresponds to no signs of AMD at all or hard drusen <63 µm) only. RS stage 1: Soft distinct drusen (≥63 µm) only or pigmentary irregularities only, no soft drusen (≥63 µm). RS stage 2: Soft indistinct drusen (≥125 µm) or reticular drusen only, soft distinct drusen (≥63 µm). RS stage 3: Soft indistinct (≥125 µm) or reticular drusen with pigmentary irregularities. RS stage 4: Atrophic or neovascular age-related macular degeneration. Patient stage is defined by the worse staged eye.
Median (IQR) of LFVFS-39 scores were 77.6 (25.4) (Range: 22 – 100) Scores were affected by sex as mean (SD) scores for males 79.9 (16.7) and females 72 (20.0) differed. (Supplementary Figure 1).

A subset of N=102 patients contributed data of the worse-seeing eye. Of the 18 missing's, 10 patients did not perform MMP. Median (IQR) VA was 20 (47)

letters for these 10 eyes and all VA were lower than 56 letters except for one patient. Additionally, in 8 patients the grid was misaligned. Visual acuity in the worse-seeing eye for these 8 patients did not exceed 52 letters.

The worse-seeing eye cohort featured n=84 patients. Mean (SD) VA was 63.5 (20.7) letters. Sixty-one eyes (73%) were graded as RS stage 4. (Table 1) Median (IQR) LFVFS-39 scores were 82 (24) (Range: 28-100).

Exploratory analysis

A simple linear regression with each candidate variable was performed. In the best-seeing eye cohort all F-test scores were statistically significant. VA performed best (R^2=.41). The retinal sensitivities were ranked MS cd log (dB) (R^2=.36), MS (dB) (R^2=.32), PRT (decimal) (R^2=.23) best to worse. (Table 2) (Figure 2).

Table 2. Simple Linear Regression with LFVFS-39

Variables	Best-seeing eye N = 102	Worse-seeing eye n = 84
	R^2	R^2
VA (letters)	.41*	.06*
MS (dB)	.32*	.07*
MS cd log (dB)	.36*	.09*
PRT (decimal)	.23*	.07*
BCEA-63 (°) ln	.10*	.04
BCEA-95 (°) ln	.09*	.04
P1 (%)	.16*	.05*
P2 (%) reflec†. ln	.07*	.02

**P* <.05. † optimal fit was achieved by reflecting P2 values and log transforming the product. Abbreviations: VA, visual acuity. MS, mean sensitivity. MS cd log, mean sensitivity candela log. PRT, percent reduced threshold. BCEA-63, bivariate contour ellipse area containing 63% of all fixation points BCEA-95, bivariate contour ellipse area containing 95% of all fixation points. P1, percentage of fixation within 1 degree. P2, percentage of fixation within 2 degrees.

Of the fixation metrics P1 (%) performed best (R^2=.16) (table 2) (Supplementary Figure 2). In the worse-seeing eye, R^2 of MS cd log (dB) was .09. All other variables performed worse. (Table 2) (Supplementary Figure 3) F-scores of BCEA-63 (°) BCEA-95 (°), and P2 (%) were not statistically significant. (Table 2) (Supplementary Figure 4) Of note, BCEA-63 (°) BCEA-95 (°), and P2 (%) were transformed prior to all analyses. Untransformed fixation metrics in the best-seeing eye cohort are visualized in Supplementary Figure 5.

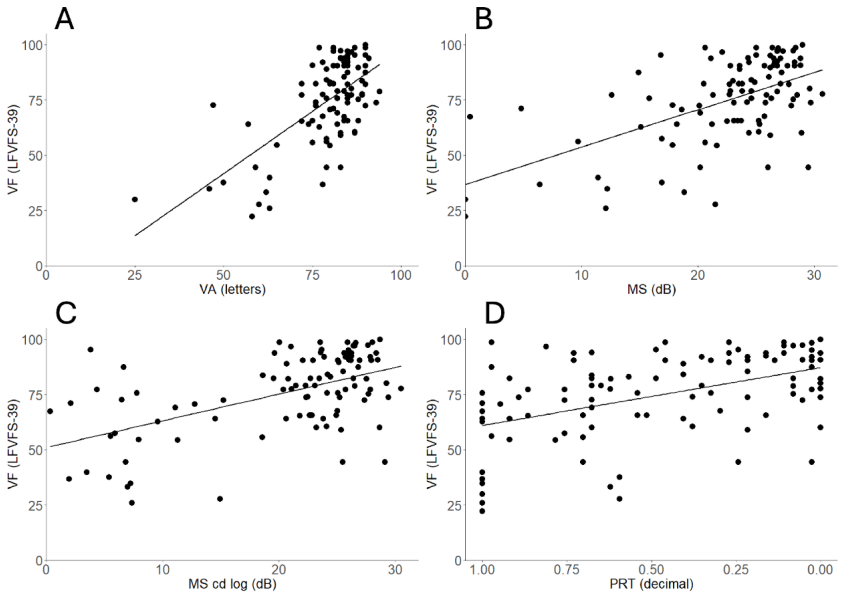

Figure 2. Visual Function and Retinal Function Measures of Best-seeing Eye

Abbreviations: LFVFS-39, long form visual function score. VA, visual acuity. MS, mean sensitivity. MS cd log, mean sensitivity candela log. PRT, percent reduced threshold.

Main results

In the best seeing-cohort, VA consistently accounted for the largest portion of variance explained in LFVFS-39 (MS; R^2 = .38, MS cd log; . R^2 = .38, PRT; R^2 =.39). (table 3) All retinal sensitivities significantly contributed to the proportion of total variance explained (MS; R^2 = .07, MS cd log; R^2 = .08, PRT; R^2 =.03).

For all three models all assumptions were met. The variance inflation factor did not exceed 1.5. None of the other candidate variables P1 (%), P2 inv. Ln (%), BCEA-63 Ln (°), age or RS stage were forward selected in any model. BCEA-63 Ln and BCEA-95 ln were statistically the same (R^2 =.99) and therefore the list of candidate variables was limited to BCEA-63. Sex accounted for approximately 5% of variance in each model. Of note, RS stage 1 and 0 were collapsed to account for power.

The MS cd log model accounted for the largest proportion of variance explained (Total adjusted R^2 = .52). The combined contribution of variance explained by VA and MS cd log was 46% (Partial adjusted R^2 = .46). Estimated (P value) were .9 (<.001) per letter and .7 (<.001) per decibel in this model. (Table 2)

Table 3. Stepwise Hierarchical Linear Regression with LFVFS-39 of Best-Seeing Eye (N=102)

	MS (dB)	MS cd log (dB)	PRT (decimal value)
Independent variables	**(*P* value)**	**(*P* value)**	**(*P* value)**
Intercept	-18.1	-5.7	1.0
VA, letters	1.0 (<.001)	.9 (<.001)	1.1 (<.001)
Retinal sensitivity	.9 (<.001)	.7(<.001)	-10.6 (.02)
Sex	-8.3 (.001)	-9.1(.001)	-9.0 (.001)
Variance			
Partial adjusted R^2*	.45	.46	.40
Total adjusted R^2†	.49	.50	.45
Stepwise R^2			
R^2 VA (*P* value)	.38(<.001)	.38(<.001)	.39(<.001)
R^2 Retinal sensitivity (*P* value)	.07(<.001)	.08(<.001)	.03(.03)
R^2 Sex (*P* value)	.05(.001)	.06(.001)	.06(.001)

Abbreviations: VA, visual acuity. MS, mean sensitivity. MS cd log, mean sensitivity candela log. PRT, percent reduced threshold.
* Partial adjusted R^2 refers to a partial model consisting of a retinal sensitivity metric and VA.
† Total adjusted R^2 refers to a model consisting of all independent variables.

In the worse-seeing eye cohort (etable 1), VA was not forward selected in the models. Only sex and retinal sensitivity were included. Total variance explained ranged from 9 to 14% (Total adjusted R^2). (supplemental Table 2) All retinal sensitivities significantly contributed to the proportion of total variance explained (MS; R^2 = .07, MS cd log; R^2 = .09, PRT; R^2 =.07). All assumptions were met. None of the other candidate variables P1 (%), RS stage 4, RS stage 3, or age were included.

In the final series including both eyes, VA of the best-seeing eye was included in each model. (supplemental Table 2) PRT, regardless of best or worse-seeing eye, was not selected. (supplementary Table 3) VA of the best-seeing eye accounted for the largest proportion of the variance explained (MS; R^2 = .30, MS cd log; R^2 = .30, PRT; R^2 =.30). In the PRT model the initial candidate variable list were subject to multicollinearity regarding BCEA-63 and P2 (variance inflation factor = 11.4). This model was rerun, leaving out BCEA-63. In the final models, all assumptions were met.

Discussion

In this secondary analysis of 102 AMD patients, VA of the best-seeing eye is more strongly related to daily vision dependent tasks (VF, LFVFS-3) than retinal sensitivity (MS, MS cd log, PRT) or fixation metrics (BCEA-63, BCEA-95, P1, P2) as measured via MMP. In the best-seeing eye cohort, combining VA and the best performing MMP retinal sensitivity (MS cd log) increases the proportion of variance explained from 38% (VA alone) to 46% (VA and MS cd log) (P <.001), suggesting limited added benefit of retinal sensitivity. The proportion of variance explained is important in the context of endpoint selection in clinical trials. While uniocular variables cannot fully capture visual function(35), these findings suggest that in cases where proportional gains or losses in VA (letters) and MMP (dB) occur, VA's higher proportion of variance explained inspires greater confidence in predicting an equivalent change in visual function. These findings are in line with previous work which underscores the relative weakness of MMP compared to VA-based measures.(36, 37)

MMP's relative underperformance might be explained twofold. First, while VA and MMP measure overlapping anatomical regions - VA measures foveal function while MMP measures macular function - both are linearly associated with VF. Therefore there might be shared variance between MMP and VA which is absorbed by VA. Secondly, it could be that foveal function is more important for VF than the extra-foveal macula reflecting cone density used for photopic vision in the human eye.(38) Therefore, if foveal function is overall more important, and VA is a more accurate estimate of foveal function than MMP, this would explain the outperformance of VA.

