Radial Shape Discrimination in eyes at high risk of developing neovascular Age-Related Macular Degeneration

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Master of Philosophy

by

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Abstract

Aims
The aim of this study was to investigate the use of a novel handheld Radial Shape Discrimination test (the hRSD test) in eyes at high risk of developing neovascular Age-Related Macular Degeneration (nAMD). The properties of the hRSD test that were investigated include the test-retest repeatability and the stability over time in clinically stable participants. The relationship between hRSD scores and other measurements of visual function (visual acuity, VA and contrast sensitivity, CS) were explored along with the relationship with retinal structural changes (foveal large drusen, ellipsoid zone disruption, EZD and central subfield thickness, CST).

Methods
A sample of 100 participants with unilateral nAMD was recruited from a UK ophthalmology clinic. The unaffected eye (the study eye, SE) had no evidence of nAMD, VA≤0.4 logMAR and was considered to be at risk of developing nAMD due to contralateral involvement. Participants performed the hRSD test on five consecutive visits, spread over approximately six months. All participants performed VA and Optical Coherence Tomography (OCT) at all visits. CS was measured in a subgroup of 34 participants. A usability questionnaire was completed at the last visit.

Results
The overall mean hRSD score in SEs was -0.56 ±0.16 (95%CI -0.60 to -0.53) logMAR for a group whose mean age was 77±7 years. Older participants had worse hRSD scores (r=0.37, p=0.0005) which corresponded to a deterioration of 0.08 logMAR per decade (slope of the linear regression). The test-retest repeatability over two consecutive visits revealed good agreement (bias=-0.004 logMAR, upper and lower limits of agreement: 0.27 and -0.28 logMAR). The coefficient of repeatability over five visits was 0.33 logMAR. There were no trends seen (no learning effects). The hRSD test was less repeatable than VA when compared by means of intraclass correlation coefficients (0.85 vs. 0.93). No correlation was seen between hRSD and VA (p=0.9) or CS (p=0.1). Of the three aspects of retinal structure investigated, only large drusen and EZD had a significant effect on hRSD scores (p=0.02 and p=0.01). Finally, the usability questionnaire revealed a very good acceptability of the test.

Discussion
The hRSD test has been suggested to be an effective mean of detecting nAMD. This study contributes to the ongoing assessment of the hRSD test in a key population with eyes at risk of nAMD. Understanding the performance of tests in the absence of disease progression is important to correctly interpret potential changes as clinically or non-clinically significant. Although the hRSD test was less repeatable than VA, the degree of variability can be considered acceptable in view of the large dynamic range seen between early and late AMD. The hRSD test has the advantage of being portable, capable of self-administration and well accepted by patients. The results from this study support the idea that the hRSD test is a better indicator of foveal integrity in the early stages of AMD compared to VA, as shown by the results of the correlations with structural parameters.

Acknowledgements

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I am most grateful to all my participants who kindly volunteered their time and shared their interest in my research. I would like to thank all the staff in the Clinical Eye Research Centre for supporting the study and for putting up with me.

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Abbreviations

ADD: near addition

AF: Auto Fluorescence

AG: Amsler Grid

AMD: Age-Related Macular Degeneration

AREDS: Age-Related Eye Disease Study

BCVA: Best Corrected Visual Acuity

CFP: Colour Fundus Photographs

CI: Confidence Interval

CNV: Choroidal Neovascularisation

CR: Coefficient of Repeatability

CST: Central Subfield Thickness

CS: Contrast Sensitivity

DR: Diabetic Retinopathy

ERM: Epiretinal Membrane

ETDRS: Early Treatment Diabetic Retinopathy Study

EZ: Ellipsoid Zone

EZD: Ellipsoid Zone Disruption

FFA: Fundus Fluorescein Angiography

FE(s): fellow eye(s)

GA: Geographic Atrophy

hRSD: Handheld Radial Shape Discrimination

ICC: intraclass correlation coefficient

IPCV: Idiopathic Polypoidal Choroidal Vasculopathy

nAMD: neovascular Age-Related Macular Degeneration

OCT: Optical Coherence Tomography

PED: Pigment Epithelium Detachment

PHP: Preferential Hyperacuity Perimeter

RAP: Retinal Angiomatous Proliferation

RF: Radial Frequency

RCT: Randomised Controlled Trial

RPE: Retinal Pigment Epithelium

RPEE: Retinal Pigment Epithelium Elevation

RSD: Radial Shape Discrimination

RVO: Retinal Vein Occlusion

SDH: Shape Discrimination Hyperacuity

SD-OCT: Spectral domain Optical Coherence Tomography

SE(s): study eye(s)

TD-OCT: Time domain Optical Coherence Tomography

VA: Visual Acuity

VEGF: Vascular Endothelial Growth Factor

VMT: Vitreo-Macular traction

Contributions

The research questions presented in this thesis were designed by me under the supervision of my primary MPhil supervisor Dr Paul Knox. The statistical plan and analysis were also performed by me, with advice from Dr Paul Knox and Dr Gabriela Czanner, Lecturer in Ophthalmic Statistics. The OCT grading protocol was developed by me with advice from Mr David Parry, Senior Ophthalmic Image Grader from the Liverpool Ophthalmic Reading Centre. I identified all eligible participants and carried out the informed consent process. The study visits were performed by me or a nurse/ health care assistant in the Clinical Eye Research Centre, St Paul's Eye Unit. I personally trained them on all study procedures. As part of routine care, nurses, health care assistants and optometrists performed VA measurements; and ophthalmic photographers obtained OCT scans. I extracted all data from the patient records and OCT scans into an electronic database created and maintained by myself.

Chapter 1 Introduction

The visual impairment caused by the late stages of age-related macular degeneration (AMD) affects the quality of life of millions of people in the UK and worldwide. Over the last decade, breakthrough developments in the treatment of neovascular AMD (nAMD), one of the forms of advanced AMD, have transformed the prognosis of patients, providing an opportunity to preserve their vision. The treatment consists of a number of injections into the eye of antiangiogenic drugs that target vascular endothelial growth factor (anti-VEGF drugs). The outcome of the treatment is affected by the level of visual impairment at commencement of treatment. This means that in those in whom the treatment starts later, once their vision has badly deteriorated, the chances of achieving an acceptable level of vision from treatment are lower, potentially affecting their ability to perform daily activities such as reading or driving.

The issue of early detection of nAMD has been recognised, and research in this field is being conducted to develop new tests that can effectively detect the earliest symptoms of nAMD. These would alert patients to seek professional advice before the symptoms became severe. Currently in the UK, the majority of patients who develop nAMD present to their General Practitioner or Optometrist with symptoms of distorted vision or small patches of blurred vision. The problem with waiting until the development of these symptoms is that, sometimes, these will not be noticed by the patient until they are quite severe or affect central vision. The Amsler grid is a simple, widely used, vision test used to detect the presence of distortions but despite its popularity it is known to lack sufficient sensitivity for detecting the earliest signs of nAMD.

A portable, smartphone-based test, the handheld radial shape discrimination (hRSD) test, has been recently developed which aims to provide an alternative to the Amsler grid. Although theoretically the test shows promise, there have not been many published studies supporting its performance in large samples of “at risk” eyes. Therefore, the objective of this study was to investigate the hRSD test in a real world clinical sample of patients at risk of developing nAMD. Several test characteristics

were studied, aiming to contribute to the knowledge on the performance of this test in this population.

The following literature review provides background information about both AMD and the hRSD test, to provide a context for the study and the results emerging from it.

1.1 Age-Related Macular Degeneration

AMD is an ophthalmic degenerative disorder that affects older people. Pathological changes occur in the retinal pigment epithelium (RPE) of the macular area, responsible for central vision. As a result, affected patients experience visual distortions and central visual field loss (Figure 1.1).

Early AMD is usually asymptomatic but the late stages of the condition can lead to loss of functional vision reducing the patient's quality of life and ultimately their independence (Mitchell and Bradley 2006). The late stages of AMD are in fact responsible for the majority of cases of visual impairment in developed countries (Congdon et al. 2004, Evans et al. 2004, Martin et al. 2011) and account for 5% of cases of blindness worldwide according to the WHO 2010 global estimates.

Of the two types of advanced AMD, namely geographic atrophy (GA) and neovascular or "wet" AMD, this study focused on the latter. Due to the lack of a large population study of AMD in the UK, a study by Owen et al. (2012) estimated the prevalence of AMD by applying published data on AMD to the UK population between 2007 and 2009. An estimated prevalence for nAMD of 2.5% was found in people aged 65 or more, increasing to 6.3% in those aged 80 or more (Owen et al. 2012). The number of people affected by nAMD in the UK is expected to increase from an estimated 414,561 cases in 2010 to 515,509 in 2020 (Minassian et al. 2011). Despite the beneficial effects of anti-VEGF therapy, 189,890 of people affected by nAMD in 2020 will suffer from sight loss as a result of nAMD (visual acuity worse than 6/12 Snellen in their better eye, Minassian et al. 2011).

The first half of this introductory chapter briefly reviews the normal structure of the retina and the pathological processes leading to the development of nAMD, the risk factors for nAMD, the in-clinic assessments (including structural and functional assessments) and the importance of early detection and treatment.

A

B

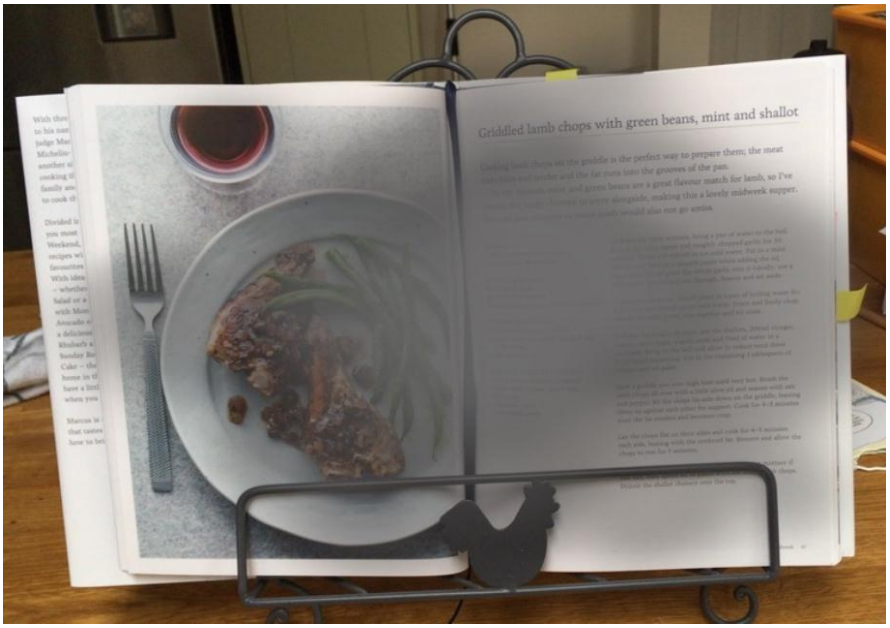

Figure 1.1 Illustration of the visual symptoms experienced by patients with late AMD. A. Visual distortions, where straight lines appear wavy. B. Central scotomas where the central part of the visual field appears blurred or absent, often described as a grey patch in vision.

1.1.1 Normal structure of the retina and pathophysiology of nAMD

The retina is a thin layer of light-sensitive tissue located at the back of the eye, anterior to the choroid. It contains photoreceptors, which are cells that have the capability of converting light into electric potentials that travel along the optic nerve towards the visual cortex for interpretation. The photoreceptors are in close relationship with the RPE (Figure 1.2A), a monolayer of pigmented cells that have an important role in photoreceptor nutrition, retinol metabolism, phagocytosis of photoreceptor outer segments and formation of the outer blood-retinal barrier (Zayit-Soudry et al. 2007).

It is believed that failure of the RPE to perform its functions leads to the clinical changes seen in AMD. Nowak (2006) suggested that there are at least four processes occurring in the RPE cells leading to AMD: 1) accumulation of lipofuscin as a result of ageing (phagocytic and metabolic insufficiency); 2) displacement of the RPE monolayer caused by drusen formation; 3) local inflammation, caused by immunologic components found in drusen; and 4) formation of new abnormal vessels resulting from an imbalance between pro-angiogenic (vascular endothelial growth factor, VEGF) and anti-angiogenic (pigment epithelium derived factor, PEDF) activity. As a result of these processes, the retinal layers are disrupted and fluid accumulates beneath and within the retina (Figure 1.2B), affecting photoreceptors function.

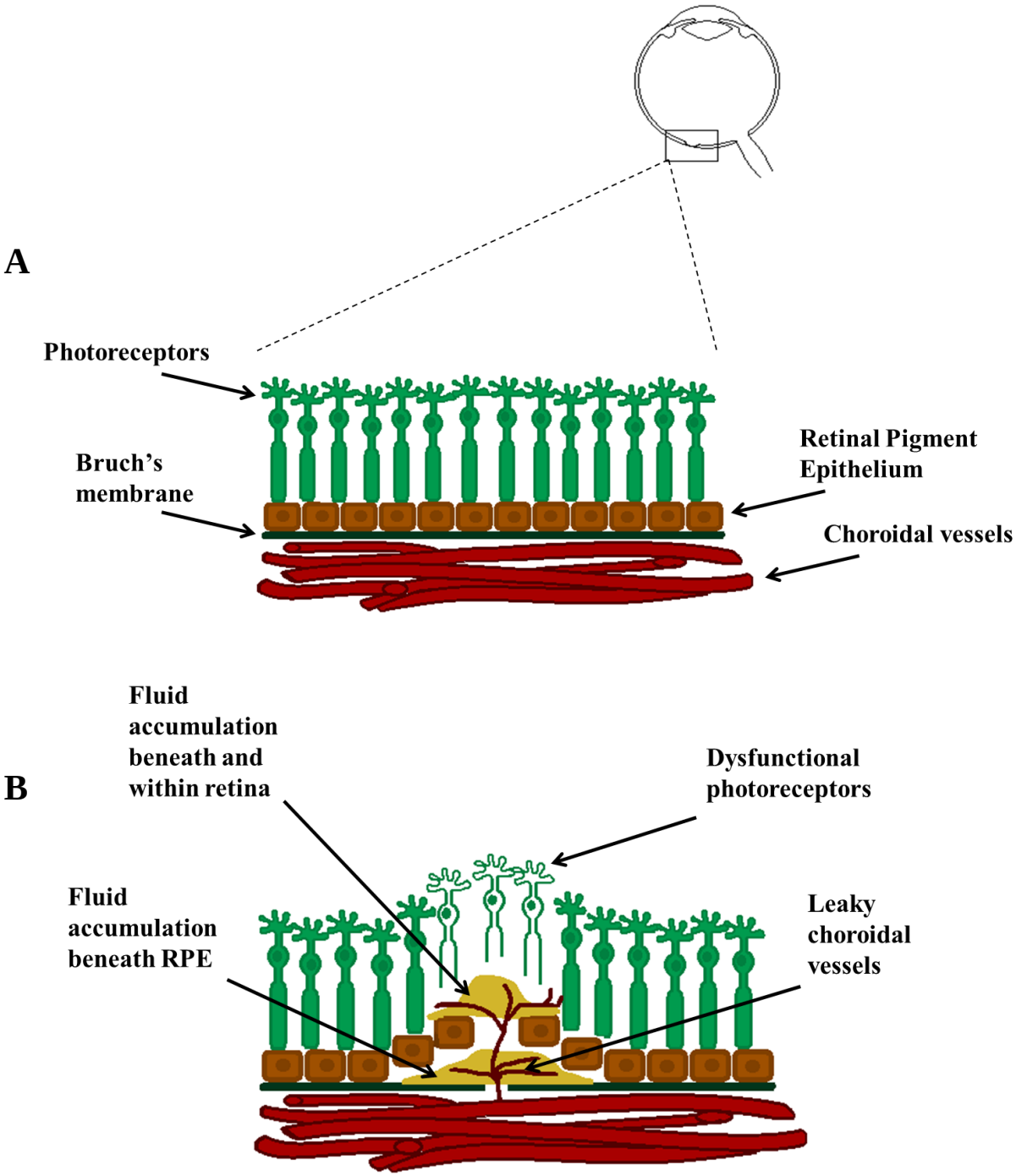

Figure 1.2 Illustration of a cross sectional view of the retina at the posterior pole of the eye, showing the relationship between the photoreceptors and the RPE in a healthy eye (A) where photoreceptors are closely related to RPE cells, separated from choroidal blood vessels by Bruch's membrane; and in an eye with nAMD (B) where disorganised leaky blood vessels braking through a weakened Bruch's membrane result in a displacement of the RPE cell layer and accumulation of fluid underneath the RPE and within the retinal layers.

1.1.2 Risk factors for nAMD

Many risk factors have been associated with a progression to the late stages of AMD. While some studies have investigated nAMD and GA separately, many others have used the terms late or advanced to include both. Some risk factors are linked to the early stages of the condition which could in turn progress onto late AMD.

1.1.2.1 Non retinal factors

The main risk factor associated with AMD is older age. Many studies have shown increased prevalence of AMD in older groups of participants, usually over 80 years old (Smith et al. 2001, Buch et al. 2005). A combined analysis of three large prevalence studies conducted in North America (the Beaver Dam Eye Study), Australia (the Blue Mountains Eye Study) and The Netherlands (the Rotterdam Study) reported that the prevalence of nAMD increased from 0.17% in subjects aged 55 to 64 to 5.76% for those older than 85 years (Smith et al. 2001).

Along with older age, having one eye with nAMD has consistently been associated to increased likelihood of developing nAMD in the second eye (Burgess et al. 1993, Wong et al. 2008, Thomas R. Friberg et al. 2012). The risk is not reduced by anti-VEGF treatment given to the affected eye (Barbazetto et al. 2010).

Smoking is frequently associated with late AMD (Evans et al. 2005, Khan et al. 2006, Seddon et al. 2006, Milton et al. 2005). Studies agree that the risk of having AMD is doubled in smokers although ex-smokers who have stopped smoking more than 20 years ago were not at higher risk than non-smokers (Evans et al. 2005, Khan et al. 2006). It has been suggested that smoking has a stronger association with nAMD than with GA (Smith et al. 2001).

Genetic factors are also known to affect the susceptibility to AMD. The two major genetic loci associated with AMD are the complement factor (CF) H on chromosome 1 and the ARMS2/HTRA1 loci on chromosome 10 (Klein et al. 2005, Yang et al. 2006, Fritsche et al. 2008, Anderson et al. 2010). Together, the CFH and HTRA1 risk variants have been suggested to increase the odds of having AMD by more than 40 times (Cameron et al. 2007). Many other known variants with smaller effects have

also been identified over the past decade, which include complement component (C) 2 (C2/ CFB), C3 and CFI, as well as several other novel variants (Fritsche et al. 2014, Fritsche et al. 2016). A particular novel variant (rs42450006 near gene MMP9) has recently been suggested to be exclusively associated with nAMD and not GA (Fritsche et al. 2016). Risk models that combine genetic, demographic and environmental factors can provide high prediction of disease onset and progression (Seddon et al. 2011).

Older age, presence of nAMD in one eye, genetic variations and smoking are risk factors strongly and consistently found to be related to the development of nAMD but the association of other risk factors is less clear. For example, cataract extraction was associated with late AMD by some authors (Klein et al. 2012) but not by others, where statistical significance was not reached (Chew et al. 2009). Other risk factors such as white race (Milton et al. 2005), sunlight exposure (Tomany et al. 2004) and refractive errors (Li et al. 2014) have been suggested in the literature as possible risk factors for early AMD, which could in turn progress to late AMD.

1.1.2.2 Retinal risk factors

Drusen are the most recognised retinal features of AMD. These deposits of yellow materials accumulate underneath the RPE and are clinical features of the early stages of AMD. It is conventionally agreed that eyes with indistinct soft, large drusen and greater total drusen area, particularly centrally located, are at a higher risk of progressing to nAMD (Wang et al. 2003, Chew et al. 2014a).

More recently, and due to the development of new imaging modalities (in particular optical coherence tomography), a new type of druse has been identified as a potential risk factor for the development of nAMD. So called “subretinal drusenoid deposits” or “reticular” drusen they are abnormal deposits which, as opposed to conventional drusen, accumulate above the RPE. Large population-based studies have found a higher incidence of nAMD in eyes with reticular drusen compared to other types of drusen (Klein et al. 2008). Nearly half (48.6%) of participants with reticular drusen developed late AMD (both nAMD and GA) within 15 years, with 37.8% of them developing late AMD within five years (Joachim et al. 2014).

It has been suggested that low levels of macular pigmentation result in an increased risk of AMD, measured post-mortem in Bone et al. (2001) and psychophysically in Beatty et al. (2001). Several clinical studies have confirmed that presence of RPE abnormalities seen by fundus photography puts patients at higher risk of progression to late AMD (Wang et al. 2003, Klein et al. 2007, Chew et al. 2014a). Recently it was recognised that pigmentary changes on their own did not increase the risk of progression to late AMD, and that the risk only increased when medium size drusen were present as well (Ferris et al. 2013). As a consequence of this finding, "AMD pigmentary abnormalities" were re-defined as hypo or hyperpigmentation present within 2 disc diameters from the centre of the macula in eyes with drusen 63µm or more in diameter (Ferris et al. 2013).

1.1.3 In-clinic assessment of AMD patients

The clinical assessment of patients with AMD includes both an evaluation of retinal structure and visual function. The following sections describe the clinical tests used to examine the structural and functional abnormalities seen in AMD patients before and after the development of nAMD.

1.1.3.1 Assessment of structural changes in AMD

Fundus imaging is an essential part of the assessment and monitoring of patients with AMD. In brief, before the diagnosis of nAMD, colour fundus photography (CFP) is most commonly used to keep records of the progression in the earlier stages of the disease in the absence of a slit-lamp examination by an ophthalmologist. This is because CFP provides high quality colour pictures of the back of the eye that can be stored for future assessment, allowing a side by side comparison between time points. Colour images capture drusen and pigmentary changes, which are features used to classify the early stages of AMD. The actual diagnosis of nAMD however should be done by means of a fundus fluorescein angiogram (FFA). Lastly, the monitoring of therapy outcomes can be done by means of optical coherence tomography (OCT).

1.1.3.1.1 Monitoring of progression prior to nAMD development

Several classification systems have been developed and modified in order to suit epidemiologic studies and clinical trials in which disease progression is monitored prior to the development of late AMD. Examples of this are the Age-Related Eye Disease Study (AREDS) Severity Scale for Age Related Macular Degeneration (Davis et al. 2005), the Wisconsin Age-Related Maculopathy Grading System (Klein et al. 1991) and the International Classification and Grading System for Age-Related Maculopathy and Age-Related Macular Degeneration (Bird et al. 1995).

In these classification systems, drusen number, location, size and area; and pigmentary changes, their location, size and area, are carefully described using measuring circles of specified diameter. The terms early and intermediate AMD are

used to describe the severity of these early changes, but are not used uniformly across the classification systems. These complex classification schemes are very useful in research studies but are time consuming and not practical in routine AMD clinics. Yet it is important to differentiate patients with intermediate AMD from those with early AMD as a patient with intermediate AMD possesses a higher risk of progression to nAMD compared to one with early AMD (Wang et al. 2003, Chew et al. 2014a). A simplified severity scale has been developed (Ferris et al. 2005) but more recently, a clinical classification was agreed using a modified Delphi process, combining scientific literature and expert opinion from a group of 26 international AMD experts (Ferris et al. 2013). The clinical classification is simplified enough to be used in routine practice by ophthalmologists using standardised equipment such as a slit lamp (Table 1.1).

Stage	Definition
No apparent ageing changes	No drusen and no AMD pigmentary abnormalities
Normal ageing changes	Small drusen (≤63μm) and no AMD pigmentary abnormalities
Early AMD	Medium drusen (63-125 μm) and no AMD pigmentary abnormalities
Intermediate AMD	Large drusen (>125 μm) and/or any AMD pigmentary abnormalities
Late AMD	nAMD and/or GA

Table 1.1 Simplified clinical classification of AMD to assess the risk of progression to late AMD proposed by Ferris et al. (2013). AMD pigmentary abnormalities are defined as hyper/hypopigmentary changes of the RPE accompanied by at least one medium druse.

The use of OCT in the early stages of AMD is less well standardised, as drusen and pigment changes have traditionally been studied by means of colour fundus imaging. OCT consists of a series of cross-sectional scans of the retina that allows identification of the different retinal layers. Figure 1.3 shows the nomenclature for the layers of the retina seen in OCT as agreed by the International Nomenclature for Optical Coherence Tomography Panel (Staurenghi et al. 2014), which will be used throughout this thesis.

The cross-sectional view of the retina means that the anatomical features seen in OCT might not be directly comparable to those seen in CFP, leading to the proposal of "new" AMD features. This area of research has especially grown since the introduction of spectral domain OCT (SD-OCT) which provides a much higher speed and better image resolution than the previously used time domain OCT (TD-OCT). The AREDS2 ancillary OCT study was the first large study to provide qualitative and quantitative investigation of SD-OCT characteristics of eyes with intermediate AMD (Leuschen et al. 2013). The study assessed various OCT features, which included drusen and their reflectivity (low, mid, high), focal hyperreflectivity and photoreceptor layer thinning above drusen. This study made a start at assessing the prevalence of these OCT lesions in eyes with intermediate AMD with the aim of developing a classification system for AMD based on OCT. Participants in the AREDS2 ancilliary OCT study were followed longitudinally for a period of four years (classification system not yet published).

New ways of measuring structural changes in SD-OCT are constantly emerging. For example recent studies have shown that the calculation of the total volume of the RPE-drusen complex can be used to distinguish between eyes with intermediate AMD and healthy eyes (Farsiu et al. 2014); and to predict progression towards nAMD (Folgar et al. 2016). Another line of research within the field has developed around the assessment of the integrity of the photoreceptor layer, which is not possible with CFP. The high resolution of SD-OCT provides a better visualisation of the photoreceptor layer, in particular a distinctive hyperreflective layer located anteriorly to the RPE-Bruch's complex initially believed to be the inner segment-outer segment junction of the photoreceptors, later defined as the ellipsoid zone (Figure 1.3). The EZ is thought to correspond to the ellipsoid component of the photoreceptors (Staurenghi et al. 2014), which hypereflect due to a high density of

mitochondria. This layer has recently garnered interest because its integrity has been identified as a good indicator of reduced visual function in pathologies that affect the macular area. For instance, studies have showed that EZ disruption was inversely associated with retinal sensitivity (measured by microperimetry) in subjects with AMD (Landa et al. 2011, Querques et al. 2012, Wu et al. 2014a). Disruption to the EZ was also found to be associated with a decrease in VA in other retinal pathologies such as epiretinal membranes (Oster et al. 2010) and diabetic macular oedema (Maheshwary et al. 2010). Even self-perceived visual function (measured with a 39 item questionnaire) was found to be significantly associated with EZD (Maheshwary et al. 2010).

International Nomenclature for OCT Meeting Consensus

Normal OCT terminology

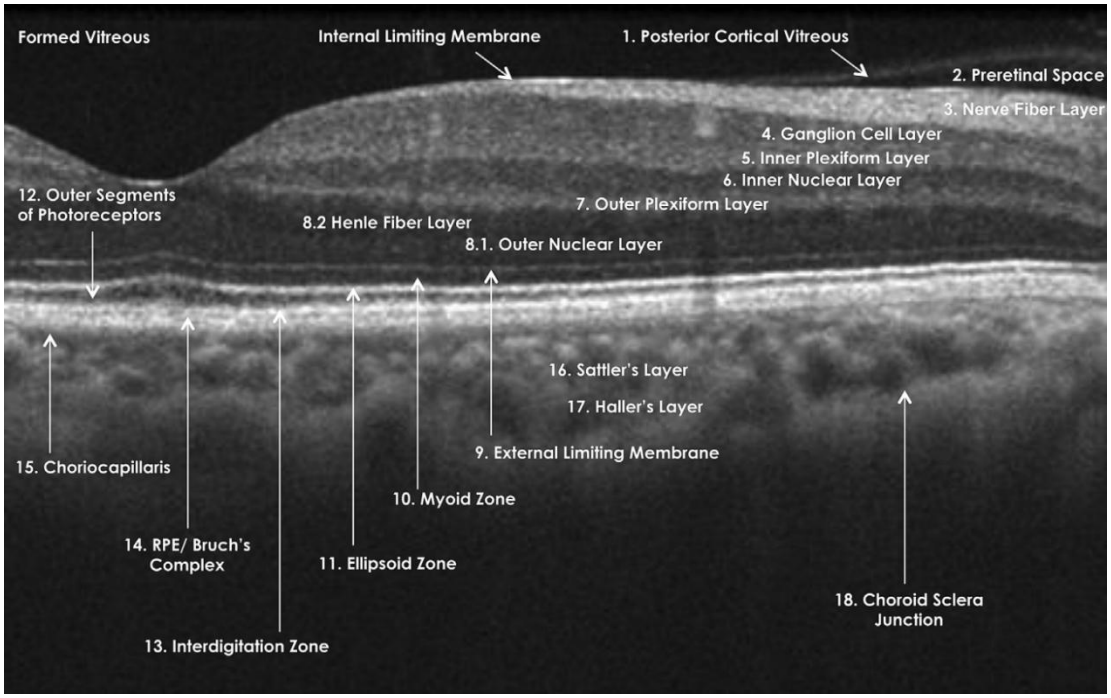

Figure 1.3 Normal OCT scan and the names of each retinal layer agreed by the International Nomenclature for Optical Coherence Tomography Panel, from Staurenghi et al. (2014). Particular attention is drawn to two layers, which will be mentioned later in this thesis. Firstly, the hypereplective layer named RPE/Bruch's complex, located directly above the choriocapillaris; and the ellipsoid zone of the photoreceptors, which is the hyperreflective layer between the myoid zone of the photoreceptors and the outer segment of the photoreceptors. Permission for reproduction in a research thesis was granted by publisher, Elsevier.

1.1.3.1.2 Diagnosis and monitoring of nAMD

The initial diagnosis of nAMD is made using the gold standard Fundus Fluorescein Angiography (FFA). To carry out an FFA, a dye (fluorescein sodium) is injected intravenously while photographs of the back of the eye are taken in rapid sequence for a period of 10 minutes. The diagnosis of nAMD is made on the basis of the presence of hyperfluorescence in the early phases and pooling and leakage of the fluorescein dye in the late phases of the FFA. An additional indocyanine green angiography (ICG) is sometimes used to differentially diagnose specific lesions. The spectrum of nAMD lesions includes classic and occult choroidal neovascularisation (CNV), idiopathic polypoidal choroidal vasculopathy (IPCV) and retinal angiomatous proliferation (RAP). Detailed definitions of these lesions can be found in Appendix 1.

FFA is expensive, time consuming, it requires pupil dilation and it needs to be carried out where facilities are available for resuscitation, as there is a small risk of serious adverse reaction to the fluorescein sodium. As newer imaging modalities improve, in particular OCT, they are also being assessed on their ability to detect the onset of nAMD compared to the gold standard FFA. Compared to TD-OCT, the higher resolution of SD-OCT (7 µm for the Spectralis OCT; Kiernan, Mieler & Hariprasad, 2010) allows better visualisation of small, subtle areas of fluid in or around the retina which might not be seen with TD-OCT, giving SD-OCT a better sensitivity for detecting active nAMD (sensitivity of TD-OCT 70%; sensitivity of SD-OCT 90-94% using FFA as gold standard; Castillo et al. 2015). However presence of fluid is not always associated with active neovascular lesions due to AMD, contributing to a lower specificity (specificity of TD-OCT 65%; specificity of SD-OCT 27-47%; Castillo et al. 2015). OCT is therefore not used for the initial diagnosis of nAMD but once this diagnosis is established with FFA, OCT can be used to monitor the progression of accumulation of fluid (The Royal College of Ophthalmologists 2013).

Although the assessment of AMD patients relies heavily on imaging of the retina, function must always be examined, as the ultimate goal of treatment is to improve patients' visual experience. This area of research lags behind compared to the above imaging technologies, as there is no clear agreement on a visual function test that can both accurately measure function in AMD patients, representing the extent of their visual defects and be widely accepted by elderly patients and easily administered in clinic (Lesmes et al. 2013). For instance, in the early stages of AMD, macular function tests such as microperimetry (Wu et al. 2016) and dark adaptometry (Jackson et al. 2014) can be used as functional biomarkers as they can reveal a functional deficit to which visual acuity is insensitive. These tests are used in many research studies trying to detect or predict progression of AMD. However they are not conventionally used in clinical practice due to the complexity and duration of the tasks, when compared to widely established and accepted tests of VA. The following section further discusses the clinical assessment of visual function in AMD.

1.1.3.2 Assessment of visual function

Visual acuity (VA) is unarguably the most common measurement of function in ophthalmology clinics but it only represents one aspect of visual function: resolution of small letters or pictures at high luminance and high contrast. Other visual function tests, such as contrast sensitivity (CS) also rely on the identification of optotypes but in lower contrast conditions which are thought to be more representative of real world conditions. Finally, measurement of central visual fields by microperimetry is a very useful measurement of function in AMD but is currently not generally used in UK clinics.

1.1.3.2.1 Resolution acuity

VA refers to the central resolution acuity of the eye (at the fovea) measured at high contrast, i.e. the smallest spatial separation between two points or lines that can be detected by the human eye. There are several ways of measuring resolution acuity, such as using square wave gratings (Jackson and Bailey 2004) or optotypes. Optotype is the name given to letters or figures used in VA charts. For adults, gratings are often used in laboratory conditions while optotypes are used clinically.

There are several designs of letter charts used clinically for the measurement of VA, one of them being the Bailey-Lovie chart. In Bailey-Lovie charts the spaces between letters and between rows are reduced progressively. There are five letters per row and each letter is given a value of 0.02 logMAR units (the logarithm of the minimum angle of resolution) so that a whole row is worth 0.1 logMAR (Bailey and Lovie-Kitchin 2013). The space between letters is equal to a letter width and the space between rows is equal to the width of the bigger row. Different optotypes can be used in Bailey-Lovie charts (HOTV letters, Landolt Cs, Tumbling Es, Lea pictures, etc, Bailey & Lovie-Kitchin, 2013).

AMD clinics often use ETDRS (Early Treatment in Diabetic Retinopathy Study) charts, which are based on Bailey-Lovie's design. ETDRS charts (Figure 2.3 in methods chapter, page 54) use Sloan letter charts placed in a standardised illuminated box usually placed at four meters from the patient. ETDRS charts can be moved

forwards for patients who cannot see the biggest letters in the chart (VA worse than 1.0 logMAR). At one metre there is a magnification factor of six (Kniestedt and Stamper 2003).

VA is routinely used as an indicator of progression or deterioration of vision and is also used to assess the outcome of treatments. The problem with VA in AMD is that early AMD lesions (drusen and pigmentary changes in the RPE) often cause a small decrease in VA (Klein et al. 1995) that is similar to the measurement variability. The coefficient of repeatability for VA increases from five letters in healthy controls (Lovie-Kitchin and Brown 2000) to 9-14 letters in non-late AMD (Patel et al. 2008, Aslam et al. 2014). Variability of measurements increases as severity of disease increases, with late AMD having a coefficient of repeatability of 17 letters (Patel et al. 2008), which might be due to the need to move the chart to one metre for some patients. Late AMD (CNV and GA) can cause a decrease in VA of several logMAR lines (Klein et al. 1995) however if the lesion spares the fovea, a relatively good level of VA can be retained. A recent study has suggested that a decrease in VA from more than 85 letters to less than 75 letters (more than two logMAR lines) in a period of one year increases the odds of developing nAMD by a factor of 20 (T. R. Friberg et al. 2012).

Overall, changes in pathology cannot be assessed or tracked by VA alone in either early or in late AMD.

1.1.3.2.2 Contrast sensitivity

CS measures the amount of contrast that a person requires to detect a target. The most common CS chart used clinically is the Pelli-Robson chart (Figure 2.4 in methods chapter, page 54), which uses letters constant in size but decreasing in contrast levels. An intersession test-retest repeatability coefficient of 6-7 letters (0.3-0.35 logCS) has been reported for Pelli-Robson CS charts in patients with stable early/intermediate AMD (Aslam et al. 2014, Patel et al. 2009). The study by Patel et al (n=91, 2009) reported that on average, CS in patients with AMD (1.05-1.2 logCS) was worse than previous reports on healthy controls (1.50-1.80 logCS). This differs from a recent larger study by Owsley et al. (2016) which found no difference in CS between early AMD and normal eyes (n=640, CS in controls: 1.61 logCS, CS in

early AMD: 1.60 logCS, p=0.21). The difference may be due to the slight difference in sample characteristics, as Patel et al. (2009) included patients intermediate AMD whereas Owsley et al. (2016) only included patients with the earliest stages of AMD.

Human contrast sensitivity is best at intermediate spatial frequencies (Owsley et al. 1983). Spatial frequency defines the level of detail and sharpness of the edges of an image. It has long been known that CS reduces with normal ageing, especially at intermediate and high spatial frequencies (Rubin et al. 1997, Owsley et al. 1983, Figure 1.4). This is mainly a result of changes in the optical properties of the eye which include smaller pupils, increased density of the lens and increased light scatter and aberrations (Pokorny et al. 1987, Glasser and Campbell 1998). Detection of contrast is particularly affected when cataracts are present (Shandiz et al. 2011).

On the whole, assessing progression of AMD by means of CS becomes difficult because many of the elderly patients with AMD are likely to have some degree of progressing lens opacity, unless cataract is surgically extracted in which case CS can return to normal (Rubin et al. 1993). However, CS can give valuable information about the overall functional vision of the patient and their quality of life (Bansback et al. 2007). This is why CS has been used to show improvement with anti-VEGF treatment for nAMD (Patel et al. 2011).

1.1.3.2.3 Other visual function tests

Reading speed, resolution acuity at low contrast/low luminance (including Smith-Kettlewell Institute Low Luminance, SKILL, cards) and contrast sensitivity at low luminance are alternative vision tests that can effectively differentiate early and intermediate AMD from healthy controls (Lott et al. 2015). Since these tests could easily be administered in clinic, their ability to predict the development of advance AMD is currently under investigation (Lott et al. 2015, Lott et al. 2016). A different test of VA has also been developed which uses high-pass filtered letters (the Moorfields Acuity Chart, MAC) which appears more sensitive to functional loss in AMD than conventional VA charts (Shah et al. 2016).

Other tests have been proposed to be useful for measuring visual function in AMD (Hogg and Chakravarthy 2006, Neelam et al. 2009) but they are currently used for

research purposes mainly. These tests are based on visual adaptation (dark adaptation and photostress test), temporal functions (temporal resolution acuity and temporal contrast sensitivity) and perimetry (in particular microperimetry).

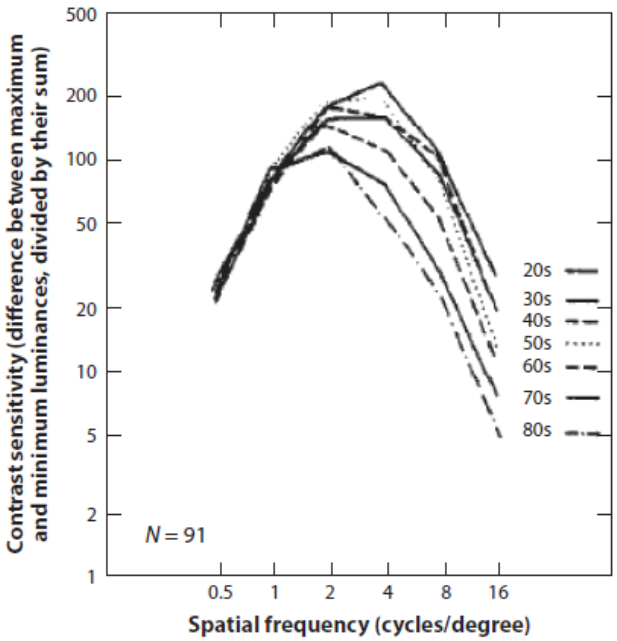

Figure 1.4 Contrast sensitivity function during adulthood. Contrast sensitivity peaks at intermediate spatial frequencies (2-4 cycles/degree) and falls off at lower (less than 2 cycles/degree) and higher spatial frequencies (more than 4 cycles/degree). Image from Owsley (2016), adapted from Owsley et al. (1983). No permission required from Annual Reviews for publication in a research thesis.

1.1.4 The importance of early treatment of nAMD

Appropriate tests for the early detection of nAMD have been given renewed importance because an effective treatment is now available in the form of anti-VEGF injections into the eye. Landmark clinical trials have shown that ranibizumab/ "Lucentis" (Rosenfeld et al. 2006, Brown et al. 2006), bevacizumab/ "Avastin" (Martin et al. 2011, Chakravarthy 2013) and aflibercept/ "Eylea" (Heier et al. 2012) are three drugs capable of reducing the harmful effects of choroidal neovascularisation in AMD.

The MARINA (Rosenfeld et al. 2006) and ANCHOR (Brown et al. 2006) studies have shown that 90-96% of patients receiving ranibizumab maintained stable vision (less than 15 letters lost). However, only 26-40% of those treated with ranibizumab improved VA by 15 letters or more. Similarly, the VIEW 1 and 2 studies (Heier et al. 2012) have shown that aflibercept is as effective as monthly ranibizumab, as 95% of patients receiving aflibercept maintained stable vision and 25-37% improved VA by 15 letters or more. The CATT (Martin et al. 2011) and IVAN (Chakravarthy 2013) studies have found that bevacizumab is as effective as ranibizumab for the treatment of nAMD. Bevacizumab is (currently) approximately 40 times cheaper than ranibizumab but is not licenced for the treatment of nAMD. A table with detailed information of the visual outcomes reported in these studies can be found in Appendix 2.

Data recently pooled from 14 UK centres revealed that the visual outcomes obtained in real world clinics might not match the results presented in the above clinical trials (Writing Committee for the UK Age-Related Macular Degeneration EMR Users Group 2014). This large multicentre study has shown that VA was maintained (less than 15 letters lost) in 90% of patients at one year, 84% at two years and 82% at three years. This might be attributable to the logistics of service delivery, leading to fewer injections and fewer appointments.

Overall, current anti-VEFG drugs are effective at preventing further vision loss in most patients, and improving vision in approximately one third of patients. The current guidelines from the National Institute for Health and Care Excellence (NICE) recommend commencing treatment with ranibizumab or aflibercept if VA is worse

than 0.3 logMAR (National Institute for Health and Care Excellence 2008). However, recent research from a multicenter study in the UK has revealed that patients with baseline VA better than 0.3 logMAR would also benefit from treatment as they can maintain a good level of VA within driving standards (mean change of 6-7 letters at two years). Although an improvement was seen in the VA of those with worse baseline VA, the final VA achieved at two years fell below driving visual standards (Lee et al. 2015). The cost-effectiveness of early commencement of ranibizumab treatment has also been assessed. An economic model by Butt et al. (2015) suggested that the cost of starting ranibizumab when VA is still 0.3 logMAR or better is within the range that the NHS is typically willing to pay for health gain. Patients would receive an average of one more injection over two years and gain 0.24 QALYs (Butt et al. 2015). The NICE guidelines for the treatment of nAMD are currently under review and expected to be published in October 2017.

It is clear from the above that early intervention in nAMD results in better visual outcomes, as current treatments are most effective at maintaining vision rather than improving it. Since early access to treatment is directly dependent on early detection, a number of tests for detecting symptoms of nAMD have recently been developed, which include the Preferential Hyperacuity Perimeter (PHP) and the Radial Shape Discrimination (RSD) test. These tests are of particular importance because they can be used not only in the clinic, but also in the community or the patient's home (i.e. for self-testing). The following section reviews the use of these two tests as well as the Amsler grid for early detection of nAMD.

1.1.5 Available tests for early detection of nAMD

Having discussed the advantages of early commencement of treatment for nAMD and taking into account the large number of people at risk of nAMD, the value of self-monitoring strategies becomes clear. The Amsler Grid chart (Amsler 1953) is commonly used to detect the onset of metamorphopsia caused by nAMD. It consists of a black and white grid with a central dot for fixation (Figure 1.5). The subject is required to monocularly fixate on the central dot and assess the rest of the grid. Any patches of the grid where the lines appear to be missing or distorted indicate an abnormal result. A wide range of sensitivities for detecting nAMD have been reported for the AG including good to excellent sensitivity (Klatt et al. 2006, Isaac et al. 2007, Nowomiejska et al. 2013) as well as sensitivity as poor as chance level (Goldstein et al. 2005, Loewenstein et al. 2003, Do et al. 2012). A recent meta-analysis revealed a pooled sensitivity of 78% (95%CI 64-87%) for detection of nAMD (Faes et al. 2014). However, the authors pointed out that many of the included studies compared patients with established nAMD to healthy controls or other groups of patients, which can result in an overestimation of the sensitivity and specificity. The implication of this limitation is that the apparent high sensitivity may not be applicable to the usual clinical situation, in which the comparison is not between healthy eyes and eyes with established disease, but between eyes with many of the age-related features known to be related to the risk of developing nAMD (section 1.1.2) and those with early, often subtle signs of nAMD.

In addition to an uncertain sensitivity, the original version of the AG does not allow quantification of the visual deficit and therefore does not allow monitoring of progression of the distortions. A newer 3-D computer version of the AG assesses central visual fields at different contrast levels. This version seems to be able to quantify the number and volume of the central visual field defects present in AMD and to distinguish between dry (non-GA) and nAMD (Robison et al. 2011) with a sensitivity of 89.7% and a specificity of 85.3%. A limitation of this cross sectional study is the fact that, as explained before, nAMD cases were not newly diagnosed, meaning that the ability of the test to detect nAMD could be an overestimation.

The Preferential Hyperacuity Perimeter (PHP, Figure 1.6A) is an FDA approved medical device that was designed for providing early detection of nAMD. The PHP evaluates the central 14° of the patient's visual field (macular field) and is based on the phenomenon of Vernier hyperacuity (Loewenstein et al. 2003). The test stimulus consists of a line of dots where some of the dots are misaligned (an artificial distortion, Figure 1.6B). The stimulus is presented on a display at different locations, horizontally and vertically, for a brief period of time. The participant, initially fixating on the central dot, has to indicate the location of the displaced dot on the line. The PHP test has shown promise in the early detection of symptoms caused by nAMD, and it seems to prompt earlier treatment promoting better visual outcomes (Lai et al. 2011, Chew et al. 2014b). However, studies have excluded patients who could not perform the test or had unreliable results, potentially biasing the patient recruitment and affecting the general applicability of the test (Pitrelli Vazquez and Knox 2015). The usability of the PHP test will be further discussed in section 4.5.

Alternative tests for the detection of distortion and scotomas have been summarised in recent reviews (Crossland and Rubin 2007, Liu et al. 2014), which include M-charts, the Macular Mapping Test, the Scanning Laser Entoptic Perimeter. In the present study, the focus of the research is on an alternative test for the detection of nAMD, the radial shape discrimination (RSD) test. The second part of this introductory chapter provides further background information of relevance to the RSD test, and a review of what is currently known about it.

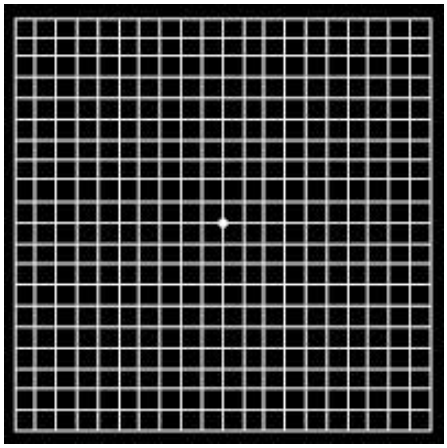

Figure 1.5 The Amsler grid. The task is to fixate on the central dot and assess the rest of the grid using the peripheral vision.

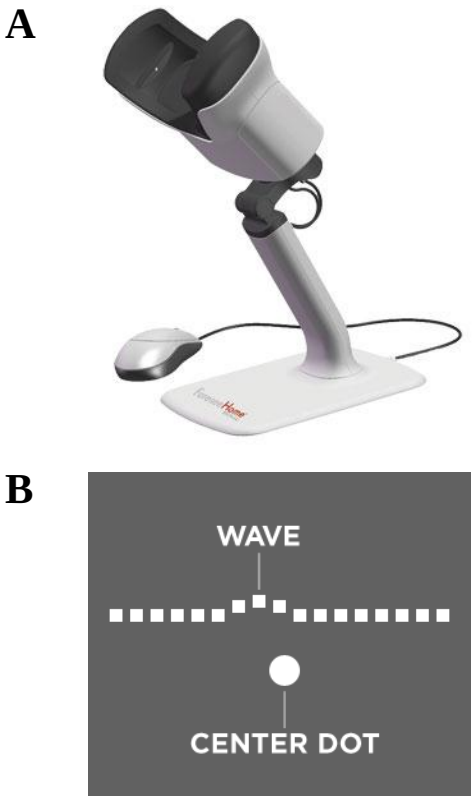

Figure 1.6 A. The Preferential Hyperacuity Perimeter (Notal Vision 2017). B The task is to fixate on the central dot and detect the presence of artificial distortions on the line. ©2017 Notal Vision.

1.2 Radial Shape Discrimination Hyperacuity

Visual hyperacuity is a term used to describe the capability of the human visual system to perform certain vision tasks obtaining thresholds that are much smaller than those reached in a resolution acuity task (traditionally thought to be 1 arc min, or 0.00 logMAR). Hyperacuity tasks are therefore not limited by the size of the foveal photoreceptors (approx. 30 arc sec) and can reach thresholds in the region of 10 arc sec (approximately -0.78 on a logMAR scale).

Hyperacuity tasks allow spatial resolution to be measured to a much more precise level than resolution acuity. It is generally accepted that two lines must be separated at least 1 arc min in order to be perceived as two (resolution acuity). This is explained by the limitations of the human eye as an optical system, a phenomenon called diffraction which causes a dot to be imaged as a patch of light on the retina. The Rayleigh criterion states that in order to be resolved, the two peaks of two spread functions caused by diffraction need to be separated by at least half the distance between the first two zero intensity points (corresponding to 1 arc min, Westheimer 2009). In other words, when the distance between the two images is smaller than 1 arc min, the two cannot be resolved and only one enlarged image is perceived.

Several visual perception tasks have been described which exhibit thresholds at hyperacuity levels. Examples include detection of a small spatial offset between targets (Vernier acuity), detection of orientation deviation, judging the relative position of a line with respect to two flank lines (bisection task) or form discrimination such as detection of curvature (Figure 1.7).

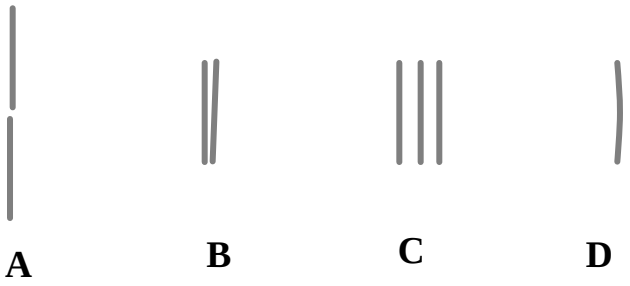

Figure 1.7 Examples of hyperacuity tasks. A. Vernier acuity, B. orientation deviation, C. bisection task, D detection of curvature.

The Radial Shape Discrimination (RSD) test is based on the ability to detect modulations on a circle using radial frequency patterns as stimuli (Wilkinson et al. 1998), a task that also falls under the category of hyperacuities. The following sections review the stimulus and task used in the test, the cortical processing behind RSD tasks, the different versions of the test and the effect of ageing and retinal disease on this particular hyperacuity task.

1.2.1 The Radial Shape Discrimination test

1.2.1.1 Stimulus and task

The stimulus used in RSD tests, called a Radial Frequency (RF) pattern, consists of a circle with a specific number of distortions or bumps around it where the number of bumps is expressed as a frequency (Figure 1.8). To create an RF pattern, a cross sectional luminance profile is applied to a circle. This profile is defined by the fourth derivative of a Gaussian (D4), where radius, contrast and spatial frequency are the defining parameters (Wilkinson et al. 1998). Once the circle is created, the radial sinusoidal deformation is generated by modifying the mean radius, modulation amplitude and radial frequency (Wilkinson et al. 1998).

In the RSD test, the subject has to make a forced choice to identify the RF pattern that contains the radial modulations (an alternative forced choice task, AFC). The patterns can be presented on the screen simultaneously (two or more patterns presented side by side, a spatial AFC task) or consecutively, where the targets, one after the other, stay on the screen for a limited period of time (a temporal AFC task). The advantage of using a spatial as opposed to a temporal task is that the subject can compare the two shapes, side by side, for a longer period of time, as opposed to a fraction of a second that the targets stay on the screen in a typical temporal task. However, temporal tasks tend to allow for better control of fixation, as the subject does not have time to move their eyes around.

During the RSD test, the average diameter of the RF patterns remains constant, but the amplitude of the modulations decreases as the subject makes correct choices and increases when mistakes are made (staircase procedure included in the software of the test). An example of modulations decreasing in size can be seen in Figure 1.8, using a spatial 3AFC.

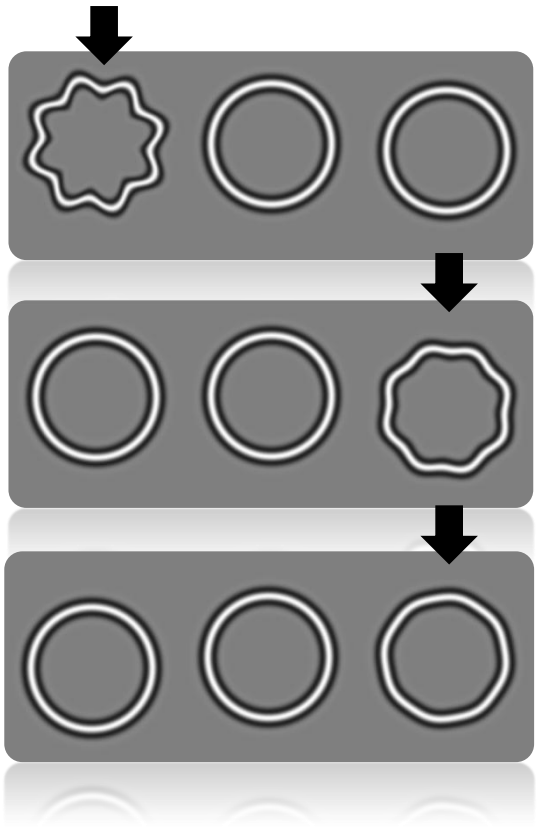

Figure 1.8 Visual stimuli used in the spatial 3AFC version of the RSD test where one of three RF patterns has a modulated contour (frequency of 8 cycles). Arrows indicate the distorted RF pattern.

1.2.1.2 Cortical processing and RSD task performance

The remarkable human ability to detect deformations in RF patterns has been suggested to be a result of a global process in which local contour information is pooled at intermediate stages prior to global processing in cortical area V4 (Wilkinson et al. 1998).

Several studies attempted to find out which local features are extracted and pooled to allow shape discrimination of RF patterns. When the RF pattern is created both orientation and position elements are varied with respect to the circular shape. With this in mind Wang and Hess (2005) designed a study to find out to what extent local orientation and local position features contribute to shape integration. The results showed that while both local orientation and positional features seem to be important in optimal shape integration, it is the former that contributes to shape integration the most (Wang and Hess 2005). This finding is not in agreement with that of Loffler et al. (2003), where gaps introduced at the peaks of the modulations resulted in a larger decrease in threshold than gaps introduced at the minimum curvature zones. Overall, it appears that neither local orientation nor local positional cues alone but a combination of both is responsible for the levels of hyperacuity observed (Wang and Hess 2005). Practically, the importance of this is that the RF patterns require a larger area of retina to be healthy in order to process the shape optimally. This makes RF patterns more vulnerable to undersampling of the retina (photoreceptors loss as a result of AMD, for example).

It has also been suggested that global pooling might happen mainly on RF patterns with lower modulation frequencies (up to five cycles in a pattern) whereas processing of RF patterns between five and ten cycles occurs by means of probability summation (as well as global processing) and after ten cycles local processes take over global processing (Loffler et al. 2003). This means that adding more than five cycles only improves performance marginally.

1.2.1.3 Versions of the RSD test

In the early laboratory version of the RSD test (desktop version) targets were displayed on a computer monitor at a testing distance of one metre (Wang et al. 2002). The stimuli were two RF patterns presented either simultaneously or consecutively (spatial or temporal 2AFC tasks). The study participants used a joystick to indicate the distorted shape. A more clinically friendly, handheld version of the RSD test (referred as hRSD from now onwards), was subsequently developed (Wang et al. 2013) in which stimuli were presented on an Apple iPod Touch (Figure 1.9). An iPod touch is a touch sensitive device, the size of a mobile phone with a screen size of 3.5 inches, where the patient indicates the distorted shape by simply touching it. In this version of the test, a spatial 3AFC task is used. The handheld version was found to be highly correlated with the laboratory desktop version ($r=0.78$, $p<0.001$) for a sample of 100 participants with healthy maculas (n=27), several degrees of AMD (n=37) and diabetic retinopathy (n=36) (Wang et al. 2013).

The most recent version of the handheld test uses four RF patterns instead of three (a spatial 4AFC) and it can be implemented in larger devices such as iPhones (4-4.7 inches) and iPads (9.7 inches, Figure 1.9). The results from the 3AFC (on an iPod touch) and the 4AFC (on an iPad) tests were also found to be highly correlated ($r=0.87$, $p<0.0001$) in a sample of 86 participants (40 with healthy maculas and 46 with various degrees of maculopathy, Bartlett et al. 2015). A mean difference of 0.06 logMAR (95%CI 0.03 to 0.08 logMAR) was found in Bland-Altman analysis, indicating that the results of the 4AFC version were slightly worse than the results obtained with the 3AFC version (Bartlett et al. 2015). Such a difference was not reported by Ku et al. (2016) who compared the 3AFC and 4AFC versions in a sample of 106 healthy participants with normal vision (mean difference -0.02 ±95%CI -0.22 to 0.19 logMAR, Ku et al. 2016).

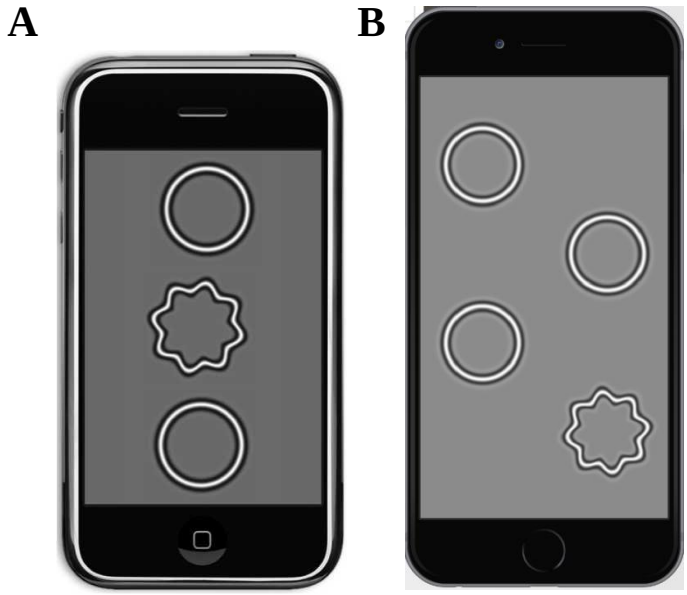

Figure 1.9 A. Apple iPod Touch (screen 3.5 inches) displaying three RF patterns, B. Apple iPhone (screen 4.7 inches) displaying four RF patterns.

The handheld version of the RSD test (hRSD) is available in the USA in the form of a smartphone/tablet application called myVisionTrack® (Vital Art and Science LLC 2011-2016). MyVisionTrack® is the first ophthalmic application to receive approval from the Food and Drug Administration (FDA, 2013) for detecting changes in visual function in maculopathies. However it is not available to use in Europe as it is not CE marked yet. Currently, myVisionTrack® can be downloaded within the US onto Apple devices (iPod, iPhone, iPad) but it requires an ophthalmologist's prescription to activate it and use it. Once the prescription is issued, patients can start using the hRSD test from home to test their vision. The results are sent to the practitioner's "portal" and an alert is triggered in the case of an abnormal finding (analysed by an internal algorithm). The clinician can then contact the patient to arrange an appointment for further testing.

After a literature search in Web of Science and PubMed (search terms: Radial shape discrimination hyperacuity) a limited list of published research articles of relevance to the clinical applications of the RSD test was found. Table 1.2 shows a list of the literature on the performance of the RSD test on either healthy controls or AMD participants (diabetics not included). Due to its recent development, very few published papers were found on the handheld version of the test. For this reason a further search was conducted in the ARVO Journal Website (Investigative Ophthalmology and Vision Science, IOVS) for recent conference abstracts reporting results on the handheld version of the test (Table 1.3). Abstracts were included where they contained information that was not available in the form of a paper but its results were relevant to the work presented in this thesis. Abstracts however, were not included if they did not include measures of statistical significance or had very limited information on the methods. The research summarised in tables 1.2 and 1.3 will be described throughout the remainder of this review chapter.

Authors	Year	Test version	Title
Wang*	2001	Desktop	Effect of ageing on shape discrimination
Wang et al.*	2002	Desktop	Shape discrimination in Age Related Macular Degeneration
Wang et al.*	2009	Desktop/ Chart	Course of development of global hyperacuity over lifespan
Chhetri et al.*	2010	Handheld	Shape discrimination test on handheld devices for patient self-test
Wang et al.*	2013	Handheld/ Desktop	Handheld shape discrimination hyperacuity test on a mobile device for remote monitoring of visual function in maculopathy
Kaiser et al.*	2013	Handheld	Feasibility of a novel remote daily monitoring system for age-related macular degeneration using mobile handheld devices
Ku et al.	2016	Handheld	Performance, usability and comparison of two versions of a new macular vision test: the handheld Radial Shape Discrimination test

Table 1.2 List of published papers where the RSD test was the study test resulting from a literature search containing the search terms "radial shape discrimination hyperacuity".

Authors	Year	Test version	Title
Wang et al.*	2011	Handheld	Sensitivity and specificity of shape discrimination hyperacuity for differentiating exudative AMD from moderate AMD
Wang et al.*	2014	Handheld	Compliance and test variability of patients with maculopathy in using an iphone-based shape discrimination hyperacuity test at home
Knox et al.	2014	Handheld	Effect of age and blur on, and test-retest variability of, a handheld radial shape deformation test
Bartlett et al.*	2015	Handheld	Comparison of myVisionTrack® vision monitor performance with 3-alternative forced-choice (3AFC) and 4AFC testing paradigms for assessing shape discrimination hyperacuity
Lott et al.	2016	Handheld	Longitudinal assessment of non-standard vision function in early to intermediate AMD: Baseline update

Table 1.3 List of conference abstracts where the RSD test was the study test from a search in the ARVO journal website (IOVS).

1.2.2 RSD hyperacuity in the ageing visual system

Visual performance is known to decrease with older age as a result of optical and neural changes. Optical changes include smaller pupil, changes in light absorption and light scatter by the lens and density of the optical media (Bron et al. 2000). Neural changes are a result of changes in the number of photoreceptors, particularly rods. A study by Curcio et al (1993) has shown an average decline in parafoveal rods of 684 rods/mm2/year. Retinal ganglion cells, particularly their axons also decline in older age (Calkins 2013). An average annual loss of 4000 optic nerve fibres has been reported in a study by Jonas et al. (1992), which included 56 eyes of 56 participants ranging from 19 to 88 years of age. A loss of cortical neurons has also been reported (Devaney and Johnson 1980).

Large longitudinal studies have shown that VA is particularly susceptible to the ageing process. A decrease in VA is seen particularly after the age of 75 (Klein et al. 2006, Hong et al. 2013). Interestingly, it has been suggested that discrimination of RF patterns is minimally altered by ageing (Wang 2001, Habak et al. 2009, Ku et al. 2016).

Wang (2001) studied the effects of normal ageing on shape discrimination using RF patterns of 8 cycles per 2π (the same radial frequency used in the present study). This case control study included 76 healthy participants with good VA up to 78 years old (groups as follows: ages 15-39, n=26; 40-59, n=22 and 60 to 78 years, n=28). A statistically significant difference was found among the mean RSD threshold of young, middle aged and senior adults (p=0.018, Wang 2001). Pair-wise comparisons revealed an 18% increase between thresholds of young and senior participants (p=0.003, Wang 2001). After adding more participants to the sample (n=236), Wang et al. (2009) used a mathematical model to describe the rate of development of RSD throughout childhood and the rate of deterioration of RSD in adulthood. The mathematical model consisted of three segments. Initially, an exponential function captured the rapid initial development of visual function early in life, which is followed by a slower development phase. Then, a horizontal line was fitted as it was assumed that visual function does not change for a period of time. Lastly, a linear function was used to represent the decline of visual function with ageing (Wang et al.

2009). The results showed that after developing rapidly in the first 5 years of life (rate of threshold improvement of 0.17 logMAR per year), adult levels of RSD hyperacuity were reached at the age of 21 years. RSD remained stable throughout adulthood until the age of 55 years. After the age of 55 a slight increase (worsening) in threshold occurred, at a rate of 0.035 logMAR per decade (Wang et al. 2009).

The stability of the RSD test in healthy ageing was recently studied by Ku et al. (2016) who found only a small increase (worsening) in threshold when using the handheld version of the RSD test. This study assessed the performance of the hRSD test in 186 healthy participants, ranging from 16 to 90 years old. A deterioration of 0.026 logMAR per decade was reported for this age range. Ku et al. (2016) did not find a significant worsening of hRSD scores in participants over 55, as had been reported by Wang et al. (2009). The authors suggested that this might be explained by the fact that 16 of their older participants had no retinal disease at all, confirmed by OCT, as opposed to self-reporting no retinal disease. These perfectly healthy retinas are unlikely to be representative of the general population of that age. It should be noted that one linear function was fitted to all the data in Ku et al. (2016) as opposed to a mathematical model that analysed different age ranges, as done by Wang (2009).

A common conclusion of the studies cited above is that the effect of age on (h)RSD test scores is smaller than the effect seen on other visual function tests, such as VA, near VA or CS (Wang 2001, Wang et al. 2009, Ku et al. 2016). The study by Wang et al (2001), which reported an 18% worsening in threshold in senior adults compared to young adults, also reported a 33% and 81% worsening in VA and CS for the same groups (Wang 2001). Wang et al. (2009) showed that the rate of deterioration of VA after the age of 55 was 0.058 logMAR per year (compared to the 0.035 logMAR per year seen for RSD). Similarly to VA, near VA worsened with age at twice the rate of deterioration seen for the hRSD test (0.051 logMAR per decade for near VA compared to 0.026 logMAR per decade for the hRSD test scores, Ku et al. 2016).

A potential reason why older age has a smaller effect on RSD than on VA might be related to the low spatial frequency used in its stimuli. The spatial frequency used in the RF patterns is 3 cpd in the handheld version of the test (Wang et al. 2013).

Contrast sensitivity peaks at around a spatial frequency of 4 cpd in young adults and this peak shifts to 2 cpd in ages older than 60 years as can be seen in Figure 1.4 (Owsley et al. 1983). This is mainly due to the loss of transparency of the crystalline lens and the development of cataracts (section 1.1.3.2.2). With older age affecting mostly high spatial frequency targets, RF patterns might be spared.

Maintaining a relatively stable test performance in older age is favourable for a test that aims to assess function in elderly subjects. The reason for this is that visual function tests that are negatively affected by the ageing process are less likely to detect true changes due to pathology. This is due to a decrease in the dynamic range of the test (the range of scores between older healthy participants and those with the condition). In other words, the test is less likely to detect poor scores due to disease in older participants whose scores are already reduced due to older age.

1.2.3 RSD hyperacuity in AMD

For the last part of this introductory chapter, the literature with regards to the RSD test in AMD is reviewed, with particular interest in certain aspects of performance of the test which are relevant to the current study.

1.2.3.1 Effect of disease severity in RSD

Tables 1.2 and 1.3 list the available literature (full articles and abstracts respectively) found on the RSD test in AMD. The research was mainly carried out by a single study group who developed the test (marked with * in tables 1.2 and 1.3).

The first study to assess the ability to detect radial shape deformation in AMD was Wang et al. (2002). Participants with AMD (n=20 participants, 35 eyes included in the analysis) were categorised into four groups depending on their retinal signs of early and intermediate AMD (Table 1.4). There was a healthy control group too (n=10). The study found a statistically significant difference between heathy control eyes and eyes with AMD using both a temporal and a spatial 2AFC task (Wang et al. 2002), with the results summarised in Table 1.5. In total, AMD groups were tested using three stimulus sizes for a spatial 2AFC task (mean radius of 0.5°, 1.0° and 2.0°) and two stimulus sizes for a temporal 2AFC (mean radius of 1.0° and 2.5°). Results showed that the mean RSD thresholds for the five groups (including controls) were significantly different from each other (ANOVA $f>5.58$, $p<0.001$) irrespective of the stimulus size (Wang et al. 2002).

	Eyes (n)	Definition	RSD (logMAR)	VA (logMAR)
Group 0	10	Normal eyes	-0.49	0.03
Group 1	13	Drusen only	-0.20	0.13
Group 2	9	Drusen/hyperpigmentation	-0.04	0.20
Group 3	7	Drusen, hyperpigmentation, hypopigmentation	+0.00	0.27
Group 4	5	Category 3 + extrafoveal GA	+0.33	0.16

Table 1.4 AMD groups from Wang et al. (2002) and their RSD score (mean RF pattern radius of 2.5°, temporal 2AFC). RSD thresholds increased as severity of AMD increased. Thresholds were reported in arc seconds but were transformed into logMAR values for consistency.

Task	Controls (n=10)	AMD (n=35)	P
Spatial 2AFC	-0.84 logMAR	-0.35 logMAR	<0.001
Temporal 2AFC	-0.75 logMAR	-0.19 logMAR	<0.001

Table 1.5 RSD thresholds from Wang et al. (2002) for healthy participants and AMD participants of any AMD category (RF pattern radius of 1°). Threshold were reported in arc seconds but were transformed into a logMAR scale for consistency.

Wang et al. (2002) used the laboratory or desktop version of the RSD test but the handheld form of the test has also been investigated. Wang et al. (2013), used a spatial 3AFC task on an iPod Touch using a stimulus size of 1° of mean radius. The study included 37 participants with AMD, 36 with diabetic retinopathy (DR) and 27 visually healthy controls. AMD participants were divided into three groups depending on disease severity (Table 1.6). In agreement with the desktop test, the thresholds obtained with the hRSD test increased as severity of AMD increased (ANOVA $p<0.0001$), with any two of the three AMD groups showing significant differences in mean RSD (pairwise comparisons, $p<0.013$, Wang et al. 2013).