Analyses with both eyes confirm the main finding that VA of the better-eye outperforms mesopic microperimetry in the better eye. However, overall model performance was worse as MMP could not be performed in a substantial number of eyes with poor vision, resulting in fewer patients overall (n = 84) and a reduced range of VF scores (R^2 was 39% for the best model in this series). Analyses with the worse-seeing eyes, while confounded by the absence of the strongest predictor (R^2 was 14% for the best model in this series), still provides an indication of the relative predictive strengths of each MMP metric as PRT failed to be forward selected and consistently underperformed in other analyses as well. (supplemental Table 2) (supplemental Table 3)

Some notes on the different analyses of retinal sensitivity. There is ongoing experimentation with alternative analysis strategies.(5, 39, 40) We are the first to perform the MS cd log transformation to our knowledge. In brief, according to the Weber-Fechner law when light intensity increases exponentially, this is perceived linearly by humans.(6) We attempted to use this to our advantage. If candela/m2 values are averaged prior to log transformation, this produces the effect of sensitizing the retinal sensitivity to scotoma formation. (Figure 1) There are theoretical limits to its applicability however, since in the case of extensive scotoma this logarithmic property will likely mask the presence of areas with relatively good functionality. Among retinal sensitivity parameters (MS, MS cd log, and PRT) MS cd log and MS performed comparably while PRT performed worse. Considering MS cd log's sensitivity to scotoma formation while retaining a strong relationship with VF, further confirmatory research into its utility is warranted. These suggestions likewise apply to scotopic microperimetry and may benefit ongoing studies such as MACUSTAR which aims to explore structure-function relationships in intermediate patients.(41)

Strengths and weaknesses

While the study has strengths including a systematic investigation of many MMP-derived variables, it also has limitations. An important limitation is the use of uniocular instead of binocular variables. In fact, in all three retinal sensitivity models of the best-seeing eye cohort, fifty percent of the variance remains unexplained. This is in part explained by the aforementioned limitation in addition to unmeasured social, cognitive and psychological factors.(42) While assuredly preferable for a more accurate estimate of visual function, statistical modelling on binocular vision represents power, interpretability and feasibility challenges. To accommodate the complex interplay of both eyes, model complexity would increase, limiting overall interpretability of findings in relation to the research question. In addition, appropriate power for all relevant interactions, was, considering our sample size, not feasible.

Similarly reflecting a pragmatic solution to a complex problem, stepwise hierarchical linear regression allows for systematic and interpretable evaluation of variances in relation to overall model performance, however it does not allow for potentially relevant interaction effects such as two eyes with highly advanced disease.(43) Lastly, the NEI-VFQ derived LFVFS-39 retains shortcomings, thus potentially contributing measurement error.(27) In addition, VF might be intrinsically noisy. Future efforts might benefit from computer adapted testing of VF such as the rigorously validated EyeQ.(44)

In conclusion, MMP is associated with daily vision-dependent tasks but VA provides a more meaningful estimation of this same construct. These results suggest that VA provides stronger evidence of potential clinical efficacy in trials than MMP. Replications of further studies are necessary to confirm these findings.

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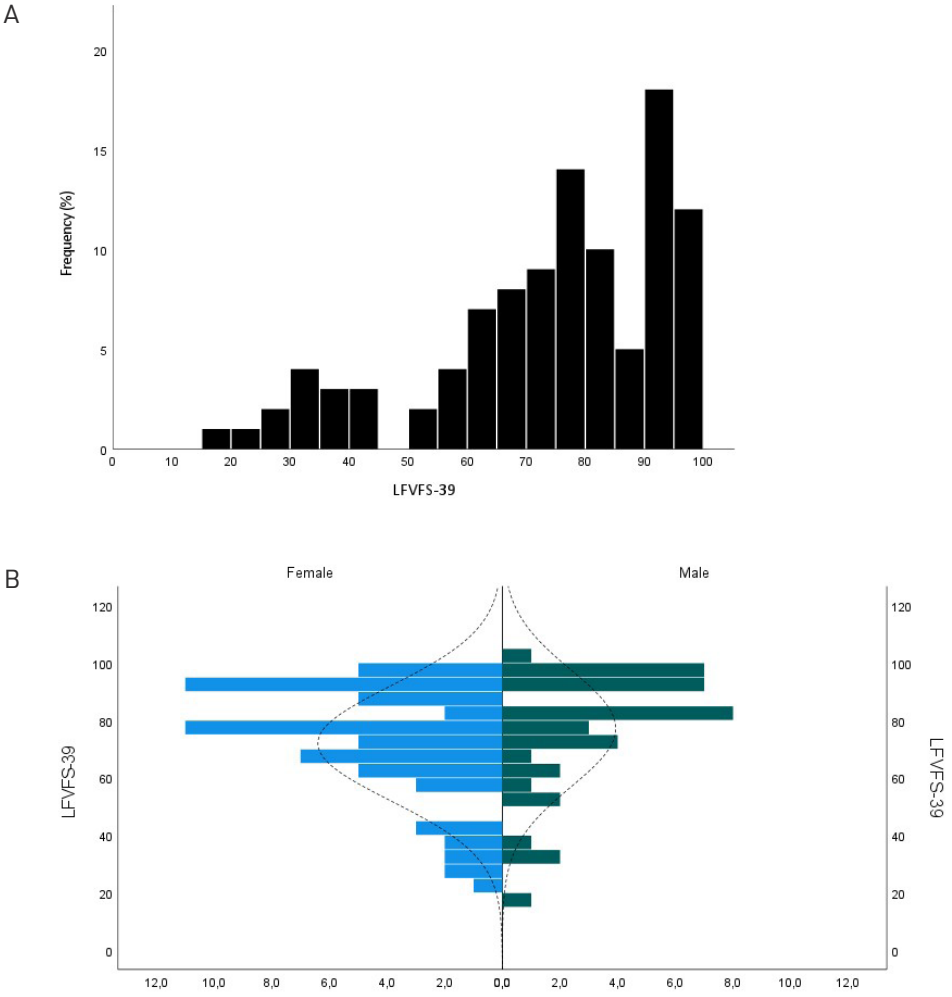
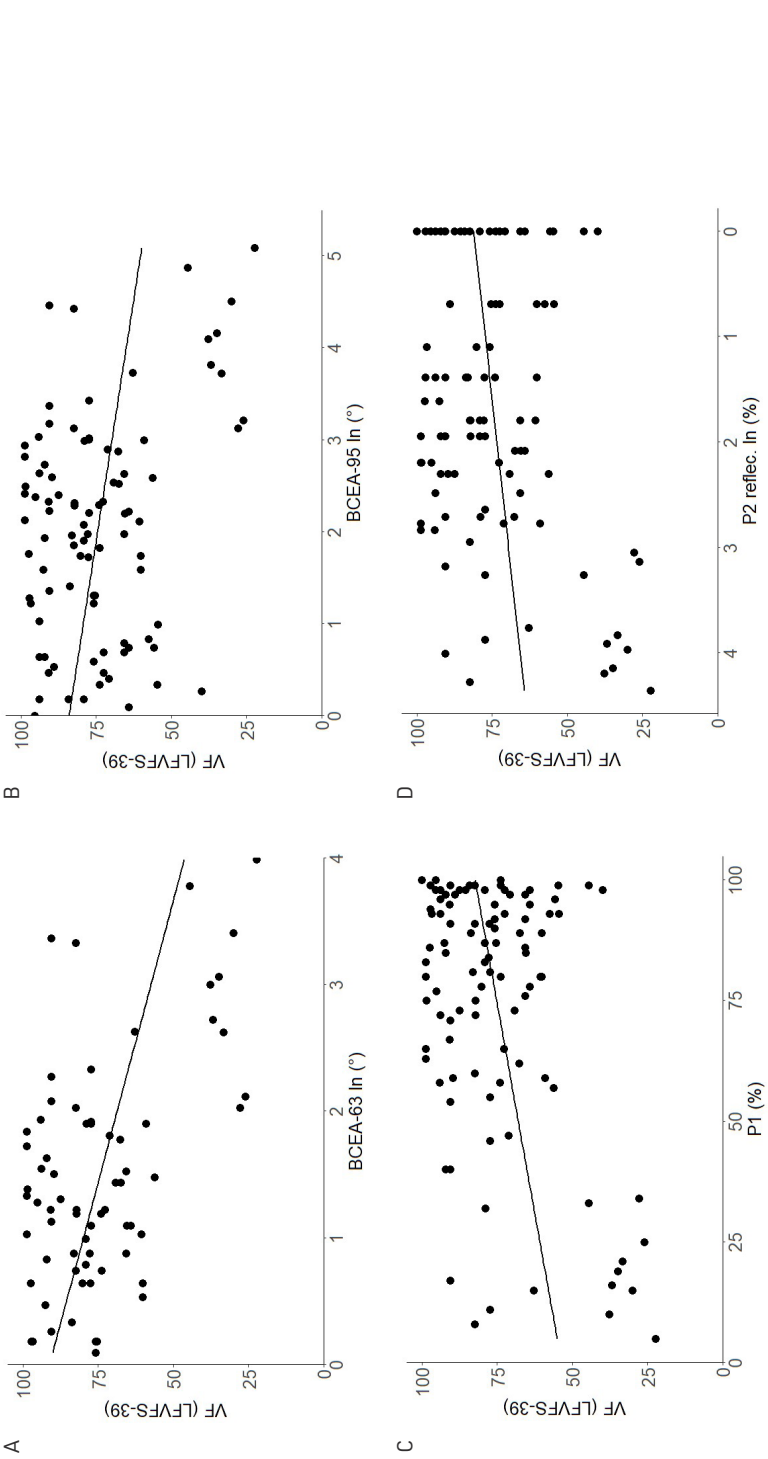
Supplementary Material