Finally, the hRSD test was used by Wang et al. (2011) to compare a small group of eyes with nAMD (n=9) to a group of eyes at high risk of developing nAMD (i.e. eyes with large drusen but no GA, n=24). In this study a two time intervals paradigm (2IFC) was used. The test had a sensitivity and specificity of 88.9% (95%CI 56.5-98.0%) and 79.2% (95%CI 59.5-90.8%) for detecting nAMD (Wang et al. 2011). The sensitivities found for VA, CS and AG in the same study were 44.4% (95% CI 18.9-73.3%), 33.3% (95% CI 12.1-64.6%) and 66.7% (95% CI 35.4-87.9%, Wang et al. 2011). Although this small study had many limitations (small sample, patients had well established nAMD as opposed to newly diagnosed nAMD and lack of FFA to confirm nAMD) it suggests that the hRSD test might be superior to VA, CS and AG for the detection of nAMD.

Overall, the handheld version of the RSD test (and previously the desktop version) have shown potential to differentiate among the different stages of AMD.

	Eyes (n)	Definition	RSD (logMAR)	VA (logMAR)
Group 1	27	Healthy control eyes	-0.68	0.02
Group 2	10	Early AMD (medium size drusen)	-0.66	0.12
Group 3	11	Intermediate AMD (large size drusen or pigment changes)	-0.36	0.24
Group 4	16	Advanced AMD (GA or nAMD)	-0.10	0.42

Table 1.6 AMD groups from Wang et al. (2013). Mean RSD and VA were extracted from Figure 5 in the paper. RSD thresholds obtained with the hRSD increased as severity of AMD increased (ANOVA $p<0.0001$).

1.2.3.2 Association with other visual functions

It is of interest to know if RSD correlates with other measurements of visual function, especially VA and CS since they are the two most commonly used visual function tests in AMD clinics (section 1.1.3.2).

Studies published so far have found no correlation between VA (measured using ETDRS charts) and the desktop RSD thresholds (neither for the spatial or the temporal 2AFC methods, p=0.065 and p=0.30 respectively, Wang et al. 2002) but a strong correlation (r=0.69, p<0.001) was found between VA and the handheld test (Wang et al. 2013). A possible reason for this discrepancy is the difference in the range of disease stage of the participants. Whilst Wang et al. (2002) included participants with non-late AMD in the correlation analysis (n=35), Wang et al. (2013) included late AMD patients as well as non-proliferative and proliferative DR (n=37). Moreover, the study eye was selected in Wang et al. (2013) in order to cover a larger range of VAs (the better eyes of the controls and the worse eyes of the maculopathy groups were selected for analysis).

The hRSD test was found to have a larger dynamic range of values compared to VA (Wang et al. 2013). A difference of 0.6 logMAR was seen between older healthy controls and participants with advanced AMD, whilst a 0.3 logMAR difference was seen in VA for the same groups (Wang et al. 2013). Currently one study is investigating whether this large dynamic range could make the test a useful predictor of disease progression (Lott et al. 2015, Lott et al. 2016). Lott et al. found a difference of 0.2 logMAR in hRSD between healthy controls and participants with intermediate AMD (n=91) and hypothesised that those with worse hRSD results could be at a higher risk of developing advanced AMD (Lott et al. 2015, Lott et al. 2016).

CS, measured with Pelli-Robson charts, was also investigated by Wang et al. (2002, 2013). A statistically significant correlation was seen between RSD and CS in both studies. For the desktop version the correlation coefficient was r=0.48, p=0.003 for the spatial 2AFC task and r=0.50, p=0.003 for the temporal 2AFC task (Wang et al. 2002). The handheld version showed a stronger association (r=0.72, p<0.0001, Wang et al. 2013). The stronger correlation seen for the handheld version of the test might

also be due to the sample differences explained before (including late stages of the diseases in Wang et al. 2013 but not in Wang et al. 2002).

1.2.3.3 Repeatability and longitudinal stability of the RSD test

The repeatability of visual function tests is affected by various sources of variability in the measurement setting (Kottner et al. 2011). For example, slight differences in room luminance could have an impact on the measurement of visual function. As well as the setting, the examiner and testing process might also have an impact on the measurements, for example giving more or less encouragement throughout the test.

To date, only one recent study has investigated the test-retest repeatability of the hRSD test in normal healthy participants (Ku et al. 2016). The intrasession test-retest repeatability was good for 74 participants aged 16 to 80 years (Bland Altman bias and limits of agreement: Bias 0.02±0.12, LoA: -0.27 to 0.22 logMAR). The intersession test-retest repeatability was also good for 30 participants who performed the hRSD test on two separate occasions 64±24 days apart however the limits of agreement were wider, which could be due to the smaller sample size (Bias: 0.04±0.3, LoA: -0.37 to 0.44 logMAR, Ku et al. 2016).

The long-term variability of the RSD test in clinically stable patients with maculopathy was studied by Wang et al. (2014), only available as a conference abstract. In this six month longitudinal study, 35 participants were instructed to use the hRSD test from home at least once a week. The test variability was reported as the average SD over the six month period. The mean SD of hRSD measurements was 0.098±0.025 logMAR (Wang et al. 2014). Results from this study however should be applied carefully to AMD patients as the study included mainly eyes with diabetic maculopathy (only 9 participants had AMD and 26 had diabetic retinopathy).

1.2.4 Usability of the RSD test

Given the portability of the mobile phone-based version of the RSD test, its feasibility as a mean of self-monitoring visual function by the patients themselves has been assessed (Kaiser et al. 2013). The test was embedded in a system (called the Health Management Tool) which sent daily audio notifications to remind the patient to take the hRSD test. Kaiser et al. (2013) studied 147 elderly patients with nAMD (mean age 77.5, range 49 to 92 years) who were provided with the test to use at home for a period of 16 weeks. The study found that 85% of patients complied with the self-testing protocol on a daily basis during the 16 week period, while 99% completed the test at least once a week (Kaiser et al. 2013). The compliance with the test over longer periods of time remains unknown. The study by Kaiser et al. (2013) reported no test failures due to inability to perform the test. This could be because, as with other studies of this kind, patient selection at recruitment was biased towards motivated participants who had an interest and/or knowledge of using hand-held electronic devices, followed by a 7-day screening period (Kaiser et al. 2013).

Surveys given to participants using the hRSD test showed that more than 90% of patients using the hRSD test at home or in clinic thought that the device and the application were easy to use (Kaiser et al. 2013, Wang et al. 2013). Other questions indicated that 83.1% of participants would be willing to use the hRSD test at least once a week, of which 44.4% were willing to use it daily (Kaiser et al. 2013, Wang et al. 2013).

1.3 Summary of the introduction and aims of this research

Undoubtedly, there is a need to improve the detection of nAMD. Early detection, which leads to early treatment, ultimately improves the vision outcome for patients (Lee et al. 2015). There have been huge improvements in the area of new treatments for nAMD (section 1.1.4) but these are most beneficial if patients present to clinic in a timely manner, ideally shortly after the onset of nAMD. The hRSD test, which has demonstrated the ability to differentiate among the different stages of AMD (Wang et al. 2013), seems to have the potential to detect nAMD lesions (Knox et al. 2016, Wang et al. 2011). Patients known to be at high risk of developing nAMD could greatly benefit from this type of monitoring.

As yet, relatively little is known about the performance of the hRSD test in eyes at high risk of developing nAMD. This is important because investigating stable participants prior to nAMD development allows to interpret changes as clinically or non-clinically significant. In this study, participants with unilateral nAMD were recruited whose unaffected eye, the study eye, did not show signs of nAMD or GA. By studying such eyes (cross-sectionally and longitudinally), this study describes the use of the hRSD test in a relatively large sample of well-defined eyes at high risk of developing nAMD. For this key sample, the following issues were addressed:

- The effect of older age and presbyopia correction on hRSD test results
- The intersession test-retest repeatability of the hRSD test
- The stability of hRSD test scores over a period of time
- The correlation between hRSD test scores and other visual function tests
- The correlation between hRSD test scores and specific retinal structural features, for which an OCT grading protocol was created
- The usability of the hRSD test

Chapter 2 Methods

2.1 Study design

A prospective observational study was carried out in order to examine a number of properties of the handheld version of the Radial Shape Discrimination (hRSD) test in elderly participants who had one eye (the study eye, SE) at risk of developing nAMD. Participants were taking part in an observational, longitudinal study designed to assess the sensitivity and specificity of the hRSD test for the detection of nAMD (the Early Detection in Macular Disease, EDiMaD, Study), with no extra visits required for the purpose of the analysis presented in this thesis. All procedures carried out followed the principles of the declaration of Helsinki and were approved by a local research ethics committee (reference number 13/NW/0449, NRES Committee North West, Preston).

2.2 Participants

2.2.1 Recruitment

Participants were recruited from a single hospital AMD service to take part in the EDiMaD study. During the recruitment period (October 2013 to January 2015) all patients attending the AMD clinic who had a diagnosis of nAMD in one eye were considered for the study. Potential participants were identified on the basis of the information available on their medical notes (previous measurements of VA and ophthalmologist assessments) and their previous OCT scans (to assess the study eye for absence of retinal pathology). All eligible individuals were given a patient information leaflet to read at home (Appendix 3) and, if they were happy to participate, a consent form (Appendix 3) was signed on their next appointment (eligibility was re-assessed on the day that the consent form was signed). When they did not require time to consider participation in the study the consent form was signed on the day. A consecutive sample of 100 participants (100% target sample) was recruited into the study when all the inclusion/ exclusion criteria were met.

2.2.2 Inclusion criteria

In order to be included in the study patients had to:

- Be over 50 years of age
- Be able to understand and perform the study tests (hRSD test and VA)
- Have a diagnosis of nAMD in one eye with no nAMD in the other eye (SE)
- Have a VA of 0.4 logMAR (6/15 Snellen) or better in the SE.

Patients were excluded if they:

- Were diabetic
- Had central GA, scars or any other sight threatening condition in the study eye, including epiretinal membranes (ERM), vitreo-macular traction (VMT) or retinal vein occlusion (RVO).

2.2.3 Participant flow

There were twelve protocol deviations in which participants were recruited into the study on the basis of the clinical notes but retinal pathology was later found, making them ineligible. Of these twelve participants, six had GA, two had ERM, two had RVO, one had a VMT and one had a longstanding, inactive shallow fibrovascular pigment epithelum detachment (PED).

Once enrolled in the study, participants continued to attend their clinical appointments as usual and were followed up until they completed five study visits on five consecutive clinical appointments. The time between appointments was not standardised across all participants but personalised at the treating ophthalmologist's discretion and dependant on appointment availability. As a general rule, patients attended the clinic every 4-8 weeks depending on the treatment they were receiving (four weeks for Lucentis or eight weeks for Eylea) and the stability of the condition in their fellow, nAMD eye. According to the medical records the SE of these participants was considered clinically stable during the study. Clinical stability in this case refers to a lack of progression towards late AMD (no conversion to nAMD or development of GA). This was confirmed, prior to analysis, by a side by side comparison of the OCT scans of the first and last study visit.

Out of the 100 participants enrolled, 15 did not complete the five study sessions for various reasons: five withdrew consent, three were lost to follow up and seven developed nAMD in their study eye. Participants who developed nAMD during the course of this study were excluded from all analysis due to the possibility of having developed subtle signs of nAMD before a diagnosis was made and treatment was given. The rest (n=85) were included in both the cross sectional and the longitudinal parts of the study, as they completed all five study visits. A Consolidated Standards of Reporting Trials (CONSORT) diagram (Bossuyt et al. 2015) was used to show the progress of participants throughout the study (Figure 2.1).

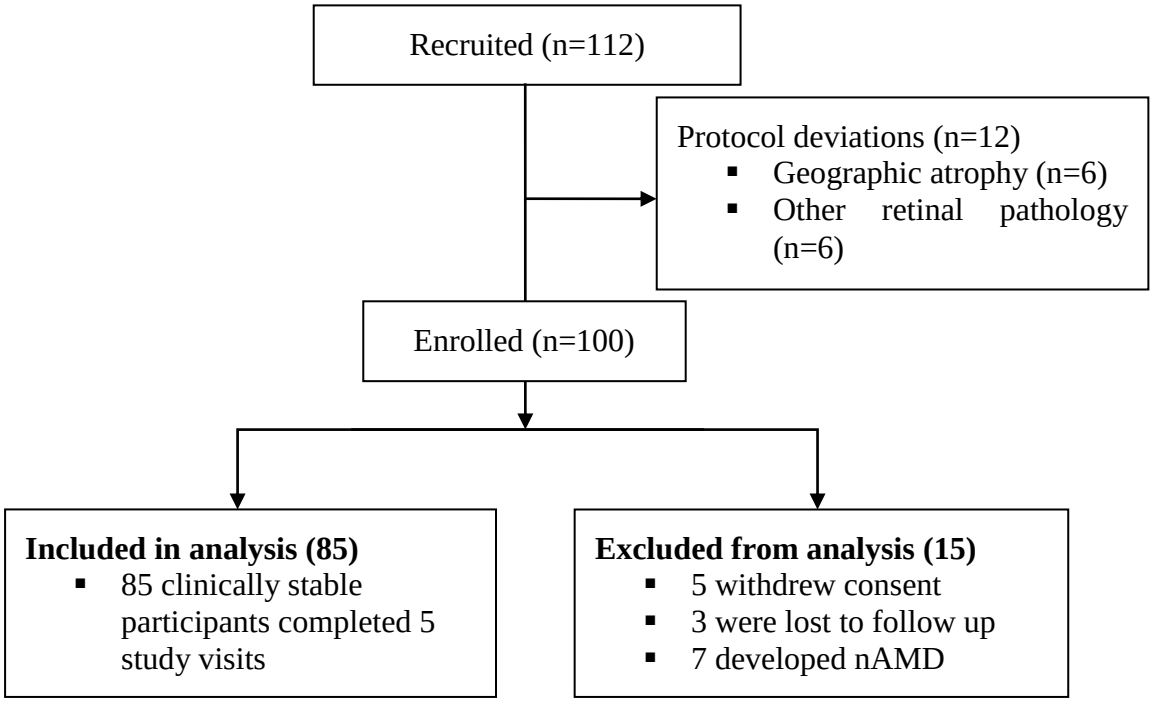

Figure 2.1 Consolidated Standards of Reporting Trials (CONSORT) diagram demonstrating study participants flow (Bossuyt et al. 2015).

2.3 Procedures and equipment

During each study visit the hRSD test, VA and OCT were performed (VA and OCT were part of the clinical appointment). The hRSD test and VA were done consecutively and always before OCT. CS was measured at specific visits as part of the clinical appointment.

2.3.1 The handheld Radial Shape Discrimination test

The version of the hRSD test used in this study runs on an Apple iPod Touch, a touch-screen electronic device the size of a mobile phone (Figure 2.2A). The test displayed a spatial 3AFC task where one out of three circles (the target) was distorted (see section 1.2.1). A spatial AFC, as opposed to a temporal one, allowed the shapes to stay on the screen until a choice was made. The participant indicated the distorted shape by touching it (Figure 2.2B). The test was performed uniocularly at the beginning of each session and the right eye was always tested first, regardless of whether this was the SE.

All participants were older than 50 years which means that they had some degree of presbyopia. As a result of this, the hRSD test was performed with reading glasses. Using a trial frame, a near addition (convex lenses) was added to the distance prescription provided by the optometrist. For the calculation of the near addition a table of age-expected additions from Antona et al. (2008) was modified to allow for ages older than 60 years (Table 2.1). This was done by taking into consideration an expected increase in near addition of 0.03D/year (Blystone 1999). The age-expected addition was used as guidance but could be refined according to individual needs.

In order to investigate the effect of uncorrected presbyopia on the hRSD test, a subsample of 30 participants performed an additional hRSD test without the appropriate near addition (due to the study being carried out in a busy AMD clinic, time and space constraints did not allow for the test to be done twice in the same session for all participants). In order to ensure that the order of the tests did not affect the results (fatigue could mean that the second test resulted in worse scores), the test

order was counterbalanced: 15 participants completed the test with correction for near first, and 15 completed it without near correction first.

The software used to run the hRSD test in the iPods was MyVisionTrack, which had all setting fixed by the manufacturer before the start of the study. The software in myVisionTrack uses a two-down, one-up staircase procedure and ends after six reversal (Wang et al. 2013). The estimated threshold was defined as the stimulus level that provided 75% correct responses (Wang et al. 2013). Only the final test result for each eye was shown at the end of the test although this was the average of two consecutive tests per eye, or three tests if the results from the first two tests differed substantially (Kaiser et al. 2013). The test returns the threshold for detecting shape deformation in a logarithmic scale (logMAR). The logMAR results are expected to fall on the negative side of the scale, as this test is a hyperacuity test. A more negative score, therefore, indicates a better score. Results from the hRSD test were collected in case report forms where there was space to write observations from the examiner. These observations included anything that could have affected the test results such as, but not limited to, poor attention, difficulty understanding instructions, the patient rushing through the test, or disruptions such as people coming into the examination room.

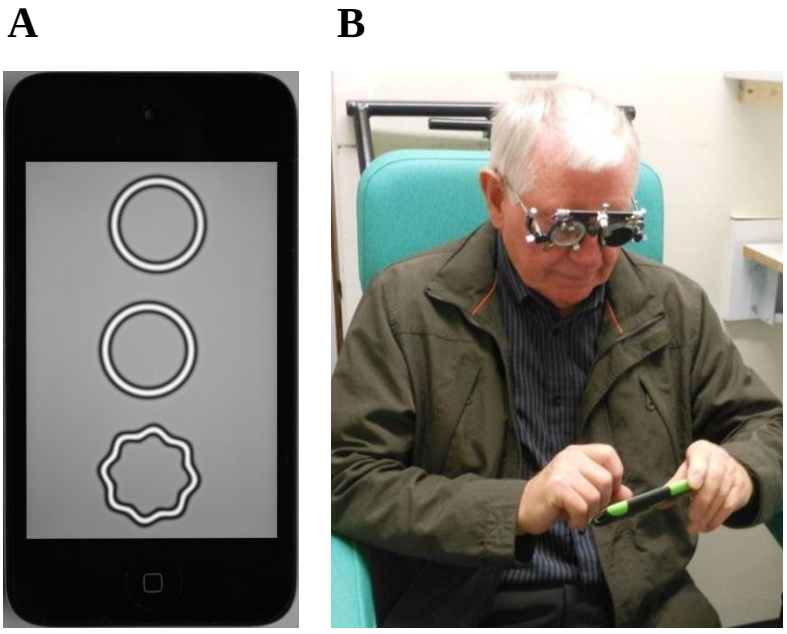

Figure 2.2 A. The hRSD test, on an iPod Touch, consisting of three RF patters of the same size, one of which is radially modulated. B. Study participant using the hRSD test during the study visit.

Age (years)	Near addition (D)
40-42	+0.75
43-45	+1.00
46-47	+1.25
48-50	+1.50
51-52	+1.75
53-55	+2.00
56-57	+2.25
58-60	+2.50
61-65	+2.50
66-70	+2.75
71-75	+3.00
>75	+3.00

Table 2.1 Age-expected near addition, modified from Antona et al. (2008) added onto the distance prescription of participants performing the hRSD test.

2.3.2 Best corrected visual acuity

VA was tested by a trained health care assistant, nurse or optometrist, using the latest refraction available from the patient records. As part of the usual clinical care, frequent refractions were performed by optometrists to ensure that patients were wearing an updated refraction for VA testing. For this reason, the terms "best corrected visual acuity, BCVA" and VA were used interchangeably throughout this thesis.

During VA examination, 4m ETDRS charts (Figure 2.3) were used with a trial frame containing their 4m optical correction. Participants were asked to start reading the letters from the top left of the chart and were encouraged to guess when unsure. When the psrticipant could not see the first 20 letters (four logMAR lines) the chart was moved to a distance of 1m and the first 30 letters were assessed (six logMAR lines). Charts 1 and 2 were used to assess the right and left eyes respectively.

The number of letters read with each eye was recorded in the medical records of the participant. The number of letters was transformed into a logMAR value by means of the following formula, from the ETDRS manual of operation (1985):

$$BCVA(logMAR) = 1.7 - 0.02 * number\ of\ letters$$

2.3.3 Contrast Sensitivity

CS was assessed by an optometrist, using Pelli-Robson charts (Figure 2.4). In Pelli-Robson charts, letter size is constant. The score, on a logarithmic scale (logCS), represents the lowest contrast at which a large letter is recognised. Similarly to measurements of VA, participants were instructed to read the letters, horizontally, from the top left, encouraged to guess when unsure. The letters on the Pelli-Robson charts are organised in sets of three (triplets) of the same contrast and the test stopped when two or more letters in a triplet were read wrong. Following the protocol used in the AMD clinic, CS was measured at 1 metre, adding +0.75D to the 4m refraction. Two charts were used, one for each eye.

Figure 2.3 ETDRS 4m VA chart, consisting of rows of high contrast letters (five per row, all the same size) which progressively reduce in size.

Figure 2.4 Pelli Robson CS chart, consisting of triplets of letters of the same size progressively reducing in contrast.

2.3.4 Optical coherence tomography

The OCT (Heidelberg Spectralis) scans were obtained by a trained imaging technician. The Spectralis OCT (Figure 2.5) is a high resolution OCT machine which is equipped with an automatic real time tracking system (TrueTrack^{TM}). The system uses the scan taken at first examination as reference and allows tracking of changes over time (Heidelberg Engineering). The protocol used to obtain the scans was the one used in clinical practice, consisting of 19 single B-scans which construct a volume centred at the fovea that extended 20°x15°.

Figure 2.5 Heidelberg Spectralis OCT used in this study.

2.4 OCT grading protocol

A grading protocol for the OCT scans was created for this study. International standardised nomenclature was used to name the different layers of the retina seen on spectral-domain OCT, Figure 1.3 (Staurenghi et al. 2014). First, the scans were graded on the overall quality of the scan. If of sufficient quality, the foveal area was located and three structural parameters were extracted: central subfield thickness, presence and height of RPE elevations (drusen) and presence and extent of ellipsoid zone disruption.

All scans were graded at baseline by a single grader (NPV).

2.4.1 Overall scan quality

The overall quality of the scan was assessed for the presence of noise, defocus and artefacts and the general integrity of the volumetric and B-scans (correct scan positioning, visibility of the whole B-scan). A grade of "good" was given when the quality was acceptable and there was no evidence of significant artefacts or noise. Scans were graded as "fair" when the scan was not considered good but there was enough evidence to proceed with the grading. Finally, "ungradable" scans where those in which the image was not of enough quality to determine a result.

2.4.2 Locating the foveal area

The foveal area was defined as a circular area, centred at the fovea centralis (small depression at the centre of the retina), which extended 1000μm in diameter, corresponding to the central ring of the ETDRS grid. The location of the foveal area was determined using the reference perpendicular line available in the "Display view" of the Heidelberg Software. The line was positioned on the B-scan that ran through the middle of the fovea and the ETDRS grid was then placed on the volumetric scan, centred directly above the presumed fovea (Figure 2.6). Once the foveal area was located, the grader proceeded to grade the structural changes in all B-scans falling within this area (Figure 2.7).

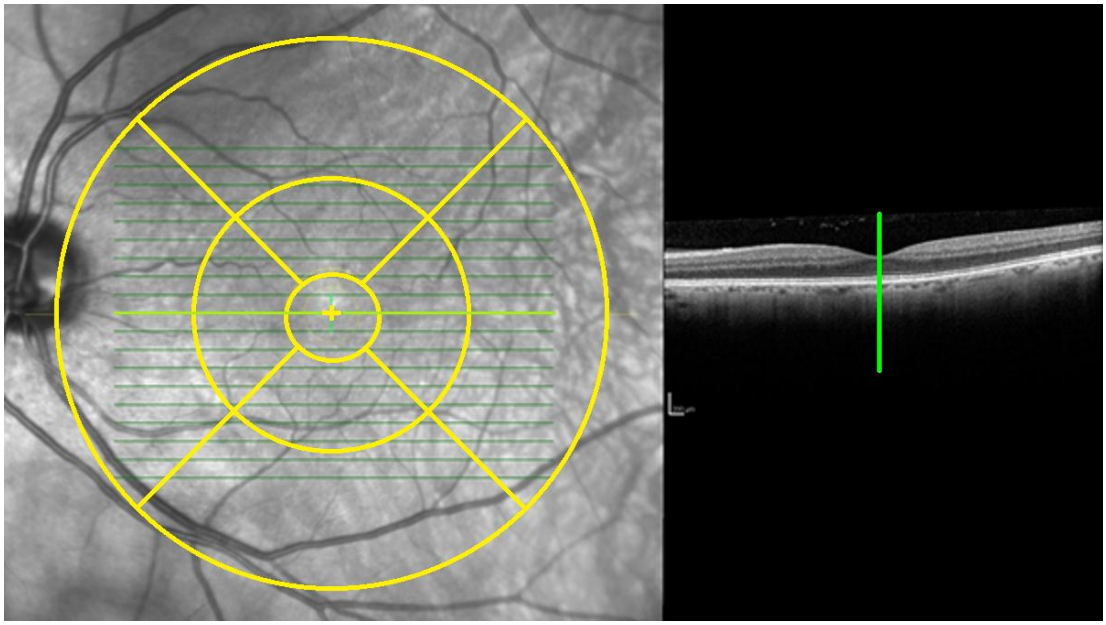

Figure 2.6 OCT scan showing the procedure used to locate the foveal area. The reference line was positioned over the fovea (green line, right) and the ETDRS grid was positioned over the volumetric scan (left), centred at the fovea.

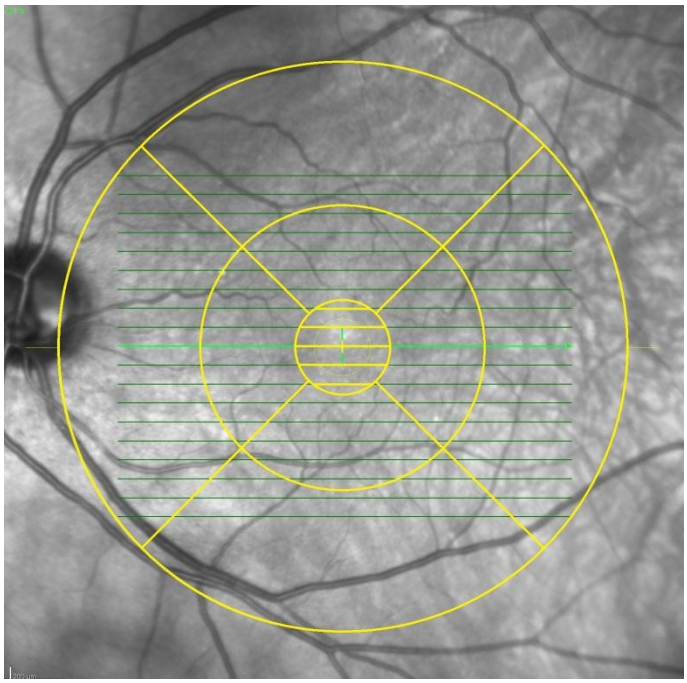

Figure 2.7 Foveal area (central ring of the ETDRS grid). All foveal B-scans falling within this area (highlighted in yellow) were graded.

2.4.3 Measurement of foveal thickness

The measurement of foveal thickness utilised in this study was the central subfield thickness (CST), defined as the average thickness of the foveal area covered by the central ring of the grid, 1mm in diameter (Figure 2.8). The CST is an automated measurement of the mean thickness of the fovea, measured from the top of the inner limiting membrane to the bottom of the RPE/Bruch's membrane complex.

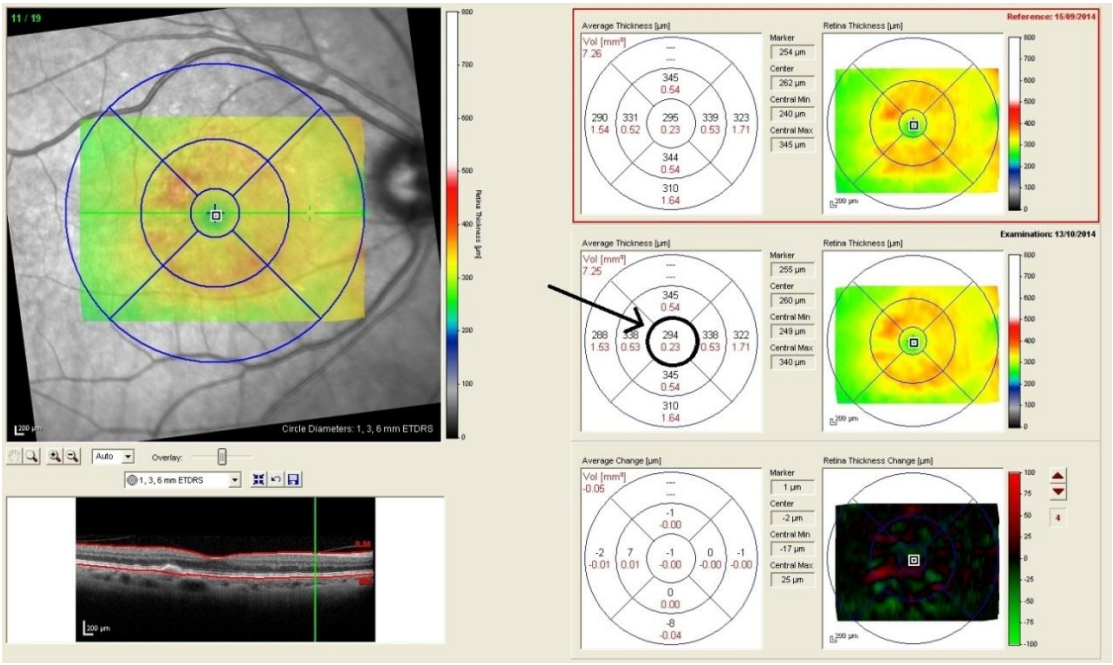

Figure 2.8 The "thickness map" view in the Spectralis software from where the CST measurement was obtained. The CST is the average thickness in the central ETDRS grid section (arrow).

2.4.4 Measurement of Retinal Pigment Epithelium Elevation

Retinal pigment epithelium elevations (RPEEs) were defined as elevations of the RPE layer from Bruch's membrane without any evidence of fluid due to nAMD (Figure 2.9A). The foveal area was assessed for the presence or absence of at least one large RPEE and if present, the maximum height of the highest RPEE was measured using the calliper from Bruch's membrane to the highest point of elevation including the RPE layer (Figure 2.9B), as was described by Hartmann et al. (2012). In this study, only large RPEEs were included, defined as greater than 70µm in maximum height. This cut-off was selected because the mean height of drusen seen in a study of early/intermediate dry AMD was 64±26µm (Hartmann et al. 2012). A comparison of a small and a large RPEE is demonstrated in Figure 2.9B.

2.4.5 Measurement of Ellipsoid zone disruption

The ellipsoid zone (previously known as the inner segment/outer segment junction) was identified in SD-OCT as the first (out of three) hyperreflective layers located on the outer aspect of the neurosensory retina (Figure 2.10). In this study, ellipsoid zone disruption (EZD) was defined as any continuous section of a scan where the EZ was either absent (Figure 2.11A) or hyporreflective/ "crumbly", yet not completely missing (Figure 2.11B). EZD might be accompanied by drusen (Figure 2.11B) or it might be seen on its own (Figure 2.11A). Focal atrophy (defined as the presence of small patches of thinning of the RPE), such as nascent GA and drusen associated atrophy, can also accompany EZD (Wu et al. 2014b) as illustrated in Figure 2.11C.

The grading of EZ status was guided by protocols used in the previous literature (Oster et al. 2010, Hartmann et al. 2012) but required to be altered because a larger area was inspected for the present study compared to these previous studies. For example in the study by Hartmann et al. (2012) only one particular retinal locus was assessed at the time (the retinal locus corresponding with microperimetry). The targets used in the hRSD test are processed across an area of the retina (the area around the fovea), requiring adequate function over this area as opposed to one particular retinal locus. In order to differentiate eyes with more or less extent of

EZD, the total length of B-scans affected by EZD was measured manually using the calliper tool. This measurement was transformed into a percentage using the total length of B-scans covering the foveal area (Figure 2.7). Although the entire fovea was not covered by the scans enclosed within the foveal area, this measurement can give a more quantitative estimate of the extent of the disruption as opposed to a simple present/absent result. This approach to measuring the extent of disruption has not been used before to our knowledge.

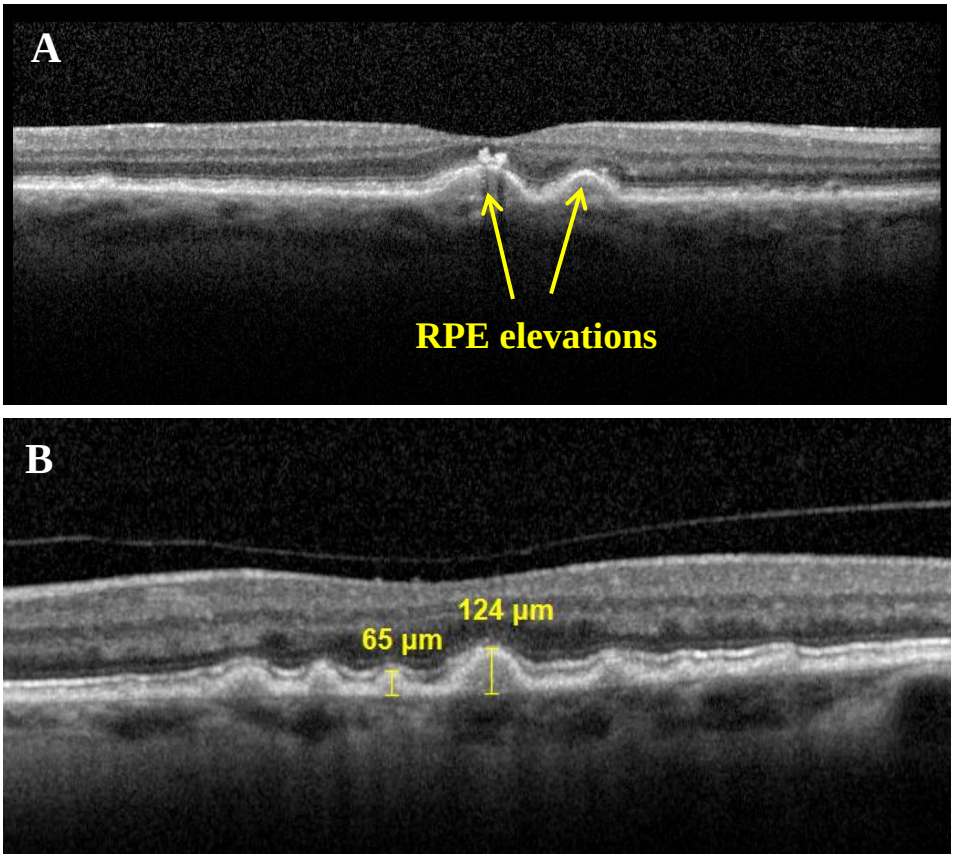

Figure 2.9 A. OCT scan showing an example of two RPE elevations where the RPE layer is separated from the underlying Bruch's membrane. B. Examples of a small (65 µm) and a large (124 µm) RPEE, measured with the calliper. Only large RPEEs (>70µm) in height we included in the grading.

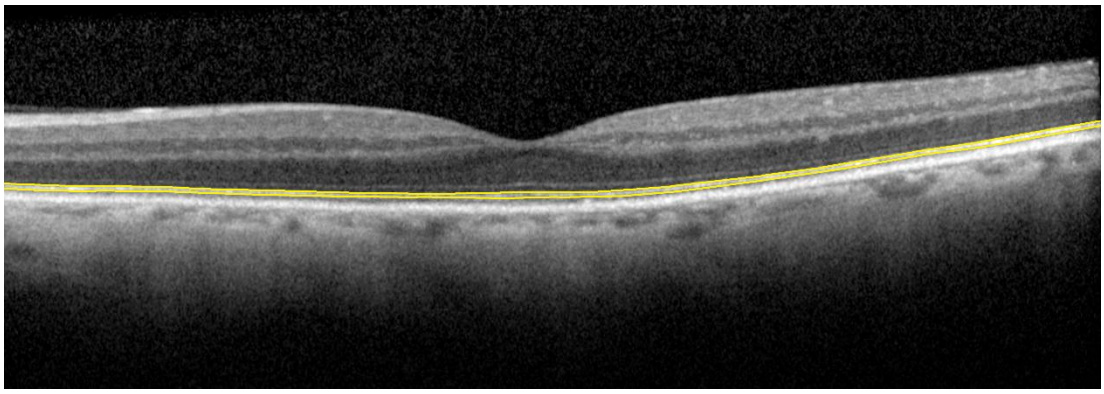

Figure 2.10 OCT scan with EZ layer highlighted in yellow.

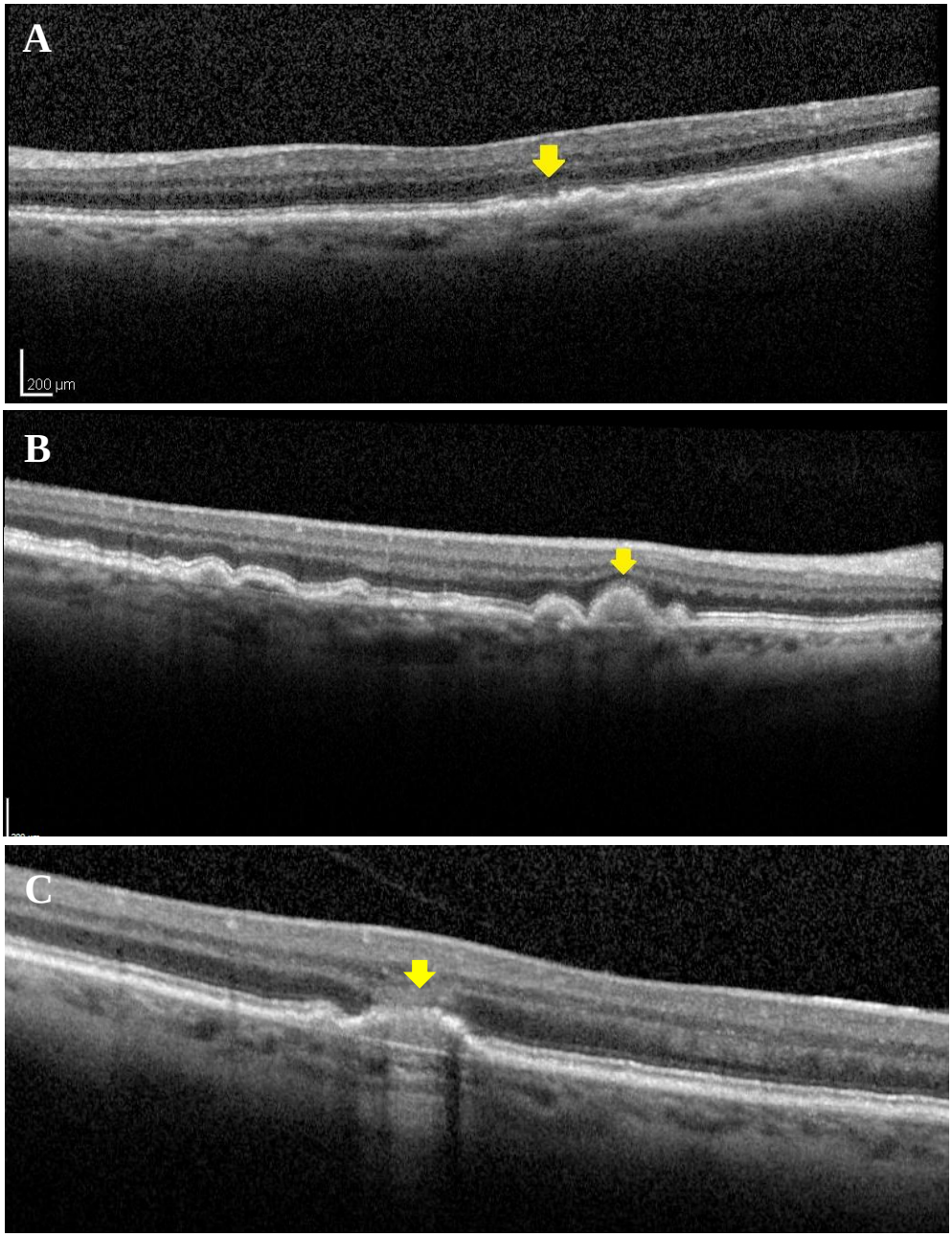

Figure 2.11 Examples of EZD (arrows). A. Absent EZ without drusen, B. Abnormal EZ above drusen, C. Absent EZ with drusen associated atrophy.

2.5 Statistics

Statistical analysis was performed with either Graphpad Prism v.6 (Graphpad Software, La Jolla, CA) or SPSS v.21 (IBM Corp, Armonk, NY) with the first being used to assess the distribution of the data (the D'Agostino & Pearson normality test) and for all graphical representations of results.

2.5.1 Sample size

A sample of 100 participants was planned for this study because this is the minimum recommended sample size for repeatability and agreement studies in ophthalmology (McAlinden et al. 2011). This recommendation is based on Bland and Altman's original formula for the calculation of the ±95%CI around the limits of agreement, expressed as $\pm 1.96\sqrt{\frac{3}{n}}s_d$, where n is the sample size and s_d is the standard deviation of the differences between two measurements (Bland and Altman 1986). For a sample of 100 participants, the ±95%CI around each limit of agreement can be calculated with a good precision of 0.34s. In view of this recommendation, several repeatability studies assessing vision tests in AMD included approximately 100 participants (Patel et al. 2008, Patel et al. 2009, Aslam et al. 2014).

A sample of 100 participants was also considered acceptable to study the relationship between retinal structural parameters and the hRSD test. The relationship between the structural OCT parameters assessed in this thesis (drusen associated RPEE and EZD) and microperimetry was assessed in a recent study that included 100 participants (Wu et al. 2014a).

2.5.2 Repeatability analysis

The study of the reliability of the hRSD test followed the Guidelines for Reporting Reliability and Agreement Studies (GRASS, Kottner et al. 2011). The literature review has described what is currently known about the reliability of the hRSD test in AMD (section 1.2.3.3) and the rationale behind this study, based on the very little

published literature. The subject population of interest were adult subjects who were at risk of developing nAMD in one eye but had not developed any signs or symptoms that might suggest the presence of nAMD in that eye; and who were on active treatment/monitoring for nAMD in their fellow eye. The unaffected eye of patients who had a diagnosis of unilateral nAMD was chosen as the study eye because these eyes are at a high risk of developing nAMD (section 1.1.2.1). This approach has previously been used by other studies assessing reliability of visual function tests in clinically stable patients with AMD (Aslam et al. 2014) which allowed for comparison. In line with the GRASS guidelines, a description of the measurement device (section 2.3.1) and a detailed description of the recruitment process, inclusion criteria and participant flow (section 2.2.3) have been included in this methods chapter.

The repeatability of the hRSD test was assessed using Bland-Altman analysis over two consecutive study visits. In the Bland-Altman method, a scatter plot is created which allows visualisation of the relationship between the measurement error and the mean of the measurements that provides an estimate of the true value (Bland and Altman 1986). The mean difference is estimated and plotted along with a reference range, defined by the so called limits of agreement, where 95% of the differences between two tests are likely to fall (assuming that the differences are normally distributed). The precision (i.e. the ±95%CI) of the mean difference and of the estimated limits of agreement can be calculated with the following formulas: $\pm 1.96\sqrt{\frac{s^2}{n}}$ and $\pm 1.96\sqrt{\frac{3s^2}{n}}$, where s is the standard deviation of the differences and n is the sample size (Bland and Altman 1986).

Since five consecutive hRSD tests during five consecutive visits were available for this study, an additional analysis of repeatability was conducted which included all five measurements per participant. In this case, the within subject standard deviation (s_w) was calculated by taking the square root of the within subject variance, obtained from a repeated measures one-way ANOVA (called residual mean square in SPSS, Bland and Altman 1996). The 95% Coefficient of Repeatability (CR) was calculated as per Bland-Altman's formula: $1.96 \times \sqrt{2s_w^2}$, where s_w is the within subject standard deviation (Bland and Altman 1996). The ±95% confidence interval (CI)

around the s_w was calculated as $1.96 \times \frac{s_w}{\sqrt{2n(m-1)}}$, where n is the number of participants and m is the number of observations per participant (Bland 2003). This approach of calculating the 95% CR for more than two measurements per participant was previously used by Patel et al. (2008, 2009) for the investigation of the repeatability of VA and CS. The repeatability analysis was also produced for VA to allow for a direct comparison within this study.

A coefficient of variation (CV) can sometimes be calculated, which allows comparing the repeatability of study tests when the tests are measured on different scales. However calculating the CV was not appropriate in the present study as the average hRSD and VA scores, on a logarithmic scale, are very close to zero which means that the coefficient of variation, which is a ratio (standard deviation divided by the mean), could result in inaccurate and misleading results. In the case of VA, not only the mean is close to zero but the scores can be positive or negative, which means that the CV cannot be calculated at all. Instead, an intraclass correlation coefficient (ICC) was calculated, which represents the correlation of measurements among study visits within each participant. The ICC provided with a dimensionless figure that allowed the comparison of the repeatability of the hRSD test to that of other measurements of visual function as assessed in a previous study by Aslam et al. (2014).

2.6 Usability questionnaire

During their last study visit, participants were asked to fill in a usability questionnaire (Appendix 4) with the following questions: 1) Before the study, how often had you used a touch screen hand-held device (such as phone, tablet or iPod)?; 2) Generally, how easy was the test to do?; 3) How easy were the instructions written on the screen to understand; 4) How easy was it to handle the device? (for example holding the iPod, pressing on the screen…); 5) If you had this test at home, would you consider using it for self-monitoring your vision between clinic appointments?; and 6) If you answered Yes to Question 5. How often would you consider doing the test at home?

2.7 Summary of methods

A large sample of participants was recruited who had unilateral nAMD. The unaffected eye (study eye) did not have nAMD, central GA or any other retinal pathology affecting the macula, and was considered to be clinically stable over the duration of the study. This tightly defined sample of participants was studied cross-sectionally and longitudinally. The use of the hRSD test in this population has so far not been described in the literature, yet these high risk eyes represent a key population that could benefit from using the test for the detection of nAMD.

The repeatability of the hRSD test was thoroughly investigated using standardised methods that allowed comparison to the repeatability of other tests such as VA and CS. An OCT grading protocol was created to quantify retinal structural changes in these high risk eyes and to assess how these changes might affect performance with the hRSD test.

Chapter 3 Results

This results chapter begins with a description of the sample of participants recruited for the study, divided into those who completed all the study visits and were included in the final analysis (included participants) and those who did not complete the study and were excluded from the final analysis (excluded participants). For the remainder of the chapter, each of the research questions outlined in section 1.3 will be addressed in separate sections.

3.1 Baseline characteristics

A consecutive sample of 100 participants was recruited for this study. Unless specifically stated, the results presented in this thesis concern only one eye of the participant: the study eye, SE. This sample was described as clinical because recruitment and study visits took place in a hospital setting whilst the participants attended hospital appointments for the treatment of nAMD to their fellow eyes.

3.1.1 Participants included in the main analysis

"Included" participants were those who met the eligibility criteria and completed all five study visits (Figure 2.1). At baseline, the mean age of included participants (n=85) was 77±7 years, ranging from 57 to 92 years. There were a higher percentage of females (64%) and only 20% had had a cataract operation to their SE prior to commencement of the study.

Age, radial shape discrimination threshold (i.e. the hRSD test) and VA were normally distributed for the SE (D'Agostino & Pearson, $p>0.05$), allowing the use of parametric statistical tests. No differences were seen in baseline age between males and females ($p=0.62$) or between those who had had a cataract surgery and those who had not ($p=0.19$).

Analysis by gender revealed no difference in baseline hRSD or in baseline VA between females and males (hRSD Females: -0.57±0.16 logMAR, Males: -0.54±0.16

logMAR, p=0.76; VA Females: 0.08±0.12, Males: 0.02±0.14, p=0.53; Figure 3.1). Neither was there a difference in hRSD or in VA between those who had cataract surgery and those who had not (hRSD Phakic: -0.57±0.16, IOL: -0.54±0.14,p=0.36; VA Phakic: 0.05±0.12, IOL: 0.08±0.15, p=0.22; Figure 3.2).

All participants were able to complete the hRSD test with their SE. At baseline, the mean±SD hRSD score for included SEs was -0.56±0.16 (±95%CI -0.60 to -0.53) logMAR. The baseline characteristics of all included participants are summarised in Table 3.1.

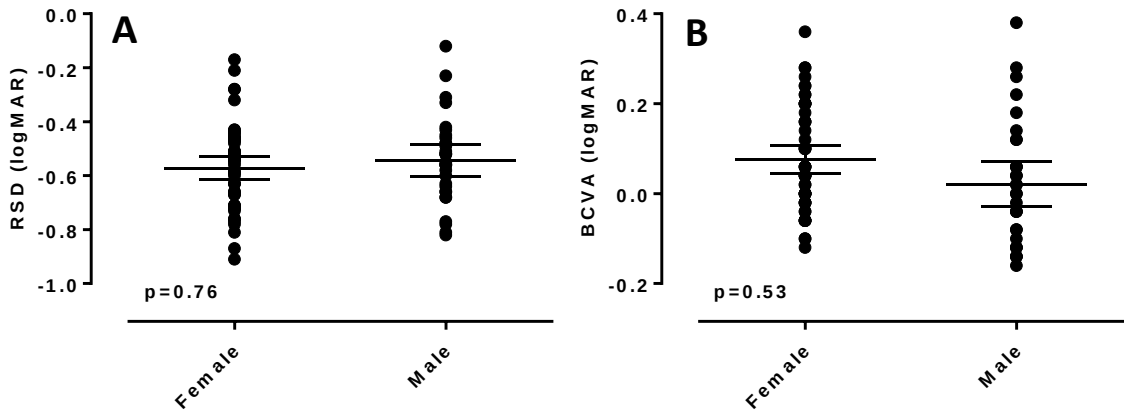

Figure 3.1 hRSD (A) and VA (B) of males and females included in the study. There were no statistically significant differences seen. Solid line indicates mean, error bars represent the ±95%CI. Dots represent individual values.

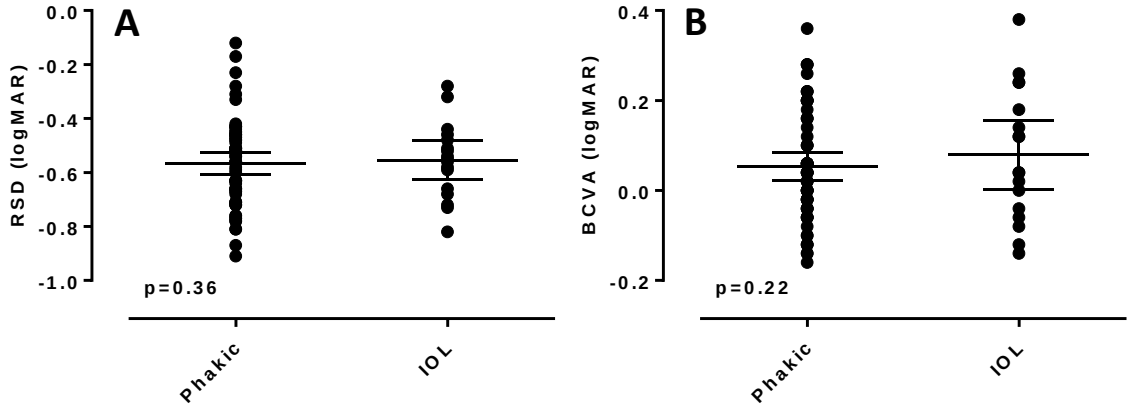

Figure 3.2 hRSD (A) and VA (B) of phakic participants (who had not had a cataract operation at baseline) and pseudophakic participants (who had had a cataract operation and intraocular lens, IOL, implanted). There were no statistically significant differences seen. Solid lines represent the mean, error bars represent ±95%CI. Dots represent individual values.

	Included n=85	Excluded n=15	p value
Age (years)			
Mean±SD	77±7	81±8	0.16
Gender			
Female	54 (64%)	13 (87%)	0.13
Study Eye			
Right	42 (49%)	8 (53%)	-
VA (logMAR)			
Mean±SD			
SE	0.06±0.13	0.1±0.13	0.28
FE	0.31±0.24	0.52±0.34	0.12
hRSD (logMAR)			
Mean±SD			
SE	-0.56±0.16	-0.45±0.23	0.02*
FE	-0.18±0.34	-0.21±0.28	0.77
Cataract surgery			
SE	17 (20%)	5 (33%)	0.31
FE	16 (19%)	3 (20%)	-

Table 3.1 Baseline characteristics of all study participants. p values indicate whether the difference between included and excluded participants was statistically significant at a significance level of 0.05 (*).

3.1.2 Participants excluded from main analysis

There were 15 participants who were excluded from the analysis because they did not complete all five study visits or were clinically unstable during the study (i.e. they developed nAMD, Figure 2.1). Of these 15, eight participants were "lost to follow up" because they withdrew consent from the study (n=5) or were discharged from their clinical appointments (n=3). The remaining seven participants, referred to as "converters", initially met the inclusion criteria but subsequently developed nAMD and were excluded from the study. All excluded and converters had at least one study visit available, which allowed for baseline comparisons.

Excluded participants were not different from included participants in age (Incl: 77±7, Exc: 81±8, p=0.16) or study eye VA (Inc: 0.06±0.13, Exc: 0.1±0.13, p=0.28). There were a higher proportion of females who were excluded (19% female vs 6% male) however the difference in proportions was not statistically significant (Fisher's exact test p=0.13). The implications of these results are that whilst a higher proportion of females were excluded in this particular sample, this cannot be generalised to the population. A similar proportion of included and excluded participants had had a cataract operation in the SE before the study (p=0.31).

The only significant difference found between included and excluded participants was the baseline hRSD test score. The mean±SD hRSD score of excluded participants was -0.45±0.23 (95%CI -0.58 to -0.32) logMAR. This mean was more positive (i.e. worse) than that of included participants (Figure 3.3). The mean difference of 0.11±0.05 (±95%CI 0.02 to 0.21) logMAR was statistically significant, t(98)=2.41, p=0.02.

The baseline characteristics of excluded participants are summarised in Table 3.1.

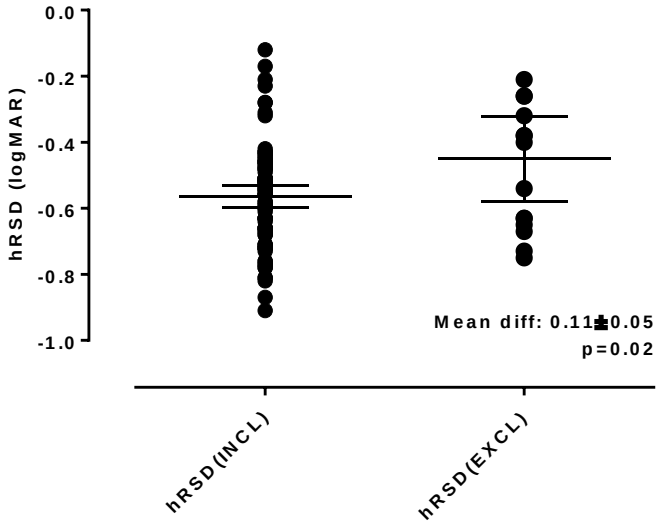

Figure 3.3 hRSD scores of included and excluded participants. A more negative hRSD score means a better score. Excluded participants performed on average statistically significantly worse. Solid line indicates the mean and error bars indicate the ±95%CIs around the mean.

3.2 Effect of older age and presbyopia on the hRSD test

The age of the participants included in the analysis ranged from 57 to 92 years. As expected in a sample of individuals at risk of developing nAMD, 85.3% were older than 70 years and almost half (45.9%) were older than 80 years of age. Pearson's coefficient revealed a statistically significant correlation between the hRSD scores and age (r=0.37, p=0.0005). Having established the presence of a relationship, linear regression analysis was used to describe the relationship between hRSD and age (Figure 3.4). The regression line had a positive slope of 0.0076±0.0021 (±95%CI 0.0035 to 0.012).

To allow for comparison, the relationship between VA and age was also calculated and included in Figure 3.4. Similarly to hRSD, there was a significant association between age and VA (Pearson's r=0.36, p=0.0007). The slope of this regression line was 0.0060±0.0017 (±95%CI 0.0026 to 0.0095). This slope, which was also positive, was not statistically different to the "hRSD on age" slope (p=0.57).

The effect of uncorrected presbyopia on hRSD test results was investigated for a subsample of 30 participants, who performed the test with and without near addition in the same session (section 2.3.1). The mean (±SD) age of the participants included in this analysis was 79±8 years, ranging from 67 to 91, with 90% of them over 70 years. This subsample was no different in age from the remaining participants that were not included in this analysis (t(89)=0.98, p=0.34) and a similar proportion (53%) were female (chi-square p=0.18). Due to their age, the majority of the participants included in this experiment used a near addition of +2.5-3D.

A paired t-test showed that on average, participants scored worse when the near addition was not used (-0.36±0.23, SE=0.04 logMAR) compared to when the near addition was in place (-0.50±0.21, SE=0.04 logMAR), t(29)=3.79, p=0.001, r=0.55. The ±95%CI of the mean difference (0.14 logMAR) was 0.07 to 0.22 logMAR, which means that the true value of the mean difference is very unlikely to be zero (Figure 3.5).

The difference in hRSD score was calculated as "hRSD without addition" minus "hRSD with addition". From Figure 3.5 one can see that the majority of the

differences fall above the zero difference mark (dashed line) indicating that the hRSD results without addition were more positive (i.e. worse) than those with near addition in the majority of cases. The hRSD results were worse without the near addition in 21 participants (70%), with a mean (±SD) deterioration in score of 0.25±0.16 logMAR. For those participants whose results were better without the near addition, a smaller 0.10±0.04 logMAR improvement in test score was seen.

In summary, the results reported in this section indicate a statistically significant correlation between age and hRSD scores for this population of “at risk” eyes and that lack of appropriate optical correction for near (i.e. not wearing the reading glasses) has a significant effect on the hRSD test results compared to the results obtained when using distance optical correction only.

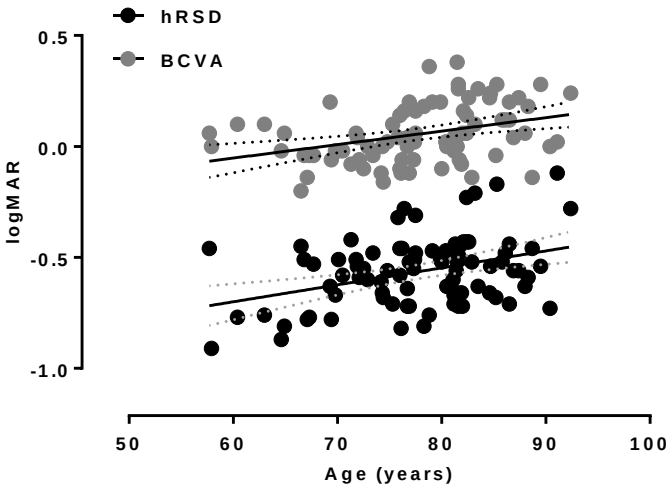

Figure 3.4 Relationship between age and hRSD (black) and VA (grey). Lines indicate the linear regression line ±95%CIs. Slopes were positive for both hRSD (0.0076±0.0021) and VA (0.0060±0.0017) and not significantly different from each other (p=0.57).

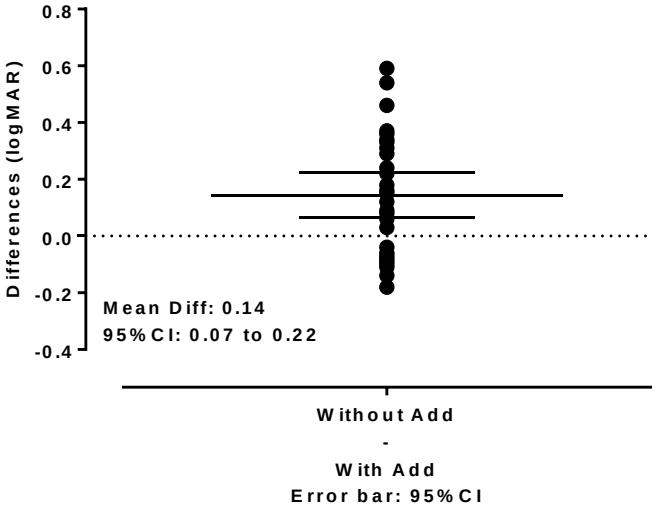

Figure 3.5 Statistically significant difference in hRSD score with and without near correction. The solid line indicates the mean difference (0.14 logMAR) and the error bars are the ±95%CIs. Dashed line (zero) indicates no difference. Underlying dots represent individual differences in hRSD score with and without near addition for each of the 30 participants.

3.3 Test-retest repeatability of the hRSD test

The test-retest (TR) repeatability is the expected variability of the test over two or more measurements. In the following sections, a combination of coefficients (section 2.5.2) will describe the intersession TR repeatability of the hRSD over two (Bland and Altman 1986) and over five study visits (Bland and Altman 1996).

3.3.1 Bland-Altman method (for two measurements)

The Bland-Altman method is commonly used for reporting TR repeatability (section 2.5.2). The Bland Altman limits of agreement were calculated for visits 2 and 3 in order to avoid any potential learning effects after the first (baseline) test but still representing the normal interval of time between AMD clinical follow-up appointments. The two hRSD tests were performed a mean of 1.7±0.6 months apart (range 0.5 to 3.4 months). All 85 participants were included in the analysis. The mean hRSD scores at visits 2 and 3 were -0.57± 0.17 (95%CI -0.60 to -0.53) and -0.56± 0.14 (95%CI -0.59 to -0.53) respectively.

The mean difference (bias) between the two tests was -0.004 (±95%CI -0.033 to 0.026) logMAR, Figure 3.6. The ±95%CI runs through zero, which means that the true mean difference is likely to be very close to zero. The limits of agreement were -0.28 to 0.27 logMAR, which represents the expected range for the difference between two test results for the same person. In order to determine the accuracy of the limits of agreement, their ±95%CIs were calculated (section 2.5.2). The ±95%CIs of the lower and upper limits of agreement were: -0.33 to -0.23 and 0.22 to 0.32 logMAR (Figure 3.6).

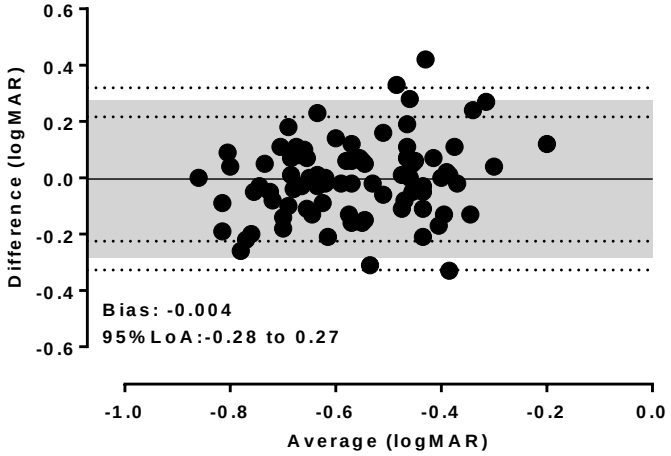

Figure 3.6 Bland-Altman plot for n=85 pairs of hRSD measurements. The solid line indicates the mean difference or bias of -0.004 (±95%CI -0.033 to 0.026 logMAR), the shaded area indicates the extent of the limits of agreement (-0.28 to 0.27 logMAR) and the dotted lines indicate the ±95%CI around each limit of agreement (-0.33 to -0.23 and 0.22 to 0.32 logMAR). LoA: limit of agreement.

3.3.2 The 95% coefficient of repeatability (for five measurements)

Since the hRSD test is likely to be used repeatedly over time, a further analysis of repeatability was performed which included five measurements per participant, as opposed to only two. As study visits were arranged on the same day as the clinical appointment, the interval between study visits was not fixed. Visits 2-5 were on average: 47±14, 99±25, 152±38 and 204±50 days from baseline respectively.

The RSD scores of all included participants (n=85) were used for the calculation of the within subject standard deviation, s_w. The mean±SD hRSD test results at each of the five study visits were: -0.56± 0.16 (95%CI -0.60 to -0.53), -0.57± 0.17 (95%CI -0.60 to -0.53), -0.56± 0.14 (95%CI -0.59 to -0.53), -0.58± 0.18 (95%CI -0.62 to -0.54) and -0.58± 0.19 (95%CI -0.62 to -0.54), Figure 3.7. A repeated-measures ANOVA demonstrated that these means were not statistically significant different, $F(4,336)=0.5422$, $p=0.68$.

The s_w is the standard deviation in each subject's measurements between tests, after any shifts in the mean were accounted for. In order to estimate the s_w as a single value across all participants it is assumed that the SD is not related to the magnitude of the measurements (i.e. poorer test results do not result in a larger variability in results). As recommended by Bland and Altman (1996), this assumption was checked by means of a scatter plot (Figure 3.8) and a rank correlation coefficient, which confirmed no relationship ($r=0.07$, $p=0.53$). The s_w was calculated by taking the square root of the within subject variance, obtained from the above repeated measures one-way ANOVA, called residual mean square in SPSS (Bland and Altman 1996). The s_w was 0.12 (±95%CI 0.11 to 0.13) logMAR (Figure 3.8) and the 95% CR, which was calculated using the s_w (section 2.5.2), was 0.33 logMAR.

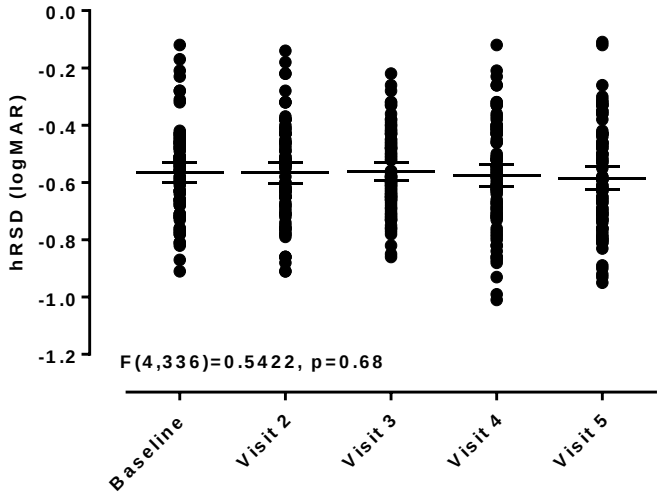

Figure 3.7 Aligned dot plot showing the hRSD scores at each visit. The means were similar at each time point. The solid line indicates the mean hRSD and the error bars are the ±95%CIs.

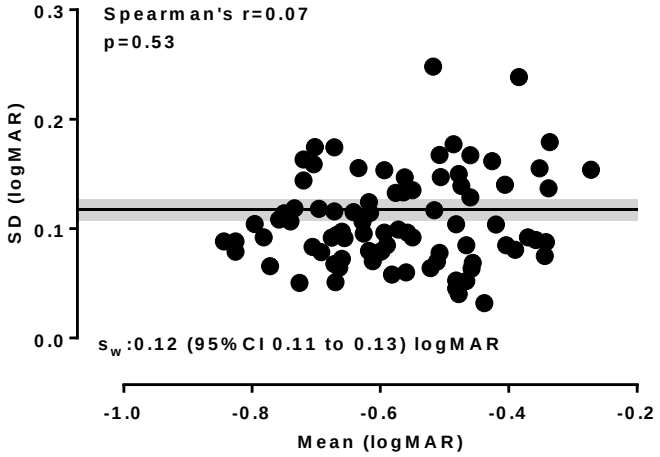

Figure 3.8 Scatterplot showing no relationship between the individual standard deviations and the magnitude of the hRSD measurement (p=0.53). Solid line indicates the within subject standard deviation (s_w). Shaded area represents the ±95%CI of s_w.

3.3.3 Intraclass correlation coefficient (ICC)

In this study, the ICC is not reported as an absolute measurement of reliability but as additional information to the above coefficients as it provides a dimensionless value for comparison to other tests. Calculated for all 85 participants as a one-way random model, the ICC was 0.54 (95%CI 0.44 to 0.63) for single measurements and 0.85 (95%CI 0.8 to 0.9) for average measurements.

A summary of the intersession repeatability results for the hRSD test can be found in Table 3.2.

	Bland-Altman limits of agreement	Within-subject standard deviation	Coefficient of repeatability	Intraclass correlation coefficient
hRSD	-0.28 to 0.27 logMAR	0.12 (95%CI 0.11 to 0.13) logMAR	0.33 logMAR	0.54 (95%CI 044 to 0.63)

Table 3.2 Summary of intersession repeatability results for the hRSD test. ICC reported for single measurements.

3.3.4 Repeatability of VA

The repeatability of VA was assessed in order to compare the results to the repeatability of the hRSD test. The mean±SD VA at the five study visits were: 0.06± 0.13 (95%CI 0.03 to 0.08), 0.06± 0.12 (95%CI 0.03 to 0.08), 0.06± 0.13 (95%CI 0.03 to 0.08), 0.06± 0.12 (95%CI 0.03 to 0.08) and 0.04± 0.12 (95%CI 0.02 to 0.07) logMAR (Figure 3.9). A repeated-measures ANOVA demonstrated that these means were not statistically significant different, $F(4,336)=0.4846$, $p=0.72$.

From the scatterplot on Figure 3.10 it is noted that although there is a positive statistically significant correlation between individual means and SDs (i.e. poorer VA was associated with larger variability in VA measurements) this correlation was weak ($r=0.263$, $p=0.015$) and the slope of the regression line was very close to zero (0.07 ±95%CI 0.007 to 0.13). Since there was no strong correlation, the s_w for the five consecutive VA measurements was calculated as a single value across all participants. The s_w of the five consecutive VA measurements was 0.067 logMAR, equivalent to 3.3 letters on the ETDRS chart. The 95% CR was 0.19 logMAR (close to two logMAR lines or 9.5 letters).

The Bland-Altman test-retest plot for visits 2 and 3 is shown in Figure 3.11. The mean difference and 95% limits of agreement were -0.0007 (-0.18 to 0.18 logMAR). These limits of agreement are equivalent to ±9 letters in the ETDRS charts. The ±95%CIs of the lower and upper limits of agreement were: -0.22 to -0.15 and 0.15 to 0.22 logMAR.