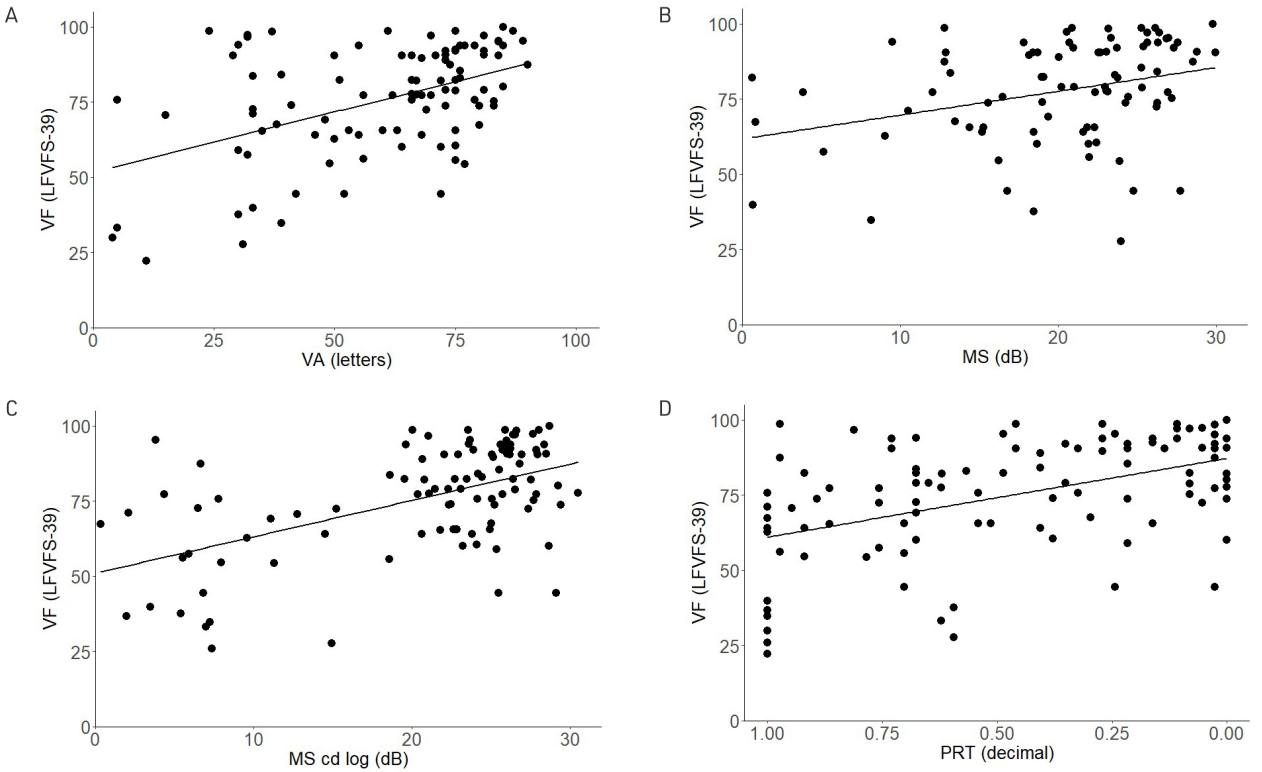
Supplementary Figure 1. Distribution of LFVS-39 Values (N=102)

Abbreviations: LFVFS-39, long form visual function score. A: y axis: frequency of values for LFVFS-39. x axis: values for LVFS-39, value 0 corresponds to being completely unable to perform daily visual tasks. 100 corresponds to being able to perform visual daily tasks. B. Population differences men and women regarding long form visual function score or LFVFS-39.



Supplementary Figure 2. Visual Function and Transformed Fixation Metrics of Best-Seeing Eye

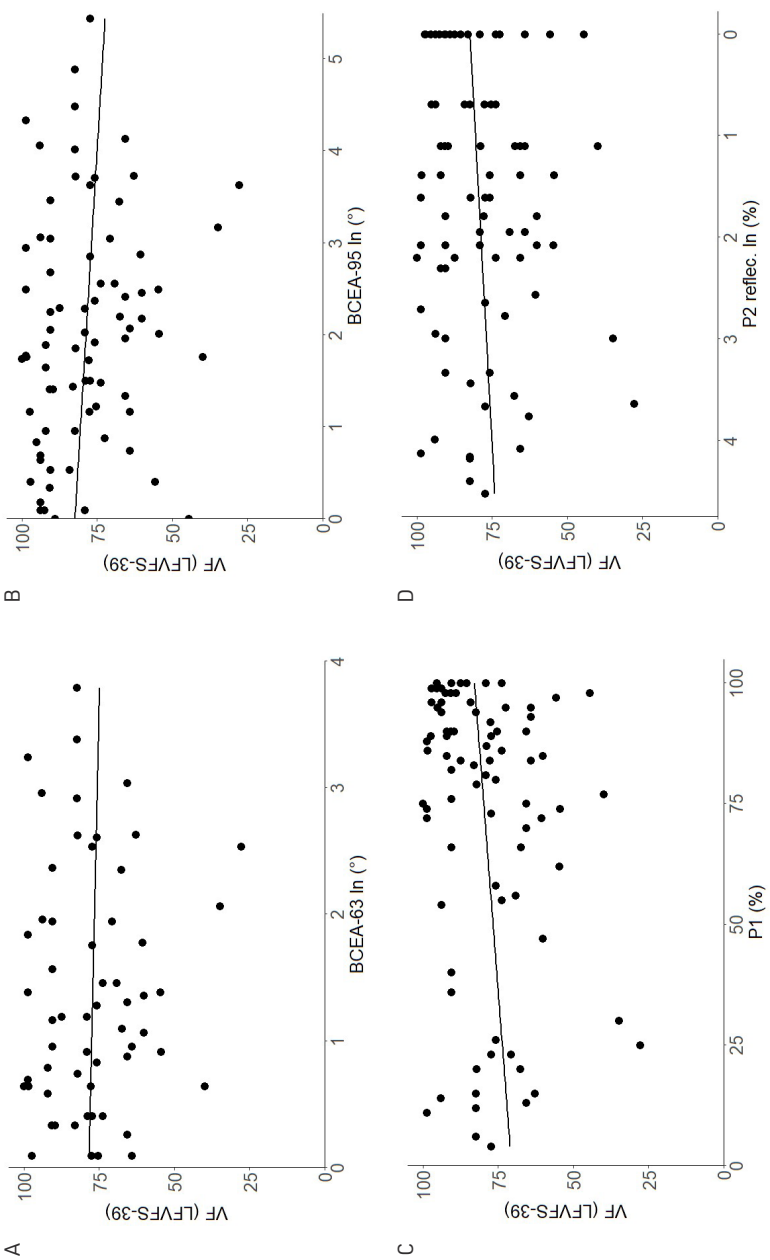
Abbreviations: LFVFS-39, long form visual function score. BCEA-63, bivariate contour ellipse area containing 63% of all fixation points. BCEA-95, bivariate contour ellipse area containing 95% of all fixation points. P1, percentage of fixation within 1 degree. P2, percentage of fixation within 2 degrees
A. LFVFS-39 and BCEA-63 natural log transformed. B. LFVFS-39 and BCEA-63 natural log transformed. C. LFVFS-39 and P1 untransformed. D. LFVFS-39 and P2, inverted and then natural log transformed.



Supplementary Figure 3. Visual Function and Retinal Function Measures of Worse-Seeing Eye

Abbreviations: LFVFS-39, long form visual function score. VA, visual acuity. MS, mean sensitivity. MS cd log, mean sensitivity candela log. PRT, percent reduced threshold.

A. LFVFS-39 and VA. B. LFVFS-39 and MS. C. LFVFS-39 and MS cd log. D. LFVFS-39 and PRT.



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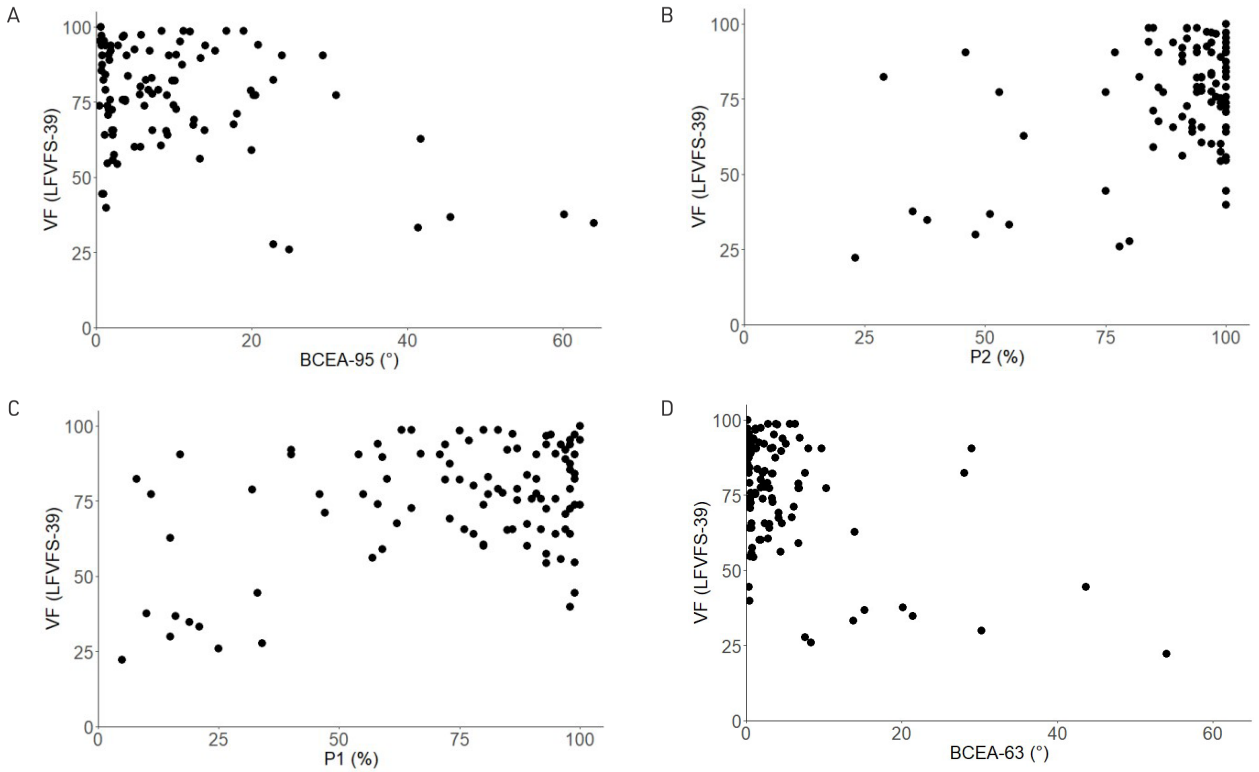

Supplementary Figure 5. Visual Function and Fixation Metrics of Best-Seeing Eye

Abbreviations: LFVFS-39, long form visual function score. BCEA-63, bivariate contour ellipse area containing 63% of all fixation points. BCEA-95, bivariate contour ellipse area containing 95% of all fixation points. P1, percentage of fixation within 1 degree. P2, percentage of fixation within 2 degrees
A. LFVFS-39 and BCEA-63. B. LFVFS-39 and BCEA-63. C. LFVFS-39 and P1. D. LFVFS-39 and P2.

General Thesis Discussion

The aim of this thesis was to study prognostic factors for age-related macular degeneration (AMD). Improved appraisal of these factors may improve clinical care through better measurement of disease progression. In addition, it is a human need to see as best as possible what lies in the future especially when our sight on the line. In this General Thesis Discussion important findings will be highlighted and contextualized. In addition, potential steps for future research connected to these findings will be explicated.

Contributions to risk assessment of age-related macular degeneration

How has this thesis added to body of knowledge on prognostic factors? In **Research Chapter One ,** we posited that choroidal thinning is of limited concern for macular atrophy. To recapitulate, intravitreal injections with anti-vascular endothelial growth factors (anti-VEGF) are the mainstay treatment for exudation secondary to neovascular AMD. One quarter of anti-VEGF treated neovascular AMD patients will develop signs of macular atrophy within 2 years, possibly related to anti-VEGF treatment. A hypothesized mechanism for atrophy induction is the effect of anti-VEGF on choroidal thickness. In our cross-sectional study, we found a non-significant 11 micron difference between anti-VEGF treated eyes and non-treated eyes in long-term follow-up neovascular AMD patients. A relationship between choroidal thinning and the number of anti-VEGF injections was furthermore not shown. In short, there is no significant choroidal thickness difference between anti-VEGF treated and non-treated long-term follow-up neovascular AMD. We therefore suggest that atrophy induction through choroidal thinning secondary to anti-VEGF injections is of limited concern.

In **Research Chapter Two** we studied patients who carry rare (Minor Allele Frequency, MAF < 1%), protein altering or splice-site variants in *CFH* and *CFI*. Rare variants in *CFH* and *CFI* are hypothesized to raise the risk of progression in AMD. To study this risk, we created a cohort which included all patients without late AMD at baseline with a follow-up of at least five years from our EUGENDA database. Our results indicate that carrier progress to late AMD faster. Rare variant carriers may therefore function as a proof-of concept cohort for drug development. This is so because in this cohort absence of observed deterioration might signal stabilisation of the disease earlier than in a slowly progressing cohort.

In **Research Chapter Three,** we studied non-exudative macular neovascularisations or NEMNV, a type of blood vessel defined as a treatment-naïve type 1 neovascularisations without exudation.(1) These vascular entities can be visible on dye based angiography and optical coherence tomography angiography, an upcoming non-invasive technique which is able to visualise retinal and choroidal blood flow.(1) There is an abundance of literature suggesting that NEMNV confer a particularly high risk of conversion to sight-threatening exudation. In our prospective cohort of 134 patients with sustained unilateral exudative AMD, we find 7 NEMNV of which the majority show vascular changes over time regardless of conversion. However, the rate of conversion *appears* to depend on the age of onset of first eye exudation. We have stratified our cohort in time since first eye exudation: <5 years versus ≥5 years. Our results show a trend toward higher conversion risk in patients with longer-existing unilateral disease (CRR: 1.3 [95% CI −0.2–3.1] in the <5-year group vs. 4.0 [95% CI 0.8–7.1] in the ≥5-year group). Importantly, patients with longer disease durations were significantly younger at the age of first eye exudation. Rather than interpreting this finding biologically (e.g. younger patients have *different* NEMNV) we instead hypothesize that in these patients, NEMNV might be detected later in the disease timeline. This contributes to the perception of particularly high conversion risk. Our results thereby provide new anchor points for research suggesting that disease timeline should be incorporated when assessing potential predictive signs of exudation in NEMNV.

NEMNV might even be protective as a type 1 MNV could sustain and nourish the overlying RPE and outer retina.(1) Systematic follow-up using for example the time since the first eye developed neovascular AMD could provide additional arguments for this hypothesis. Our findings have important implications for clinical practice as they suggest there is little merit in providing more frequent check-ups in patients with NEMNV without knowing the duration of presence of NEMNV. Instead, upon discovering a NEMNV in a patient, a more personalised approach could be taken based on shared-decision making; more frequent check-ups might detect leakage earlier and might arguably result in better outcomes. However, the risk is currently unclear and more frequent visits result in a higher patient burden as well as increasing costs of care.

Re-imaging the Second-Eye Progression Study in the age of artificial intelligence

Research Chapter Three represents a significant departure from the initial aim and scope of what has, throughout past years of research been referred to as the *Second-Eye Progression Study*. Indeed, rather than concluding that existing risk estimates of NEMNV are unsatisfactory, initial ambitions were to provide concrete, novel risk markers for progression to neovascular AMD. This proved unfeasible on account of length-biased sampling which will be explained below.

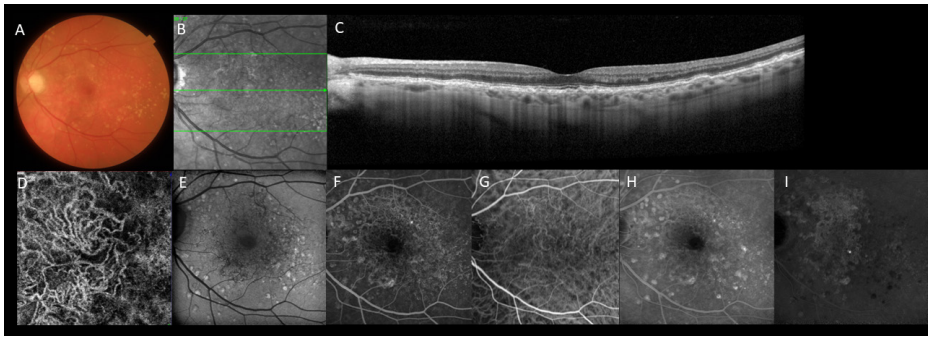

Figure 1. Multimodal Imaging of an Eye which *Might* Develop a Macular Neovascularisation
Example of 67-year-old woman with baseline imaging (patient VI). A. Colour fundus photography with drusen. B. infrared image. B. corresponding foveal B-scan on spectral domain optical coherence tomography (SD-OCT) without presence of sub- or intraretinal fluid. D. Optical coherence angiography image showing vascular structure, image settings: projection artifact removal, contrast 1:7, reference retinal pigment epithelium with a depth of -27 micron and thickness of 40 micron. E. Fundus autofluorescence showing pigment epithelial changes. F. Fluorescein angiography (FAG) at T=1 min not showing leakage. G. Indocyanine green angiography (ICG) with visible vascular structure. H. FAG at T 10 min. I. ICG at T10 min.

We planned to follow up patients with unilateral MNV secondary to AMD of which a proportion would develop MNV in the fellow eye thus providing extensive multimodal imaging of the situation preceding this event. By comparing the images of would-be progressors to non-progressors, the risks of developing MNV would hopefully be elucidated. However, this proved to be unfeasible due to bias induced by sampling. The non-progressors are meaningfully different (this will be expanded on) and are observed at different points in their clinical course. In figure 1, for instance, the patient did **not** develop exudation during follow-up over 1.5 years *despite* the presence of a vascular structure.