Finally, similarly to the analysis of hRSD, the ICC was calculated for VA measurements. The ICC, calculated for all 85 participants as a one-way random model, was 0.73 (95%CI 0.66 to 0.80) for single measures and 0.93 (95%CI 0.91 to 0.95) for average measures.

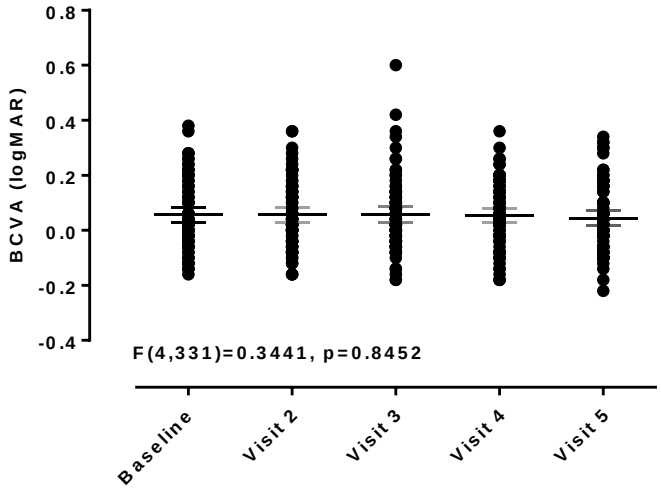

Figure 3.9 Aligned dot plot showing the VA at each visit. No statistically significant difference found among the time points. The solid lines represent the means ±95%CIs (error bars).

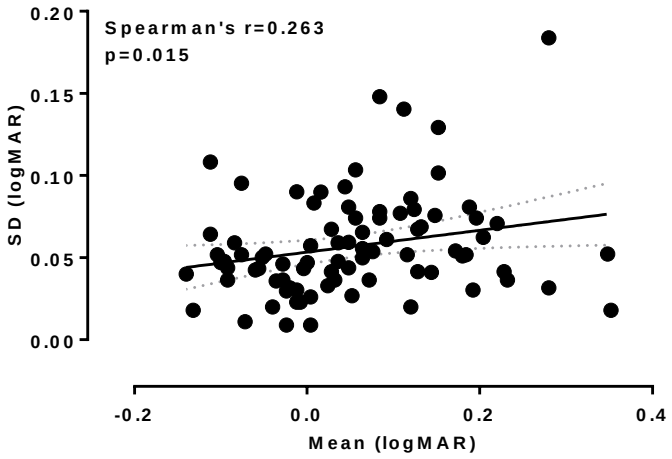

Figure 3.10 Scatterplot showing a weak relationship between the individual standard deviations and the magnitude of the VA measurements.

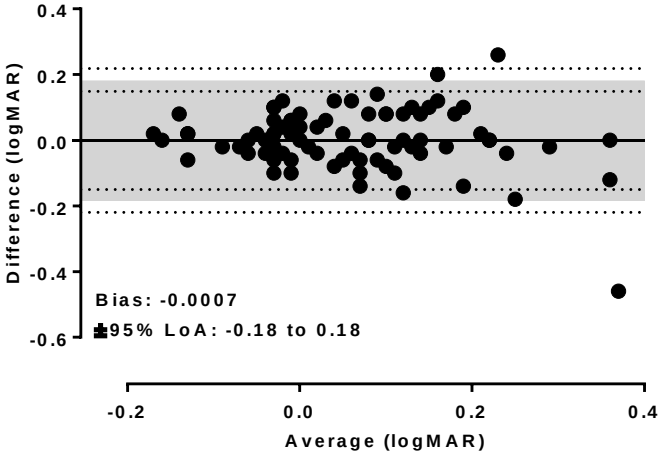

Figure 3.11 Bland-Altman plot for n=85 pairs of VA measurements. Solid line indicates mean difference or bias of -0.0007 (±95%CI -0.021 to 0.019 logMAR), shaded area indicates the extent of the limits of agreement (-0.18 to 0.18 logMAR) and dotted lines indicate the ±95%CI around each limit of agreement (-0.22 to -0.15 and 0.15 to 0.22 logMAR). LoA: limit of agreement.

3.3.5 Sensitivity analysis on the effect of missing data

Missing data can sometimes represent a problem in the analysis of repeated measures ANOVA (used to calculate the CR), as the whole participant needs to be excluded from the calculation. In this study a total of 425 hRSD tests were done (five tests for 85 participants) and only ten (2.3%) were missing. These missing values occurred randomly, usually because a participant was mistakenly dilated before the hRSD test was carried out. The ten missed tests corresponded to ten different participants and occurred at random study visits (one in the second visit, two in the third visit, three in the fourth visit and four in the last visit). In order to be able to include all participants in the analysis, and in view of the very small proportion of missing values, the missing hRSD test scores were imputed by calculating the average of the remaining four tests for the particular participant. In order to ensure that imputing the missing values did not have an effect on the repeatability analysis, the s_w, Bland-Altman limits of agreement and ICC were repeated excluding the ten participants who had a missing value, i.e. using a sample of 75 participants. The results remained unchanged as the s_w was 0.12 logMAR; the Bland-Altman bias and limits of agreement were -0.006 (-0.29 to 0.27) logMAR and the ICC was 0.52 and 0.84 for single and average measures. There were no missing VA data.

3.3.6 Summary of repeatability

The repeatability of the hRSD test was assessed for a large clinical sample of stable participants. Clinical stability was defined as no progression to late AMD (neither GA nor nAMD) or any other sight threatening diagnosis. The repeatability was assessed over two visits (Bland-Altman method) and was confirmed with the calculation of the 95% CR method, which included five measurements of RSD. The ICCs provide a means of comparing the test-retest repeatability of the hRSD test and VA within the study and in relation to other studies.

3.4 Stability over time

Participants taking part in this study were attending clinical appointments for the monitoring and treatment of their fellow eyes. The mean interval between clinical appointments was 1.7±0.6 months. The five study visits extended over 6.7±1.6 months (range 4-11 months).

In order to evaluate individual performance, each participant's hRSD test results and the corresponding time from baseline were used to produce 85 regression lines (one for each participant). The sign of the slope of each regression line was assessed on its sign (positive/negative) and whether it was statistically significantly different from zero, which might reveal potential trends over time, for example a continuous improvement or deterioration in scores.

Overall, 60% of regressions had a negative slope (mean -0.00078±0.00068 logMAR), meaning that their scores improved over time if only very slightly. Only a small number (n=5) of these negative slopes were statistically significantly different from zero. The remaining 40% had a positive slope (mean 0.00086±0.00062 logMAR), of which only two were statistically significantly different from zero. Figure 3.12 shows the regression lines for stable participants (A) and for those who showed a significant trend over time (C), along with corresponding profile plots summarising the performance as mean ±SD across the five study visits (B and D). Only data from the first 20 participants were plotted in Figure 3.12A as plotting data from all 85 participants resulted in a blur that did not allow visualising individual lines.

The seven participants who showed a significant trend over time represent only 8% of the total sample confirming that hRSD performance was stable in the great majority of participants. The OCTs of the two participants in whom hRSD significantly deteriorated over time were re-evaluated but no clinically significant changes were seen in terms of foveal thickness, drusen, RPE atrophy or general integrity of the retinal layers (Figure 3.13).

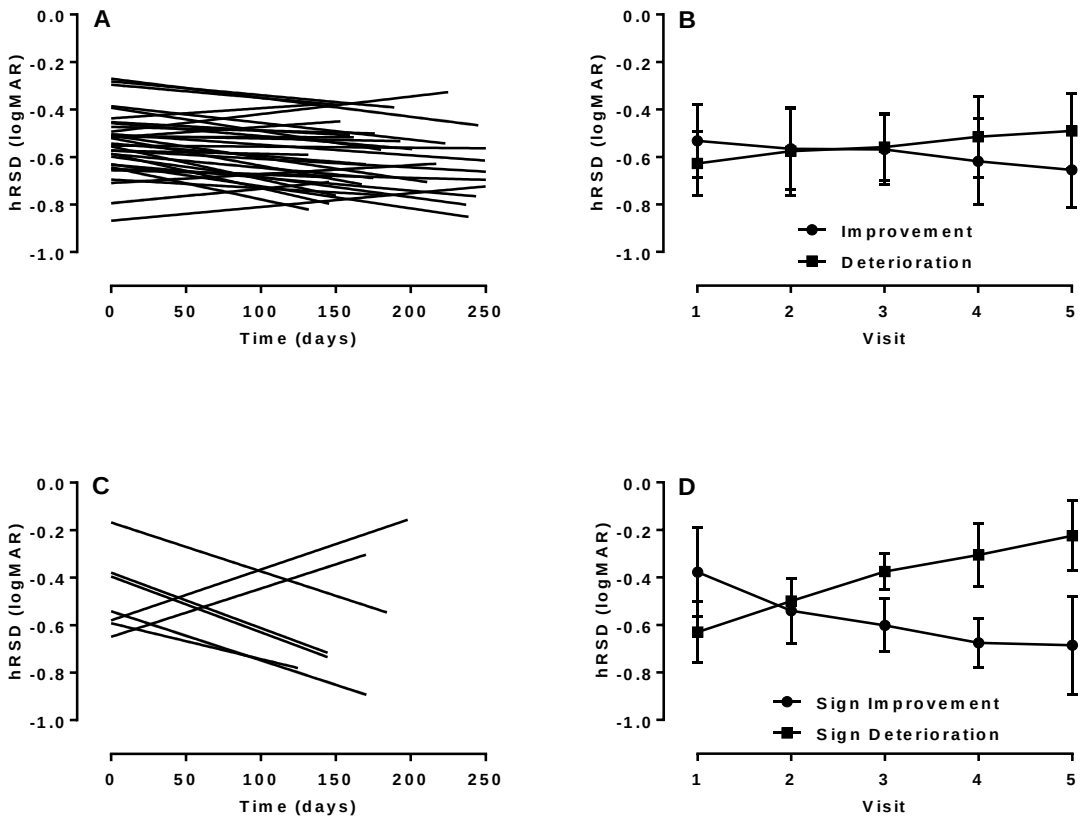

Figure 3.12 Individual regression lines and profile plots for participants who showed stable hRSD performance over time (A, B) and for those who showed a statistically significant change in hRSD over time (C, D). A. Stable participants, (n=78) had slopes that were statistically not significantly different from zero (46 improvements and 32 deteriorations). C. Non-stable participants (n=7) had slopes that were different from zero (five improvements and two deteriorations). B and D. Profile plots showing the mean (solid line) and SD (error bars) at each study visit of participants whose RSD was stable (B) and those whose RSD significantly changed over time (D).

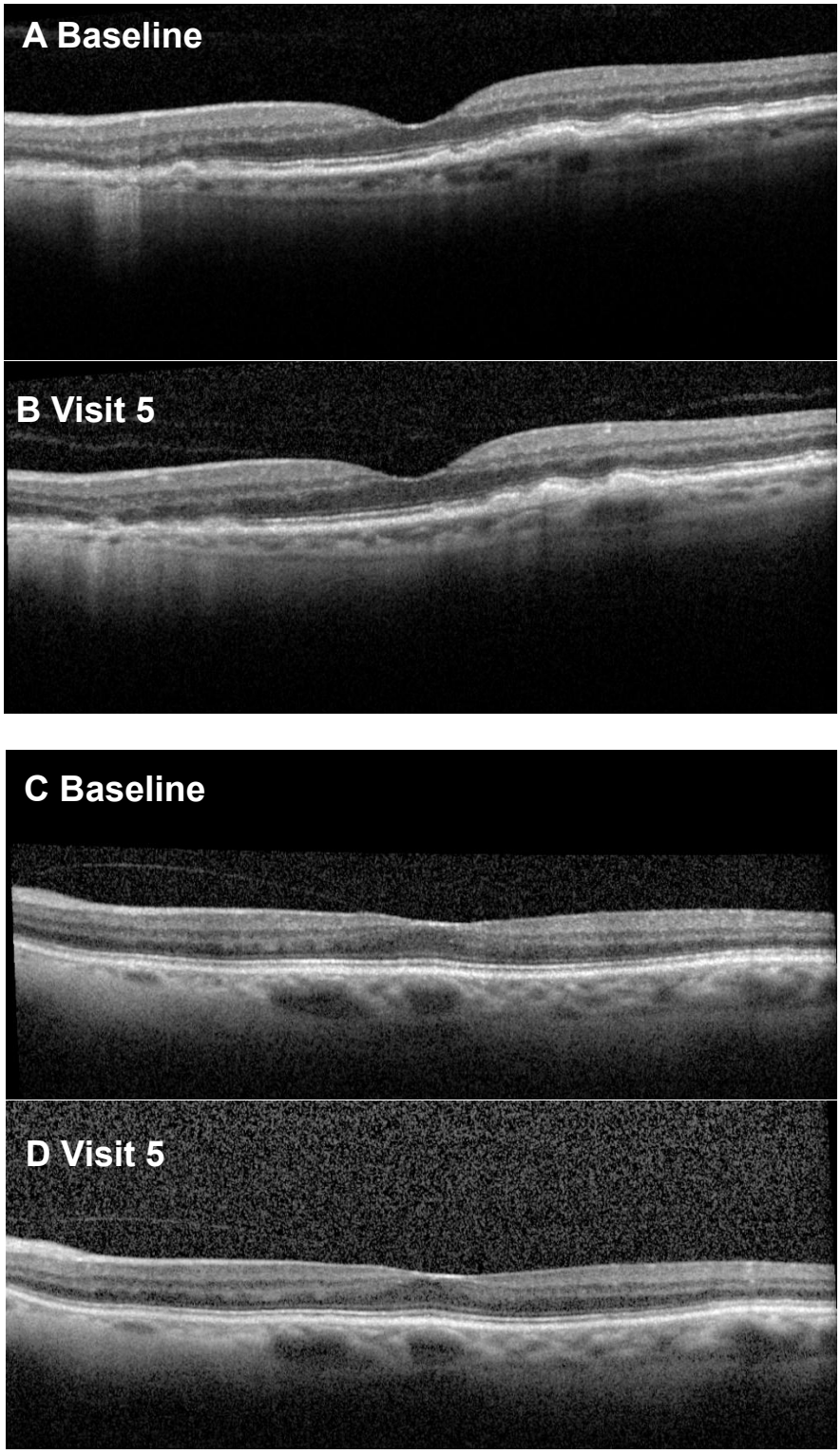

Figure 3.13 OCT scans for baseline (A and C) and visit 5 (B and D) corresponding to the two participants shown in Figure 3.12 C whose scores significantly deteriorated over time. No clinically significant change can be seen between the two OCT images of either participant that could explain for the significant worsening of hRSD score.

3.5 Structure and function

VA and OCT are two investigative techniques used in AMD clinics to assess visual function and structure, respectively. In this study, the relationship between VA and RSD and the relationship between specific OCT parameters and RSD were explored. A number of participants also had measurements of CS available.

3.5.1 RSD and other measurements of visual function

The relationship between VA and hRSD scores was analysed for SEs firstly as they were the main focus of this study, but FEs were also analysed separately (section 3.7). In view of the association between older age and hRSD in our sample of participants (section 3.2) and the association between VA and older age, which is accentuated in early AMD (Sjöstrand et al. 2011), partial correlations were used to reveal the unique relationship between hRSD and VA when the effect of age is accounted for. The baseline measurements of VA and hRSD scores in SEs were normally distributed (D'Agostino & Pearson p=0.12 and p=0.31 respectively) allowing the use of parametric correlations. The demographic characteristics were those discussed in section 3.1.1. The mean VA in SEs at baseline was 0.06±0.13 logMAR (±95%CI 0.03 to 0.08). There were no participants with VA worse than 0.4 logMAR as this was an exclusion criterion. A partial correlation revealed no association between hRSD and VA when the effect of age on both these variables was accounted for (partial r=0.01, p=0.9), Figure 3.14.

CS was not routinely assessed at every clinical appointment, thus 34 CS measurements of 34 participants were available for this analysis. CS was measured at any of the five study visits, therefore the corresponding hRSD test results on the same day were used for this correlation. CS also met the assumption of normality in the SEs (D'Agostino & Pearson p=0.72). The statistical approach was the same as that used for VA. The mean CS for SEs was 1.63±0.23 (95%CI 1.55 to 1.71) logCS and the mean hRSD for this subsample of 34 participants was -0.53±0.18 logMAR. There was no significant association between CS and hRSD (partial r=-0.28, p=0.109), Figure 3.15. Interestingly, a statistically significant relationship was found

when age was not used as a covariate (r=-0.35, p=0.04). This is an important finding and will be considered further in the discussion (section 4.4.1).

In summary, the hRSD scores was not related to either VA or CS in this sample of at risk eyes.

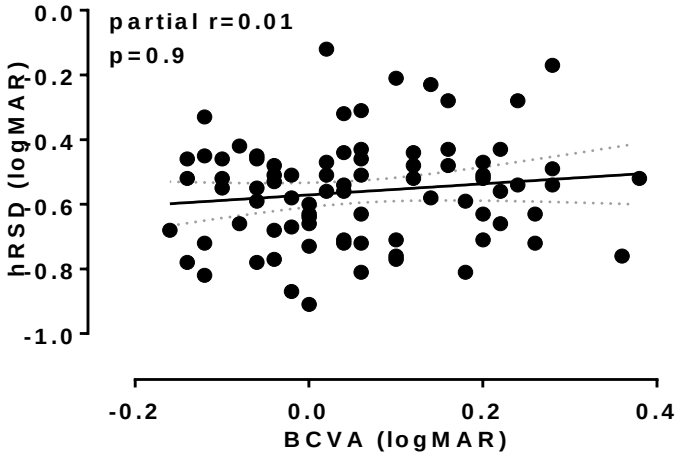

Figure 3.14 No statistically significant relationship seen between hRSD scores and VA in SEs. Line represents the linear regression line and its ±95%CIs.

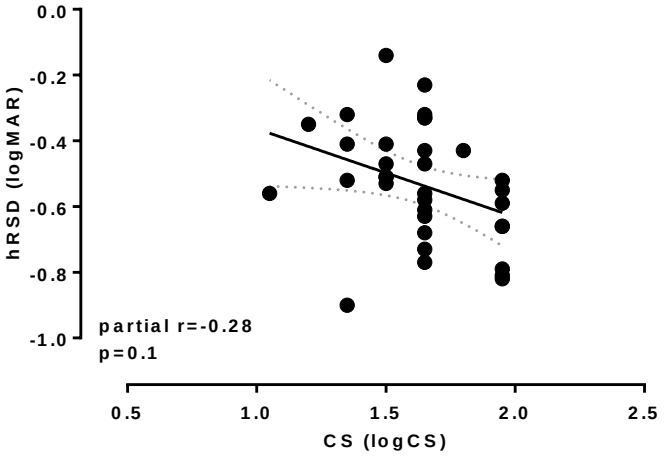

Figure 3.15 No statistically significant relationship seen between hRSD scores and CS in SEs. Line represents the linear regression line and its ±95%CIs.

3.5.2 RSD and retinal structural changes

The relationship between hRSD scores and structural changes in the retina was investigated by means of OCT scans. An OCT grading protocol was developed for the purpose of this study (section 2.4) and three aspects of the retinal structure were measured: the presence and size of RPEEs, the disruption to the photoreceptor layer by assessing the presence and extent of EZD, and the central foveal thickness. It was hypothesised that participants with more foveal disruption to the EZ, and/or foveal RPEEs would have a worse hRSD score.

All OCT scans were available for grading. The majority of scans were of good quality, with only eleven (14%) having an overall fair quality. Sufficient evidence was available from fair scans to be graded for all parameters (Figure 3.16A). Only seven scans (9%) were ungradable for the presence or absence of foveal EZD (Figure 3.16B). These scans had an overall lack of hyperreflectivity of the EZ which meant that there was not sufficient evidence to determine its integrity.

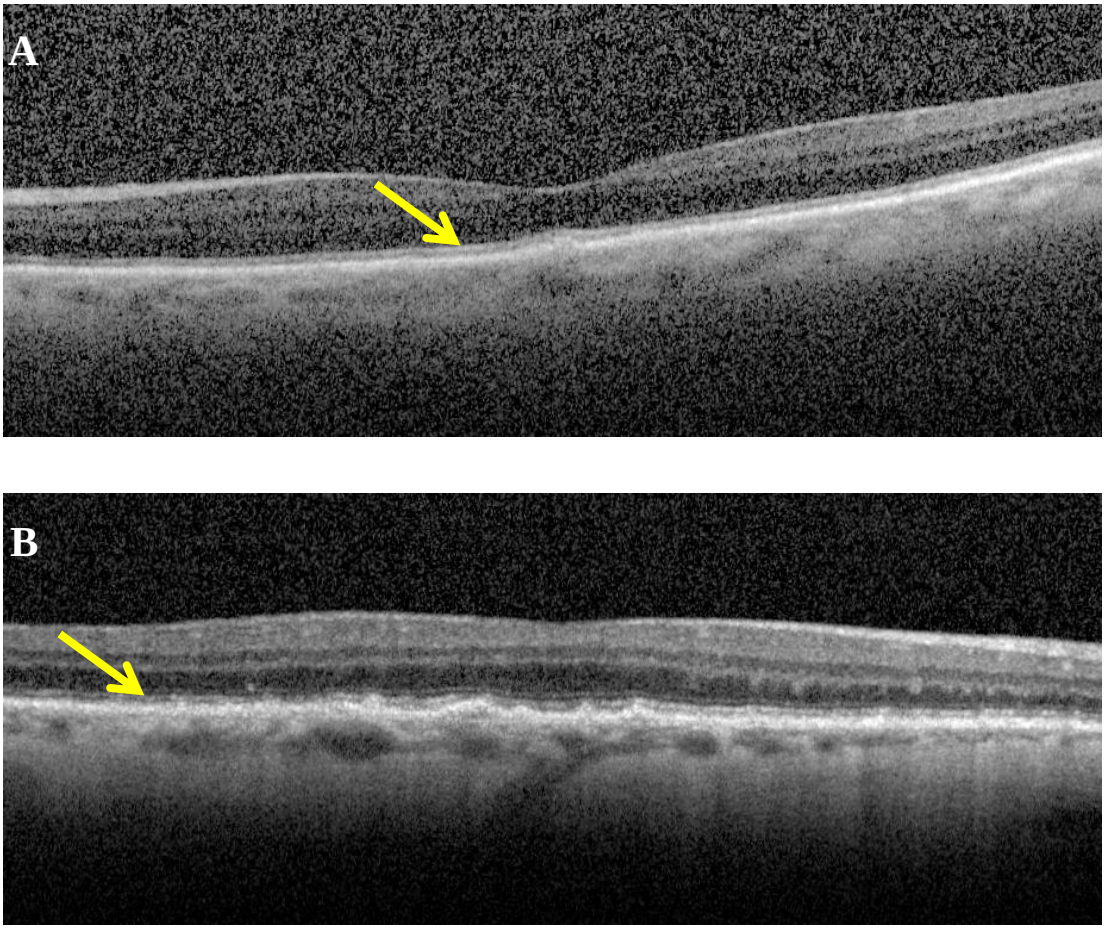

Figure 3.16 A. Example of a fair quality OCT where the EZ was still visible and gradable, B. Example of a scan with an overall lack of reflectivity of the EZ that prevented the grading of EZD. The arrows indicate the position of the EZ (hyperreflective layer above the RPE/Bruch's complex).

3.5.2.1 The hRSD test and Retinal Pigment Epithelium Elevations (RPEEs)

The grading protocol for determining the presence of large RPEEs in the foveal area was described in section 2.4.4. Scans of all 85 SEs were available and included in the grading. None were ungradable for the presence of RPEEs.

The mean hRSD scores of participants with at least one large RPEE at the foveal area (n=37, 43.5%) was -0.53±0.15 (95%CI -0.58 to -0.48) logMAR. This mean was higher (worse) than that of participants without any foveal RPEE, -0.59±0.16 (95%CI -0.64 to -0.54) logMAR. Analysis of variance using age as a covariate (ANCOVA) revealed that these two means were statistically significantly different: $F(1,82)=5.88$, $p=0.02$, partial $\eta^2=0.067$, Figure 3.17. The effect size (partial eta squared, η^2) was very small, which means that RPEE only explains a small proportion of the total variance in hRSD (which is not explained by age).

Of the 37 participants who were found to have at least one RPEE at the fovea, the mean maximum height of the RPEEs was 113±29 µm (95%CI 103 to 122 µm), with the largest one measuring 200µm in height. A partial correlation was used to find whether larger RPEEs were related to worse hRSD scores. Such association was not found (partial $r=0.13$, $p=0.43$), Figure 3.18.

When the analysis was repeated for VA, there was no difference in mean VA between those with (0.04±0.12 logMAR) and without (0.07±0.13 logMAR) foveal RPEE, $F(1,82)=0.98$, $p=0.33$, Figure 3.19.

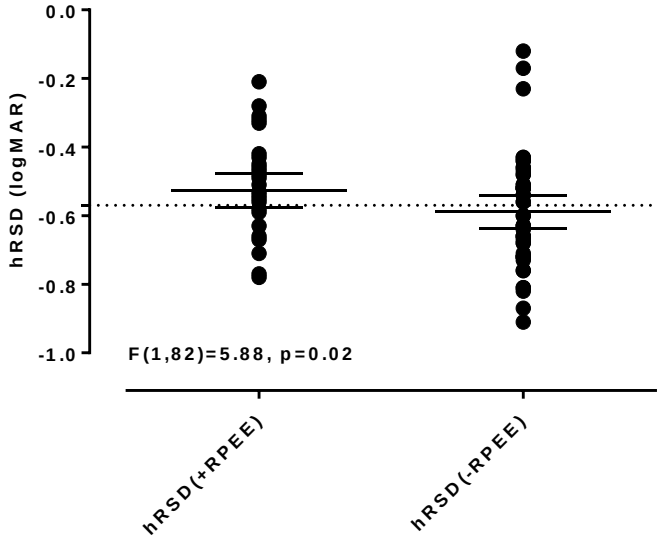

Figure 3.17 Mean hRSD (solid lines) ±95%CIs (error bars) for participants with and without foveal RPEE. Participants with at least one large RPEE had statistically significantly worse hRSD. For reference, dashed line indicates the overall mean hRSD of -0.57 logMAR for all participants.

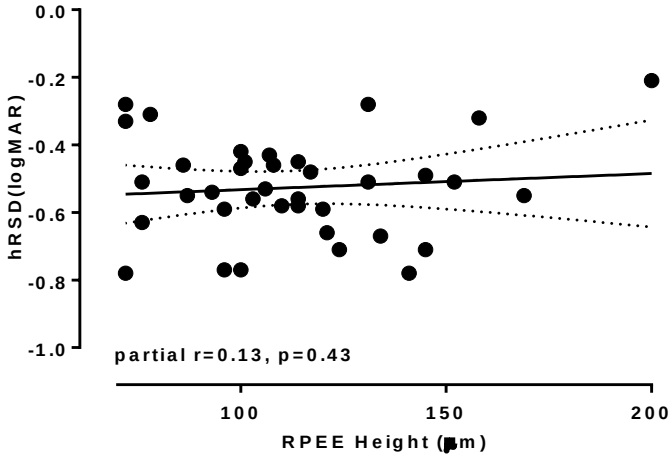

Figure 3.18 No statistically significant relationship seen between hRSD scores and maximum height of the RPEEs. Line represents the linear regression and its ±95%CI.

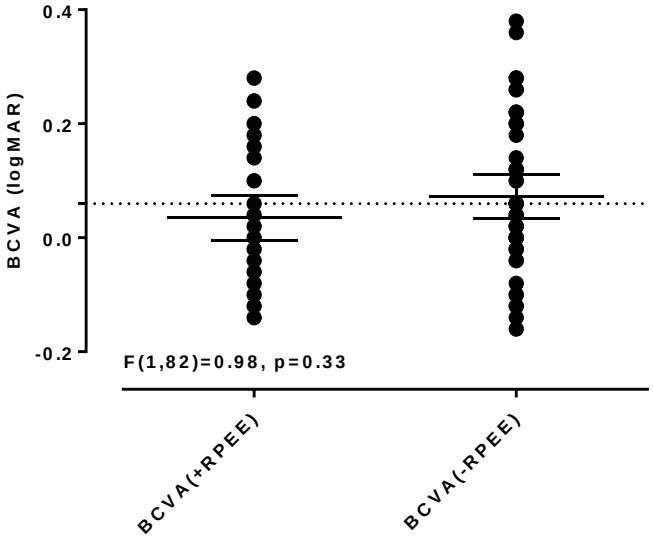

Figure 3.19 Mean VA (solid line) ±95%CIs (error bars) for participants with and without foveal RPEEs. The difference was not statistically significant. For reference, dashed line indicates the overall mean VA of 0.06±0.13 logMAR for all participants.

3.5.2.2 The hRSD test and Ellipsoid Zone Disruption (EZD)

The lack of hyperreflectivity to the EZ was assessed for all scans falling within the foveal area. This allowed reporting not only of whether the presence of EZD has a negative effect on hRSD scores, but also whether greater extent of foveal EZD is related to poorer scores (section 2.4.5). OCT scans in Figure 3.20 provide examples of abnormal EZ close to the fovea and how this was measured. In Figure 3.20A, there were two segments of EZ clearly missing, measuring 245 and 162µm in length. Figure 3.20B illustrates how the measurements of EZD were obtained when drusen were present as well. The calliper was used to measure the length of the scan with abnormal EZ (a linear measurement), cutting through the drusen.

Of the 85 SEs graded for EZD, seven (9%) showed an overall lack of hyperreflectivity of the EZ that prevented an assessment of its integrity. Therefore, 78 scans were included in the analysis. The seven participants whose data was not included in this analysis were older (77.2±7.6 vs 83.6±5.8 years, $t(83)=2.19$, $p=0.03$).

Participants whose EZ was completely normal ("-EZD", n=33) had a mean hRSD score of -0.62±0.15 (96%CI -0.67 to -0.57) logMAR. Those with disruption to the EZ (any extent, n=45) had a mean hRSD score of -0.53±0.16 (95%CI -0.58 to -0.47) logMAR. The mean difference of 0.09 logMAR (95%CI 0.16 to 0.02) was statistically significant, ANCOVA $F(1,75)=7.01$, $p=0.01$, partial $\eta^2=0.086$, Figure 3.21. Similarly to RPEEs, the effect size (partial eta squared, η^2) was very small, which means that EZD explained a small proportion of the total variance in hRSD scores.

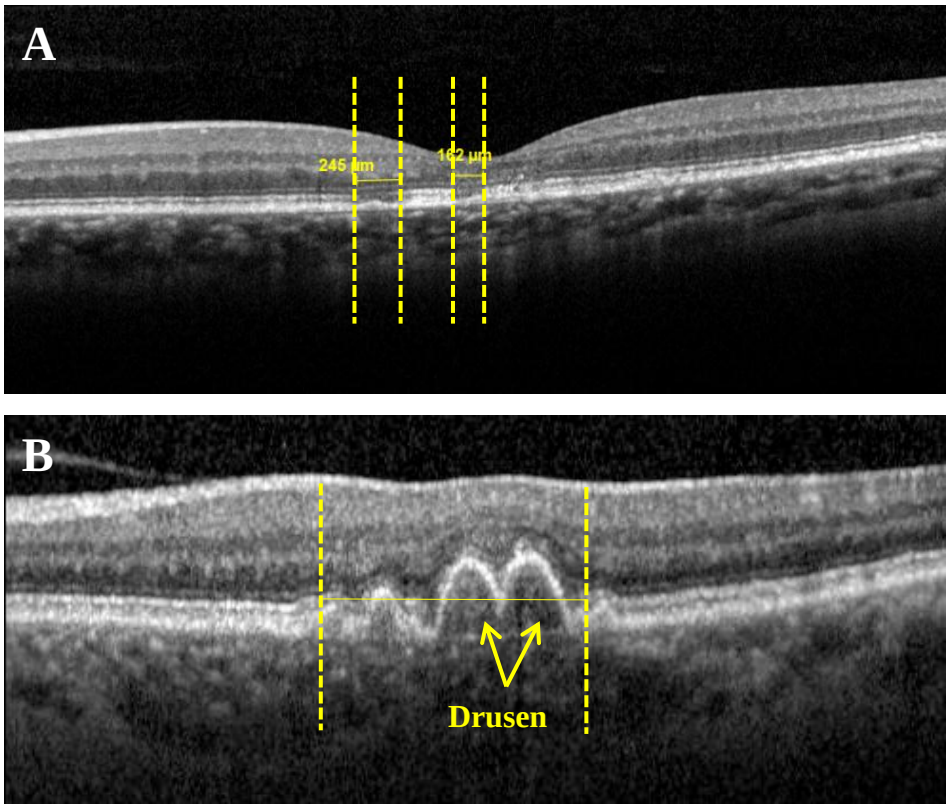

Figure 3.20 Examples of EZD demarcated by the dotted lines. A. Two segments of EZ clearly missing, measuring 245 and 162µm in length, B. Measurement of EZD when drusen were present. A linear measurement was obtained cutting through the drusen.

With regards to the extent of EZD, a partial Spearman correlation coefficient was calculated because the extent of EZD was not normally distributed (D'Agostino & Pearson p=0.0002). There was no statistically significant association between hRSD score and total foveal EZD when the effect of age on hRSD was accounted for (semi partial Spearman's r=0.26, p=0.087), Figure 3.22.

When the group comparison analysis was repeated for VA, there was no difference in VA between those without and with EZD (0.05±0.14 logMAR vs 0.55±0.12 logMAR respectively), F(1,75)=0.002, p=0.97, Figure 3.23.

Given the above results, the last part of this analysis was to determine whether a combination of both RPEE and EZD can explain more of the hRSD variability than each separately. For this, participants were grouped into three groups: "intact fovea", "RPEE/EZD only" and "combined RPEE+EZD". RPEE only and EZD only were grouped into the same category because there were only three participants with RPEE alone (without EZD). The result of the ANCOVA used to compare the three groups (with age as covariate) was statistically significant, F(2,74)= 4.368, p=0.016, partial η^2=0.106. Pairwise comparisons revealed that the only statistically significant difference among the groups was found between intact fovea and "combined RPEE+EZD". The mean difference was -0.11±95%CI -0.21 to -0.02, p=0.03, Figure 3.24.

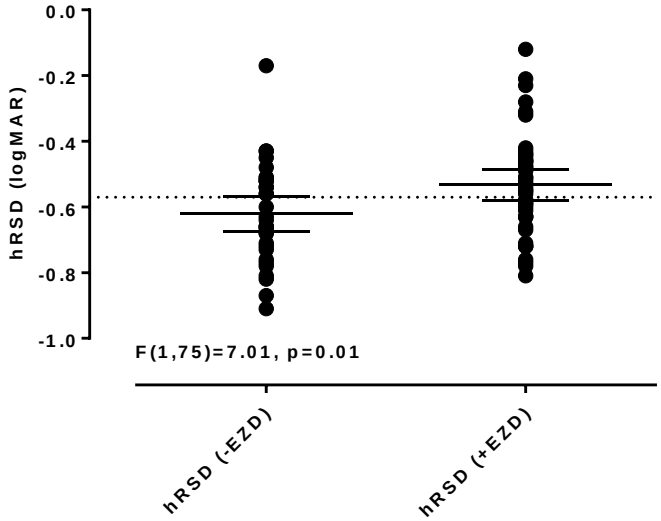

Figure 3.21 Mean hRSD (solid lines) ±95%CIs (error bars) in SEs with and without foveal EZD. SEs with EZD performed statistically significantly worse that those without EZD. For reference, dashed line indicates the overall mean hRSD of -0.57 logMAR for all participants.

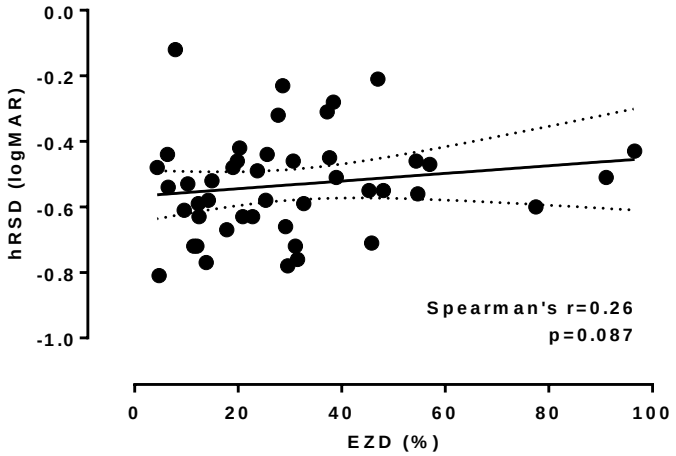

Figure 3.22 No relationship between hRSD scores and EZD extent. A partial Spearman's r was calculated due to the non-parametric distribution of the EZD data. Line indicates the linear regression line and its ±95%CIs.

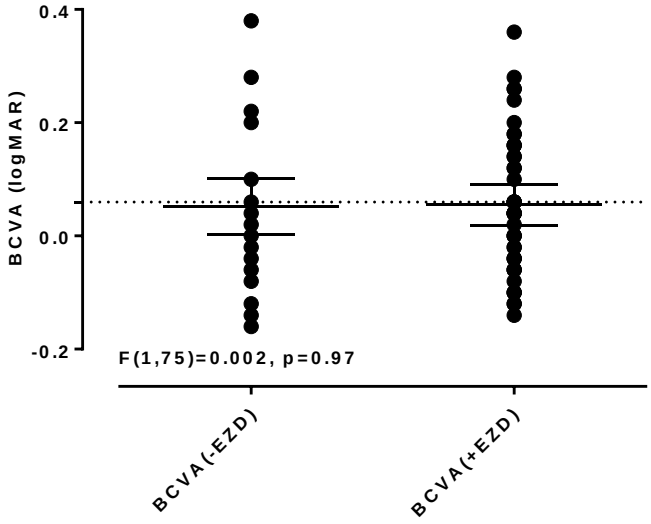

Figure 3.23 Mean VA (solid line) ±95%CIs (error bars) for participants with and without foveal EZD. The difference was not statistically significant. For reference, dashed line indicates the overall mean VA of 0.06±0.13 logMAR for all participants.

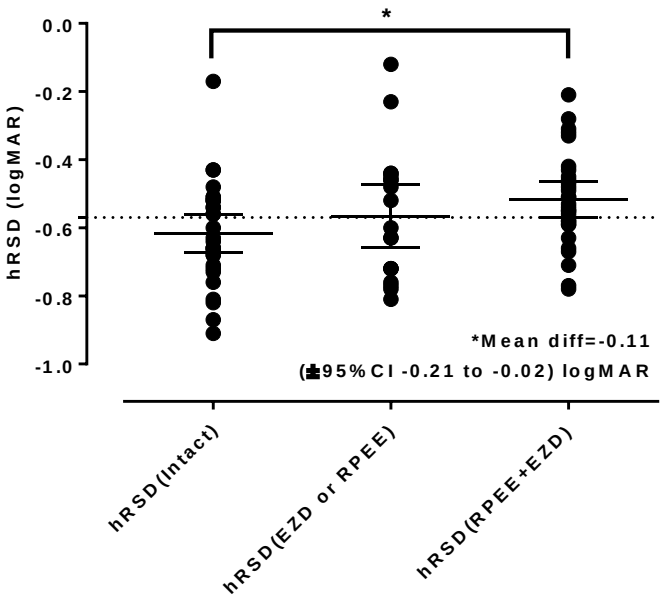

Figure 3.24 Mean hRSD (solid line) ±95%CIs (error bars) for three groups of participants (intact foveal area; presence of either RPEE or EZD alone; combination of both RPEE and EZD). Dashed line indicates the overall mean hRSD of -0.57 logMAR for all participants. Pairwise comparisons indicated that only the "intact fovea" and the "RPEE+EZD" groups were statistically different (*).

3.5.2.3 The hRSD test and foveal thickness

The central subfield thickness (CST) is an average of the foveal thickness at the foveal area, automatically calculated by the Heidelberg Spectralis software and measured from the internal limiting membrane to the posterior margin of the RPE.

All 85 SEs were included in the CST analysis. The mean±SD CST was 282±23 (95%CI 277 to 287) µm, ranging from a minimum of 192µm to a maximum of 362µm. Males had a slightly thicker fovea (289±23 vs 278±23µm, t(83)=-2.075, p=0.04).

A partial Spearman correlation coefficient was calculated to determine if there was an association between foveal thickness and hRSD, to account for the effect of age on hRSD and the skewed distribution seen in CST (D'Agostino & Pearson p=0.005). No statistically significant correlation was found between CST and hRSD (partial Spearman's r=-0.12, p=0.25, Figure 3.25).

Overall, the key message from the results in this section is that the presence of large RPEEs and EZD close to the fovea had a small, yet statistically significant effect on hRSD scores. Interestingly this effect was not seen for VA.

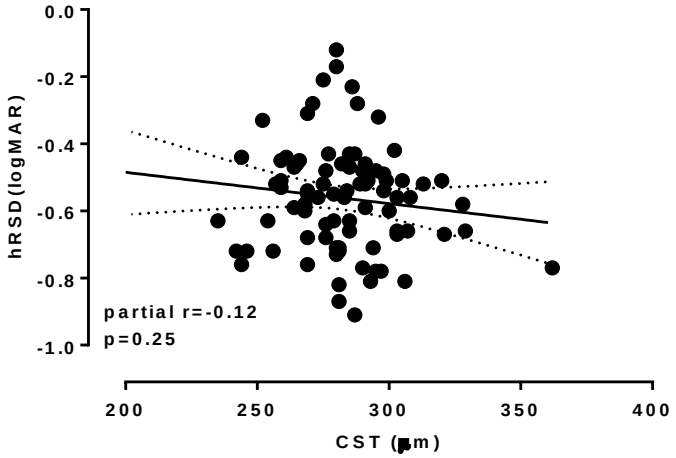

Figure 3.25 No relationship seen between hRSD scores and CST. A partial Spearman's r was calculated due to the non-parametric distribution of the CST data. Line indicates the linear regression line and its ±95%CIs.

3.6 Usability of the hRSD test

A usability questionnaire was given to participants after completion of the study. Due to the busy nature of the clinics, the pressures on time and room availability, only 72 participants completed the questionnaire.

Of all participants who completed the questionnaire, 37.5% had never used a touch screen electronic device like the iPod used for the hRSD test, with only 16.7% using one very often. Nevertheless, the majority thought that the test was generally "very easy" or "easy" to use (37.5% and 44.4% respectively). The remaining 16.7% and 1.4% thought that the test was difficult/ very difficult (Figure 3.26).

The majority of participants thought that the instructions were either "very easy" or "easy" to understand (66.7% and 31.9% respectively). Also, all participants thought that the device was "very easy" or "easy" to handle (75% and 25% respectively, Figure 3.27).

Question five asked whether participants would consider using the hRSD test at home to monitor their vision between clinical appointments. Only 8.5% of participants reported that they would not consider using it, with 78.9% willing to use it and 12.7% who could not decide. Finally, question six asked participants how often they would be willing to use the device from home (if they answered "yes" to question five). No indication was given as to the recommended frequency in order to obtain an unbiased answer. Only 19.6% of participants responded that they would use the hRSD test daily, 53.6% responded that they would use it on a weekly basis. Every fortnight and monthly was chosen by 14.3% and 12.5% of participants (Figure 3.28).

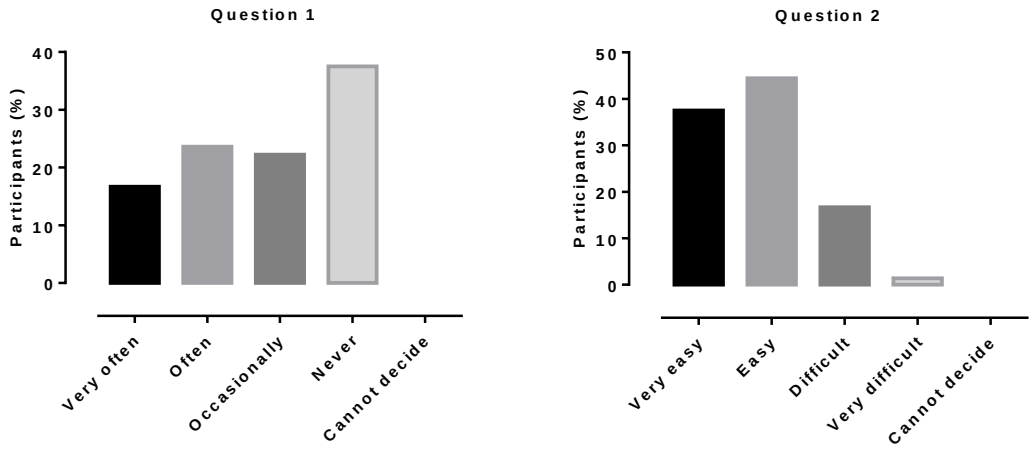

Figure 3.26 Answers to questionnaire given at the end of the study in percentages. Question 1: Before the study, how often had you used a touch screen hand-held device (such as phone, tablet or iPod)? Question 2: Generally, how easy was the test to do?

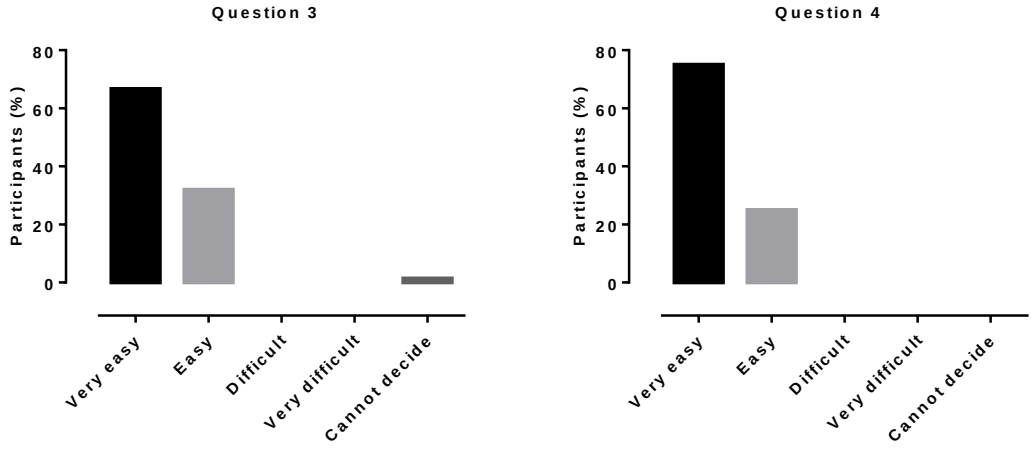

Figure 3.27 Answers to questionnaire given at the end of the study in percentages. Question 3: How easy were the instructions written on the screen to understand? Question 4: How easy was it to handle the device? (for example holding the iPod, pressing on the screen…).

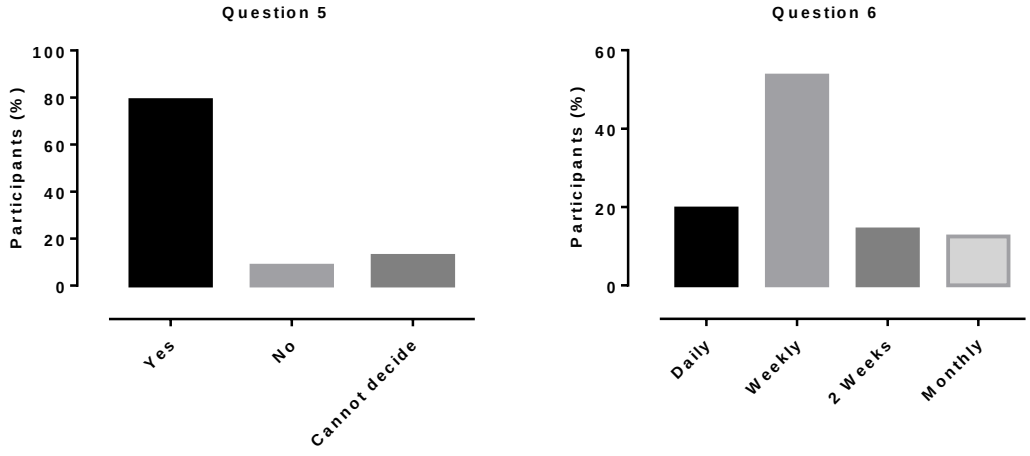

Figure 3.28 Answers to questionnaire given at the end of the study in percentages. Question 5: If you had this test at home, would you consider using it for self-monitoring your vision between clinic appointments? Question 6: If you answered Yes to Question 5. How often would you consider doing the test at home?

3.7 Radial shape discrimination in fellow eyes

Although it was not the main focus of this study, one last piece of analysis was carried out for the fellow eyes (FEs) to highlight the difference in hRSD scores between the two eyes.

The hRSD test results at baseline were normally distributed for the SE (D'Agostino & Pearson $p>0.05$) but not for the FE ($p<0.0001$, Figure 3.29). Consequently non-parametric tests were used (Kruskal-Wallis to compare group mean among lesion types and the Wilcoxon matched-pairs signed rank to compare RSD between SEs and FEs). Summary statistics are also reported as means and standard deviations to allow for comparison.

The hRSD test was always attempted for both eyes however there were twelve participants (14%) who were not able to complete the hRSD test with their fellow (nAMD) eye because they had very poor vision or distortions that prevented them from completing the test. The VA of the FEs of those participants who could not complete the hRSD test was 0.8 (IQR 0.67 to 1.02) logMAR (mean ±SD: 0.84±0.28 logMAR).

Recruited participants had been coming to the AMD clinic to receive treatment to their nAMD eye for a mean period of 1.5 years (ranging from 0 to 5.8 years). At commencement of treatment, 35 participants (41%) had a classic CNV lesion, 35 (41%) had occult CNV, eleven (13%) had a RAP lesion, three (4%) had IPCV and the lesion type was unavailable for one participant. There was no significant difference in hRSD score among lesion types, $H(3)=1.23$, $p=0.75$.

Those who managed to complete the hRSD test with their FE had a median hRSD score of -0.22 (IQR -0.41 to -0.07) logMAR (mean±SD: -0.18±0.34 logMAR). This contrasts with a median of -0.56 (IQR -0.48 to -0.47) logMAR in the SEs of these participants (Figure 3.30A). The median paired difference of 0.39 (±96.56%CI 0.22 to 0.51) logMAR was statistically significant (Wilcoxon test, $p<0.0001$, Figure 3.30 B). Unsurprisingly the VA of FEs at baseline was also statistically different from the VA in SEs ($p<0.0001$, Figure 3.30 C). The median paired difference was 0.26 (±95.25%CI 0.18 to 0.36) logMAR (Figure 3.30D).

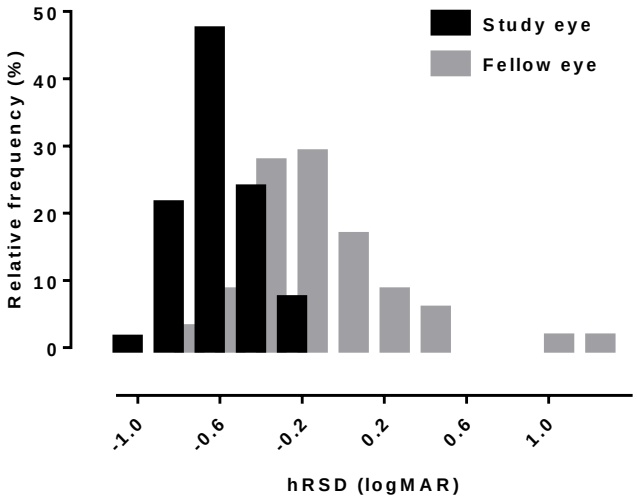

Figure 3.29 Relative frequency histogram of hRSD scores in SEs (black) and FEs (grey). The SEs showed a normal distribution whilst the distribution of the FEs was slightly skewed ($p<0.0001$). The hRSD data in FEs was more widely spread, ranging from normal scores to positive scores (very poor).

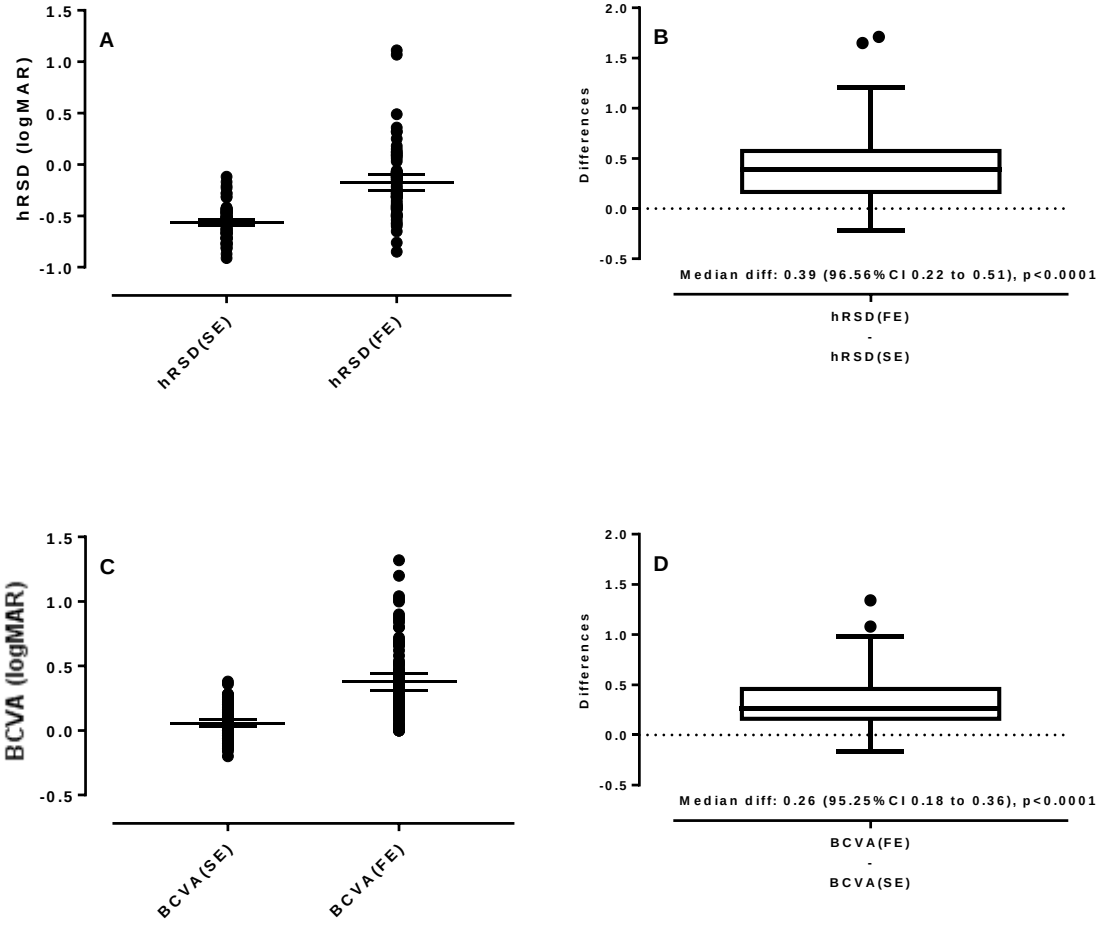

Figure 3.30 Aligned dot plots and Tukey plots showing the paired differences in hRSD (A, B) and VA (C, D) between study and fellow eyes. Dashed line in Tukey plots indicates zero difference. The median differences (calculated as FE minus SE) fall above zero, which indicates that the FEs had more positive (i.e. worse) scores than the SEs. Solid line indicates the median difference, the limits of the boxes indicate the 25th and 75th percentiles and the whiskers indicate the 75th percentile plus 1.5 times IQR (as defined by Tukey's method).

The correlation between study and fellow eyes was assessed by means of an intraclass correlation coefficient (ICC), to obtain a measure of the relationship between paired measurements from the right and left eyes of the same participant. The ICC (one-way random) was not significant (ICC -0.31, p=0.997, Figure 3.31).

The mean VA of FEs was 0.31±0.24 logMAR. As opposed to the SEs, there was no limit to the VA of the FE in the inclusion criteria, which means that VA in FEs ranged from 0.00 to 1.04 logMAR. The relationship between hRSD and VA in FEs was calculated using a partial correlation, instead of a non-parametric partial rank correlation. This was done because SPSS does not give the option for a partial rank correlation. A statistically significant correlation was found between hRSD and VA of FEs after the effect of age was removed (partial r=0.446, p<0.0001, Figure 3.32). As mentioned above, twelve participants could not perform the test with the FE due to visual distortions and scotomas. These participants, who would have scored very poorly on the hRSD test, also had a very poor VA (mean VA of 0.84±0.28 logMAR), which means that including these participants would have increased the strength of the correlation. In order to confirm this result by means of a non-parametric test, a semi partial correlation was done between hRSD and age to create a residual variable which was age adjusted. A Spearman correlation coefficient was then calculated between this residual variable and VA. The results agreed with the above parametric correlation (semi partial Spearman's r=0.43, p<0.01).

Of the 34 participants who had measurements of CS, seven could not perform the hRSD with their FE due to impaired vision. The mean CS of these seven FEs was 1.16±0.33 logCS, lower than the mean CS of the whole sample (1.63±0.23 logCS). The association between hRSD and CS in FEs was just short of significance when calculated as a partial correlation (partial r=-0.39, p=0.051, Figure 3.33) but it was statistically significant when calculated as a semi-partial Spearman correlation (semi-partial Spearman r=-0.36, p=0.038).

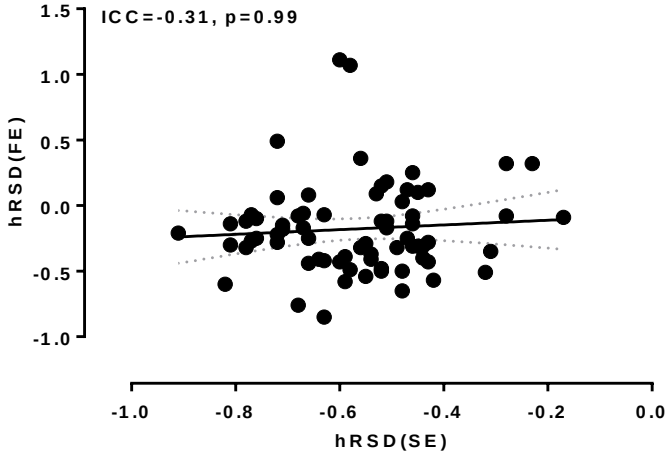

Figure 3.31 No relationship between hRSD scores in study and fellow eyes of the same participants. Solid line indicates the linear regression line and its ±95%CIs. ICC=Intraclass correlation coefficient.

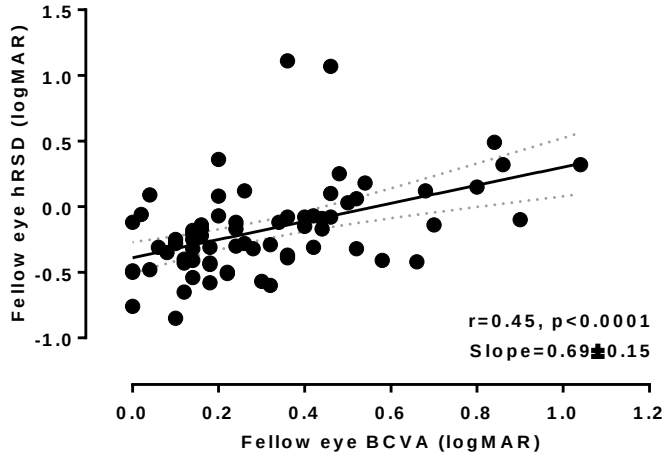

Figure 3.32 Moderate statistically significant relationship between hRSD scores and VA in FEs. Solid line indicates the linear regression line and its ±95%CI.

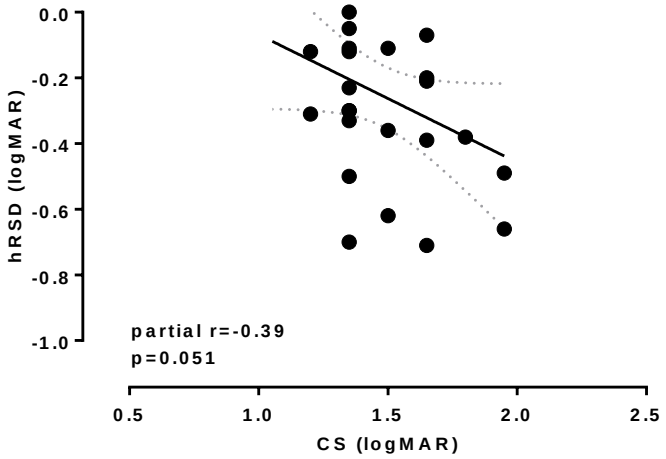

Figure 3.33 Non conclusive result on the relationship between hRSD scores and CS in FEs, as the correlation was not significant when calculated as a partial correlation (partial r=-0.39, p=0.051) but it was statistically significant when calculated as a semi-partial Spearman correlation (semi-partial Spearman r=-0.36, p=0.038).

3.8 Summary of results

The results reported in this thesis apply exclusively to participants at risk of nAMD, excluding other maculopathies such as diabetic macular oedema. GA, the much more prevalent type of late AMD, was also excluded. The results include a description of the flow of participants and the baseline characteristics of included and excluded participants, which maximises transparency and allows for better interpretation of the results (Bossuyt et al. 2015).

Having in mind the target population that would benefit from using the hRSD test, the effect of age and presbyopia were studied. A significant correlation was found between older age and hRSD scores, therefore subsequent analysis was done using age as a covariate. Results from the presbyopia experiment showed that the lack of near addition can result in a significant worsening in hRSD scores.

The repeatability of the hRSD test was thoroughly investigated and several coefficients were reported. This is to date the first study to report the repeatability of the hRSD test in participants at risk of nAMD. This analysis not only will help differentiate clinically significant changes from measurement variability for the hRSD test but it provides with dimensionless repeatability coefficients for the comparison with other visual function tests studied elsewhere. The stability of the hRSD test over time was assessed over a period of approximate six months for each individual participant. Overall, clinically stable participants showed stable hRSD results over time, with only seven cases (8%) showing statistically significant changes that were not explained by changes in the retina.

These results also described the relationship between hRSD, VA and CS, the most frequently used clinical tests to measure function in AMD clinics. There was no association between VA/CS and RSD.

OCT analysis sought to establish whether specific OCT features could be related to hRSD test results. While there was no relationship between hRSD and measurements of central foveal thickness, there was a statistically significant difference in hRSD scores between those with and without large RPEEs, as well as with and without EZD.

The usability questionnaire revealed a very positive response to the hRSD test, with nearly all participants pleased with the test and the device. When participants were asked how often they would use the test at home, the majority responded that they would happily use it once a week.

There was a significant difference in hRSD scores between study and fellow eyes. The median difference was larger than that seen for VA (0.39 vs 0.26 logMAR), suggesting a larger functional deficit in the treated eye.

Overall, the results presented above provide useful information about the performance of the hRSD test in an elderly clinical population at risk of developing nAMD. The following section discusses the clinical significance of the above findings and the similarities and discrepancies to the previous literature.

Chapter 4 Discussion

Having reviewed the importance of early detection and prompt treatment of nAMD (section 1.1.4) and the potential of the hRSD test for measuring visual function in AMD (section 1.2.3), there is a clear possibility that the hRSD test could have a role in the early detection of nAMD. At present, however, there is a lack of published data around the performance of the test, especially in a key population at high risk of developing nAMD.

The main complaint from patients who develop nAMD is the sudden appearance of visual distortions. Therefore, the AG (section 1.1.5) is often used for the detection of symptoms caused by nAMD. Figure 4.1A provides an illustration of the AG in the presence of unilateral nAMD (left eye). To produce this illustration, one of the participants taking part in this study covered each eye in turn. The affected eye was used to observe the image (unaffected eye occluded) and the unaffected eye was used to draw the illustration of the distorted image (affected eye occluded). Studies have shown that the AG lacks sensitivity for the detection of new nAMD (section 1.1.5) which means that many patients developing nAMD might not be detected soon after nAMD develops, when they would benefit most from treatment (section 1.1.4).

The hRSD test is one of the alternative tests that have been developed to prompt early detection of nAMD (section 1.2.1). Theoretically, optimal processing of the RF patterns requires the initial retinal image to be formed on a structurally and functionally intact photoreceptor mosaic. Retinal pathology that disrupts the organisational integrity of the photoreceptors mosaic would affect the global processing of the RF pattern (Wang et al. 2002). Figure 4.1B shows an illustration of the RF patterns in the hRSD test as seen by a study participant with nAMD in the left eye. Previous studies assessing the hRSD test focused on healthy participants (Ku et al. 2016) or a mixture of AMD and diabetic participants (Wang et al. 2013). The results from the present study provide important data on the performance of the hRSD test in a clinical sample of “at risk” eyes of participants who already had nAMD in their fellow eye. The sample collected was relatively large and the performance of the hRSD test in this tightly defined group was studied in great detail, cross-sectionally and longitudinally. The eyes included in this study had

retinal changes resulting from ageing and early AMD lesions, such as drusen and pigmentary changes but showed no clinically significant progression during the length of the study. This type of eye would benefit the most from using the hRSD test for the early detection of nAMD, as they are at the greatest risk. This is also a key population in which clinical decisions are being made on a daily basis. Firstly, this study established the average hRSD scores, the intersession test-retest repeatability and the stability of the test over time in clinically stable patients. Secondly, the relationship between hRSD test scores and other clinical measurements of function and structure were explored. Finally, the usability of the test was assessed by means of a short questionnaire.

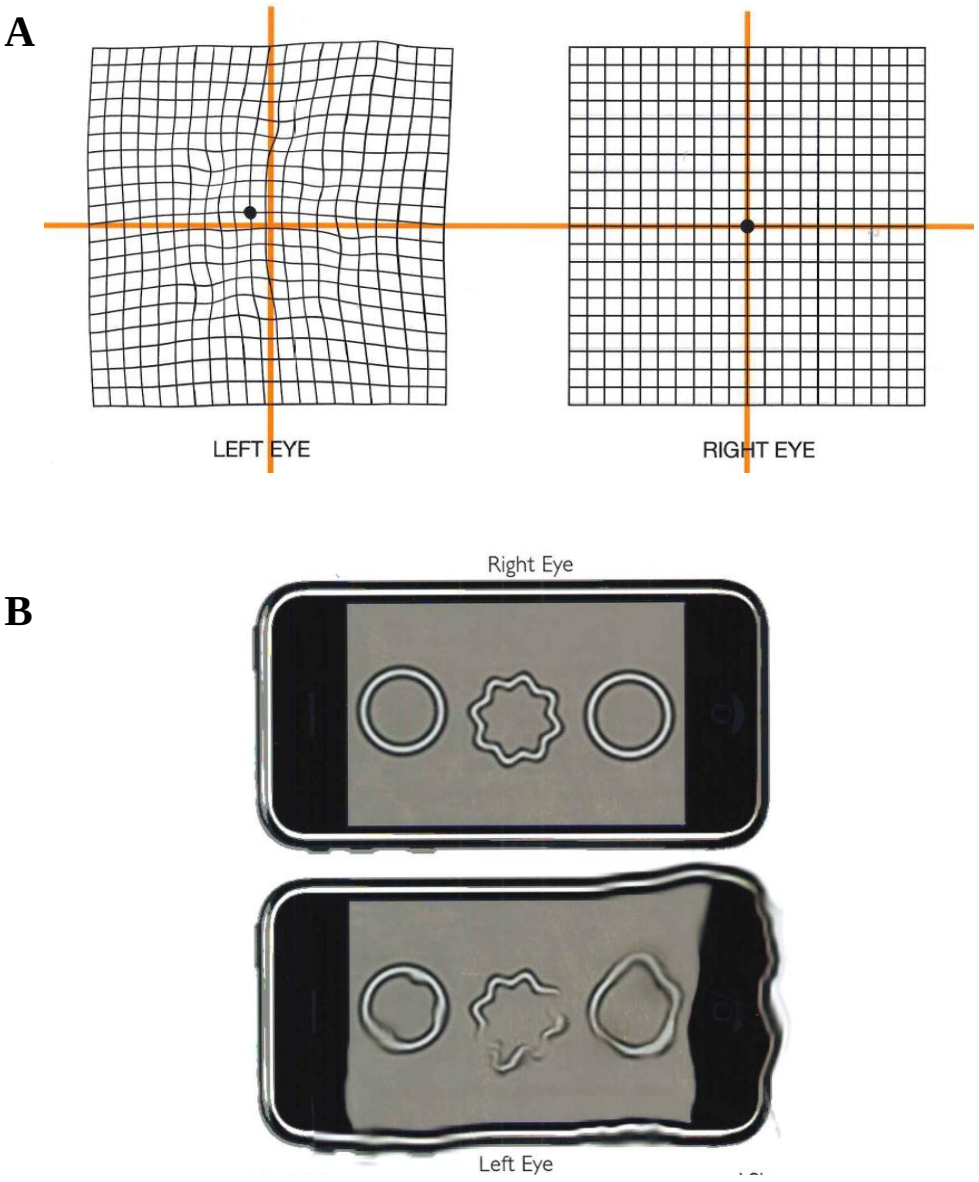

Figure 4.1 Illustration of the Amsler grid (A) and the hRSD test (B) produced by a participant taking part in the present study with unilateral left nAMD. The illustration of the left eye was carried out by observing the object with the left eye (good eye occluded) and drawing the image using the right eye (affected eye occluded). Permission was obtained from the participant to use this illustration in this thesis.

4.1 The study population

The participants included in this study already had nAMD in one eye and were therefore at risk of developing it in their second eye (the study eye or "high risk" eye). This idea of using the non-affected eye of patients with unilateral nAMD has been used in previous studies (Do et al. 2012, Aslam et al. 2014) because patients are already attending clinics for assessment and treatment of their first eye, providing an easily accessible population for investigating new tests. Their unaffected eye is being monitored due to the strong evidence suggesting that second eyes are at a high risk of developing nAMD (section 1.1.2). Caution should be used, however, when interpreting the results from this study for a population that has two at-risk eyes (i.e. without unilateral nAMD) as the extent to which uniocular treatment can affect the fellow (non-nAMD) eye has not been established. An increased risk of GA with anti-VEGF treatment was suggested by the CATT trial (Grunwald et al. 2014); however this referred to the treated eye. A protective effect of anti-VEGF treatment for the fellow untreated eye (i.e. a reduction in the likelihood of developing nAMD in the second eye at 2 years) was not found either (Barbazetto et al. 2010).