To appreciate why our ambitions were not met, it is necessary to appreciate the impact of length-biased sampling. Length-biased sampling means that patients with longer disease histories are more likely to be included. (Figure 2)

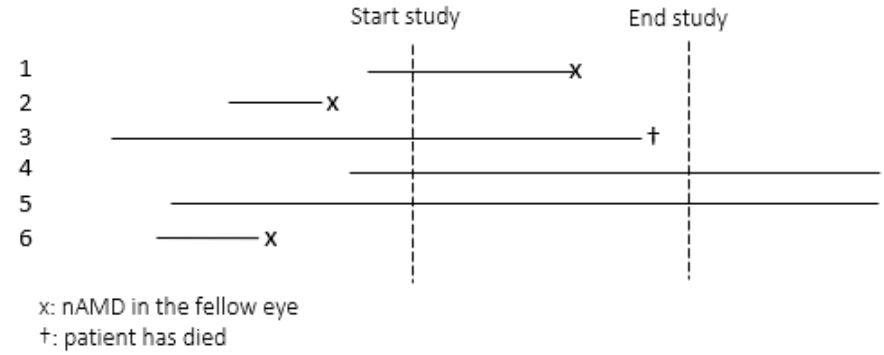

Figure 2. The mechanism of Length-Biased Sampling

Figure 2 shows the mechanism of length-biased sampling and how it results in overinclusion of patients with longer disease histories.

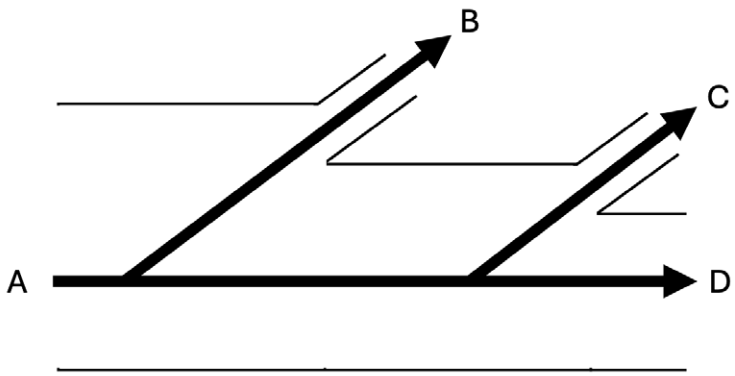

Figure 3. Diagram Illustrating the Impact of Length-biased Sampling

Suppose a student wishes to explore the relationship between finishing an event such as the Zevenheuvelenloop and training. She hypothesizes that more training decreases the chance of not-finishing. She decides to sample the day of the race by approaching both finishers and did-not-finishers and then collecting data. However, out of convenience, poor planning or a combination of both, she arrives with the race already in full swing. The sample she collects will feature a majority of runners who have already completed a substantial portion of the race. This is the situation created by length-biased sampling. In the diagram this is represented by C and D.

In figure 3 a simple example is shown which illustrates the impact of length-biased sampling on causal relationships. Considering that even runners who end up DNF will still have run most of the course, a true relationship between training and outcome will most likely not be borne out of the analysis. This is because runners who do not finish late in the reason, most likely do so for other reasons than lack of training (B ≠ C). This example illustrates how length-biased samples results in samples in which even straightforward causal relationships might not be found and alternatively, we might imagine, induce spurious ones. Length-biased sampling, thus, is a form of selection bias.

However, one might object, we currently live in an age of artificial intelligence. One might therefore wonder whether some extremely gifted mathematician or otherwise technically qualified person could be able to somehow "correct" for the bias discussed in the previous paragraphs. The answer is: unlikely. A brief exploration of these modelling techniques will support this insight;

AI algorithms are a heterogenous group of statistical techniques (random forests, deep learning, neural nets, boosting, support vector machines, etc) which according to Bradley Efron might collectively called pure prediction algorithms.(2) Despite their popularity, it is often little appreciated how different these algorithms are compared to traditional methods of statistical estimation (that which Galit Shmueli in a similar paper calls "explanation"). (3, 4) In a somewhat simplified manner, the main difference between pure prediction algorithms and traditional statistics is outlined in table 1 below.

Table 1. Main Differences Between Estimation and Prediction Algorithms

	Traditional regression methods	**Pure prediction algorithms (e.g. random forest, neural network)**
1.	Aimed at scientific truth (eternal truths)	Empirical prediction accuracy (short term accuracy)
2.	Parametric modelling (causality)	Nonparametric (black box)
3.	Parsimonious modelling (researchers choose covariates)	Anti-parsimony (algorithm chooses predictors)
4.	P << N (homogenous data)	P >> N (mixed data)
5.	Scientific knowledge is inferred from model	Predictions are valid if applied to similar situations as training set (training/test set paradigm)

A key difference as outlined by Efron is aim. Traditional statistical methods such as a linear regression model are aimed at discovering eternal truths. Efron shows how some of Newtons fundamental insights into the laws of nature were developed by applying statistical methods. Real world data is noisy, and statistical modelling can provide a structure on which this "noise" may rest. Pure predictive modelling is entirely different; it aims to provide as accurate predictions as possible yet does not concern itself with causal relationships.

Further differences are closely related to the aforementioned difference in aim. Traditional methods use parametric modelling which rest on causal theories, while predictive modelling functions as a black box. This is important; Efron provides an example of a predictive model for prostate cancer which was able to make equally accurate predictions after removing the most predictive genes. This phenomenon is referred to as *weak learners.* In other words, the small contribution of many (!) predictors is what ultimately provides the accurate prediction; this flies in the face of common understanding of most natural phenomena which are assumed to be caused by a few key causal factors.

Researchers also choose most covariates in traditional methods, doing so from a theoretical perspective on mechanism, while in pure predictive modelling the algorithm itself performs this task. For this reason, traditional regression data features large numbers of patients and relatively few covariates. Pure predictive modelling is again entirely different in that its datasets are often huge and might feature an astonishing amount of covariates. Pure predictive modelling handles these covariates unflinchingly.

Considering current trends in medical science, it is not unlikely that data from the *Second-Eye Progression Study* might find its way into some sort of pure predictive modelling setting. Predictions derived from this dataset are solely accurate in the unlikely event that the real world situation mirrors that of the training set. This is again unlikely because length-biased sampling resulted in overinclusion of patients with sustained unilateral neovascular AMD. Moreover, most patients were relatively young at onset of neovascular AMD (**Research Chapter Three)** In clinical practice this is necessarily different. Moreover, pure-predictive modelling is uniquely susceptible to what lab technicians might call batch-effects, subtle differences in outcomes based on the manner in which the data was collected. In short, pure predictive modelling will unlikely provide meaningfully accurate risk predictions for patients in clinical practice on data of the *Second-Eye Progression study.*

Measuring disease progression and why surrogate endpoints are generally a bad idea

Prognostics encompass biomarkers which may be used to predict events or measure disease progression. The term biomarker refers to reproducible, patient-derived datapoints. (5) Ideally, a biomarker would exist such that a single marker could accurately reflect disease severity. Such a biomarker would follow the natural history of any disease closely thus providing an easy window into individual disease course in clinical practice.

Presently no such biomarker exists in AMD. AMD instead, progresses over many years and its course seems to bifurcate into two quite distinct end stages (atrophic vs. neovascular). Presently, there is no single marker which allows for continuous monitoring from early to end stage AMD. (see Figure 4)

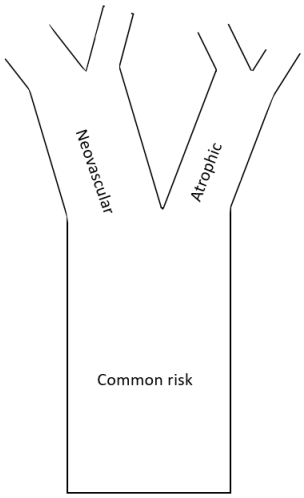

Figure 4. Common Tree of Age-related Macular Degeneration

The diagram shows (albeit in a highly simplified manner) the different clinical courses which patients with age-related macular degeneration may experience. Out of a common trunk of risk (development of drusen) there may follow distinct end stages (neovascular)(atrophic). In some cases, both phenotypes may develop. As the trunk grows, so AMD-signs change.

Biomarkers may function as an endpoint in a trial. The idea of an endpoint is derived from the tradition of evidence-based medicine (EBM) which rose to prominence at the end of the 20th century. Though typically associated with appraisal of hierarchically defined evidence, EBM also strives towards unambiguity in its research findings, stressing the importance of clearly defined endpoints which are important to patients.(6) Indeed, endpoints were conceived to combat subjective physician-based interpretations of disease progression.(7) In many ways, survival represents the ultimate endpoint as its clearly defined, measurable, and aligns with the patient's incentive. In other

words, an endpoint should be *clinically meaningful* which means that it is a reflection of how the patient *feels, functions* or *survives.* AMD is considered a strictly ophthalmic disease; therefore, survival is less apt as 'our' endpoint. Instead, AMD in its advanced stages results in visual impairment which negatively impacts the patient his ability to function and diminishes quality of life. (see figure 5)

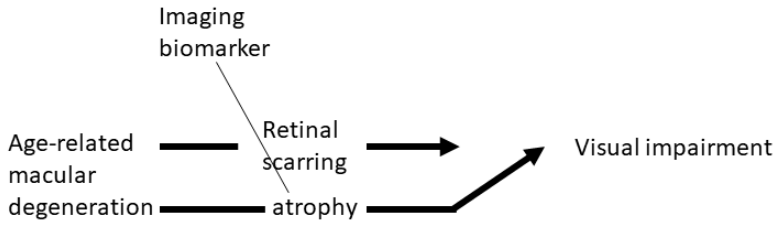

Figure 5. The Trouble with Surrogate Endpoints

This diagram is a redevelopment of a similar diagram by Fleming, et al. 2012. It shows how in age-related macular degeneration multiple causal pathways may lead to visual impairment. Interventions measured on a surrogate (e.g. geographic atrophy size) - which represents only one pathway - leaves the door open for alternative routes that lead to the same result: visual impairment. It is for this precise reason that a strong correlation between atrophy and visual impairment is not enough to validate a surrogate endpoint.

A biomarker by its nature cannot represent a true endpoint like survival, however it can function as a *surrogate*. It seems intuitive that for any biomarker to qualify as surrogate endpoint, additional requirements have to be met. Most importantly, it would be necessary that any biomarker is causally implicated in the disease process such that modification of the marker would reflect a true change in the disease for better or worse. This requirement for causality is highly important yet it might also be insufficient. In AMD in particular, there are multiple causal pathways which result in visual impairment.(8)

While seemingly abstract, the aforementioned points bear directly on current ophthalmic drug development. In 2024 the EMA rejected intravitreal injections with pegcetacoplan for dry advanced AMD – pegcetacoplan binds and inhibits complement protein C3, and is thus hypothesized to mitigate a key causal pathway in the development of GA – despite successful phase 3 trials.(9) This is remarkable considering that pegcetacoplan *was* accepted by the FDA earlier. In fact, the EMA stated lack of functional evidence as its main reason for rejection. Lack of demonstrated impact on everyday functioning is considered by the EMA to be obligatory before granting access to the European market.

Contributions to measuring disease progression in age-related macular degeneration

The paragraphs above provide powerful arguments why trial endpoints and – perhaps more so than is currently in vogue – study endpoints should focus on retinal functionality rather than biomarkers. To sum up, function is what matters to patients and it should therefore matter most to scientist wishing to study disease progression. Moreover, function functions as a resultant measure of diverse causal paths, bypassing the multiple causal-pathway problem posited in figure 5.

Contributions to measuring disease progression

In **Research Chapter Two** studying rare variants, we addressed the issue of monitoring disease progression over a short (one year) period. During the short-term progression study 44 patients were mapped out in terms of retinal function (visual acuity and mesopic microperimetry, MMP). We observed that mean retinal sensitivity changed significantly during a period of 12 and 20 months for eyes with late AMD whereas no such change was observed for visual acuity. We could not however, objectify any significant decrease in mean retinal sensitivity for non-late eyes, which is likely a result of the small number of non-late eyes.

In **Research Chapter Four** we further investigated MMP. This is a psycho-physical test in which light stimuli of varying intensity are presented to specific retinal locations.(10) The threshold at which the patient perceives the light stimulus is then determined. The result is any number of thresholds in candela/m2, usually expressed in decibel (dB) which communicate retinal sensitivity. Because MMP allows for different calculations of its threshold data, we have the opportunity to manipulate and fine-tune how the resultant potential endpoint behaves.

In **Research Chapter Four** three analysis strategies were calculated: the mean of 37 sensitivity thresholds (MS), the percent-reduced threshold (PRT), and the log-transformed candela mean (MS cd log). Sample size requirements were calculated for 12- and 24-month follow-ups using a paired one-sided T-test ($\alpha = .05$, power = 0.80). A relatively small sample size indicates that a decline in a certain analysis strategy is easier to detect than a decline in others. This is important for trials, but also clinical practice. Our results indicate that all MMP analysis strategies outperformed VA, and MS required the least number of patients to show significant changes. Translated to clinical practice

this means that the in MS communicates the most information about the state of the measured retina compared to other analysis strategies.

In **Research Chapter Five** the three analysis strategies MS, PRT, and the log-transformed candela mean MS cd log were again studied. However, in this chapter daily vision dependent tasks (VF), measured using questionnaire served as dependent variable. Through a combination of models, we compared the aforementioned MMP-derived variables to visual acuity, and found in a sample of N = 102 AMD patients that visual acuity provides a better indication of the degree of visual impairment compared to MMP. Therefore, visual acuity provides stronger evidence of potential clinical efficacy in trials than MMP.

Future directions to improve functional outcomes

At first glance, **Research Chapter Four** and **Research Chapter Five** may appear to be at odds with one another. On the one hand, MMP is better able to capture functional decline over relatively short time periods compared to visual acuity. On the other hand, it appears to do a relatively poor job of conveying how the patient *feels*, *functions*, and *survives*. While initially confusing, this discrepancy is readily explained by the distinction between anatomical deterioration and performance in daily, vision-dependent tasks—essentially, the difference between what the clinician observes and what the patient experiences.

Using an argument-based approach to appraise potential trial endpoints, visual acuity remains the preferable choice, as it more accurately reflects visual impairment and correlates better with patient-reported outcomes.(14) However, visual acuity is slow to change, increasing the cost and duration of clinical trials, and it assesses only a limited portion of the retina. Therefore, the objective becomes clear: in order to justify selecting MMP as an endpoint, MMP must be improved to a point where it can compete with visual acuity.

There are three key areas in which MMP might be substantially improved:

Measurement Variance

In test-retest experiments, MMP has shown considerable pointwise variability. This variability is also unequal across decibel (dB) values, meaning that some dB readings are less reliable than others. Upon immediate retesting, these values may vary significantly. To address this issue, MMP could be

enhanced with adaptive testing: decibel values within critical ranges could be remeasured intrasession, as many times as necessary, to provide a more stable estimate of the true value.

Grid Optimization

The standard microperimetry grid could be refined to better target retinal areas critical for daily visual functioning. While the fovea is clearly central, other macular quadrants may vary in importance. For example, the upper temporal retina corresponds to the lower nasal visual field, which plays a key role in fall prevention. Increasing measurement density in such strategically important areas could yield a more clinically relevant metric.

Reducing Patient Burden

Increasing grid density and repeating measurements inevitably raise patient burden. As Chapter F.4 discusses, higher burden reduces the likelihood that patients can complete the test successfully, thereby limiting its applicability and introducing selection bias. To mitigate this, a stepwise elimination of low-yield test loci could be considered, or test loci could be redistributed in a more efficient and patient-friendly manner.

In sum, MMP may be further developed to convey clinically relevant information comparable to current trial endpoints—most notably, visual acuity—while retaining its ability to sensitively measure AMD-related deterioration over relatively short time frames. By addressing these areas, MMP might evolve from a promising but limited tool into a robust, patient-aligned endpoint capable of advancing both research and clinical care in AMD.

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Appendices

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Dutch Summary

Introductie

De introductie beschrijft leeftijdsgebonden maculadegeneratie (LMD); de meest voorkomende oorzaak van irreversibele blindheid bij ouderen met een nadelige impact op alledaags functioneren. Gezien de omvang van het onderwerp is een volledige uiteenzetting van het ziektebeeld onmogelijk. In plaats daarvan zijn enkele hoog-impact studies genoemd in zoverre ze betrekking hebben tot de centrale doelstelling van de thesis: het beschrijven en onderzoeken van (nieuwe), klinische en genetische prognostische factoren voor LMD.

Onderzoekhoofdstuk Een

In dit hoofdstuk is met behulp van een cross-sectioneel, within-patient studie-design de invloed van langdurige behandeling met anti-VEGF (*vaatgroei-remmers*) op de dikte van het choroïd gemeten. Het choroïd, oftewel vaatvlies, voorziet voor een groot deel de zeer metabool actieve, bovenliggende retina. Behandeling met anti-VEGF blijkt met name na de oplaadfase tot verdunning van het choroïd te leiden hetgeen begrijpelijkerwijs bij behandelaars tot zorgen over mogelijke atrofiëring van de retina heeft geleid. Op basis van de gedane metingen blijkt het verschil tussen choroïden van behandelde ogen vs. niet-behandelde ogen beperkt tot een niet-statische significante 11 micron, hetgeen in het licht van de normale dikte van het choroïd weinig opzienbarend is. De conclusie luidt dan ook dat behandelaren atrofie-inductie niet als argument tegen behandeling van exsudatieve LMD hoeven aan te voeren.

Onderzoekhoofdstuk Twee

In hoofdstuk twee is de mogelijke invloed van zeldzame, eiwitveranderende, genetische varianten in complement factor H of complement factor I op progressie tot late LMD onderzocht. Complement factor H en I zijn twee belangrijke genen die beide eiwitten tot expressie brengen die voor complementsysteemdeactivatie zorgen. Gezien de belangrijke rol van het complementsysteem bij LMD werd verwacht dat patiënten met een dergelijke variant *sneller* progressie zouden doormaken dan een groep patiënten waar dergelijke varianten nauwelijks voorkomen (referentiegroep). Dit bleek inderdaad het geval op basis van onze prospectieve studie. Op basis van deze resultaten lijkt het zinnig voor medicijnontwikkelaars om zeldzame-variantdragers apart bestuderen; de snellere progressie maakt het ontwikkelen van medicijnen efficiënter en mogelijk ook het verwachte therapie-effect groter!

Onderzoekhoofdstuk Drie

In hoofdstuk drie is de zogenoemde *non-exudative macular neovascularisation* (NEMNV) bestudeerd. Deze structuren worden gezien als de meest voorspellend risicofactor voor het ontstaan van exsudatieve LMD. Bestaande studies in unilaterale neovasculaire LMD patiënten hebben tal van factoren die aan exsudatie van NEMNV voorafgaan/bijdragen onderzocht. Echter, *hoelang* de NEMNV bestaat ten opzichte van het begin van LMD is daarbij veronachtzaamt. In dit hoofdstuk hebben we het risico van NEMNV op conversie onderzocht en daarbij onderscheid gemaakt tussen patiënten wiens eerste oog *kort geleden* (<5 jaar) en *lang geleden* (≥5) exsudatief werd. Op basis van onze resultaten blijkt het aanmerkelijk risico hoger te zijn voor de *lang geleden* groep. We denken daarom dat deze NEMNV op een onwelgevallig moment in beeld zijn gekomen en niet dat er per se factoren aan de NEMNV te zien waren die hintten op deze uitkomst. Dit wordt onderstreept door de secundaire analyse waaruit blijkt dat nagenoeg alle NEMNV tijdens het beloop van de studie groeiden en veranderden – ongeacht of ze exsudatief zijn geworden.