There were relatively few exclusion criteria adopted in this study. For example, participants were not excluded if they had cataracts or glaucoma, two common ocular conditions among older adults (Evans et al. 2004). This was deliberate; it means that the sample of participants is likely to be a fair representation of the general population at risk of developing nAMD in their second eye and that the results are likely to be applicable to what is currently a large population in the UK. All participants who met the criteria were included, regardless of their previous experience or abilities using mobile phone technologies, as opposed to only including participants who could use the device or who could pass a device tutorial as was done in many studies assessing the PHP test (section 1.1.5). Again, the advantage of not excluding such participants is that the sample is more representative of the general elderly population, many of whom might have never used a smart phone or a tablet before.

4.1.1 Comparative RSD scores across studies

RSD is a hyperacuity, which by definition means that the scores obtained with RSD tests are smaller (i.e. more negative) than those observed for resolution acuity. The limit of resolution acuity expected for a normal healthy eye is around -0.1 to 0.00 logMAR (Elliott et al. 1995), whereas the average RSD threshold (using the 3AFC handheld version of the test) in healthy participants is -0.77±14 logMAR (n=186, mean age=42±17 years, Ku et al. 2016). Compared to participants with healthy vision, elderly participants with AMD are expected to have worse visual function. The only study to report RSD results in the logMAR scale, using the handheld version of the test, for patients with AMD was Wang et al. (2013), who reported a mean test result of -0.67 and -0.37 logMAR for eyes with early and intermediate AMD respectively (from Figure 5 in paper). Whilst the early AMD group had nearly normal hRSD score, the mean hRSD of the group with intermediate AMD was considerably lower. The mean hRSD test results found in the present study was -0.56±0.16 logMAR which, considering that SEs had a broad range of lesions (from no AMD to intermediate AMD), seems in accordance with Wang's results. The average hRSD scores so far reported in the literature are plotted in Figure 4.2. In eyes with late AMD (GA or nAMD), the presence of active neovascularisation, scar tissue or atrophy can severely disrupt the retinal structure leading to poor vision. The fellow eyes of participants included in this study (i.e. those being treated for nAMD) generated hRSD scores that were very close to those reported by Wang et al. (2013) for their "late AMD" group, which included both nAMD and GA (-0.18 and -0.1 logMAR). Both of these means were considerably lower than the means for the rest of the groups (healthy, early AMD, intermediate AMD and our SEs, Figure 4.2). Figure 4.2 also shows the cut-off value of -0.37 logMAR suggested for the differentiation between intermediate AMD and nAMD (Wang et al. 2011), with our treated eyes accordingly falling above the line and our SEs falling below the line.

Overall, the mean hRSD test results found in the present study are consistent with previous studies that used the handheld version of the test. A definite trend is seen where healthy eyes have better test scores than eyes with intermediate AMD, which in turn score better than eyes with late AMD.

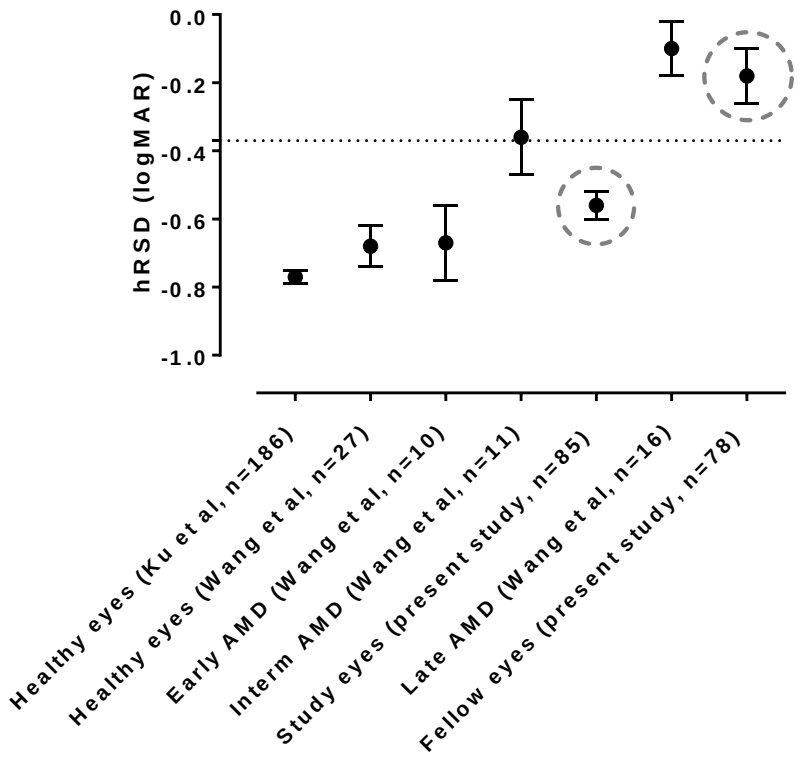

Figure 4.2 Summary of hRSD test results for different participant groups from Ku et al. (2016), Wang et al. (2013) and the present study. Note that mean and error bars from Wang et al. were approximated from Figure 5 in their paper. Error bars represent the ±95%CI. Dotted line indicates the -0.37 logMAR suggested to be an appropriate cut-off value for differentiating intermediate AMD from late AMD (Wang et al. 2011).

4.2 The hRSD test in older age

After the fifth decade of life, changes occur to the optics of the eye and transparency of the ocular media which can result in degraded vision. VA and CS are known to be particularly susceptible to age-related changes (Owsley et al. 1983, Elliott et al. 1995). Furthermore, early AMD can accelerate the deterioration of vision compared to healthy ageing, as it is the case for VA (Sjöstrand et al. 2011). Three studies have shown that, overall, the detection of deformations on RF patterns is relatively stable regardless of the effects of ageing in the eye (Ku et al. 2016, Wang et al. 2009, Wang 2001, section 1.2.2), which suggest that the hRSD test could be useful at detecting retinal pathology.

Whilst previous studies on healthy participants included a wide age range (Wang et al. 2009, Ku et al. 2016, Wang 2001), the present study only included participants who were older than 50 years (as AMD is an age-related pathology). Finding older participants with completely healthy vision is a difficult task as age-related maculopathy is a prevalent condition amongst older adults (Augood et al. 2006), so Wang et al. (2009) recruited participants up to the age of 78 years only and Ku et al. (2016) managed to include a group of 16 eyes with no ocular pathology (confirmed with OCT) who were 64 to 90 years of age. Our participants were considerably older, with an average age of 77 years and 46% of them being older than 80 years. Although the SEs did not have the target condition (nAMD), they cannot be considered healthy either, as early/intermediate AMD changes were likely to be present such as drusen and pigmentary changes. The analysis of age on hRSD test results showed that older participants performed significantly worse than younger ones (section 3.2). The rate of deterioration (i.e. the slope of the linear regression line) was 0.0076 which translates into an increase (worsening) in hRSD scores of 0.076 logMAR per decade of life. This increase is substantially greater (double) than that previously reported for a group of healthy participants older than 55 years, where a rate of worsening of 0.0035 logMAR per decade was seen (Wang et al. 2009). Both studies (present study and Wang et al. 2009) used linear regression analysis to assess the effect of age on hRSD scores, however the linear function in Wang et al. (2009) was part of a mathematical model (section 1.2.2). The difference in slope is not surprising since the participants included in the present study were

older and had a SE that was at high risk of nAMD as opposed to being healthy controls.

Interestingly, RSD worsened at a very similar rate to VA in SEs (0.06 logMAR per decade), as the regression slopes were not statistically different from each other (Figure 3.4). The slope found for VA was very close to that found by Wang et al. (2009) for healthy participants (0.058 logMAR per decade). What this result indicates is that RSD worsened with age at a faster rate in our sample of AMD eyes than previously reported for healthy aged eyes. Meanwhile, VA worsened with age at a similar rate as previously reported for healthy aged eyes. With RSD deteriorating more rapidly with age, a worse RSD score can be expected of older eyes with AMD changes compared to healthy eyes. On the contrary, with VA deteriorating at a similar rate between healthy and AMD eyes, a poor VA result could not distinguish a healthy eye from an eye with AMD. This results support the suggestion that the hRSD test might be a better test at detecting AMD related retinal changes.

Due to the moderate, statistically significant association between older age and hRSD scores ($r=0.37$, $p=0.0005$), the analysis reported in this thesis was adjusted for the effect of the participant age. An interesting issue originating from the analysis of the relationship between CS and hRSD was the reversal of results when age was not adjusted for. Whilst no relationship was seen with age used as a covariate, a statistically significant relationship was seen when age was not accounted for. This will be further discussed in section 4.4.1.

4.2.1 Presbyopia

Because the hRSD test is held at arm's length, approximately at the reading distance, it was suspected that the hRSD test scores would be affected by uncorrected presbyopia.

Starting at around the age of 40, presbyopia occurs as a result of age-related changes to the accommodative system of the eye. As the lens progressively loses its elasticity and with it, its ability to change shape, focusing on objects located close to the eye becomes increasingly difficult. As a result an optical correction is required in which convex lenses are added to the distance prescription, a "near addition" in order to

correct the defocus. As a minimum age of 50 years was an inclusion criterion, all the study participants needed some degree of near addition.

Optical defocus, such as that caused by the lack of near addition, causes a reduction in the contrast of the image formed on the retina, which mainly affects high spatial frequencies (Norton et al. 2002), i.e. small letter print. It could be hypothesised that the hRSD test, which has a low peak spatial frequency of 3cpd (Wang et al. 2013), might not be dramatically affected by optical defocus. A previous study suggested that hRSD thresholds were only slightly affected by small amounts of optical defocus (Knox et al. 2014) in healthy adults without macular disease. This study used convex lenses to create a myopic shift in refraction (image formed in front of the retina) of approximately one dioptre. The type of blur experienced with presbyopia is different to the type of artificial blur created by Knox et al., as the defocus is larger (up to three dioptres) and in a hyperopic direction (image formed behind the retina). This difference is important, as hyperopic/presbyopic refractive errors affect vision close up whereas myopic refractive errors affect vision in the distance.

As the effect of optical defocus caused by uncorrected presbyopia on the hRSD test was unknown this was specifically investigated for a subgroup of participants. The results (section 3.2) showed that the blur on the RF patterns experienced by uncorrected presbyopic participants with some degree of macular disease can have a significant impact on shape discrimination performance. For this experiment the same group of participants were tested with and without the appropriate near addition in the same session in a random order. A statistically significant worsening in the mean hRSD score was seen when the near addition was removed. Similarly to Knox et al. (2014), 70% of participants experienced worsening of hRSD test scores in conditions of blur (when near addition was not used). However, the mean change in hRSD score was 0.25±0.16 logMAR in the present study, which is larger than the 0.18±0.14 logMAR difference seen in Knox et al. (2014). The worsening in hRSD scores seen when presbyopia was not corrected for was not only statistically but clinically significant.

The practical importance of this experiment is that it demonstrated that reading glasses should be worn whilst using the hRSD test, even though the stimuli have relatively low spatial frequency. Although this might sound obvious, many patients

might feel that reading glasses are not needed as they can easily detect the targets with just their distance prescription; however they should be instructed to use their reading glasses to use the test. Likewise, patients using varifocal/bifocal lenses (with the optical correction for near and distance on the same lens, distance at the top, near at the bottom) should be instructed to look through the near section whilst performing the hRSD test. The issue around glasses choice whilst using the hRSD test had not been previously discussed in the literature; however it needed clarification as subjects at risk of AMD are all in presbyopic age.

Another practical consideration relates to the scenario where the hRSD test is used to prompt early detection of nAMD. In this scenario, the hRSD test would be used in an unsupervised manner, potentially in the patient's home. The results from this study suggest that not using a near addition to correct presbyopia could lead to an increase in false positive results. This was calculated using the cut-off value of -0.37 logMAR suggested by Wang et al. to differentiate between non-nAMD and nAMD (Wang et al. 2011). In our sample, 30% of the participants fell above the cut-off value of -0.37, implying a false positive result since as a matter of design, the participants in this study did not have nAMD in the SE. The percentage of participants falling above this cut-off would increase by 25% if near addition was not used. This is clinically important, as it means that in 1 in 4 of those who failed the test could have failed due to the lack of near addition and not the presence of nAMD.

In summary, hRSD test scores increased significantly with age in the non-affected eye of participants with unilateral nAMD, which contrasts with previous reports on healthy eyes. This means that age must be taken into account when interpreting the results of the hRSD test in patients with early stages of AMD and that future studies assessing the hRSD test in AMD should account for the effect of age in the analysis. Furthermore, it is very important that patients are carefully instructed to wear the correct spectacles (the reading correction) when using the test, as not doing so could have a negative effect in their hRSD score.

4.3 The hRSD test reliability

There are several important aspects to consider around the clinical application of the hRSD test, one of which is the reliability of the test. Reliability is not a fixed property of a test, but a result of interactions between the examiner, the test, the environment and the process of testing (Kottner et al. 2011). Therefore, it is important to establish whether the hRSD test can perform consistently over time and across different circumstances. A test which exhibits high variability that is clearly not related to the state of the retina might not be useful clinically.

Since the hRSD test seeks to detect visual changes related to disturbances in the macular area of the retina as might be caused by nAMD, it might be necessary to use the test a number of times in the absence of nAMD until CNV develops (as opposed to just the one off test). Two key properties of a good test used in this fashion would be to a) perform consistently in those without disease progression and b) to have enough sensitivity to detect the development of new disease. In this study the former was investigated by assessing the repeatability and stability of the hRSD over a period of time in eyes at risk of developing nAMD. These two types of variability were examined separately because they provide different information about the reliability of the test. On one hand, the test-retest repeatability shows the extent of random change (noise) in scores from measure to measure. On the other hand, examining the stability of the test results over time allows the identification of patterns such as those expected from learning or training. Whilst the test-retest analysis was performed by means of an analysis of variance (ANOVA) and Bland-Altman analysis, the stability over time analysis examined the pattern across individual regression lines of hRSD scores over time (section 3.3).

4.3.1 Test-retest repeatability

Knowledge of reliability of visual function tests is important in order to clinically interpret any fluctuations in vision of a particular individual. There are several areas of ophthalmology where visual function is measured longitudinally. An example of this is the use of visual field tests every 6-12 months to monitor progression of glaucoma patients. In AMD, longitudinal measurements of VA are collected to

monitor the treatment of nAMD, with appointments being as frequent as one month apart. The AG, often used from the home, provides longitudinal monitoring if not quite measurement of high risk eyes (section 1.1.5). If the hRSD test was to be used from home to monitor high risk eyes (Kaiser et al. 2013, Wang et al. 2014) longitudinal quantifiable measurements would be obtained, potentially as frequently as twice a week. A key aspect of test performance would be a low variability of test results in the absence of a change in retinal state.

Currently, only one study provides some insight into the variability of the hRSD test in patients with maculopathy. The study (n=35 eyes) reported an average standard deviation of hRSD measurements of 0.098±0.025 logMAR for a period of over six months (Wang et al. 2014). This result must be interpreted with caution, as only nine participants had AMD (various degrees) and the rest had DR. A breakdown of repeatability for each condition was not provided. Diabetic patients tend to be younger and potentially more familiar with electronic devices and have different retinal changes to those seen in AMD. This study was only reported as a conference abstract, with limited information on the methods and analysis. No studies to date have adequately reported the intersession repeatability of the hRSD test in AMD patients, highlighting the importance of the present study. The guidelines followed in this study (Kottner et al. 2011) recommend including a large, representative sample of participants and a detailed description of the methodology in order to provide enough information to allow accurate interpretation of the results.

The results presented in this thesis include the intersession test-retest repeatability of the hRSD test, calculated using the Bland Altman method. In this method the mean difference between two measurements is estimated along with the limits of agreement, defined as the maximum range of measurements where 95% of the differences between two test results for the same person are expected. A test result outside this range would be considered abnormal. Overall, the hRSD test showed moderate intersession repeatability, however the bias and limits of agreement found with the Bland-Altman method (bias: -0.0004, LoA: -0.28 to 0.27 logMAR) were very similar to the bias and limits of agreement found when the intersession test-retest repeatability was calculated for healthy, younger adults without known retinal pathology (Knox et al. 2014). For the healthy participants, the limits of agreement for two hRSD tests performed some time apart (18 subjects, age 38±16,

mean of 72 days between tests, from 10 to 169 days) were -0.27 to 0.26 logMAR (Knox et al. 2014). This is an important finding, as retinal disease can sometimes negatively affect the repeatability of measurements of vision. For instance, the expected variability in VA measurements for visually normal individuals is one logMAR line (Lovie-Kitchin and Brown 2000) whereas eyes with small or intermediate drusen but no late AMD have a twice as large intersession coefficient of repeatability (9-10 letters or 2 logMAR lines, Patel et al. 2008).

The longitudinal design of the present study allowed the calculation of re-test repeatability over more than two time points. Taking data from five time points over approximately six months, an intra-subject standard deviation was calculated by means of a repeated-measures ANOVA in order to account for measurements coming from the same individual, which are usually more correlated than measurements coming from independent subjects. The within subject standard deviation was 0.12 (±95%CI 0.11 to 0.13) logMAR, slightly higher than previously reported (0.098 logMAR, Wang et al. 2014). In the present study, the within subject standard deviation was used to calculate the 95% coefficient of repeatability (CR). The CR (0.33 logMAR, for five study visits) confirmed the results from the Bland Altman limits of agreement (for two visits), as it was only slightly greater than the upper and lower limits of agreement. The slight difference could be due to the greater number of tests included. In view of both the limits of agreement and the CR, a change of 0.3 logMAR or more is unlikely to be related to measurement variability and should be considered as a clinically significant change for eyes at high risk of developing nAMD. This cut-off is greater than that recommended by Wang et al. (2012) for younger eyes with diabetic retinopathy (0.2 logMAR, information available from the poster presented at ARVO 2012).

The repeatability of the hRSD test was then compared to that of VA. The repeatability of VA found in this study (CR: 0.19 logMAR or 9.5 letters) agrees with a previous study that included eyes with early AMD (CR: 8.9 letters, Patel et al. 2008) and was slightly better than the repeatability reported in another study which assessed the fellow eye of nAMD patients (CR: 14 letters, Aslam et al. 2014). This might be due to the fact that, unlike the study by Aslam et al. (2014), our VA assessors were not masked. Nurses and optometrists testing VA were aware of the previous measurements, potentially encouraging patients to achieve at least previous

measurements of VA. The repeatability of RSD was compared to the repeatability of VA by means of an ICC, which confirmed that the hRSD test was less repeatable than VA (ICC 0.54 vs. 0.73) in our SEs. The reason for not comparing the coefficients of repeatability directly to each other was that although both tests measure its stimuli in angular size and are recorded as logMAR values, there is a difference in the nature of the tasks. Whilst VA is defined by the smallest visual angle that allows resolution of a letter (i.e. differentiating each stroke in the letter in order to resolve it and identify it), RSD is defined by the smallest visual angle that allows perception of the modulation on the RF patterns (measured as the angle subtended by the peak of the radial modulation and the unmodulated circle). For this reason, the logMAR values obtained for each test (hRSD and VA) were not directly comparable.

As well as VA, is was possible to compare the intersession repeatability of the hRSD test to the repeatability of other vision tests such as Pelli-Robson CS, reading acuity, reading speed and critical print size from a study by Aslam et al. (2014). This study was used for comparison because it included a similar group of at-risk eyes (n=83, fellow eye of patients with uniocular nAMD) to assess the intersession repeatability over two clinical appointments (four weeks apart). The repeatability statistics used by Aslam et al. (2014) were similar to those used in the present study including Bland-Altman plots, the coefficient of repeatability and the ICC. The results from Aslam et al. (2014) are summarised in Table 4.1, with the addition of the repeatability results for the hRSD test and VA from the present study. Overall, the hRSD test was less repeatable than VA (agreeing with our own results) but showed better repeatability than the other visual functions assessed in Aslam et al. (2014).

		B-A Limits of agreement	Repeatability coefficient	ICC (average meas.)
Aslam et al., 2014	Distance VA (letters)	14.1 to -16.5	14	0.91 (0.87 to 0.95)
	Pelli Robson CS (letters)	6.5 to -7.7	6.76	0.84 (0.76 to 0.90)
	Reading acuity	0.71 to -0.69	0.59	0.69 (0.55 to 0.80)
	Reading speed (words/min)	105.7 to -109.5	113.4	0.68 (0.53 to 0.79)
	Critical print size	0.61 to -0.69	0.63	0.68 (0.53 to 0.79)
Present study	hRSD (logMAR)	-0.28 to 0.27	0.33	0.85 (0.8 to 0.9)
	VA (logMAR)	-0.18 to 0.18	0.19	0.93 (0.91 to 0.95)

Table 4.1 Repeatability results from Aslam et al. (2014) for VA, CS, reading acuity, reading speed and critical print size as well as the repeatability results for hRSD and VA found in the present study.

Several reasons could explain why the repeatability of the hRSD test was found to be inferior to that of VA. Firstly, patients attending eye clinics are familiar with the procedure of VA testing, where the instructions are simply to "read down the letters on the chart". The hRSD test is also a relatively straightforward task, as will be discussed in section 4.5, but it is an unfamiliar one. It is therefore possible that elderly participants are less confident at performing the hRSD test compared to VA. If this was the case, a learning effect would be expected. Better scores would be obtained after a series of tests resulting from increasing familiarity with the test and the task. This was not seen in this study, where clinically stable participants performed consistently over five tests (section 3.4, discussed in 4.3.2). Secondly, it is possible that the hyperacuity levels reached by the hRSD test are intrinsically accompanied by a greater variability of measurements, merely because of the high precision of measurements. The higher cortical processes involved in shape discrimination (extra estriate cortical area V4, see section 1.2.1.2) might add noise to the measurements because more stages are involved in the processing of the shape compared to VA (which occurs mainly at the primary visual cortex, area V1). This might explain why reading tests were also less repeatable, as reading involves more than just fine vision, but also cognitive abilities and accurate eye movements, involving several other cortical processes.

In order to interpret the repeatability results accurately, one needs to look at the clinical context in which the measurements are to be used. A large dynamic range of hRSD scores (0.6 logMAR) was seen in the study by Wang et al. (2013) between early and advanced AMD. In view of this, a coefficient of repeatability of 0.3 logMAR seems acceptable for eyes at risk of developing nAMD. The difference between the average hRSD scores of the SEs (-0.56 logMAR) and the average hRSD scores in late AMD (-0.10 logMAR in Wang et al. 2013) is still comparably larger than what was found to be normal test variability. Therefore, although the repeatability of the hRSD test was inferior to that of VA, this might be compensated by the larger dynamic range of test results between non-nAMD and nAMD, the fact that the repeatability is not affected by AMD (compared to healthy controls) and the closer relationship of the hRSD test scores to retinal structural changes (section 3.5.2).

4.3.2 Stability over time

When repeated measurements are taken it is also important to evaluate whether there are any patterns in test results over time. When no clinical changes are seen, a good test should show no systematic change towards better (for example due to learning effects) or worse results (for example due to loss of motivation) and should instead stay at a stable level. If gradual changes occur, these could mask a real deterioration due to a gradual loss of vision. A gradual deterioration due to fatigue or loss of interest was not expected in this study, as the hRSD test was performed 1-2 months apart. However the presence of learning effects, where a participant obtains better results with the test over time due to an increased familiarity and practice with the test was investigated.

The results showed that the performance of the hRSD test over a period of approximately six months in clinically stable participants was constant, with no trends towards improvement or deterioration over time (section 3.4). Calculating individual regression lines for each participant allowed assessment of stability at an individual level, as opposed to obtaining a group mean. Now that it has been established that the hRSD test performs consistently in clinically stable participants, future longitudinal studies could assess the ability of the hRSD test to track retinal changes, for example from intermediate to late AMD, i.e. the performance with the test in patients with sight threatening disease progression.

4.4 Relationship between the hRSD test and other clinical tests

The results presented in section 3.5 covered the analysis on the relationship between the hRSD test and other clinical tests routinely used in AMD clinics: VA, CS and measurements of retinal structure with OCT. The intention was to find out whether participants with worse VA/CS would also have worse hRSD scores; and whether participants with more foveal structural changes in the retina showed worse hRSD scores.

4.4.1 Relationship with other visual function tests

The measurement of VA by means of a letter chart is the most widely used test to assess vision in ophthalmology. This method allows measuring the level of resolution at the fovea and is particularly useful when the main symptom is blurred vision. VA is used in AMD studies to assess the outcome of treatments (Holz et al. 2015, Chong 2016) as nAMD can severely disrupt VA. However new nAMD lesions can sometimes be subtle and located away from the fovea, initially sparing VA. In the case of early AMD, measuring function by means of VA is even more problematic because the worsening in VA caused by the early stages of AMD can be just a few letters (Klein et al. 1995).

The idea of global processing of RF patterns was initially introduced by Wilkinson et al. (1998), who suggested that the excellent ability to accurately recognise RF patters cannot be explained by local orientation or curvature analysis alone but instead by a global pooling of contour information at intermediate levels of form vision, processed in cortical area V4 (section 1.2.1.2). Since shape discrimination requires integration over a wider area of retina, the hRSD test could be a better indicator of macular function than VA, potentially capturing a more widespread disruption to the retinal mosaic.

The mean baseline VA of the SEs (0.06±0.13 logMAR) was similar to the VA seen in a group of participants with signs of early AMD (0.1 logMAR, Patel et al. 2008). The only previous report of the relationship between the hRSD test and VA was by Wang et al. (2013), which included a group of 37 patients with AMD. In the study

eye the hRSD test scores were highly correlated with VA ($r=0.69$, $p<0.0001$), with a slope (of the best fit line from a regression analysis of hRSD and VA) of 0.95. A slope that close to unity, suggested that changes in hRSD are comparable to those in VA (Wang et al. 2013).

The present study found no significant correlation between VA and hRSD scores, and in fact as can be seen from Figure 3.14, participants with normal VA in the SE (between -0.1 to 0.1 logMAR) showed a wide range of hRSD scores, from excellent to poor. This differs with the results from Wang et al. (2013), which could be due to the differences found between the two populations included. Whilst the present study excluded eyes with late AMD (GA or nAMD) and VA worse than 0.4 logMAR, the study by Wang et al. (2013) accepted participants with any level of AMD (including late AMD) and VA as poor as 0.7 logMAR. A wider range of VA might explain why a correlation was seen in Wang et al. (2013). In fact, when the correlation analysis was repeated for our FEs, which had no limits in VA (section 3.7) a moderate positive correlation was found.

A previous study using the desktop 2AFC version of the test (Wang et al. 2002) included a sample of participants that was comparable to the present study. Wang et al. (2002) included eyes with signs of early and intermediate AMD only, without late AMD and with VA better than 0.4 logMAR (i.e. similar to our SEs). Similarly to our results, Wang et al. (2002) did not find a correlation between the desktop RSD test and VA. The authors hypothesised that RSD might provide more information about the integrity of the retinal mosaic in AMD compared to VA, which our results support.

To examine this issue further, two participants were selected, one with a good hRSD score and the other with a poor hRSD score, but both with a VA of 0.1 logMAR. The OCT scans from these two participants (Figure 4.3) showed that the participant with the worse hRSD score (-0.21 logMAR) had a large elevation of the RPE in the foveal area whilst the participant with the better hRSD score (-0.76 logMAR) had a normal OCT scan at the foveal area. OCT scans from two further participants who had a VA of -0.12 logMAR were also evaluated (Figure 4.4). One had a hRSD score of -0.33 logMAR and the other showed a score of -0.82 logMAR. In this case, a subtle abnormality was seen near the foveal area in the OCT of the participant with poorer

hRSD score (abnormal material above RPE) and a normal OCT scan (although of overall poorer quality) was seen for the participant with better hRSD score. It indeed appears that the hRSD test could be more representative of the status of the retina at the foveal area than VA, making the dissociation between hRSD scores and VA a positive finding. More insight into this hypothesis is discussed in the following section, which aimed to investigate whether the hRSD test correlated with specific OCT features better than VA did.

With regards to the CS analysis, the results showed that the hRSD scores of a subsample of 34 SEs were not correlated with CS measurements. Interestingly, if age was not used as a covariate, the result of the correlation became statistically significant (section 3.5.1). Previous studies had found a statistically significant correlation between RSD and CS (Wang et al. 2002, Wang et al. 2013). On the one hand, it is possible that adding age as a covariate to our fairly small sample (n=34) might have reduced the statistical power of our correlation. However, on the other hand, not adjusting for age when there is a well-known effect of age on CS (section 1.1.3.2.2) could have strengthened the correlations seen in previous studies. The results from the CS analysis were not conclusive. Further studies assessing age-adjusted CS and hRSD with a larger sample size could clarify this issue.

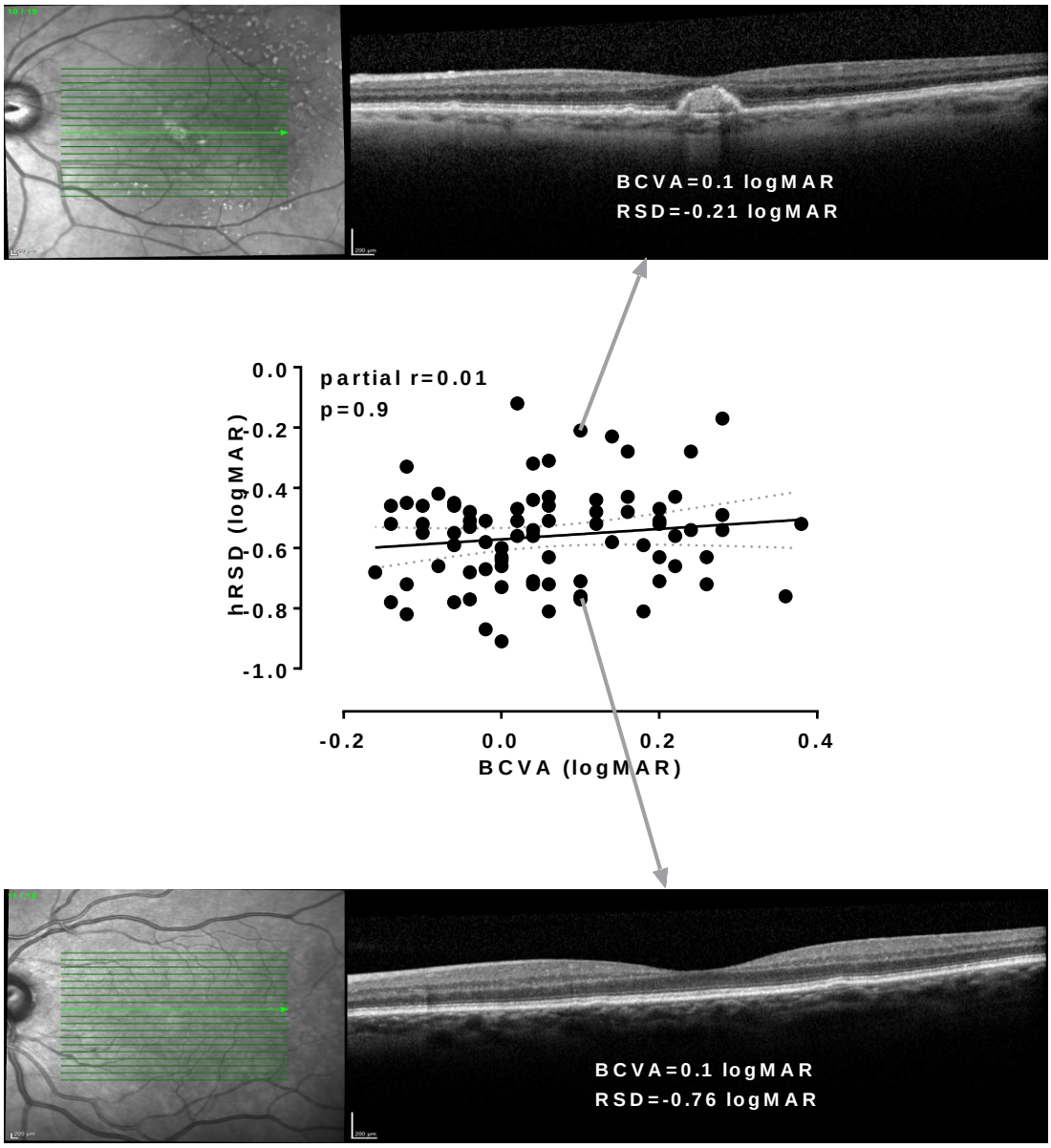

Figure 4.3 Scatterplot showing the relationship between VA and hRSD scores. For a given VA of 0.1 logMAR, two participants can obtain very different results with the hRSD test. The top OCT scan (participant with normal VA but poor hRSD score) shows a large elevation of the RPE in the foveal area whereas the bottom OCT scan (participant with normal VA and normal hRSD score) shows a normal OCT scan for this population.

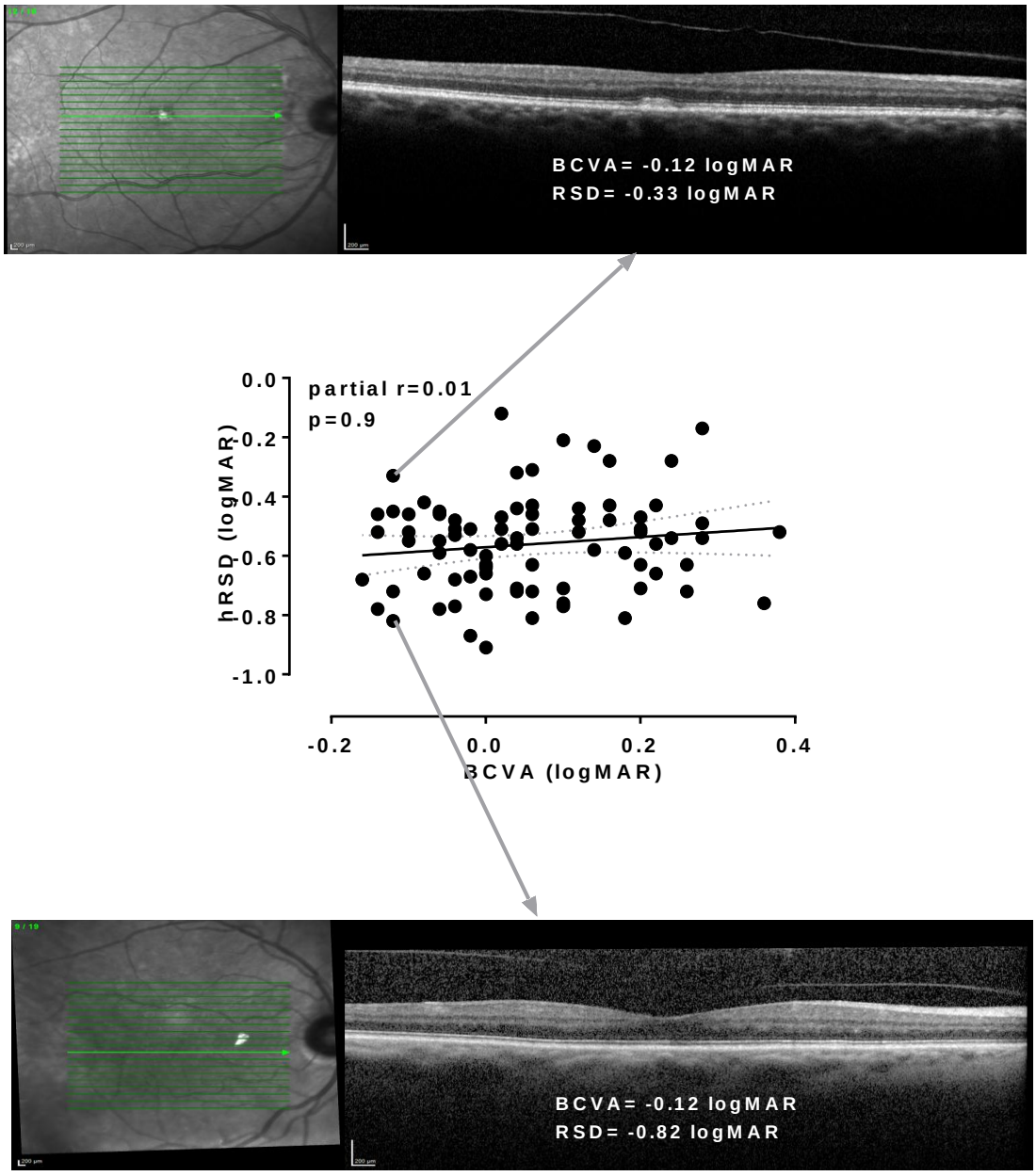

Figure 4.4 Scatterplot showing the relationship between VA and hRSD scores. Two OCT scans are shown for two participants with a given VA of -0.12 logMAR. The top OCT scan (participant with excellent VA but poor hRSD score) shows an abnormality above the RPE near the foveal area whereas the bottom OCT (participant with same VA and normal hRSD score) shows a normal OCT scan for this population.