Onderzoekhoofdstuk Vier

Hoofdstuk vier en vijf zijn beide gericht op mesopische microperimetrie – een functietest die de lichtgevoeligheid van het netvlies meet. Mesopische microperimetrie is een relatief nieuw onderzoek met veel verschillende output waarvan niet geheel duidelijk is hoe deze output het beste geanalyseerd dient te worden in klinischetrialverband. Eerder onderzoek liet zien dat mesopische microperimetrie wél eerder achteruitgang van LMD laat zien dan visus (bestaande uitkomstmaat). We hebben het niet-neovasculaire oog van onze unilaterale LMD populatie die gedurende twee jaar in beeld is geweest op verschillende manieren geanalyseerd; door het gemiddelde van de metingen te nemen (MS), door de metingen in hun grondeenheid (candela per vierkante meter) te middelen alvorens een logtransformatie toe te passen (MS cd log), door een percentage van metingen onder een afkapwaarde te nemen (PRT). Het blijkt zo te zijn dat het gemiddelde (MS) het meest efficiënt is in veranderingen statistisch zichtbaar maken. Dat houdt dus in dat MS de beste manier is LMD over een lange periode te monitoren.

Onderzoekhoofdstuk Vijf

In hoofdstuk vijf is de relatie tussen mesopische microperimetrie en visus-gerelateerde kwaliteit van leven onderzocht. Voortbordurend op hoofdstuk vier, is het is erg belangrijk dat een uitkomstmaat voor trials niet alleen snel bepaalde veranderingen kan laten zien, maar dat deze verandering ook klinisch

betekenisvol is. In deze cross-sectionele studie is mesopische microperimetrie (opnieuw met MS, MS cd log en PRT) vergeleken met visus in hun vermogen om visus-gerelateerde kwaliteit van leven (met een vragenlijst gemeten) te voorspellen. Het blijkt dat visus beter in staat is om te voorspellen in welke mate een patiënt beperkt voor zijn alledaagse bezigheden, maar dat MS wel de betere maat is van de drie microperimetrie uitkomstmaten.

Discussie

De discussie blikt terug op de belangrijkste bevindingen van de onderzoekhoofdstukken en recontextualiseert enkele bevindingen. Met name de methodologische uitdaging rondom onderzoekhoofdstuk drie zijn uiteengezet. Daarnaast wordt stelling genomen in een debat over mogelijke eindpunten in LMD-therapiestudies (functionele eindpunten of anatomische eindpunten) waar mesopische microperimetrie mogelijk een sleutelrol speelt.

Data Management

Ethics and privacy

This thesis is based on the results of human studies, which were conducted in accordance with the principles of the Declaration of Helsinki. The Medical Research Ethics Committee Oost-Nederland or CMO Radboudumc have given a positive advice to conduct these studies to the head of the Department of Ophthalmology, Radboudumc, Nijmegen, the Netherlands, who formally approved the conduct of these studies. A statement that the study was not subject to the Dutch Medical Research Involving Human Subjects Act (WMO), was obtained from the recognized Medical Ethics Review Committee METC Oost-Nederland (2021-13187) (Biobank protocol version 1.0 d.d. 14 September 2021). Research funders had no role in in the design or conduct of this research.

All data in this thesis is subject to strict privacy standards due to their sensitive nature. All data (raw and processed) is pseudonymised with a study ID through PIMS (PIMS (radboudumc.nl)). Access is controlled via the data manager of the department.

Informed consent was obtained from participants to collect and process their data for this research project. Consent was also obtained for sharing the (pseudonymized) data after research.

The sensitivity and confidentiality of the raw qualitative data (i.e. interviews, forum groups) makes sharing of the data without compromising confidentiality and privacy impossible, therefore consent for sharing of the raw data was not asked from the participants. Written informed consents of participants are stored in a locked archive at the Department of Ophthalmology, Radboudumc, Nijmegen, the Netherlands.

Data collection and storage

The table below details where the data and research documentation for each chapter can be found. All data remain available for at least 10 years after termination of the studies. All data have been collected through in-house patient visits using equipment and materials of the Radboudumc department of ophthalmology. Due to their highly sensitive nature, data can only be shared upon request to the owners of the data collections.

Research Chapter (RC)	Storage
RC 1	1. Questionnaire (Biobank): data can be found in an online-protected secured castor database named AMD_EUGENDA (Castor EDC) 2. Retinal Images (Biobank): all images are stored https://research-ohk.radboudumc.nl/
RC 2	1. Plasma/serum/DNA (Biobank): stored in the -80°C freezers at the Radboud Biobank overseen by the Department of Laboratory Medicine, Radboudumc, Nijmegen, the Netherlands and belong to the sub-biobank of the Department of Ophthalmology, Radboudumc, Nijmegen, the Netherlands. 2. Questionnaire (Biobank). AMD_EUGENDA. (Castor EDC) 3. Retinal Images (Biobank), https://research-ohk.radboudumc.nl/ 4. Mesopic microperimetry: raw data is stored after pseudonymisation on devices at the Radboudumc medical centre (MAIA, s-MAIA).
RC 3	1. Questionnaire (Biobank). AMD_EUGENDA. (Castor EDC) 2. Clinical data (including visual acuity) (Biobank). AMD_EUGENDA. (Castor EDC) 3. Retinal Images (Biobank) https://research-ohk.radboudumc.nl/.
RC 4	1. Questionnaire (Biobank). AMD_EUGENDA. (Castor EDC) 2. Clinical data (including visual acuity) (Biobank). AMD_EUGENDA. (Castor EDC) 3. Retinal Images (Biobank). https://research-ohk.radboudumc.nl/.
RC 5	1. Questionnaire (Biobank). AMD_EUGENDA. (Castor EDC) 2. Clinical data (including visual acuity). AMD_EUGENDA. (Castor EDC) 3. Retinal Images (Biobank). https://research-ohk.radboudumc.nl/.

Findable and accessible

The table below details where the data and research documentation for each chapter can be found on the Radboud Data Repository (RDR). All data archived as a Data Sharing Collection remain available for at least 10 years after termination of the studies.

	DAC (closed access)	RDC (closed access)	DSC (restricted access)
RC1	Raw choroid thickness measurements are in(.ppt), DOI 10.34973/2gt0-m470	Included patients, processed data and statistical procedures/ syntax including read me files are at: DOI 10.34973/ jdvr-zk38	Published or definitive version of manuscript & processed data is: DOI 10.34973/j59x-d282
RC2	1. Mesopic microperimetry: a copy of raw data files up to august 23 (.spss) DOI 10.34973/vg20-5j31 2. Geographic atrophy grading is in (.ppt) in DOI 10.34973/hm9a-6361	Included patients, processed data and statistical procedures/ syntax including read me files are at: DOI 10.34973/05ak-6j41	Published version or definitive of manuscript & processed data is at: DOI 10.34973/qzr7-j052
RC3	Graded retinal images in (.ppt) at: DOI 10.34973/ f6jj-h384	Included patients, processed data and statistical procedures/ syntax including read me files are at: DOI 10.34973/ sbhp-dk76	Published version or definitive of manuscript & processed data is at DOI: 10.34973/hbdm-fj44
RC4	Mesopic microperimetry a copy of raw data files up to august 23 in in DOI 10.34973/ehth-8959	Included patients, processed data and statistical procedures/ syntax including read me files are at: DOI 10.34973/ nacy-he75	Published version or definitive of manuscript & processed data is at DOI: 10.34973/t8kb-x644
RC5	1. Mesopic microperimetry, a copy of raw data files up to august 23 (.spss) DOI 10.34973/hm9a-6361	Included patients, processed data and statistical procedures/ syntax including read me files are at: DOI 10.34973/ pffm-cv80	Published version or definitive of manuscript & processed data is at: DOI 10.34973/dw55-vg81

Curriculum Vitae

Francesco Cinque was born on the first of February 1995 in Boxmeer. He attended high school at *College Den Hulster* in Venlo where he graduated in 2013 for his Nature & Health profile alongside an International Baccalaurate diploma in English.

Francesco went on to study Medicine and Philosophy at *Radboud University* in Nijmegen. He earned his medical degree *cum laude* in 2020 and continued with his research on age-related macular degeneration that same year. In 2024, he completed his master of arts in Philosophy by writing a thesis on relationships in modernity.

In addition, he was secretary for the 2022 Dutch Ophthalmology conference, an annual national conference to connect young ophthalmology researchers. He co-authored an article with Dr. Yara Lechanteur published in *Het Nederlands Tijdschrift voor de Geneeskunde* in 2022, aimed at a broader audience.

In his free time, Francesco enjoys doing sports and playing jazz guitar, reflecting his deep passion for music.

Portfolio

Francesco Cinque
Donders Graduate School
PhD contract: 1-8-2021 – 1-5-2024.