4.4.2 Relationship with structural parameters

Many of the SEs in the present study were expected to have some degree of AMD-related retinal changes due to age and the presence of contralateral nAMD. These changes were assessed by SD-OCT. In particular, three SD-OCT features were graded: elevations of the RPE layer, photoreceptor layer abnormality and foveal thickness.

4.4.2.1 The relationship between large RPE elevations and the hRSD test

Drusen were evaluated in this study because they are the main feature of the early stages of AMD (section 1.1.2.2). A higher risk of progression towards nAMD exists when drusen are large, confluent and centrally located (Wang et al. 2003, Chew et al. 2014a). Whilst well-defined grading protocols exist for grading drusen using CFP (Davis et al. 2005), there is currently no standardised way of measuring drusen using OCT. In this study drusen were defined as separations of the RPE from Bruch's membrane or thickening of the RPE, covering both drusen and pigment epithelium detachments (Leuschen et al. 2013). In view of this definition, the terms elevation of the RPE and drusen have been used interchangeably throughout this thesis. Colour fundus photographs provide an en face image of the retina, allowing quantification of drusen size (measured in terms of drusen diameter) and the total area that they occupy. With OCT, these measurements are not directly obtained as the image provided is a cross-section of the retina. This requires a different kind of grading (section 1.1.3.1.1). The cross-sectional view provided by OCT also allows measuring the height of drusen, i.e. by how much the RPE is separated from Bruch's membrane. In the present study only the presence/absence of drusen located close to the fovea were measured, to assess whether this had any effect on hRSD scores at all. Only elevations of the RPE greater than 70µm in height were included because large drusen (i.e. those measuring more than 125µm in diameter using CFP) have been found to have an average height of 63µm assessed by SD-OCT (Hartmann et al. 2012). By doing this, we assume that mainly large elevations of the RPE were included in the analysis, which are better detected with SD-OCT (Jain et al. 2010)

and present a definite risk factor for the progression of the disease as opposed to small drusen that could represent normal ageing (Ferris et al. 2013).

Although drusen alone are unlikely to affect VA (Leuschen et al. 2013), this does not mean that visual function is completely normal in AMD patients with drusen. Wu et al. (2014a) demonstrated a significant inverse association between drusen (seen in SD-OCT) and retinal sensitivity (measured by microperimetry) in areas of the visual field corresponding with the location of drusen. The results from section 3.5.2.1 showed that the presence of large RPEEs in the foveal area was associated with higher (worse) hRSD scores. Although the difference was statistically significant, it was clinically very small (0.06 logMAR). This, confirmed by a small effect size from the ANCOVA analysis, means that the presence of large RPEEs only accounts for a small proportion of the total variation in hRSD scores seen in this population. This finding is expected, as the hRSD test was not expected to differentiate between eyes with and without large drusen at the fovea. Instead, the analysis was performed to investigate whether the wide range of hRSD scores seen in our sample could be explained, at least in part, by the presence of AMD-related features close to the fovea, namely large central drusen, which was indeed the case. What is more interesting from this analysis was the fact that no difference in VA was observed between those with and without large foveal RPEEs. This finding once again supports the hypothesis that measuring vision using the hRSD test is more representative of the status of the central retina, compared to VA, (Wang et al. 2002).

This study has shown that the presence of at least one large elevation of the RPE in the foveal area has an impact in the ability to detect deformations in RF patterns. For those participants who had large RPEEs, there was no relationship found between the height of the RPEE and the hRSD score, although this analysis, which was age adjusted, included only 37 participants, limiting the statistical power of the partial correlations. Future studies could make use of automatic segmentation of drusen (Chiu et al. 2012) to quantify the number and total volume of the elevations of the RPE. For example studies could assess the total volume of the RPE-drusen complex, as defined by Farsiu et al. (2014) and Folgar et al. (2016), to assess whether a greater extent of elevated RPE correlates with poorer hRSD scores. Only the foveal ring in the ETDRS grid (1 mm in diameter) was graded in this study to see if any effect was present at all and because the retinal image of RF patterns used in the hRSD test are

unlikely to fall on areas larger than the central 1 mm around the fovea due to the stimulus size and distance to the eye.

4.4.2.2 The relationship between disruption to the ellipsoid zone and the hRSD test

Since the integrity of the photoreceptor layer is associated with visual function (section 1.1.3.1.1) it was interesting to find out whether this predictor of visual function was at all related to the performance with the hRSD test.

In the early stages of AMD, drusen can cause structural and molecular abnormalities in the surrounding photoreceptors (Johnson et al. 2003) that compromise their function. The photoreceptors overlying drusen suffer a change in orientation and shape, which is seen in SD-OCT as a lack of reflectivity of the EZ. Hartmann et al. (2012) observed that larger drusen were related to a greater disruption to the EZ and that the integrity of the EZ over drusen changed over time as drusen progressed or regressed (Hartmann et al. 2012). Reticular pseudodrusen, a recently described type of drusenoid deposits located underneath the retina (section 1.1.2.2), can also cause a disruption to the overlying EZ (Zweifel et al. 2010, Mrejen et al. 2014, Querques et al. 2011).

It is not yet clear what causes the absence of hyper-reflectivity in the EZ seen in SD-OCT. It could be due to disorganisation, change in orientation or density or a complete absence of the photoreceptors or their segments. Whether EZD is accompanied by drusen, reticular pseudodrusen or by no drusen at all, disturbances to the photoreceptor mosaic can certainly have an effect on their function. Assuming that a greater amount of EZD corresponds with a greater disorganisation and dysfunction of the photoreceptors, then greater disruption to the EZ might be associated with a poorer radial shape discrimination ability in the hRSD test. The relationship between EZD and VA was also investigated for comparison.

In order to assess the presence and extent of EZD in the SEs, a grading protocol was developed (section 2.4.5). The results of the analysis revealed a small, yet statistically significant, worsening in hRSD scores in eyes with EZD. The difference was again, clinically small but it has value in explaining some of the variability seen

in hRSD scores in this sample. More importantly, and similarly to the case of the elevations of the RPE, there was no difference in VA between those with and without EZD.

Large RPEEs and EZD often occur simultaneously (Hartmann et al. 2012, Wu et al. 2014a) however drusen can sometimes have an intact EZ overlying them, and likewise the EZ can be disrupted without the presence of drusen beneath, for example if drusen have regressed (Hartmann et al. 2012). The mean difference in hRSD score between those with an intact foveal area (no RPEE, no EZD) and those with both RPEE and EZD was only marginally larger than the mean difference seen between those with and without EZD, irrespective of drusen (0.09 vs 0.11 logMAR). Wu et al. (2014a) suggested that combining measurements of EZ integrity and RPEE can provide a powerful predictor of retinal function (from multiple linear regression analysis). However combining the two did not reveal a bigger difference in hRSD scores in our study than using EZD alone.

It is worth recalling at this point that the protocol for obtaining SD-OCT used in this study included 19 B-scans. This number of B-scans is acceptable for clinical practice but is lower than used in previous studies using SD-OCT, where 49 B-scans (Wu et al. 2014a, Wu et al. 2014b), 96 B-scans (Hartmann et al. 2012) and up to 128 scans (Landa et al. 2011, Querques et al. 2012) were used. Having such a small number of scans and still finding a statistically significant difference makes these results even more noteworthy.

Overall, although the effect sizes were small, this study found evidence linking hRSD test performance to disruption of retinal structure at the fovea. In contrast, VA was completely insensitive to such foveal changes. This, again, suggests that hRSD test results might be more representative of the status of the central retina.

4.4.2.3 The relationship between central subfield thickness and the hRSD test

The final structural measurement assessed in this study was the foveal thickness. Measurements of foveal thickness are routinely taken for eyes that are being monitored for nAMD, as an increase in thickness may indicate fluid build-up, potentially requiring treatment. The CST is an average thickness measurement of all points falling within the central 1mm ETDRS ring. Obtaining the CST is quick as it is an automatic quantitative measurement provided by the SD-OCT software (section 2.4.3). Although manual corrections of the layer segmentation are sometimes needed for nAMD (Keane et al. 2009), this was not the case for the (non-nAMD) SEs.

Healthy eyes of adults (up to the 6^{th}-8^{th} decade of life) have a CST between 270-290µm (Grover et al. 2009, Wolf-Schnurrbusch et al. 2009, Chopovska et al. 2011) measured using Spectralis SD-OCT. A literature search indicates a lack of literature on normative foveal thickness, measured with Spectralis OCT, in subjects with early and intermediate AMD. A decrease in foveal thickness has been reported in early AMD compared to controls (Wood et al. 2011) however this was not using SD-OCT.

In the present study, the CST of the SEs was 282±23µm, which agrees with the CST previously reported for normal healthy participants. This suggests that there is no increase or decrease in the mean foveal thickness in the fellow eye of participants with unilateral nAMD, compared to healthy controls. There was no association between the hRSD test scores and CST for participants who had no macular oedema or thickening due to nAMD. Wang et al. (2013) reported a significant correlation between the hRSD test and CST ($r=0.58$, $p<0.0001$), when participants with all levels of AMD were included. As the author pointed out, the hRSD measurements varied significantly among participants with normal CST (Wang et al. 2013), which this study confirms.

In view of the findings discussed in this section, future studies assessing the relationship between the hRSD test and structural changes in SD-OCT in early/intermediate AMD should examine features such as presence of large drusen or photoreceptor abnormalities, as opposed to the overall thickness of the retina.

4.5 Usability of the hRSD test

The data discussed so far suggest that the hRSD test shows reasonable repeatability and that test results may closely mirror retinal structural change. However, if participants found the test difficult to understand or use, this would weaken its applicability, particularly away from the clinic or study settings, which is why collecting data on the usability of the test is important.

The hRSD test currently runs on handheld touch-screen devices (Apple iPod Touch, iPad and iPhone) and it has a user friendly interface appropriate for people with sight impairment, including large, high contrast print and instructions that are played audibly by the device. A study by Kaiser et al. (2013) has demonstrated that unsupervised use of the hRSD test in elderly patients with nAMD is feasible, with over 80% of participants complying with at least a daily test and only 1% not complying with at least weekly testing. In the present study, 79% of participants reported that they would consider using the test at home for monitoring their vision in between clinical appointments. However, participants were more likely to comply with weekly testing as opposed to daily testing. There are considerable differences between the study by Kaiser et al. (2013) and our study. Firstly, they were carried out in different countries (US vs UK) with different health systems and pressures. Our participants might not feel the need to use the hRSD test as often because they are already being monitored every 1-2 months during their standard NHS clinical appointments. The underlying type of AMD was also different, as Kaiser et al. (2013) included unilateral and bilateral nAMD, with the latter showing slightly better compliance (figures or mean difference not reported). Compliance is in fact a well-known problem of the AG. In a study based in the UK, where patients were provided with and instructed to use the AG on a weekly basis to help detect development of nAMD, only 29% of patients that had developed nAMD presented themselves to the dedicated emergency department because they noticed changes with the AG (Zaidi et al. 2004). An advantage of using an electronic device is that reminders can be set up to encourage the user to perform the test.

While a large proportion of participants in the current study had never used a touch screen device such as an iPod, tablet or touch-screen phone (37.5%) the majority

(82%) thought that the test was in general, very easy/easy to do. Overall, 99% of participants agreed that the instructions were easy/ very easy to understand and 100% felt that the device was easy/ very easy to handle. These results are similar to previous reports, where more than 90% of participants thought that the test was easy to use (Wang et al. 2013, Kaiser et al. 2013). It should be mentioned that filling in a questionnaire in the clinic instead of anonymously at home might bias responses. Although patients were encouraged to give an open and honest answer, some, especially the elderly, might respond more positively to the questions to please staff.

In the present study, no participants had to be excluded due to inability to understand or use the hRSD test. The apparent acceptability of the hRSD test contrasts with that of the PHP test (section 1.1.5). In the home-use version of the PHP (Foresee Home), users have to view the visual stimulus on a screen and manipulate a mouse that is out of their sight. In a study by Loewenstein et al. (2010), patients who did not pass a mouse tutorial to use the PHP test were excluded. Despite this, 13% of the recruited participants still had to be subsequently excluded due to unreliable results. In the prospective part of Loewenstein et al. (2010), in which experience with a computer mouse was part of the inclusion criteria, 15% of the participants were excluded due to not passing the tutorial and another 8% had unreliable results. Similarly, Chew et al. (2014b) reported that 15% of patients screened could not use the PHP test due to pre-existing visual field defects in their study eyes. Of those who were randomised into the PHP arm, 8% could not establish a baseline measurement and 14% returned the device before the end of the study (Chew et al. 2014b). A recent study reported that only 69.5% of a group of participants with intermediate AMD qualified to use the PHP test from home (Thomas et al. 2015). To qualify to use the test from home participants had to pass a reliability test (more than 70% of responses correct) and a qualification test (achieve a threshold score below 0.34, which is the threshold to differentiate nAMD from non-nAMD, Thomas et al. 2015).

Generally, the higher the proportion of participants who can use the test, the higher the number of people who could benefit from using it to potentially prevent vision loss. Users are more likely to produce reliable results with an uncomplicated device and task combination. In this respect, the hRSD test would be ideal to be used from the patients' homes due to its ease of use.

4.6 Limitations of the study

In the present study, all SEs were considered "at risk" of nAMD due to contralateral disease. However SEs were not stratified into early and intermediate AMD, known to have a different risk of progression (section 1.1.2.2). This was not possible because CFP was not available therefore established grading protocols (section 1.1.3.1.1) could not be used. Not enough information was available from the clinical records to categorise the SEs into early and intermediate AMD. Recorded information on SEs, where there was any, consisted of an assessment of the presence or absence of nAMD. This limitation was overcome by assessing retinal status by means of grading OCT scans.

Clinical stability was defined as being at risk of nAMD but without development of nAMD or any other sight threatening retinal pathology. OCT scans at baseline and study end were checked for the absence of clinically significant changes but were not formally graded, which could have potentially overlooked small structural changes (e.g. in drusen number/ size/ volume). Similarly, worsening of lens opacities (progression of cataracts) was not assessed, which could have affected visual function. As discussed in section 1.2.2, the RF patterns used in the hRSD test are unlikely to be affected by lens opacities given their low spatial frequency. In fact no difference in hRSD score was seen between phakic and pseudophakic participants (section 3.1.1).

The repeatability results reported in this study apply to the in-clinic use of the hRSD test, under supervision. Before starting the test the examiner reminded the participant how to use the test and answered any questions they might have. It must not be assumed that the repeatability would remain the same when the test is used without supervision.

Finally, a general limitation of this study is the fact that the cognitive function of our participants was not assessed. A large range of cognitive statuses can be expected from an elderly sample of patients attending hospital. Similarly, the presence of other comorbidities could affect the general health and general mood of the patient. These limitations not only affect the hRSD test but every subjective measurement of vision.

4.7 Potential uses of the hRSD test and areas for future research

There are at least two potential uses of the hRSD test in the context of the detection of nAMD. One would be to include the hRSD test in a screening programme for AMD (this currently does not exist) and the other would be to use the test from home to detect the development or reactivation of nAMD lesions. In either scenario, the hRSD test would be used as a means of triggering the need for the patient to undertake further diagnostic tests. For this purpose, it is important for the test to show a high sensitivity, even at the expense of a higher rate of false positives. Over detecting changes potentially related to nAMD would be preferable than missing the detection of nAMD.

If the hRSD test was used in the context of screening, high risk asymptomatic individuals would be tested in order to detect potential signs of nAMD. This could be done by means of an AMD specific screening program or at opportunistic screening taking place during visits to the GP or opticians. The introduction of a screening programme for AMD has been studied by the Health Technology Assessment Programme (Karnon et al. 2008). A screening programme for AMD was, at the time, considered not to be cost-effective as there were many areas of uncertainty, including the effectiveness of the treatment. This however has changed since the introduction of anti-VEGF injections. Other areas of uncertainty were related to the effectiveness of preventive interventions in early AMD. Since then, more research has been carried out on the long term effects of the AREDS vitamin supplements (Chew et al. 2013a) and modifications to the original formula (Chew et al. 2013b). Another area of uncertainty identified with their model was around the effectiveness of the screening test. Experts agreed that covering one eye and observing an object with fixed horizontal lines, for example a window frame (the environmental Amsler Grid) would be an appropriate way to self-test vision (Karnon et al. 2008). Should disturbances, blurring or distortion be detected by self-testing from home, individuals should see an Optometrist who could examine the fundus for the presence of signs of nAMD. Optometrists can refer suspects cases of nAMD directly to specialised units with facilities for the investigation and treatment of nAMD by means of a rapid referral pathway (The Royal College of Ophthalmologists 2013).

A problem of using a subjective test (such as the hRSD test) in screening is that other sight threatening retinal pathologies could affect the test results. The extent to which pathologies such as ERM, VMT or GA affect performance with the hRSD test is currently not known. More likely, the hRSD test could be a useful screening tool to detect sight threatening maculopathies as opposed to being nAMD specific.

Alternatively, there are several properties of the hRSD test that make it suitable for home monitoring of vision and detection of nAMD. It is portable and deployed on an inexpensive platform that does not require regular calibration or maintenance and it does not require strict testing conditions, such as specific levels of room illumination. The instructions are simple, written on the screen and also played audibly by the device. The problem of non-compliance might be reduced when using electronic devices as notifications can be sent to the patient when a certain length of time has passed without using the test. Finally, if internet connection or access to Wi-Fi is available, the results of the test could be sent to the clinician for interpretation, lessening the risk of misinterpretation by the patient. As the population ages, it is more likely that patients themselves would own a smartphone with internet connection.

As well as aiding in the initial detection of nAMD, the hRSD test could potentially be used to monitor the reactivation of pre-existing neovascular membranes. The hRSD scores seen in many of the nAMD eyes included in the present study were already quite poor, potentially affecting the capability of the test to detect any further worsening in scores. There were a proportion of participants (14%) who could not complete the hRSD test at all with their nAMD eye due to poor vision. This would potentially limit the use of the hRSD test for monitoring re-activation of nAMD to those who can produce a baseline with the test. Future research using FFA (the gold standard for diagnosing nAMD, section 1.1.3.1.2) could explore whether the performance with the test in nAMD eyes is limited by factors such as the lesion size and location.

Although the hRSD test has a number of advantages over the alternatives, it is the accuracy of the test to detect the development of nAMD that has not yet been established. In this study, the eyes with nAMD had a significantly worse score than the SEs. Although the mean difference of -0.39 logMAR between study and treated

eyes was statistically significant, a considerable overlap can be seen on the histograms in Figure 3.29, which illustrates the challenge in differentiating between these two groups by means of a single cut-off value.

The Early Detection in Macular Disease (EDiMaD) Study is an extension of the work presented in this thesis aiming to establish the sensitivity and specificity of the hRSD test for the detection of new nAMD lesions. The EDiMaD study enrolled 202 participants, which were followed up longitudinally for a period of up to two years (data collection finished in January 2017). Of the 202 participants without nAMD in their SE at baseline, 18 (9%) developed nAMD in their SE (confirmed by masked FFA grading). The preliminary results of the EDiMaD study show a sensitivity and specificity of the hRSD test to detect the development of nAMD of 78% and 53% for a cut-off value of -0.60 logMAR (Figure 4.5, unpublished results presented at ARVO, May 2017). By using a longitudinal study design, the performance of the hRSD test was assessed at the time of (or shortly after) the initial appearance of CNV. At this point, many neovascular lesions are small and parafoveal, potentially causing little functional changes. This might explain why the sensitivity and specificity of the hRSD test to detect new nAMD were lower than reported by Wang et al. (2011) who used a cross-sectional case control design with participants with established nAMD (sensitivity and specificity of 89% and 79% for a cut-off value of -0.37 logMAR, Wang et al. 2011).

Using the same longitudinal design of following up the unaffected eye of participants with nAMD, a study by Do et al. (2012) has assessed the diagnostic accuracy of the AG and the PHP test (section 1.1.5). The hRSD test assessed longitudinally appears to have similar sensitivity to the PHP test and better sensitivity than the AG to detect the development of nAMD (Table 4.2). In contrast to Do et al. 2012, the 95% CI of the sensitivity obtained in the EDiMaD study was narrower and the lower limit of the 95% CI was above 50%. In view of these promising results, future studies could assess whether using the hRSD test prompts early diagnosis of nAMD and whether this ultimately results in better visual outcomes. Ultimately, a parallel group study assessing the different tests (as well as a control arm without any monitoring test but the presentation of visual symptoms) would be useful to establish whether any of these self-administered tests, used remotely is better than the others.

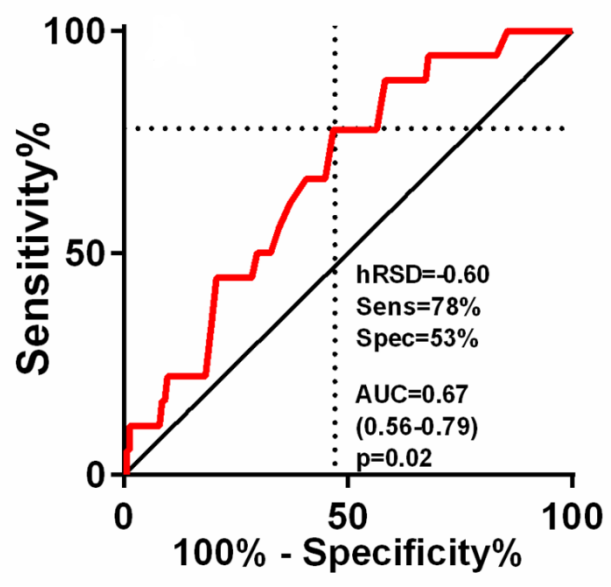

Figure 4.5 ROC curve showing the sensitivity and specificity of the hRSD test for the detection of new nAMD. Unpublished results from the EDiMaD study, presented at ARVO, May 2017.

Study	Test	Sensitivity (%)	95% CI (%)
Do et al. (2012)	AG	50	19-81
	PHP	70	35-93
EDiMaD study	hRSD	78	52-94

Table 4.2 Sensitivity of the AG, the PHP test (Do et al. 2012) and the hRSD test (EDiMaD study)

Chapter 5 Research summary and Conclusions

The results of this study contribute to the ongoing assessment and external validation of the hRSD test in AMD. A number of recommendations for future research flow from these results.

Firstly, the average and range of hRSD scores seen in our clinically stable participants provide comparative data for future studies assessing a similar population. Although older age does not seem to have an impact on the hRSD test scores in healthy controls (section 1.2.2), this study has shown that this is not the case for AMD participants, for whom the deterioration of hRSD scores in older age was significant. Future studies assessing the hRSD test in AMD participants should take this into account and adjust their analysis accordingly.

Secondly, in view of the repeatability results (assessed for repeated measurements over a period of approximately six months) a change of 0.3 logMAR for a given participant is proposed as a clinically significant change in hRSD scores. This value should be validated by future longitudinal studies.

Thirdly, the lack of relationship between RSD and VA along with the significant correlation found between foveal structural changes and RSD, not seen for VA, suggest that the hRSD test might provide a better functional measurement of the structural integrity of the foveal area. The hRSD test should be considered as a clinically friendly measure of function in future studies and trials assessing the early stages of AMD.

The OCT grading protocol, created specifically for this study, allows measuring the extent of photoreceptor layer disruption over a particular area as a percentage of the B-scans affected as opposed to a simple yes/no assessment. Further development in this area of research could consist of combining this approach with the type of grading used by Hartmann et al. (2012) where the severity of EZD was assessed. Assessing both severity and extent of EZD could be complex but it may provide a good structural variable to assess correlation with any visual function test. The results found here also encourage investigating the relationship between the hRSD

test and drusen in more detail, for example by using the calculation of the RPE-drusen complex.

There is an evident benefit from closely monitoring high risk patients by means of a test such as the hRSD test. A longitudinal study by Lott et al. (2016) is under way, in which the hRSD is being compared to a battery of other visual function tests, to assess their ability to predict progression towards advanced AMD. Future studies will be needed to determine whether remote monitoring with the hRSD test results in better visual outcomes for patients developing nAMD and whether the hRSD test is as efficient or superior to alternative tests. The feasibility and cost-effectiveness of incorporating such monitoring strategy into the NHS in the UK would also need to be considered.

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Appendixes

Appendix 1: Classification of CNV on the basis of FFA and ICG. These definitions are used in the AMD clinic where the current study was carried out. PED: pigment epithelium detachment. IRN: intra retinal neovascularisation. SRN: subretinal neovascularisation.

Lesion type		Definition
Classic CNV		Well demarcated area of hyper fluorescence that appears in the early transit phase and increases in intensity and size throughout the FFA. Progressive pooling and leakage in late phase.
Occult CNV	Fibrovascular pigment epithelium detachment (PED)	Area of irregular elevation of the RPE and stippled early hyper fluorescence (within 1-2'). Dye pooling in late phase.
	Late leakage of undetermined source	Poorly demarcated area of hyper fluorescence that appears late in the FFA (after 2') with some late leakage and dye pooling
IPCV		Early ICG: hypo fluorescence of the lesion area and surroundings Late ICG: Reversal of the fluorescence Very late ICG: disappearance of the dye
RAP		Focal hyperfluorescence spot (hot spot) in all ICG stages. A vessel might be seen reaching the hyperfluorescent area

Appendix 2: Percentage of participants maintaining VA (less than 15 letters lost) and improving VA (more than 15 letters gained) in each clinical trial. *available as decrease of 5-14 letters for the CATT trial (Martin et al. 2011), n/a: not available.

Trial	Duration (months)	Treatment and Control	Regime	Less than 15 letters lost (%)	More than 15 letters gained (%)
MARINA	24	Ranibizumab		92.0	26.1
		0.3 or 0.5		90.0	33.3
		Placebo		52.9	3.8
ANCHOR	12	Ranibizumab		94.3	35.7
				96.4	40.3
		PDT		64.3	5.6
CATT	12	Ranibizumab	Monthly	6.7*	34.2
		0.5	As needed	6.8*	31.3
		Bevacizumab	Monthly	8.1*	24.9
		1.25	As needed	8.5*	28.0
IVAN	24	Ranibizumab	Monthly	n/a	n/a
		0.5	As needed	n/a	n/a
		Bevacizumab	Monthly	n/a	n/a
		1.25	As needed	n/a	n/a
VIEW 1 and 2	12	Ranibizumab 0.5	Monthly	94.4	30.9
		Aflibercept 0.5g	Monthly	95.9	24.9
		Aflibercept 2g	Monthly	95.1	37.5
		Aflibercept 2g	Monthly for 3m, then bi-monthly	95.1	30.6

Appendix 3: Patient information leaflet and informed consent form for the EDiMaD study, approved by North West Preston NRES committee (reference number 13/NW/0449).

Patient information leaflet and inform consent form

Study Title: Early Detection in Macular Disease

Participant Information Sheet Version: EDPI 1.2 Date:2/7/13

Principal Investigator: Dr Paul C. Knox
Eye & Vision Science

Tel: 0151 794 5736
Email: pcknox@liv.ac.uk

You are being invited to participate in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and feel free to ask us if you would like more information or if there is anything that you do not understand. Please also feel free to discuss this with friends or relatives if you wish. You do not have to accept this invitation and should only agree to take part if you want to.

What is the purpose of the study?

We wish to find out whether an eye test presented on a small electronic device (an Apple Ipod touch) is able to detect the very earliest signs of wet age-related macular degeneration (AMD)

Why have I been chosen to take part?

We have invited you to take part because you already have wet AMD in one eye which is being treated.

Do I have to take part?

Taking part is entirely voluntary and even if you decide to take part you are free to withdraw at anytime without explanation and without incurring any

disadvantage. If you choose not to take part, or if you later withdraw, your medical care will not be affected.

What will happen if I take part?

If you decide to take part, you will continue to attend for your AMD assessment and treatment in the normal way. Each visit will begin by completing the new test – this will take a maximum of 20 minutes and will often be quicker. The test runs on an Ipod Touch, a device about the size of a mobile phone. On the device screen you will see three large circles. One of these will be slightly distorted with “bumps” appearing round its edge. You will simply touch the screen of the device to say which of the three circles is distorted. Over a number of attempts the size of the distortions will get smaller, until you will have to guess which circle is distorted. When the device has worked out how big the distortions have to be for you to see them, it will calculate a score which we will record. Each eye is done in turn with the other eye patched during the test. Once the test is completed, you will continue as normal with your other assessments and treatment, under the care of your normal doctor.

Expenses and / or payments

There are no payments available for taking part in this study.

Are there any risks in taking part?

There are no risks posed to you by doing the new test.

Are there any benefits in taking part?

There will be no direct benefits to you from taking part. However, our findings may benefit future patients.

What if I am unhappy or if there is a problem?

If you are unhappy, or if there is a problem, please let us know. You can contact the Principal Investigator, Dr Knox (0151 794 5736, pcknox@liv.ac.uk) and he will try to help. If you remain unhappy or have a complaint which you feel you cannot deal with in this way, you can contact the University of Liverpool Research Governance Officer on 0151 794 8290 (ethics@liv.ac.uk). When contacting the Research Governance Officer, please provide details of the title of the study, the researcher(s) involved, and the details of the complaint you wish to make.

Will my participation be kept confidential?

The results of the tests will be kept on secure, password protected computers. The test results will be kept for up to 10 years. Only anonymised data, that cannot be related to you personally, will be released or discussed publicly at scientific meeting or in research publications.

Will my taking part be covered by an insurance scheme?
This research is covered by both University of Liverpool and NHS insurance policies.

What will happen to the results of the study?

Eventually the results of the study will be published in the form of abstracts at scientific meetings and research papers.

What will happen if I want to stop taking part?

You can withdraw from this research study at anytime, without explanation. If data has already been collected, we will ask you if we can continue to use it.

Who has reviewed the study?
This study has been reviewed by the NRES Committee North West – Preston.

Who can I contact if I have further questions?

Principal Investigator:
Dr Paul C. Knox, 0151 794 5736, pcknox@liv.ac.uk

Thank you for reading this.

CONSENT FORM

Title of Research Project: **Early Detection in Macular Disease**

Name of Principal Investigator: **Dr Paul C. Knox**

Version: 1.2
Date: 2/7/2013

Please initial box

1. I confirm that I have read and understood the Participant Information sheet (Version: EDPI 1.2; date: 2/7/2013) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected. ☐
3. I give permission for my medical notes to be reviewed as part of this study. ☐
4. I understand that medical notes and data collected from the study may be looked at by regulatory authorities and by persons from the Trust where it is relevant to my taking part in this research. I give permission for these individuals to have access to this information. ☐
5. I agree to take part in the above study. ☐

_____	_____	_____
Participant Name	Date	Signature

_____	_____	_____
Name of Person taking consent	Date	Signature

See over for the contact details of Principal Investigator.

Dr Paul C. Knox
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Thompson Yates Building
Brownlow Hill
Liverpool L69 3GB

Tel: 0151 794 5736
Email:pcknox@liv.ac.uk

Participant Number:

Appendix 4: Usability questionnaire given to study participants at the end of the study

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Dear participant,

Thank you for taking part in the study. We would appreciate if you could complete this short questionnaire to tell us about your experience with the test.

If you have any questions, please ask us.

1. **Before the study, how often had you used a touch screen hand-held device (such as phone, tablet or iPod)?**

   ☐ Very often

   ☐ Often

   ☐ Occasionally

   ☐ Never

   ☐ Cannot decide

2. **Generally, how easy was the test to do?**

   ☐ Very easy

   ☐ Easy

   ☐ Difficult

☐ Very difficult

☐ Cannot decide

3. **How easy were the instructions written on the screen to understand?**

   ☐ Very easy

   ☐ Easy

   ☐ Difficult

   ☐ Very difficult

   ☐ Cannot decide

   If not easy please specify:

4. **How easy was it to handle the device? (for example holding the iPod, pressing on the screen…)**

   ☐ Very easy

   ☐ Easy

   ☐ Difficult

   ☐ Very difficult

   ☐ Cannot decide

   If not easy please specify:

5. **If you had this test at home, would you consider using it for self-monitoring your vision between clinic appointments?**

   ☐ Yes

   ☐ No

   ☐ Cannot decide

6. **If you answered Yes to Question 5. How often would you consider doing the test at home?**

   ☐ Daily

   ☐ Weekly

   ☐ Every 2 weeks

   ☐ Monthly

7. **Any other comments**

Thank you.

List of publications

Papers

Pitrelli Vazquez, N. and Knox, P. C. (2015) 'Assessment of visual distortions in age-related macular degeneration: emergence of new approaches', *British and Irish Orthoptics Journal*, 12, 9-15

Ku, J. Y., Milling, A. F., **Pitrelli Vazquez, N.** and Knox, P. C. (2016) 'Performance, usability and comparison of two versions of a new macular vision test: the handheld Radial Shape Discrimination test', *PeerJ*, 4, e2650

Conference abstracts

Knox, P. C., **Pitrelli Vazquez, N.**, Harding, S. P. and Heimann, H., Czanner, G. (2017) 'Diagnostic performance of the handheld radial shape discrimination test for detecting recent onset neovascular age-related macular degeneration', *ARVO Annual Meeting Abstract, Investigative Ophthalmology & Visual Science*, Poster 2345

Pitrelli Vazquez, N., Harding, S. P. and Heimann, H., Knox, P. C. (2016) 'Relationship between foveal ellipsoid zone integrity and central visual function in age related macular degeneration', *ARVO Annual Meeting Abstract, Investigative Ophthalmology & Visual Science*, 57(12), 4969

Knox, P. C., **Pitrelli Vazquez, N.**, Harding, S. P. and Heimann, H. (2016) 'Detection of new neovascular AMD in at-risk eyes using a handheld radial shape discrimination test in a clinical population', *ARVO Annual Meeting Abstract, Investigative Ophthalmology & Visual Science*, 57(12), 2663

Pitrelli Vazquez, N., Harding, S. P. and Heimann, H., Knox, P. C. (2015) 'Stability of performance of a handheld radial shape discrimination test in patients at risk of developing neovascular AMD', *ARVO Annual Meeting Abstract, Investigative Ophthalmology & Visual Science*, 56(7), 2220

Pitrelli Vazquez, N., Knox, P. C. (2014) 'A handheld radial shape discrimination hyperacuity test: assessment of variability in a clinical population' *Ophthalmic and Physiological Optics*, 34, 639