Courses & Workshops

Course	Organizer	Hours
Graduate School Introduction Day (2021)	Donders Graduate School	7
Graduate School Day (2021)	Donders Graduate School	7
Graduate School Day 2 (2023)	Donders Graduate School	7
Scientific Integrity Course (2022)	Donders Graduate School	7
eBROK (2021)	NFU	42
Statistiek voor promovendi met SPSS (2021)	Radboud University	60
Design and Illustration (2021)	Radboud University	28
Scientific writing for PhD candidates (2022)	Radboud University	96
Analysing longitudinal and multilevel data using R (2023)	Radboud University	96
Principles of research in Medicine and Epidemiology (2022)	Netherlands Institute Health Sciences	19
Introduction to data-analysis (2022)	Netherlands Institute Health Sciences	19
Practical Philosophy-1 (2022)	Radboud University	280
Practical Philosophy-2 (2023)	Radboud University	280
Public Philosophy (2023)	Radboud University	140
Philosophical tools (2024)	Radboud University	140
Master's Thesis (2024)	Radboud University	560

Conferences

Course	Location
ARVO (2022)*	Denver
ARVO (2023)*	New Orleans
E3 (2023)^	Nijmegen
International Symposium on AMD (2023)^	Baden-Baden
DOPS (2023)^	Nijmegen

*poster presentation, ^oral presentation

Teaching activities

Teaching activity	Study program
Tutor practicum BMS-19 (2022-2023)	Master of biomedical sciences
Internship supervision	
Student 1 (2020)	Master Medicine (3 months)

Committees & other activities

Organizer DOPS (2022)
Subinvestigator various trials (2022-2024)

Outreach & Societal impact

Droge leeftijdsgebonden maculadegeneratie: achtergrond en toekomstperspectief, written by Francesco Cinque and Yara Lechanteur for MaculaVisie (quartal magazine for MaculaVereniging) (2022)
Nieuwe Inzichten in de Behandeling van Leeftijdsgebonden Maculadegeneratie, written by Francesco Cinque, Carel Hoyng, Yara Lechanteur for Nederlands Tijdschrift van de Geneeskunde. (2022)

Dankwoord

Hier begint het meest gelezen onderdeel van ieder proefschrift. Ik wil graag iedereen bedanken die me heeft geholpen om dit proefschrift tot een goed einde te brengen. Sommige groepen en mensen wil daarbij in het bijzonder bedanken.

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Met dank aan mijn promotoren prof dr. Caroline Klaver, prof. dr. Carel Hoyng en mijn copromotor dr. Yara Lechanteur.

Yara, onze lange werkrelatie heeft zich ontvouwd tot een zeer effectieve samenwerking. Jouw grondigheid kent zijn weerga niet en gedurende het traject heb ik altijd op een rechtvaardig oordeel van jou over mijn werk kunnen rekenen.

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Met dank aan mijn familie, moeder en broertje, mijn trouwe vrienden en Simone voor jullie niet-aflatende steun.

In het bijzonder bedankt aan mijn zusje Elena voor je inzicht in statistiek en methodologie. Zonder jouw welwillendheid om klankbord te spelen, was het me nooit gelukt om mijn gedachten van hun warrigheid te ontdoen. Ik hoop dat je je talent voor de wetenschap net zo hoog waardeert als ik dat doe. Eveneens dank aan Amir dat je me voor zoveel faux-pas hebt behoed en je weergaloze gevoel voor humor.

Ook ben ik mijn paranimfen zeer dankbaar; Jeroen, jij hebt een fijn luisterend oor geboden - vaak onder genot van een cappuccino - en hebt me daarmee enorm vooruit geholpen. Ook dank aan Laurens, jij wist met jouw scherpe aanwijzingen – op de bank zittend met een glas goede wijn en met die karakteristieke nonchalance die jou zo bijzonder maakt – mijn manuscripten voor een wisse afwijzing te behoeden. Verder, achter iedere paranimf gaat een misschien wel belangrijkere schaduwparanimf schuil en daarom wil ik Marjolein ook bedanken voor je organisatietalent en alle gezelligheid.

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Tot slot, ten tijde van dit schrijven is het feest nog niet helemaal voorbij maar lijkt de vijfde akte wel aangebroken. Het is lastig om daar woorden aan te geven; een echte terugblik is voorbarig en alle clichés over promoveren ten spijt ("it takes a village" (beetje kruiperig), "het is net als je rijbewijs" (hopelijk niet want ik hou niet van autorijden), "een antidankwoord" iets te ribfluwelig past alles net niet. Daarom laat ik het bij een citaat van W.F. Hermans uit zijn boek *Nooit Meer Slapen* (1966) waarin de hoofdpersoon zijn frustratie over promoveren als volgt uitdrukt:

> *"Ik geef toe aan een onbedwingbare neiging mijn fantasie te laten gaan over de verschrikkelijkste van alle mogelijkheden: dat het allemaal voor niemendal zou blijken te zien."*

Over het Donders Instituut

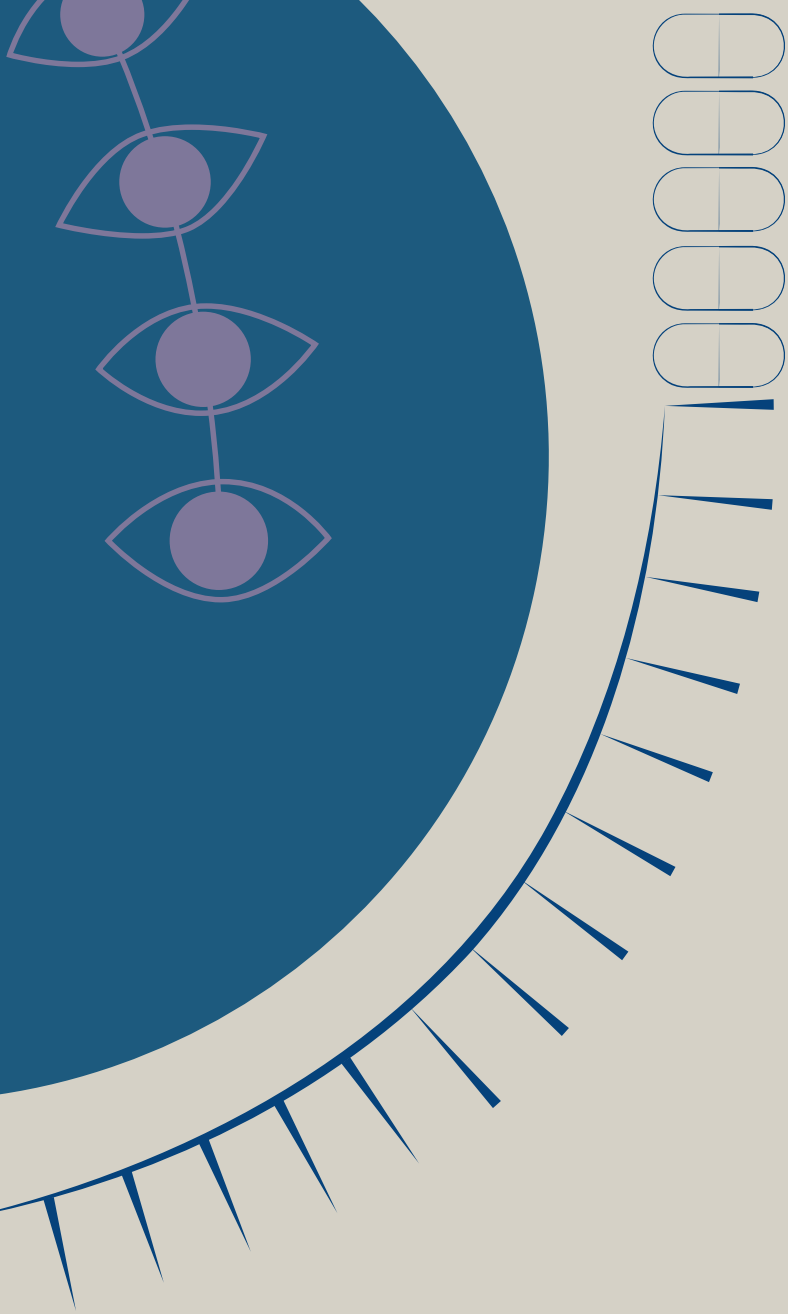
Donders Graduate School For a successful research Institute, it is vital to train the next generation of scientists. To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the Donders Graduate School in 2009. The mission of the Donders Graduate School is to guide our graduates to become skilled academics who are equipped for a wide range of professions. To achieve this, we do our utmost to ensure that our PhD candidates receive support and supervision of the highest quality.

Since 2009, the Donders Graduate School has grown into a vibrant community of highly talented national and international PhD candidates, with over 500 PhD candidates enrolled. Their backgrounds cover a wide range of disciplines, from physics to psychology, medicine to psycholinguistics, and biology to artificial intelligence. Similarly, their interdisciplinary research covers genetic, molecular, and cellular processes at one end and computational, system-level neuroscience with cognitive and behavioural analysis at the other end. We ask all PhD candidates within the Donders Graduate School to publish their PhD thesis in de Donders Thesis Series. This series currently includes over 600 PhD theses from our PhD graduates and thereby provides a comprehensive overview of the diverse types of research performed at the Donders Institute. A complete overview of the Donders Thesis Series can be found on our website: https://www.ru.nl/donders/donders-series

The Donders Graduate School tracks the careers of our PhD graduates carefully. In general, the PhD graduates end up at high-quality positions in different sectors, for a complete overview see https://www.ru.nl/donders/destination-our-former-phd. A large proportion of our PhD alumni continue in academia (>50%). Most of them first work as a postdoc before growing into more senior research positions. They work at top institutes worldwide, such as University of Oxford, University of Cambridge, Stanford University, Princeton University, UCL London, MPI Leipzig, Karolinska Institute, UC Berkeley, EPFL Lausanne, and many others. In addition, a large group of PhD graduates continue in clinical positions, sometimes combining it with academic research. Clinical positions can be divided into medical doctors, for instance, in genetics, geriatrics, psychiatry, or neurology, and in psychologists, for instance as healthcare psychologist, clinical neuropsychologist, or clinical psychologist. Furthermore, there are PhD graduates who continue to work as researchers outside academia, for instance at non-profit or government organizations,

or in pharmaceutical companies. There are also PhD graduates who work in education, such as teachers in high school, or as lecturers in higher education. Others continue in a wide range of positions, such as policy advisors, project managers, consultants, data scientists, web- or software developers, business owners, regulatory affairs specialists, engineers, managers, or IT architects. As such, the career paths of Donders PhD graduates span a broad range of sectors and professions, but the common factor is that they almost all have become successful professionals.

For more information on the Donders Graduate School, as well as past and upcoming defences please visit:
http://www.ru.nl/donders/graduate-school/phd



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